2016

Carbon dioxide receptor genes and their expression profile in *Diabrotica virgifera virgifera*

Thais B. Rodrigues
Ministry of Education of Brazil, Brasília, thaisbarros.bio@gmail.com

Etsuko N. Moriyama
University of Nebraska-Lincoln, emoriyama2@unl.edu

Hang Wang
University of Nebraska-Lincoln

Chitvan Khajuria
University of Nebraska-Lincoln, ckhajuria2@unl.edu

Blair D. Siegfried
University of Nebraska-Lincoln, bsiegfried1@ufl.edu

BMC Research Notes

RESEARCH ARTICLE

Open Access

CrossMark

Carbon dioxide receptor genes and their expression profile in *Diabrotica virgifera virgifera*

Thais B. Rodrigues[1,2*], Etsuko N. Moriyama[3], Hang Wang[4], Chitvan Khajuria[4] and Blair D. Siegfried[4]

Abstract

Background: *Diabrotica virgifera virgifera*, western corn rootworm, is one of the most devastating species in North America. *D. v. virgifera* neonates crawl through the soil to locate the roots on which they feed. Carbon dioxide (CO_2) is one of the important volatile cues that attract *D. v. virgifera* larvae to roots.

Results: In this study, we identified three putative *D. v. virgifera* gustatory receptor genes (*Dvv_Gr1*, *Dvv_Gr2*, and *Dvv_Gr3*). Phylogenetic analyses confirmed their orthologous relationships with known insect CO_2 receptor genes from *Drosophila*, mosquitoes, and *Tribolium*. The phylogenetic reconstruction of insect CO_2 receptor proteins and the gene expression profiles were analyzed. Quantitative analysis of gene expression indicated that the patterns of expression of these three candidate genes vary among larval tissues (i.e., head, integument, fat body, and midgut) and different development stages (i.e., egg, three larval stages, adult male and female).

Conclusion: The *Dvv_Gr2* gene exhibited highest expression in heads and neonates, suggesting its importance in allowing neonate larvae to orient to its host plant. Similar expression patterns across tissues and developmental stages for *Dvv_Gr1* and *Dvv_Gr3* suggest a potentially different role. Findings from this study will allow further exploration of the functional role of specific CO_2 receptor proteins in *D. v. virgifera*.

Keywords: *D. v. virgifera*, Western corn rootworm, CO_2 receptors, Gustatory receptors, qRT-PCR, Phylogenetic analyses, Gr

Background

Many insects are able to detect carbon dioxide (CO_2) in the environment for a variety of purposes, such as the location of their vertebrate hosts by hematophagous insects [1] evaluation of floral quality by lepidopterans [2], and the regulation of potentially lethal CO_2 concentrations by social insects in colonies [3]. Insect herbivores, such as the western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), use CO_2 as an important host finding cue [4].

Diabrotica virgifera virgifera is one of the most devastating corn pests in North America [5]. The common name, western corn rootworm, refers to the larval life stage that feeds on corn roots, which moves through the soil to find roots of a suitable host [6]. Neonates that

hatch in the spring from overwintering eggs must crawl through the soil to locate the roots on which they feed. It has been suggested that CO_2 emitted by corn roots is one of the important volatile cues that attract *D. v. virgifera* larvae to corn roots [4].

In general, a chemical signal from the environment is converted to an electrical signal that can be interpreted by the insect nervous system due the binding of a ligand to a receptor protein [7]. Most of these chemosensory proteins are recognized as members of two evolutionarily related chemosensory receptor families; the odorant receptors (ORs) and gustatory receptors (GRs) [8–10]. Three groups of GR receptors (GR1–3) appear to contribute to the detection of CO_2 in insects [11]. In *Drosophila melanogaster*, *DmGR21a* and *DmGR63a* (belonging to the Gr1 and Gr3 groups, respectively) are co-expressed in olfactory receptor neurons of the sensilla on the antennae that are sensitive to CO_2 and both proteins are required for CO_2 detection [12–14]. In mosquitos and other insects, a third group of *Gr* genes is also identified

*Correspondence: thaisbarros.bio@gmail.com
[1] CAPES Foundation, Ministry of Education of Brazil, Brasília, DF 70040-020, Brazil
Full list of author information is available at the end of the article

Rodrigues *et al. BMC Res Notes* (2016) 9:18

Page 2 of 7

and designated as *Gr2* [11]. Although all three *Gr* genes are expressed in the sensilla located on the maxillary palps in mosquitoes, expression of only *Gr1* and *Gr3* is required for CO_2 perception [13, 15, 16]. The orthologs of three CO_2 receptor genes have been identified from a lepidopteran species (the silk moth *Bombyx mori*) and from several coleopteran species (the red flour beetle *Tribolium castaneum*, the mountain pine beetle *Dendroctonus ponderosae*, and the European spruce bark beetle *Ips typographus*) [11, 17]. Interestingly, in *D. ponderosae*, the *Gr2* gene was only identified from the draft genome and from larval RNAseq data, but not from antennal transcriptomes [17, 18].

In this study, we have taken significant steps to further investigate CO_2 receptor genes in *D. v. virgifera*. We identified three putative CO_2 receptor genes from a larval *D. v. virgifera* transcriptome [19] and we characterized the expression of those genes in different *D. v. virgifera* tissues and developmental stages.

Methods

Identification of CO_2 receptor genes from the D. v. virgifera transcriptomes

Protein sequences of the following CO_2 receptor genes were obtained from the National Center for Biotechnology Information database: DmGr21a (NM_078724.6) and DmGr63a (NM_001144411.1) from *D. melanogaster*, GPRGR24 (DQ989013.1), GPRGR22 (DQ989011.1), and GPRGR23 (XM_312786.3) from *Anopheles gambiae*, and TcGr1 (AM292331.2), TcGr2 (XM_008193301.1), and TcGr3 (XM_001814609.2) from *T. castaneum*. These protein sequences were used as the queries for tblastn similarity searches [20] against the combined transcriptome obtained from *D. v. virgifera* eggs, neonates, and midgut of 3rd instar larvae [19] with 1×10^{-100} as the E-value threshold to identify CO_2 receptor gene candidates in *D. v. virgifera*.

In order to confirm our assembled CO_2 receptor transcript sequences and examine their exon–intron structures, we also compared the protein sequences of the three CO_2 receptor candidates we obtained against the draft *D. v. virgifera* genome sequences (Hugh M. Robertson, personal communication) using tblastn similarity search. Prediction of membrane protein topology was achieved using TOPCONS [21].

Phylogenetic reconstruction of insect CO_2 receptor proteins

Multiple alignments of CO_2 receptor protein sequences were generated using MAFFT (ver. 7.215) with the L-INS-i algorithm [22]. The maximum-likelihood phylogenetic tree was reconstructed using PhyML (ver. 3.0)

[23] with the LG substitution model. Non-parametric bootstrap analysis was performed with 1000 pseudoreplicates [24].

Expression studies of the three Dvv_Gr genes

Insect

The adults and eggs of a non-diapause strain of *D. v. virgifera* used in this study were purchased from Crop Characteristics (Farmington, MN). The adults were held in rearing cages with artificial diet and maintained in a growth chamber with 23 ± 1 °C and 75 ± 5 % relative humidity. The freshly laid eggs received in petri dish were wrapped with foil and kept in an incubator at 27 ± 1 °C and 75 ± 5 % relative humidity until hatching.

Sample collection

The gene expression profiles of the three putative CO_2 receptors genes were analyzed in two different experiments involving four different tissues and six developmental stages. Five 3rd instars were dissected for samples from integument, midgut, fat body and head with thorax. The same tissues from five 3rd instar larvae were pooled as a single replicate. All collected tissues and whole bodies from different development stages were snap-frozen in liquid nitrogen and stored at −80 °C until used. The samples for different development stages included pooled samples of eggs, 1st (30 larvae), 2nd (15 larvae) and 3rd (6 larvae) instar, and individual female and male adults. Each treatment condition was replicated three times.

RNA extraction and cDNA synthesis

Total RNA was extracted using RNeasy Mini Kit (Qiagen) according to the manufacture's instructions. The RNA integrity was confirmed on 1 % agarose electrophoresis gels and NanoDrop-1000 (Thermo) before cDNA synthesis. RNA (1000 ng) from each sample was used to synthesize the cDNA using the QuantiTect Reverse Transcription kit (QIAGEN) according to manufacturer's instructions. The cDNAs were quantified using a NanoDrop-1000 and stored in −20 °C until used.

Primer design and efficiency test

Based on the nucleotide sequences of the three *Dvv_Gr* genes, as well as two reference genes, EF1a (elongation factor 1a) and beta-actin [25], the primers for qPCR were designed using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/). The primer efficiency test (E) and correlation coefficients (R^2) were calculated and qRT-PCR assays were performed using Fast SYBR Green Master Mix (Applied Biosystems, Cat. 4385612) on Applied Biosystems® 7500 Real-Time PCR Systems at default setting (Table 1).

Rodrigues *et al. BMC Res Notes* (2016) 9:18

Page 3 of 7

Table 1 General information of the primers for qPCR analyzes

Gene name	Primer Sequence (5′–3′)	Amplicon (bp)	E (%)	R^2
Dvv_Gr1	Forward: GTGGCACAG CATTGCTTA	221	94	0.970
	Reverse: CTATACGCCCT GCCCAAC			
Dvv_Gr2	Forward: GAACTAAGC GAGCTCCTCCA	192	108.8	0.989
	Reverse: CAGAAGCACCAT GCAATACG			
Dvv_Gr3	Forward: CTGGATGAAT GACCATGCAC	184	104.6	0.992
	Reverse: ATCCTCGGGGAT GCTTATCT			

E amplification efficiency, R^2 correlation coefficients

Real-time quantitative PCR (qRT-PCR) and data analysis

The qPCR experiments were conducted with SYBR Green PCR Master Mix kit following the manufacturer's instructions. Briefly, the PCR mixture contained 1 μL synthesized cDNA (~35 ng), 0.2 μL of each primer (10 μM), 5 μL of the SYBR green PCR master mix and 3.6 μL of ddH$_2$O. All reactions were carried out in triplicate per template in a final volume of 10 μL. qRT-PCR reactions were performed on the 7500 Fast Real-Time PCR system (Applied Biosystems) with the following cycling conditions: one cycle at 95 °C (20 s), followed by 40 cycles of denaturation at 95 °C (3 s), annealing and extension at 60 °C for 30 s. At the end of each qRT-PCR reaction, a melting curve was generated to confirm a single peak and rule out the possibility of primer-dimer and non-specific product formation. The EF1a (elongation factor 1a) and actin genes were used as endogenous controls for tissue and stage experiments, respectively [25]. Third instar larvae were selected as reference stage for comparisons in both experiments.

The $2^{-\Delta\Delta Ct}$ method [26] was used to calculate the relative expression level of target gene in the samples as compared to control sample. The one-way analysis of variance (ANOVA) was used for statistical analysis and Tukey test (at $P < 0.05$) for statistical significance with Sigma Plot Program (version 12.0).

Results

Identification of D. v. virgifera CO_2 receptor genes

Three CO_2 receptor gene candidates were identified from the combined transcriptome assembled from egg, neonates, and midgut of 3rd instar larvae from *D. v. virgifera* [19]. The maximum-likelihood phylogenetic analysis at the amino-acid level including CO_2 receptors from *D. melanogaster*, *A. gambiae*, and *T. castaneum* showed clear orthologous relationships for the three candidate genes (Fig. 1). We therefore named these *D. v. virgifera* genes as *Dvv_Gr1*, *Dvv_Gr2*, and *Dvv_Gr3* following the convention proposed by Robertson and Kent [11]. All three CO_2 receptor proteins were predicted to have seven transmembrane regions with intercellular N-terminals. This topology, which is opposite to those of the regular 7-transmembrane G-protein coupled receptors, is consistent with what has been reported for other insect chemoreceptors [27].

Structures of D. v. virgifera CO_2 receptor genes

We confirmed the assembled sequences of the three CO_2 receptor transcripts against the draft *D. v. virgifera* genome sequences (Hugh M. Robertson, personal communication). This comparison also enabled us to identify intron–exon structures of each CO_2 receptor gene. While the *Dvv_Gr2* gene structure is consistent with the *Tribolium* ortholog (*TcGr2*), the other two *Dvv_Gr* genes have more introns compared to their *Tribolium* orthologs (Fig. 2).

Expression studies of the three Dvv_Gr genes

The expression levels of three *D. v. virgifera* CO_2 receptor gene candidates were quantified and compared among different tissues and development stages. No significant difference was observed in expression levels of *Dvv_Gr1* and *Dvv_G3* among different tissues including larval integument, midgut, fatbody and head (Fig. 3a, b). In contrast, the expression of *Dvv_Gr2* varied significantly among different tissues and was expressed almost five-fold higher in the head as compared to expression in integument and midgut (Fig. 3c).

No significant differences in expression of *Dvv_Gr1* among different developmental stages were observed although third instars appeared to exhibit higher expression compared to second instars (Fig. 4a). *Dvv_Gr3* did not vary in expression level among the developmental stages analyzed (Fig. 4b). In contrast, the level of expression of *Dvv_Gr2* gene in eggs and first instar larvae was significantly (fivefold) higher, compared to the other instars and adults (Fig. 4c).

Discussion

Many studies have been conducted to identify and validate the function of chemosensory receptors in insects and their role in allowing insects to perceive their environment [11, 13, 16, 17, 28–31]. The three genes (*Dvv_Gr1*, *Dvv_Gr2*, and *Dvv_Gr3*) were identified from a *D. v. virgifera* transcriptome based on significant similarity to CO_2 receptor genes from *D. melanogaster*, *A. gambiae*, and *T. castaneum* and confirm their existence in western corn rootworms. Importantly, the relative expression of
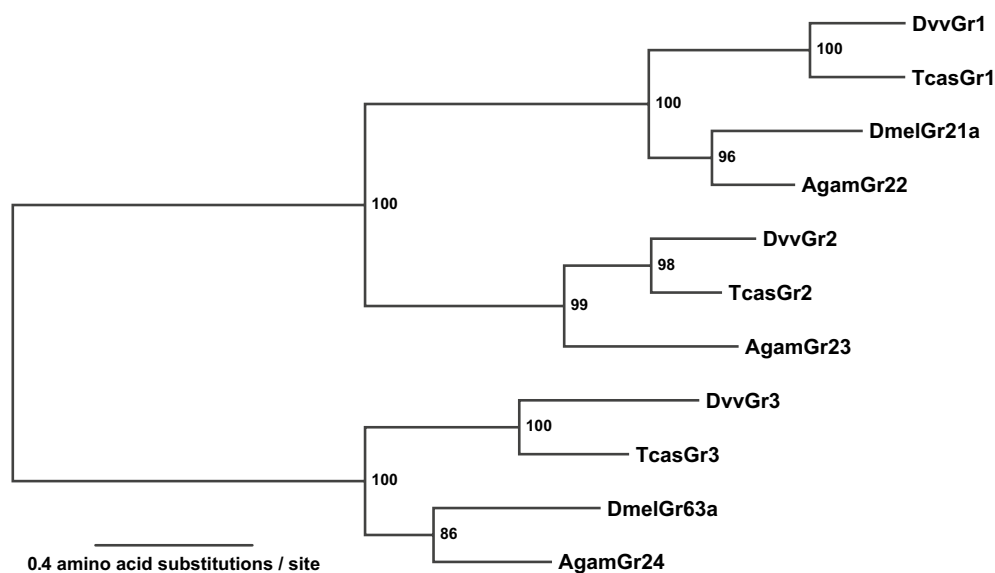
Rodrigues *et al. BMC Res Notes* (2016) 9:18

Page 4 of 7

Fig. 1 The maximum-likelihood phylogeny of insect CO_2 receptor proteins. Three CO_2 receptor proteins identified from *D. v. virgifera* (Dvv) were compared against orthologous proteins from *T. castaneum* (Tcas), *D. melanogaster* (Dmel), and *A. gambiae* (Agam). For the *T. castaneum* proteins, the naming convention proposed by Robertson and Kent [11] is used. The phylogeny is the consensus tree based on bootstrap analysis with 1000 pseudoreplicates. The *numbers* at nodes show the bootstrap supporting values (%)
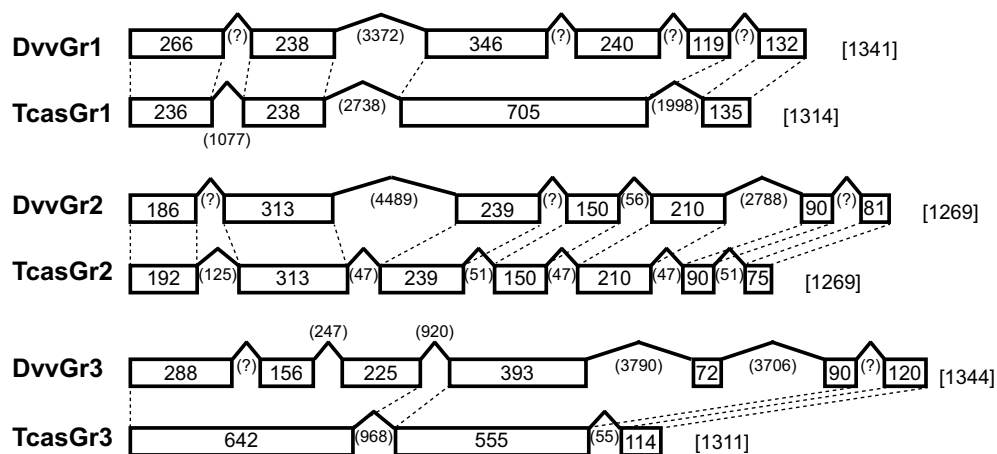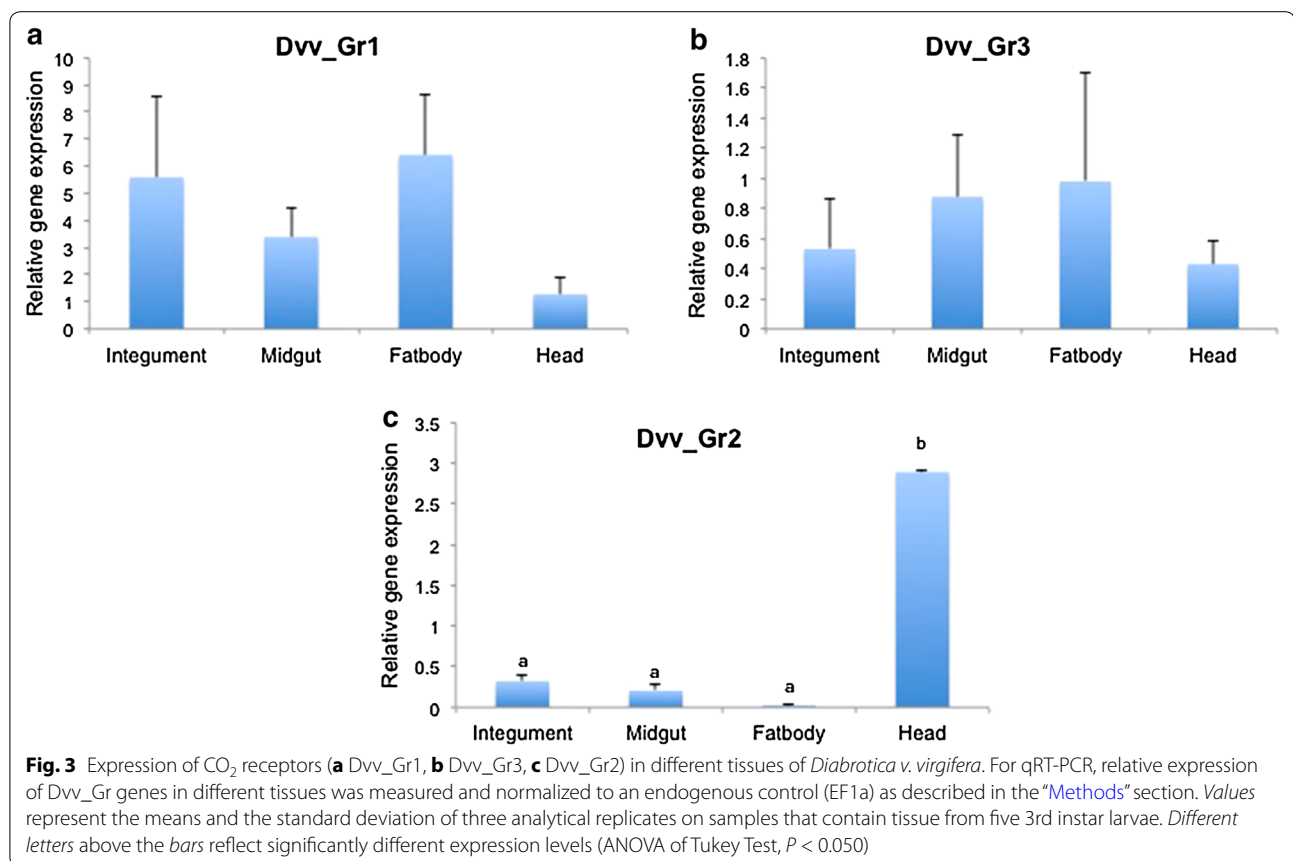

Fig. 2 Comparison of CO_2 receptor gene structures between *D. v. virgifera* and *T. castaneum*. Exons and introns located within the coding regions are depicted by boxes and peaks with their lengths (bp), respectively. The total length (bp) of each coding region is shown in *square brackets*. *D. v. virgifera* gene structures were determined by comparing transcript sequences and the draft genome sequence (Hugh Robertson, personal communication). Exon–intron structures for *T. cantaneum* CO_2 receptor genes are based on the annotations available in the BeetleBase [39]: TC030102 (TcasGr1), TC030103 (TcasGr2), and TC030104 (TcasGr3)

these genes among different larval tissues and developmental stages suggest a possible role for at least one of these genes in orientation to CO_2 and potentially host finding. The entire larval stage of *D. v. virgifera* is spent underground feeding on roots. Neonates must crawl relatively long distances through the soil to locate roots of a suitable host after hatching [5]. Previous research has shown that neonates are attracted to carbon dioxide in the soil, which may serve as a mechanism of host finding [6]. *Gr1* and *Gr3* orthologs in *Drosophila* (*Dm21a* and *Dm63a*) were found to mediate carbon dioxide detection in adults [12–14]. However, for the three CO_2 receptor orthologs identified from the *T. castaneum* genome (*TcasGr1*, *TcasGr2*, and *TcasGr3*) [32], their function has yet to be documented. No expression differences were observed among the four different *D. v. virgifera* tissues

Rodrigues *et al. BMC Res Notes* (2016) 9:18

Page 5 of 7

Fig. 3 Expression of CO_2 receptors (**a** Dvv_Gr1, **b** Dvv_Gr3, **c** Dvv_Gr2) in different tissues of *Diabrotica v. virgifera*. For qRT-PCR, relative expression of Dvv_Gr genes in different tissues was measured and normalized to an endogenous control (EF1a) as described in the "Methods" section. *Values represent the means and the standard deviation of three analytical replicates on samples that contain tissue from five 3rd instar larvae. Different letters above the bars reflect significantly different expression levels (ANOVA of Tukey Test, P < 0.050)*

for *Dvv_Gr1* and *Dvv_Gr3* genes (Fig. 3a, b). However, *Dvv_Gr2* was highly expressed in the head as compared to fat body, integument, and midgut (Fig. 3c). The higher expression of *Dvv_Gr2* in the head may suggest localization of the receptor to chemosensory organs associated with mouthparts and a specific role for this gene as a carbon dioxide receptor in *D. v. virgifera* larvae. Similar expression patterns from the two CO_2 receptor genes from *Drosophila* where expression is localized in olfactory receptor neurons of the sensilla on the antennae have been previously noted [13]. Similarly, all three CO_2 receptor genes in mosquitoes are expressed on the maxillary palps [13, 15, 16].
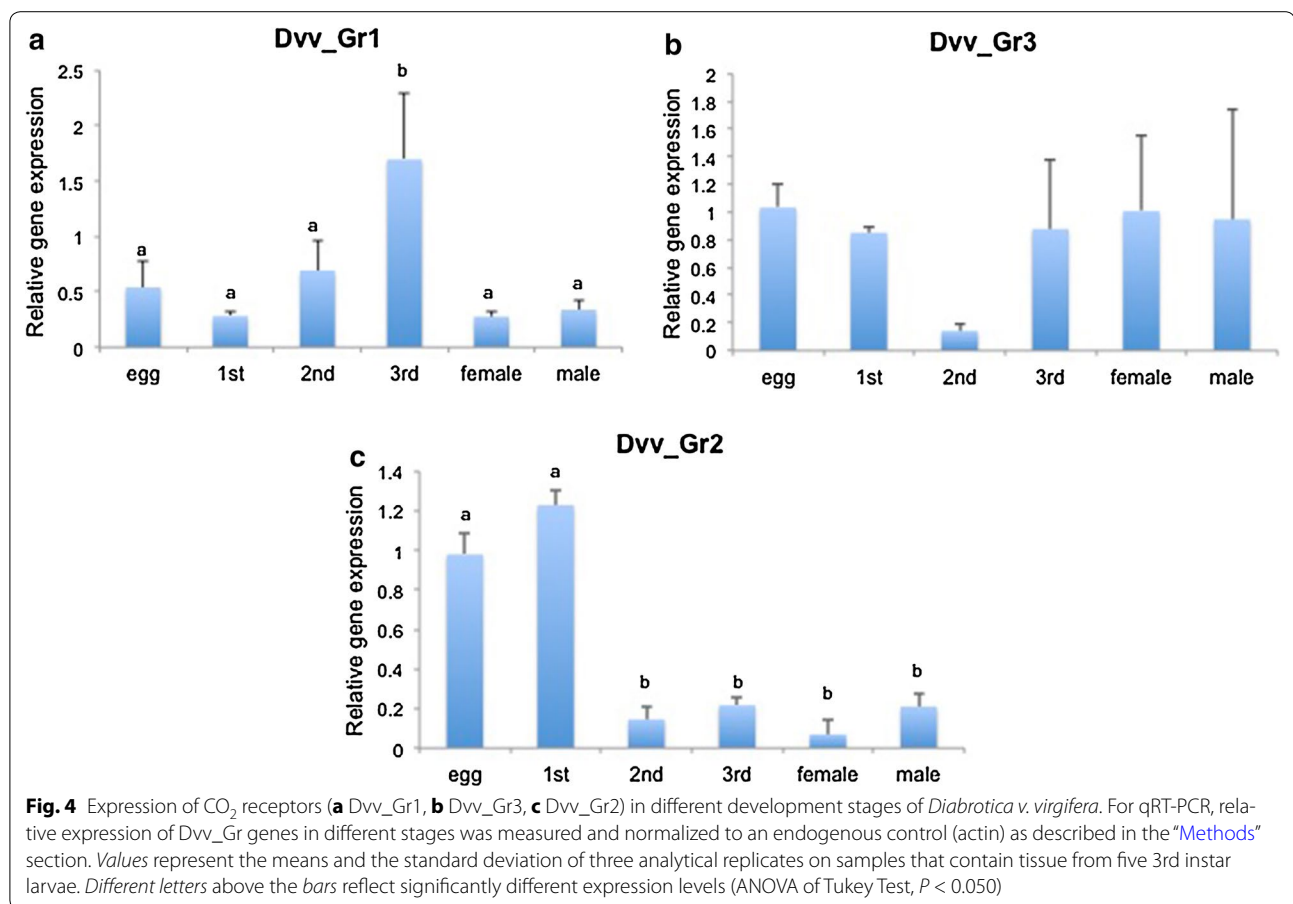
Erdelyan et al. [16] reported that in *Aedes aegypti* and *Culex pipiens quinquefasciatus,* the *Gr1* and *Gr3* genes were expressed at higher levels in adults than in larvae and pupae. For blood-feeding mosquitoes, CO_2 is a chemical stimulus emitted in the breath of animal hosts and produces host-seeking behaviors in adult mosquitos [33, 34]. In contrast, CO_2 is used by *D. v. virgifera* larvae to locate the roots of growing corn plants for feeding [6, 35]. Therefore, the relatively high expression of *Dvv_Gr2* gene in the head might indicate a possible role for this

gustatory receptor gene that mediates CO_2 detection in *D. v. virgifera* larvae.

The level of expression of the *Dvv_Gr2* gene in eggs and first instar larvae was higher than in other development stages (Fig. 4c). CO_2 is given off by growing corn roots in the soil or potentially other sources of CO_2 that are associated with plant growth, and neonate larvae that hatch in the spring from overwintering eggs must crawl through the soil to locate the roots on which they feed [6]. Higher expression of *Dvv_Gr2* gene in eggs and first instars is consistent with a possible role in host finding, which is different from mosquitoes that need to orient to hosts in the adult stage [16]. Interestingly, for *D. ponderosae*, the two *Gr* genes (*Gr1* and *Gr3*) were identified from an antenna-specific transcriptome but *Gr2* was only identified from a draft genome (Keeling et al., in press) and from larval RNAseq data [17]. The specific expression of *Gr2* in larvae further suggests a role in orientation of neonates to CO_2 detection in *D. v. virgifera*.

Conclusion
Specific genes potentially involved in CO_2 perception in *D. v. virgifera* have been identified and were differentially

Rodrigues *et al. BMC Res Notes* (2016) 9:18

Page 6 of 7

Fig. 4 Expression of CO_2 receptors (**a** Dvv_Gr1, **b** Dvv_Gr3, **c** Dvv_Gr2) in different development stages of *Diabrotica v. virgifera*. For qRT-PCR, relative expression of Dvv_Gr genes in different stages was measured and normalized to an endogenous control (actin) as described in the "Methods" section. *Values* represent the means and the standard deviation of three analytical replicates on samples that contain tissue from five 3rd instar larvae. *Different letters* above the *bars* reflect significantly different expression levels (ANOVA of Tukey Test, $P < 0.050$)

expressed among development stages and tissues. Based on expression results, *Dvv_Gr2* may be more important in host orientation of neonates. It should be noted that these results contrast those from mosquitoes and fruit flies where *Gr1* and *Gr3* have been identified as playing a more important role in CO_2 perception. Differences in receptors between adults and larvae may explain such results. Additional studies to validate the relative importance of these genes in larval host orientation will provide insight into the relative roles for these gustatory receptors in rootworm larvae. Previous success with RNA interference in both adult and larval rootworms [36–38] should provide an effective tool for validating functions for these putative receptors through loss of function assays.

The importance of CO_2 as an orientation cue for neonates is well documented in rootworm larvae [6] and may provide a potential mechanism to protect corn plants from rootworm damage. The identification of specific genes responsible for CO_2 perception may provide important information for designing rootworm specific management approaches that disrupt rootworm host finding.

Availability of supporting data
The data sets supporting the results of this article are included within the article.

Authors' contributions
TBR performed experiments, data acquisition, analysis and interpretation, statistical analysis, participated in the design of the study, and manuscript drafting. ENM performed bioinformatic experiments, contributed to data acquisition, analysis and interpretation, and manuscript drafting. HW contributed to experiments, data acquisition, analysis and interpretation, participated in the design of the study, and critical revision of the manuscript. CK contributed to data analysis and interpretation, statistical analysis and participated in the design of the study. BDS contributed to data interpretation, directed and designed the study, and critical revision of the manuscript. All authors read and approved the final manuscript.

Author details
[1] CAPES Foundation, Ministry of Education of Brazil, Brasília, DF 70040-020, Brazil. [2] Federal University of Lavras, Lavras, Minas Gerais, Brazil. [3] School of Biological Sciences and Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA. [4] Department of Entomology, University of Nebraska-Lincoln, Lincoln, NE 68583, USA.

Competing interests
The authors declare that they have no competing interests.

Rodrigues *et al. BMC Res Notes* (2016) 9:18

Page 7 of 7

References

1. Bowen MF. The sensory physiology of host-seeking behavior in mosquitoes. Ann Rev Entomol. 1991;36:139–58.
2. Thom C, Guerenstein PG, Mechaber WL, Hildebrand JG. Floral CO_2 reveals flower profitability to moths. J Chem Ecol. 2004;30(6):1285–128830.
3. Kleineidam C, Tautz J. Perception of carbon dioxide and other "air condition" parameters in the leaf cutting ant *Atta cephalotes*. Naturwissenschaften. 1996;83:566–8.
4. Bernklau EJ, Bjostad LB. Behavioral responses of first-instar western corn rootworm (Coleoptera: Chrysomelidae): to carbon dioxide in a glass bead bioassay. J Econ Entomol. 1998;91(2):445–56.
5. Sappington TW, Siegfried BD, Guillemaud T. Coordinated *Diabrotica* genetics research: accelerating progress on an urgent insect pest problem. Am Entomol. 2006;52(2):90–7.
6. Short DE, Luedtke RJ. Larval migration of the western corn rootworm. J Econ Entomol. 1970;63:325–6.
7. Sato K, Touhara K. Insect olfaction: receptors, signal transduction, and behavior. Results Probl Cell Differ. 2009;47:121–38.
8. Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron. 1999;22(2):327–38.
9. Clyne PJ, Warr CG, Carlson JR. Candidate taste receptors in *Drosophila*. Science. 2000;287(5459):1830–4.
10. Robertson HM, Warr CG, Carlson JR. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc Natl Acad Sci USA. 2003;100(Suppl 2):14537–42.
11. Robertson HM, Kent LB. Evolution of the gene lineage encoding the carbon dioxide receptor in insects. J Insect Sci. 2009;9(19):1–14.
12. Suh GS, Wong AM, Hergarden AC, Wang JW, Simon AF, Benzer S, et al. A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila*. Nature. 2004;431(7010):854–9.
13. Jones WD, Cayirlioglu P, Kadow IG, Vosshall LB. Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. Nature. 2007;445(7123):86–90.
14. Kwon JY, Dahanukar A, Weiss LA, Carlson JR. The molecular basis of CO_2 reception in Drosophila. Proc Natl Acad Sci USA. 2007;104(9):3574–8.
15. Lu T, Qiu YT, Wang G, Kwon JY, Rutzler M, Kwon HW, et al. Odor coding by maxillary palp neurons of the malaria vector mosquito *Anopheles gambiae*. Curr Biol. 2007;17(18):1533–44.
16. Erdelyan CNG, Mahood TH, Bader TSY, Whyard S. Functional validation of the carbon dioxide receptor genes in *Aedes aegypti* mosquitoes using RNA interference. Insect Mol Biol. 2012;21(1):119–27.
17. Andersson MN, Grosse-Wilde E, Keeling CI, Bengtsson JM, Yuen MM, Li M, Hillbur Y, Bohlmann J, Hansson BS, Schlyter F. Antennal transcriptome analysis of the chemosensory gene families in the tree killing bark beetles, *Ips typographus* and *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytinae). BMC Genom. 2013;14(198):1–16.
18. Keeling CI, Yuen MMS, Liao NY, Docking TR, Chan SK, Taylor GA, et al. Draft genome of the mountain pine beetle, dendroctonus ponderosae Hopkins, a major forest pest. Genome Biol. 2013;14(3):R27.
19. Eyun SI, Wang H, Pauchet Y, Ffrench-Constant RH, Benson AK, Valencia-Jimenez A, et al. Molecular evolution of glycoside hydrolase genes in the western corn rootworm (*Diabrotica virgifera virgifera*). PLoS ONE. 2014;9(4):e94052.
20. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421.
21. Bernsel A, Viklund H, Hennerdal A, Elofsson A. TOPCONS: consensus prediction of membrane protein topology. Nucleic Acids Res. 2009;37(Supp 2):W465–8.
22. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.
23. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010;59:307–21.
24. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783–91.
25. Rodrigues TB, Khajuria C, Wang H, Matz N, Cunha Cardoso D, et al. Validation of reference housekeeping genes for gene expression studies in Western Corn rootworm (*Diabrotica virgifera virgifera*). PLoS One. 2014;9(10):e109825.
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25:402–8.
27. Benton R, Sachse S, Michnick SW, Vosshall LB. Atypical membrane topology and heteromeric function of drosophila odorant receptors in vivo. PLoS Biol. 2006;4(2):e20.
28. Zhang HJ, Anderson AR, Trowell SC, Luo AR, Xiang ZH, Xia QY. Topological and functional characterization of an insect gustatory receptor. PLoS One. 2011;6:e24111.
29. Howlett N, Dauber KL, Shukla A, Morton B, Glendinning JI, Brent E, et al. Identification of chemosensory receptor genes in *Manduca sexta* and knockdown by RNA interference. BMC Genom. 2012;13:211.
30. Li KM, Ren LY, Zhang YJ, Wu KM, Guo YY. Knockdown of microplitis mediator odorant receptor involved in the sensitive detection of two chemicals. J Chem Ecol. 2012;38(3):287–94.
31. Dong X, Zhong G, Hu M, Yi X, Zhao H, Wang W. Molecular cloning and functional identification of an insect odorant receptor gene in *Spodoptera litura* (F.) for the botanical insecticide rhodojaponin III. J Insect Physiol. 2013;59(1):26–32.
32. Tribolium Genome Sequencing Consortium. The genome of the model beetle and pest *Tribolium castaneum*. Nature. 2008;452:949–55.
33. Gillies MT. The role of carbon dioxide in host-finding in mosquitoes (Diptera: Culicidae): a review. Bull Entomol Res. 1980;70(4):525–32.
34. Takken W, Knols BG. Odor-mediated behavior of Afrotropical malaria mosquitoes. Annu Rev Entomol. 1999;44:131–57.
35. Strnad SP, Bergman MK, Fulton WC. First-instar western corn rootworm (Coleoptera: Chrysomelidae) response to carbon dioxide. Environ Entomol. 1986;15:839–42.
36. Khajuria C, Vélez AM, Rangasamy M, Wang H, Fishilevich E, Frey ML, et al. Parental RNA interference of genes involved in embryonic development of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Insect Biochem Mol Biol. 2015;63:54–62.
37. Li H, Khajuria C, Rangasamy M, Gandra P, Fitter M, Geng C, et al. Long dsRNA but not siRNA initiates RNAi in western corn rootworm larvae and adults. J Appl Entomol. 2015;139:432–45.
38. Rangasamy M, Siegfried BD. Validation of RNA interference in western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) adults. Pest Manag Sci. 2012;68:587–91.
39. Kim HS, Murphy T, Xia J, Caragea D, Park Y, Beeman RW, Lorenzen MD, Butcher S, Manak JR, Brown SJ. BeetleBase in 2010: revisions to provide comprehensive genomic information for Tribolium castaneum. Nucleic Acids Res. 2010;38:D437–42.