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Differential expression of the CrV1 haemocyte inactivation-associated polydnavirus gene in the African maize stem borer *Busseola fusca* (Fuller) parasitized by two biotypes of the endoparasitoid *Cotesia sesamiae* (Cameron)

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Abstract

Polydnaviruses are rarely studied for their natural variation in immune suppressive abilities. The polydnavirus harboring braconid *Cotesia sesamiae*, a widespread endoparasitoid of *Busseola fusca* and *Sesamia calamistis* in sub-Saharan Africa exists as two biotypes. In Kenya, the western biotype completes development in *B. fusca* larvae. However, eggs of the coastal *C. sesamiae* are encapsulated in this host and ultimately, no parasitoids emerge from parasitized *B. fusca* larvae. Both biotypes develop successfully in *S. calamistis* larvae. Encapsulation activity by *B. fusca* larvae towards eggs of the avirulent *C. sesamiae* was detectable six hours post-parasitization. The differences in encapsulation of virulent and avirulent strains were associated with differences in nucleotide sequences and expression of a CrV1 polydnavirus (PDV) gene, which is associated with haemocyte inactivation in the *Cotesia rubecula*/*Pieris rapae* system. CrV1 expression was faint or absent in fat body and haemolymph samples from *B. fusca* parasitized by the avirulent *C. sesamiae*, which exhibited encapsulation of eggs. Expression was high in fat body and haemolymph samples from both *B. fusca* and *S. calamistis* larvae parasitized by the virulent *C. sesamiae*, encapsulation in the former peaking at the same time points as CrV1 expression in the latter. Non synonymous difference in CrV1 gene sequences between virulent and avirulent wasp suggests that variations in *B. fusca* parasitism by *C. sesamiae* may be due to qualitative differences in CrV1-haemocyte interactions.

Keywords: CrV1 gene; *Cotesia sesamiae*; *Busseola fusca*; Gene expression

1. Introduction

Polydnavirus (PDV) are parasitoid symbionts produced in the female wasp calyx gland and injected into the host along with the eggs during oviposition. PDV genes are expressed in the host and are responsible for immune suppression and host physiological regulations to the benefit of the parasitoid during the course of its development (Turnbull and Webb, 2002). PDV-host molecular interactions have been studied as model systems to investigate parasitoid–host immune interactions for decades (Glatz et al., 2004). The PDV genome of *Cotesia congregata* (Say) (Hymenoptera: Braconidae) has been sequenced (Espagne et al., 2004) and this has opened avenues to a better understanding of insect immunity against parasitoids. Although PDV have been considered as key factors determining parasitoid host range (Whitfield, 1994) and their effects have been extensively studied experimentally, their natural variation has rarely been investigated. Studies have not been conducted on mechanisms that may explain natural differences in parasitoid virulence.

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PDV harboring parasitoids *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) vary naturally in their abilities to suppress *Busseola fusca* Fuller (Lepidoptera: Noctuidae), one of its main natural hosts in sub-Saharan Africa. Variation in the successful development of *C. sesamiae* in *B. fusca* has been documented in East and southern Africa (Ngi-Song et al., 1998; Mochiah et al., 2001; Chinwada et al., 2003; Gitau, 2006). In Kenya, the two types of populations of *C. sesamiae* have been described as biotypes (Ngi-Song et al., 1998). The biotype from the East and coast areas (namely Kitui and Mombasa) does not complete development in *B. fusca* and parasitoid eggs are encapsulated soon after oviposition. The biotype from the inland and western areas (namely Meru and Kitale) is able to suppress the *B. fusca* immune system and parasitoid eggs evade encapsulation (Fig. 1; Gitau, 2006). The difference in virulence between the two biotypes may perhaps be due to PDV since the injection of virulent wasp calyx fluid in avirulent-wasp-infected hosts restores parasitoid development (Mochiah et al., 2002). Thus far, *B. fusca* is the only stem borer that shows refractoriness towards the avirulent *C. sesamiae* populations. Currently, a project is underway with the aim of redistributing *C. sesamiae* in regions of sub-Saharan Africa (Schulthess et al., 1997; Schulthess pers. comm.). It is imperative that the correct biotype is selected for releases into the regions where the suitable stem borer hosts exist.

As in the case of other Braconid parasitoids, *C. sesamiae* manipulates host physiology in order to facilitate development inside its hosts. The parasitoids transmit maternal factors, including symbiotic PDV (Kroemer and Webb, 2004) and venom gland protein secretions (Asgari et al., 2003) into the body of their host along with egg(s) during oviposition. The presence of PDV is asymptomatic to the wasp. Following infection of cells in host tissues, PDV-encoded genes are expressed and their products alter host physiology, enabling successful development of the endoparasitoid wasps (Whitfield and Asgari, 2003). The PDV-associated gene products act by suppressing the host immune response (Asgari et al., 1996), most often by targeting haemocytes. The two most commonly

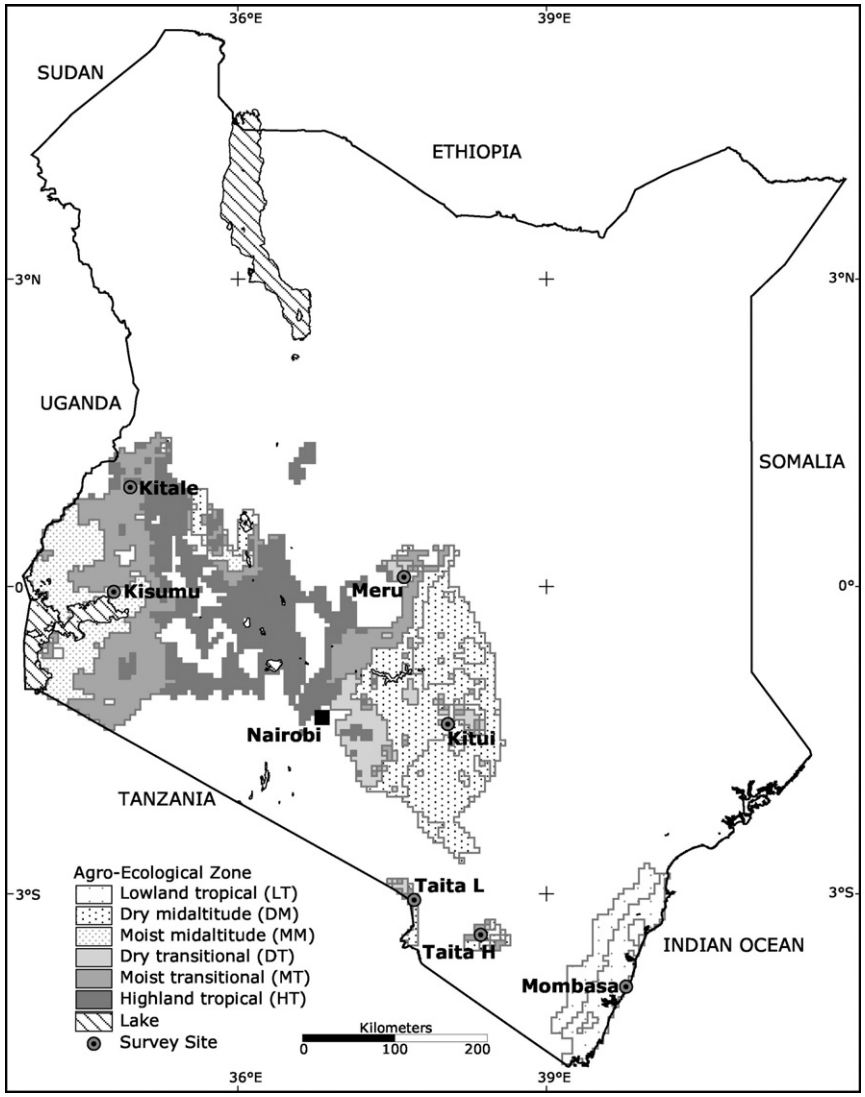

Fig. 1. Map of Kenya showing the regions where *C. sesamiae* and *B. fusca* were collected.

observed pathologies in infected caterpillars are suppression of immunity and regulation of host development (Beckage and Gelman, 2004). PDV also interfere with the host endocrine system causing developmental arrest and physiological disorders (Strand and Pech, 1995; Beckage and Gelman, 2004). Success of the parasitoid hence depends upon PDV and virulence characteristics as well as the permissiveness of the host (Rodriguez-Perez et al., 2006).

In the parasitoid *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae), the bracovirus (CrBV) genes are expressed in the host larvae, *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), over a relatively short time period, from 4 to 12 h post-parasitization (pp) (Asgari et al., 1996). Only four major expressed CrBV gene products have been detected in this larval host (Asgari et al., 1996; Glatz et al., 2003). Of the four major CrBV gene products detected in *P. rapae* tissues, the *C. rubecula* virus 1 (CrV1) gene is the best characterized so far. The CrV1 gene product is a secreted glycoprotein that has been implicated in depolymerization of the actin cytoskeleton of host haemocytes leading to haemocyte inactivation (Asgari et al., 1996). Homologues of CrV1 have been found to occur in at least six *Cotesia* species (Whitfield, 2000). In contrast to PDV gene products in other systems such as *Campoletis sonorensis* (Cameron) (Hymenoptera: Braconidae) ichnovirus (CsIV), over 35 genes comprising several gene families are estimated to be expressed in the host *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) (Turnbull and Webb, 2002).

Different mechanisms may explain the difference in virulence between *C. sesamiae* geographic strains against *B. fusca* which include the PDV or other factors present in the calyx fluid (Gitau, 2006). If it is related to the virus, the difference in virulence characteristics could be due to a lack of expression of essential genes or to differences with allelic variation in the genes, some alleles being less efficient which opens up other new study areas for the future. Viral genes involved in immune suppression have not been explored in the *C. sesamiae–B. fusca* system. Variation in PDV expression patterns between parasitoid strains in relation to natural variation in immune suppressive ability have not been studied in any system. The presence of two biotypes of the *C. sesamiae* species provides an excellent model in understanding the role(s) of PDV in host immune suppression and host physiology. This study compared the expression patterns of *C. sesamiae* bracovirus (CsBV) CrV1 gene in *B. fusca* larvae parasitized by the avirulent and virulent biotypes. The molecular mechanisms that govern natural variation in virulence and the forces that govern their evolution are discussed.

2. Materials and methods

2.1. Insects

2.1.1. Collection and rearing of insects

C. sesamiae parasitoids were collected from five geographic locations within Kenya (Fig. 1). The locations were selected to acquire both parasitoids and stem borers from a range of elevations and distributions where both *C. sesamiae* and *B. fusca* were known to occur (Onga'mo et al., 2006). Twenty maize plants were collected from at least 10 farmers' fields at least a kilometer apart. Plants showing signs of stem borer attack were dissected and larvae and parasitoid cocoons recovered were placed individually in glass vials (7.5 cm × 2.5 cm). The collected samples were then transferred to the laboratory and maintained at 25 ± 2 °C, relative humidity of 70 ± 10% and 12 L: 12 D photoperiod regime. The larvae were observed until they died, pupated or parasitoids emerged. Adult *Cotesia* were identified to species level by the shape of the male genitalia or sculpturing of the propodeum for broods with only females (Kimani-Njogu and Overholt, 1997). The adult *C. sesamiae* that emerged were allowed to mate in a vial under light for 12 h and a colony was initiated with progeny pooled from at least three cocoons from each geographic location. All colonies of *C. sesamiae* were maintained on species of stem borer larvae from which they were collected and adults were fed on a 20% honey/water solution. *Busseola fusca* and *S. calamistis* hosts were obtained from the Animal Rearing and Containment Unit (ARCU) at the ICIPE and were reared according to the method described by Onyango and Ochieng-Odero (1994). Rearing of parasitoids was started 24 h post-emergence by offering the females host larvae for oviposition using the hand-stinging method (Overholt et al., 1994). Parasitized larvae were placed on artificial diet until cocoon formation after which cocoons were harvested and placed in clean vials until emergence of adults, which were thereafter used in bioassays.

2.2. Egg encapsulation

One or two day old *C. sesamiae* females from the five geographical regions (Kitale, Meru, Kitui, Taita and Mombasa) (see Fig. 1) were exposed to fourth instar *B. fusca* using the hand stinging method (Overholt et al., 1997) and thereafter placed on artificial diet. The virulence status of these *C. sesamiae* populations had previously been characterized as avirulent (Ngi-Song et al., 1998; Mochiah et al., 2001; Gitau, 2006). The parasitized stem borer larvae were then dissected 6, 12, 24 h post-parasitism (pp) at × 40 magnification under the microscope. *Cotesia sesamiae* eggs found inside were counted and recorded. Encapsulated eggs were recorded if a ring of immune response cells surrounding the eggs were visible under the dissecting microscope (Fig. 2). Four replications of five larvae each were dissected for each of the *C. sesamiae* strains. The CsVr derived from Kitale (virulent) were used as a positive control as encapsulated eggs could not be found.

2.3. Data analysis

Data analysis on the number of eggs encapsulated was carried out by analysis of variance (ANOVA) using the

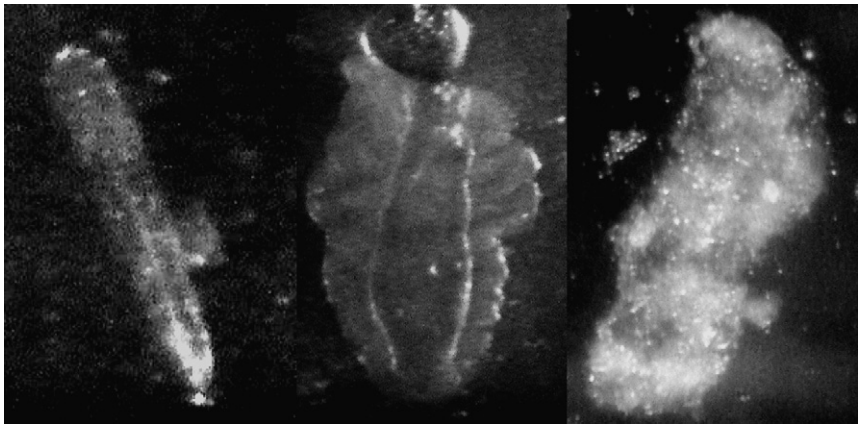

Fig. 2. Encapsulation of *C. sesamiae* egg (magnification 40 ×) at (1) 24 h post-parasitism (pp) in the permissive host *Sesamiae calamistis*; (2) 24–36 h pp in the refractory host, *Busseola fusca*, where the egg is partially covered; and (3) 24 h pp in the refractory host, *B. fusca*, where the egg is completely covered.

general linear model (Proc glm) (SAS Institute, 2002). Due to absence of encapsulated eggs in the 6 h time point and presence of zeros in the data, count data on the number of eggs encapsulated was transformed using $\log_{10}(n+1)$ before ANOVA to normalize data. Means were compared using the Student Newman Keul's test.

2.4. Virus purification and viral DNA isolation

CsPDV was purified from the calyx fluid of *C. sesamiae* females as described by Beckage et al. (1994). Ovaries were dissected on ice blocks and immediately placed in phosphate buffered saline (PBS) solution (pH 7.0). At least 100 females of both virulent and avirulent *C. sesamiae* biotypes were dissected in total for CsBV DNA. The calyx fluid was filtered through a 0.45-μm filter to remove eggs and cell debris and stored at −20 °C prior to DNA extraction.

2.5. Fat body and haemolymph sample preparation for RACE and RT-PCR

Fourth instar *B. fusca* parasitized as described above were maintained on artificial diet and kept at 25 °C until they were dissected 6, 24, or 48 h later. Parasitized hosts *S. calamistis* (permissive to both CsVr and CsAv) and *B. fusca* (refractory to CsAv) were examined, with non-parasitized *B. fusca* larvae serving as a negative control. Before extraction of fat body tissues and haemolymph, larvae were washed in 70% alcohol, rinsed in distilled water three times and dried on sterile paper tissue. The proleg of each larva was snipped off using dissecting scissors and haemolymph was collected into a sterile microcentrifuge which contained 50 μl of RNA stabilization solution (RNAlater™, Ambion). Fat body was isolated from the same larvae and other tissues discarded. To prevent contamination with haemolymph, fat body tissues were rinsed in PBS five times prior to being dipped in 50 μl of RNAlater solution. All samples were preserved at −20 °C until RNA extraction. Similarly, the tissues extracted from the permissive host *S. calamistis* and were used for RT-PCR as the positive control.

2.6. RNA isolation

Approximately 50 mg fat body and 1.5 ml of haemolymph samples were separately homogenized in the presence of 1 ml Trizol (Invitrogen) with the Thermo Savant Fastprep 120 instrument using matrix 'A' tubes (Q-Biogene) for 30 s, setting 4.5. Supernatants were transferred to sterile 1.5 ml tubes and incubated for 5 min at room temperature. Samples were extracted in an equal volume (200 ml) of chloroform and incubated for 3 min at room temperature. Samples were centrifuged at 10,000 rpm for 15 min at 4 °C and the aqueous phase was precipitated with 100% isopropanol. Total RNA pellets were washed once with 75% ethanol and resuspended in water; all solutions were DEPC-treated. RNA yield and purity was quantified at an absorbency of 260–280 nm. RNAs were used immediately for RACE and RT-PCR or stored at 80 °C after addition of 40 U/μl RNase inhibitor (Ambion).

2.7. RACE assays

To analyze CsBV CrV1 gene sequence and structure, first-strand 'RACE ready' cDNAs were synthesized separately for 5′- and 3′-RACE reactions from larval RNAs 24 h pp using the BD SMART RACE cDNA amplification kit (BD Biosciences) with the supplied modified oligo(dT) and BD SMART II A oligonucleotide primers according to the manufacturer's instructions. Gene-specific primers were designed for 5′- and 3′-RACE second-strand cDNA synthesis reactions on the basis of conserved CrV1 sequences. These RACE primers were: CskCrV1-5′R 5′-GGAGAGGAACGATGGGTCAGCGG-3′, and CskCrV1-3′R 5′-GCTGTCATTCAAATAGACGATG-3′. Both 5′- and 3′-RACE gene specific products were generated with cycling conditions of 25 cycles of 94 °C for 30 s, 68 °C for 30 s and 72 °C for 3 min followed by 16 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 5 min

and a final elongation at 72 °C for 7 min, and examined by electrophoresis on 1% agarose gels containing ethidium bromide. RACE reactions yielded strong single products that were sequenced directly. For sequencing, products were concentrated by speed vacuum, separated on 1.5% NuSieve agarose gel (FMC) in modified TAE (0.04 M tris/acetate and 0.1 mM EDTA) and excised for sequencing using the ABI BigDye version 3 kit with corresponding gene-specific primers. Cycle-sequencing conditions were 35 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Automatic sequencing was carried out on an ABI 3100. The 5′- and 3′-RACE product sequences were assembled (DNASTAR, SeqManII component) and compared with DNA sequences to identify features of the gene transcript. Sequence alignments were created using the Clustal V method (DNASTAR, Megalign component). CrV1 gene sequences have been deposited in GenBank under accession numbers **EF141828** and **EF141829**.

2.8. RT-PCR assays

CrV1 gene transcription in fat body and haemolymph tissues was examined at 6 and 24 h pp time points. Primers for RT-PCR were designed based on aligned sequences of RACE transcripts that were specific for CsBV and located within conserved exon sequences. The primers used for RT-PCR were: CsKCrV1F 5′-TCGTCAAAAGTGCG-TTTGTGC-3′; and CrV1R 5′-ACTCCTTCAACGCTGG-GTTCCTTG-3′ (Dupas et al., 2006) the latter of which was found to be a conserved sequence among *C. sesamiae*, *C. rubecula*, and *C. congregata* CrV1 genes at this locus. Total RNAs were treated with DNA-free (Ambion) to eliminate DNA contamination prior to synthesis of the first strand cDNA. Treated RNAs were used as template in non-quantitative RT-PCR, which was performed using the RETROscript kit (Ambion) for two-step RT-PCR with heat denaturation according to the manufacturer's protocol. Cycling conditions were 95 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 2 min with a final elongation at 72 °C for 7 min. Duplicate reactions using haemolymph and fat body RNA templates from both *B. fusca* and *S. calamistis* without reverse transcription were performed to verify the absence of viral DNA contamination within RNA samples. Products were separated by electrophoresis on 1% agarose gels containing ethidium bromide and visualized under UV light.

3. Results

3.1. Egg encapsulation

B. fusca larvae parasitized by the CsVr population from Kitale did not have encapsulated eggs (Fig. 2, Table 1). In larvae parasitized by CsAv populations, the highest numbers of encapsulated eggs were observed at 24 and 48 h pp time points (Table 1). The most encapsulated eggs were observed in *B. fusca* larvae parasitized by *C. sesamiae* from Mombasa (CsMombasa) ($F = 4.37$; $df = 15, 319$; $P < 0.0001$). The lowest number of eggs encapsulated was found at 6 h pp in all four *C. sesamiae* populations tested, with highest numbers of encapsulated eggs apparent at 24 h pp. Encapsulated eggs were not found in *S. calamistis* larvae parasitized by the CsAv populations.

Table 1
Total number of *C. sesamiae* eggs (±SE) encapsulated in *B. fusca* larvae at four time points post-parasitism

C. sesamiae	6 h	12 h	24 h	48 h
Kitale	0b	0c	0c	0c
Kitui	3±0.09a	4±0.18a	7b±0.08b	5±0.09b
Mombasa	2±0.07a	6±0.15a	20±0.29a	15±0.26a
Taita	0b	2±0.07b	7±0.15b	7±0.17b
F	2.79	1.84	6.54	3.69
df	3,76	3,76	3,76	3,76
P	0.046	0.147	0.0005	0.0154

Means followed by the same letters in the same column are not significantly different at $P \leqslant 0.05$ (Student Newman Keuls multiple comparison test).

3.2. Organization and transcription of the CsBV CrV1 gene in B. fusca and S. calamistis larvae

Analysis of *C. sesamiae* from Kitale (CsKitale) BV CrV1 gene transcripts obtained by 5′- and 3′-RACE and genomic DNA (Figs. 3 and 4) indicated the gene possesses a short exon 1 encoding only a signal peptide, followed by a 115 base intron, joined to a larger exon 2 encoding the majority of the gene. This structure is typical of CrV1 genes from other PDV CrV1 genes, including that from *C. rubecula* and *C. congregata*. From alignment of these sequences (Fig. 4, CcBV CrV1 not shown), it was also evident there is a variable region of the gene, with a 71 bp gap in CrBV CrV1 present at this locus. Comparison of transcript (t) and genomic (g) sequence of the CrV1 gene from coastal CsMombasa and inland CsKitale BV CrV1 genes in the parasitized hosts (Fig. 4) showed an identical gene structure between the two biotypes. However, there were a total of eight distinct base differences between them, the majority of which would result in non-synonymous amino acid substitution in the translated protein product. Both were different from CrBV CrV1, with CsKitale CrV1 sharing more base similarities and being more closely related to *C. rubecula* CrV1 than was CsMombasa CrV1.

3.3. Expression of C. sesamiae CrV1 gene transcripts by RT-PCR

Non-quantitative RT-PCR was used with gene-specific RT-PCR primers to examine CrV1 expression patterns in both haemolymph and fat body tissues from both *B. fusca* and *S. calamistis* parasitized by *C. sesamiae* from the different geographical regions of Kenya. Using specific primers designed within the CrV1 exon regions, temporal

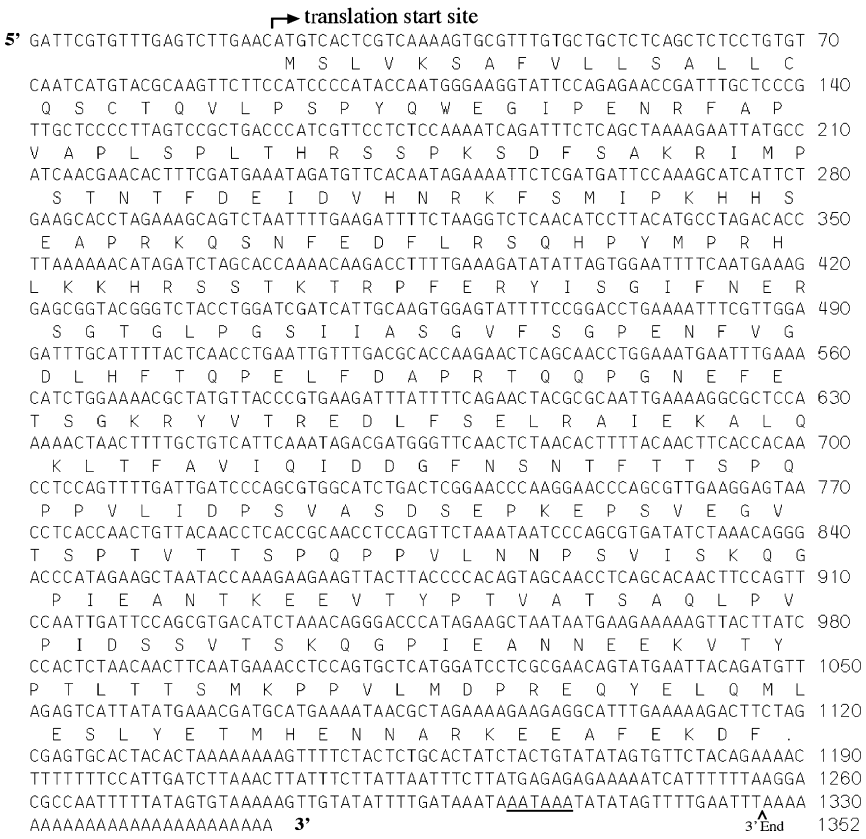

Fig. 3. CrV1 transcript sequence from *Cotesia sesamiae* from Kitale (CsKitale). Putative amino acid translation is given. Poly adenylation signal is underlined.

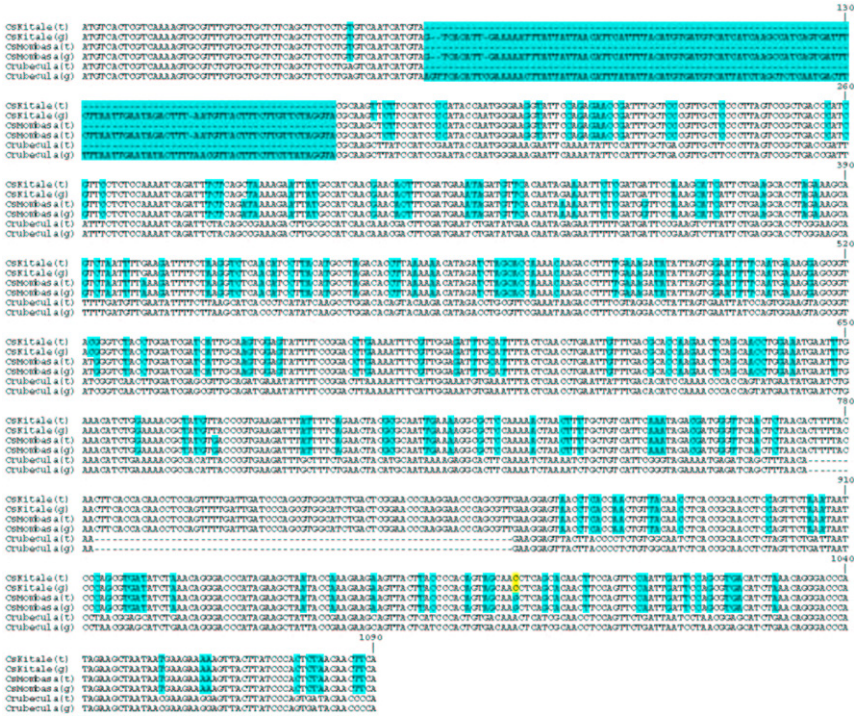

Fig. 4. CrV1 gene sequence alignment from genomic DNA (g) or transcript cDNA (t) from *Cotesia sesamiae* from Kitale (CsKitale), Mombasa (CsMombasa) and *Cotesia rubecula* (Crubecula) (GenBank sequences **EF141828** and **EF141829**). Nucleotide sequences differing from *C rubecula* are shaded.

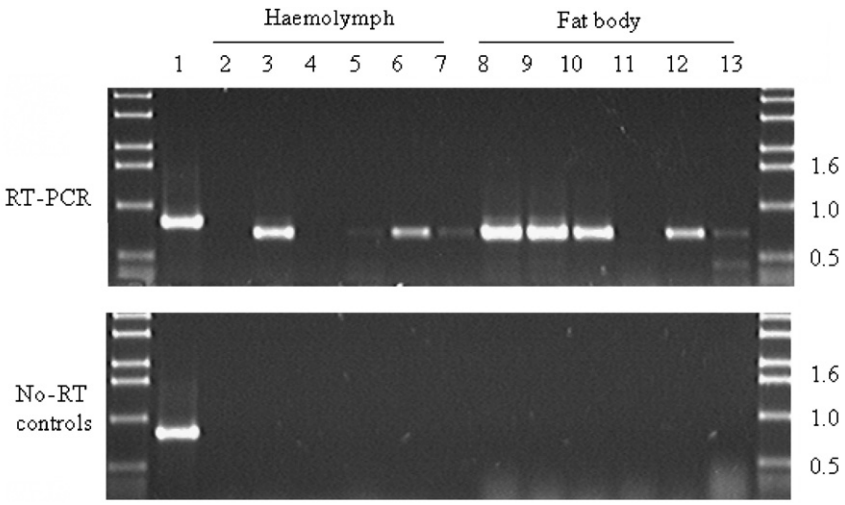

Fig. 5. RT-PCR products using template obtained 6 h post-parasitization from: (1) *C. sesamiae* (Cs) BV DNA (control); haemolymph samples from (2) CsKitale on host *Sesamiae calamistis* (SC), (3) CsMombasa on SC, (4) CsKitui on *Busseola fusca* (BF), (5) CsMombasa on BF, (6) CsMeru on BF, and (7) CsKitale on BF; Fat body samples from (8) CsKitale on BF, (9) CsMeru on BF, (10) CsMombasa on SC, (11) CsMombasa on BF, (12) CsKitale on SC, and (13) CsKitui on BF.

expression patterns in the two parasitized host were assessed (Fig. 5). The absence of contaminating DNA in RNA samples was verified by inclusion of templates that were DNase-treated but not reverse-transcribed (Fig. 5). Amplified CrV1 product was readily generated for CsKitale on *B. fusca*, *C. sesamiae* from Meru (CsMeru) on *B. fusca* and CsMombasa on *S. calamistis* for both haemolymph and fat body samples. Amplified product was also readily generated for CsKitale on *S. calamistis*, but only for the fat body sample. No or very faint amplified product was detected for the avirulent CsMombasa on *B. fusca* and *C. sesamiae* from Kitui (CsKitui) on *B. fusca* for both fat body and haemolymph samples. The larger expected fragment was generated from CsBV DNA control. The same RT-PCR amplification patterns were obtained at 24 and 48 h pp time points (data not shown).

4. Discussion

In previous studies, PDV expression had been compared between permissive and non-permissive hosts in other systems (Cui et al., 2000), but never between virulent and avirulent wasps of the same species in the same host. Temporal patterns of PDV gene expression have been examined in several *Cotesia*–lepidopteran host systems (Harwood et al., 1994; Asgari et al., 1996; Yamanaka et al., 1996). In the host *P. rapae*, the CrV1 gene was identified as one of the viral transcripts expressed from *C. rubecula* BV (CrBV) (Asgari et al., 1996; Glatz et al., 2004). Its expression occurred from 4 to 12 h pp and caused inactivation of the haemocytes in *P. rapae* (Glatz et al., 2004). In contrast, the *Cotesia congregata* (CcBV) CrV1 gene expressed in host *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae) appeared to be expressed transiently and did not persist in the infected host. Our results with the *C. sesamiae*–stem borer system showed that the *C. sesamiae* CrV1 gene expression in both *B. fusca* and *S. calamistis* larvae was detected in both host fat body tissues and haemolymph, with the CrV1 gene transcript detected early (6 h pp) and abundantly by 24 h pp. Lower expression levels of the CrV1 gene observed in haemocytes in the case of the virulent CsKitale may be due to the depletion of haemocytes associated with immune suppression, since haemocyte depletion was observed in this specific infestation by Mochiah et al. (2003).

Encapsulation of *C. sesamiae* eggs in *B. fusca* parasitized by CsAv was readily detected at 24 h pp. Observation of the encapsulated eggs was detectable as early as 6 h pp, but less common at 48 h pp. This may suggest that encapsulated eggs were regularly cleared from the haemolymph. CrV1 gene expression clearly occurs at the same time as encapsulation, as has been observed in other systems (Strand and Pech, 1995). The CsBV genes appear to be expressed early, peaking at 24–48 h pp. Encapsulated eggs were observed within this time; it remains to be investigated whether this expression in *B. fusca* larvae continues until cocoon formation. The gene seems to be sufficiently expressed but not efficient against its target probably due to allelic variation (Dupas et al., 2006). The result showing differential expression suggests that expression level is important, but the difference in sequence may suggest that the efficiency of expression varies between strains which can contribute to variation in virulence between the *C. sesamiae* biotypes.

In *M. sexta* larvae parasitized by *C. congregata*, production of six CcBV transcripts begins as early as 30 min pp in host fat body and haemocytes and gene expression persists until parasitoid emergence (Strand et al., 1992). At 24 h pp, in the same system, Beckage et al. (1994) observed that numbers of host haemocytes of naturally parasitized or virus-injected larvae were significantly altered compared with those of non-parasitized

larvae. A CcBV CrV1 homolog was localized in infected host haemocytes using polyclonal CrV1 antibodies, suggesting that a CcBV CrV1-like gene product was responsible in part for the impaired immune response of the host. Our transcript sequences showed the CrV1 gene expressed in vivo from the coastal population of *C. sesamiae* PDV possessed numerous non-synonymous base substitutions that would result in a different translated protein. It is possible that such a gene may not serve its function in immunosuppression of *B. fusca* either by failure of the protein product to be expressed at sufficient levels, failure to attach to host haemocyte receptors or by mechanisms yet undetermined. The transcript sequences are consistent with analyses by Dupas et al. (2006) which showed CrV1 gene sequences differed at the genomic DNA level in *C. sesamiae* populations from coastal and inland Kenya. Results from these two studies confirm that the CrV1 allelic status differs between the CsVr and CsAv biotypes.

Host permissiveness has been associated with pathology of host immunocytes during parasitism in some systems. Cui et al. (2000) showed that expression levels of the *Campoletis sonorensis* (Cameron) (Hymenoptera: Braconidae) PDV (CsIV) cysteine-rich gene at later times post-parasitization could be correlated to the permissiveness of a range of larval hosts. Lovallo et al. (2002) also demonstrated haemocyte aggregation in permissive *M. sexta* larvae parasitized by *C. congregata*, which did not occur (or occurred to a limited degree) in non-habitual refractory hosts where encapsulation resulted. Most recently, Rodriguez-Perez et al. (2006) described the cellular events associated with *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) parasitization of permissive host stem borer (*Diatraea saccharalis*) Fabricius (Lepidoptera, Pyralidae) and refractory (*M. sexta*) hosts and suggested haemocyte apoptosis is induced in the refractory host where egg encapsulation is triggered. The effect of the host in variation of successful parasitism of *B. fusca* by *C. sesamiae* cannot be discounted. *Sesamia calamistis* was permissive to both the CsVr and CsAV while *B. fusca* evidently exhibited disparities and hence results shed light on the refractoriness of *B. fusca* towards *C. sesamiae* biotypes. Furthermore, Mochiah et al. (2003) showed that the total numbers of haemocytes in *B. fusca* larvae parasitized at several time points by the inland CsVr populations were lower than those in larvae parasitized by coastal CsAv populations. The numbers of circulating haemocytes in the larvae parasitized by the CsAv population increased linearly from two to 72 h. Gitau et al. (2006) in addition showed that ovarian proteins of the CsVr and CsAV are different and cause differential changes in haemolymph and fat body tissues of *B. fusca* larvae several hours after parasitization. These observations, in combination with the current CsBV CrV1 gene expression analyses suggest that the associated PDV of the two *C. sesamiae* populations are different at several levels and host responses contribute to these differences. The exact contribution and interaction between variations of all these factors is currently being analyzed (Gitau et al., unpublished) with future work focused on genetic crosses and correlation analysis between allelic variations in PDV, calyx proteins and parasitoid performance in segregating generations.

Why has virulence been maintained in some *C. sesamiae* populations only? It can be speculated that PDV genes of the CsAv were maintained in highlands where *B. fusca* is abundant and counter selected in the course of evolution in areas where *B. fusca* was rare due to genetic costs (Gitau, 2006; Le Rü et al., 2006). Differences between avirulent and virulent PDV are associated to both expression patterns and gene sequence of CrV1 gene. This suggests that both expression level and the quality of molecular interactions with host haemocytes contribute to the evolution of virulence in this system. In highly co-evolved host-parasitoid systems, it is anticipated that expression of PDV genes in host haemocytes induces their apoptosis and prevents encapsulation of the parasitoid eggs or other alterations of haemolymph phenoloxidase activity (Sugumaran and Kanost, 1993; Nappi et al., 2000). These findings from the *C. sesamiae*/*B. fusca* system open up new opportunities for research on wasp factors of stem borer parasitoids in Africa. Host populations and host immune responses to *C. sesamiae* populations are clearly important factors capable of limiting parasitoid success. In areas where *C. sesamiae* and *B. fusca* occur in sympatry, it is unclear how populations are regulated. Further analysis into the molecular mechanisms involved in immunosuppression and developmental arrest in targeted pest populations will be necessary. New molecular targets for the development of pest control products that target host immunity or impact stem borer development are needed in order to find lasting solutions to control of cereal stem borers in Africa.

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