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Calyx fluid proteins of two *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) biotypes in Kenya: implications to biological control of the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)

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Abstract. *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) is an indigenous larval endoparasitoid of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in sub-Saharan Africa. In Kenya, reports suggest that *C. sesamiae* occurs as two biotypes. Biotype avirulent to *B. fusca* gets encapsulated by haemocytes in this host and is unable to complete development. Biotype virulent to *B. fusca* is able to overcome immune defences. Factors present in the calyx fluid such as the PolyDNAviruses (PDV), venom and calyx fluid proteins have been implicated in the variation of *C. sesamiae* virulence against *B. fusca*. In the present study, calyx fluid proteins of the two *C. sesamiae* biotypes were compared using 2-D gel electrophoresis. More protein spots were observed in the virulent parasitoid calyx fluid, but some proteins were specifically observed in the avirulent parasitoid calyx fluid while others were observed in both. To study changes in proteins due to parasitism of *B. fusca* larvae by the two strains, SDS-PAGE gel were performed on fat body tissues and the haemolymph at three time points. Differences between the two strains were observed in both the fat body and haemolymph tissues. Parasitism-specific protein bands were detectable in fat body tissues of *B. fusca* larvae parasitized by the two *C. sesamiae* strains. These proteins were absent in unparasitized larvae. Implications for using *C. sesamiae* as a biocontrol agent of *B. fusca* in Africa are discussed.

Résumé. Protéines des fluides du calyx de deux biotypes de *Cotesia sesamiae* (Cameron) (Hymenoptera : Braconidae) au Kenya: implications pour le contrôle biologique du foreur *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae). *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) est un parasitoïde larvaire indigène de *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) en Afrique sub-saharienne. Au Kenya *C. sesamiae* existe sous forme de deux biotypes. Le biotype avirulent pour *B. fusca*, encapsulé par les hémocytes de cet hôte est incapable de s'y développer. Le biotype virulent est capable de contourner ses défenses immunitaires de *B. fusca*. Des facteurs présents dans le fluide du calyx de la guêpe ont été impliqués dans ces variations de virulence. Dans la présente étude, les protéines du calyx de guêpes appartenant aux deux biotypes furent comparées sur gel d'électrophorèse bidimensionnelle. Globalement, plus de spots protéiques furent observés chez la souche virulente, mais certains furent observés spécifiquement chez le biotype virulent, d'autres enfin, chez les deux biotypes du parasitoïde. Afin de comparer la localisation dans la larve et dans le temps des changements protéiques dus aux infestations par les deux biotypes, des gels SDS-PAGE furent réalisés à partir d'extraits de l'hémolymph et du corps gras prélevés à trois intervalles de temps après l'infestation. Les différences entre les souches furent observées pour les deux tissus. Certaines protéines, spécifiques du parasitisme, absentes des larves non parasitées, furent observées dans le corps gras des larves parasitées par les deux souches de parasitoïde. Les implications pour l'utilisation de *C. sesamiae* comme agent de lutte biologique sont discutées.

Keywords: *Busseola fusca*, *Cotesia sesamiae*, Stem borer, Endoparasitoid, Africa.

Hymenoptera endoparasitoids spend their life cycle inside other insects, generally Lepidoptera hosts. The host immune system can perceive the parasitoid's egg as foreign, and respond by mounting an encapsulation reaction that can lead to the egg's

death (Ratcliffe 1993). A variety of mechanisms have been developed by parasitoids to overcome the defence reactions of their natural hosts. The most well studied mechanism is immune suppression induced by symbiotic viruses known as PolyDNAviruses (PDV) (Asgari & Schmidt 1994a; Hayakawa & Yazaki 1997; Beckage 1998; Drezen *et al.* 2000).

Many hymenopteran parasitoids contain viruses and other components in their ovaries which are co-

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injected together with the eggs and specifically interfere with the hosts' internal defence mechanisms (Fleming 1992; Stolz 1992). They also manipulate the hosts' physiology in order to accommodate and favour the developing parasitoid larvae (Vinson 1990). Parasitoid females in several genera of parasitoid wasps from Ichneumonidae and Braconidae families produce PDVs in the calyx gland of their ovaries that are injected into the host during parasitism and are disrupting to the host immune response. The PDV DNA is also present as integrated proviruses in the parasitoids chromosomes (Savary *et al.* 1997; Belle *et al.* 2002). Certain PDV genes are transcribed and translated and exert effects on the lepidopteran host, including disruption of host immune systems and protein synthesis in ways that favour parasitoid survival. The calyx fluid injected into the host along with the eggs also contains ovarian and venom proteins.

It is known that some host insects react quickly to foreign objects that are introduced into their haemolymph (Ratcliffe 1993). The time that is required to express viral genes in the host cells (Theilmann & Summers 1986) and to change the immune status of the host (Stoltz 1986) probably exceeds the time it takes to encapsulate the egg of the parasitoid. Calyx fluid proteins have been known to offer early protection of eggs before PDV expression. In the parasitoid *Cotesia rubecula* (Marshall 1885) (Hymenoptera: Braconidae), early protection of eggs by calyx fluid proteins has been shown in the host *Pieris rapae* L. 1758 (Lepidoptera: Pieridae) (Asgari & Schmidt 1994b). Calyx fluid proteins and egg surface proteins hence could provide passive protection to the parasitoid eggs from encapsulation by the host. Although the mechanisms involved in immune suppression have been studied extensively in many systems, the factors involved in its natural variation remain little studied, especially for PDV carrying wasps.

Cotesia sesamiae (Cameron 1891) (Hymenoptera: Braconidae) is a gregarious koinobiont endoparasitoid that is widespread in Africa (Mohyuddin 1990; Polaszek & Walker 1991) and attacks mid- to late- instar stem borer larvae. This parasitoid attacks several lepidopteran stem borer larvae including *Sesamia calamistis* Hampson 1910 (Noctuidae), *Busseola fusca* (Fuller 1901) (Noctuidae), *Chilo partellus* (Swinhoe 1885) (Crambidae) and *Chilo orichalcociliellus* (Strand 1911) (Crambidae) (Mohyuddin 1971; Polaszek & Walker 1991). Among the complex of stem borers on maize and sorghum in sub-Saharan Africa, only *B. fusca* are able to mount an immune response against *C. sesamiae*. A study by Ngi-Song *et al.* (1998) showed that *C. sesamiae* from the Kenyan coast does not develop in *B. fusca*, whereas

C. sesamiae from Kitale successfully develops in *B. fusca*. The fact that *C. sesamiae* exists in two biotypes that react differently to *B. fusca* immune reactions raises a few questions about the physiological differences that may exist between the two parasitoid populations.

Studies by Mochiah *et al.* (2002) showed that eggs of *C. sesamiae* from Kenyan coast that normally do not develop in *B. fusca*, developed when the host was injected with calyx fluid of *C. sesamiae* from Kitale prior to oviposition. This indicates that factors in the calyx fluid are responsible for disarming the immune system of *B. fusca* and that the factors from the two *C. sesamiae* biotypes are physiologically and genetically different. PDV expression has been detected in haemocytes, fat bodies and other tissues in some parasitoid systems as early as four hours post-parasitism (Webb & Luckhart 1994). Calyx fluid proteins and viral proteins play a vital role in the encapsulation response of the host in the presence of a functional PDV (Hayakawa 1994; Asgari & Schmidt 1994b). Venom and ovarian proteins are introduced directly into the haemolymph during parasitization where they may target the haemocytes or other components of the host immune system.

There is renewed interest in the redistribution of *C. sesamiae* as a biological control agent of stem borers in Africa (Schulthess *et al.* 1997). The location from where *C. sesamiae* would be drawn from during the releases and the strain of the parasitoid to be used need to be known depending on the investment in resistance of the target host species. In order to identify parasitoid or host proteins involved in the variation in *C. sesamiae* virulence, we compared calyx protein migration patterns in the two *C. sesamiae* biotypes as well as in *B. fusca* larvae parasitized by the two biotypes as opposed to unparasitized ones.

Materials and methods

Insects collection and rearing

Insects were collected from farmers' fields in Kitale and Mombasa, Kenya (fig. 1). Plants that exhibited signs of stem borer attack or feeding were randomly picked, dissected and all the stem borer larvae and parasitoid cocoons found in the stems placed individually in glass vials (7.5 cm x 2.5 cm). The larvae were provided with a piece of maize stem or artificial diet (Onyango & Ochieng-Odero 1994). The collected material was transported to the laboratory in ICIPE, Nairobi and the larvae were observed for cocoon formation and parasitoids emergence. Adult *Cotesia* spp. that emerged from cocoons were identified using the shape of male genitalia or the propodia in all-female broods (Kimani-Njogu & Overholt 1997). Upon identification, *Cotesia sesamiae* progeny were allowed to mate under light in a vial. *C. sesamiae* females from Kitale were reared on *B. fusca*, while Mombasa *C. sesamiae* were reared on *S. calamistis* larvae. The stem borers were hosts from which the

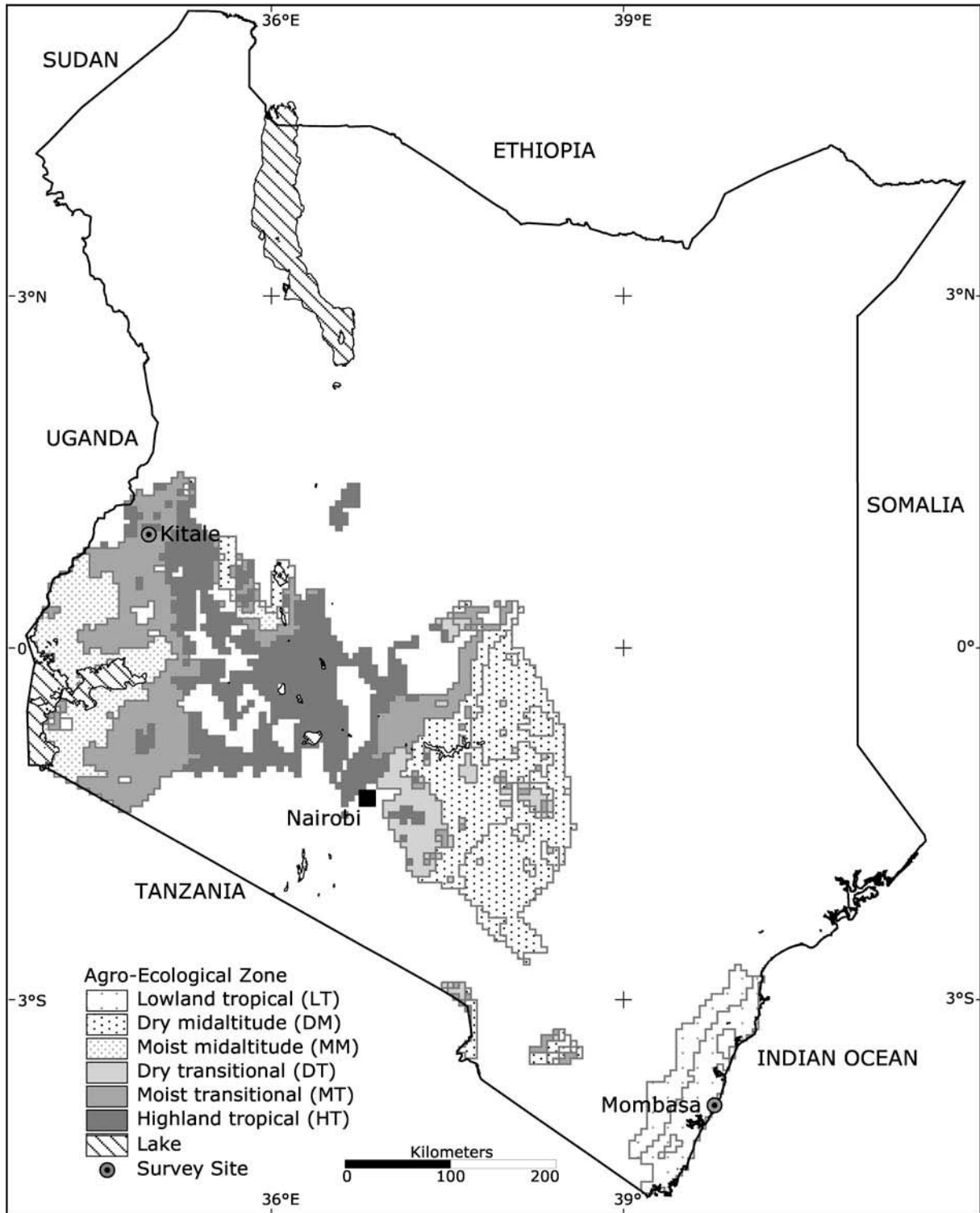


Figure 1
Map of Kenya showing the two geographic locations where *Cotesia sesamiae* and *Buseola fusca* were collected. The coastal Mombasa *C. sesamiae* population was collected from Mtwapa.

parasitoids emerged from. Larvae were placed in artificial diet at 25 ± 1 °C until cocoon formation and later wasp's emergence. Progeny that emerged were used for the bioassays.

Collection of calyx fluid

Mated two- to three- days old *C. sesamiae* females from Kitale and Mombasa were used for the experiment. 50 female wasps were selected from the rearing cages, put in a vial and

immobilized on ice prior to dissection. A drop of phosphate buffer saline (PBS pH 7.0) was placed on a Petri dish and dissection carried out on ice blocks. Using sharp dissecting forceps, the intersegmental membranes between the posterior abdominal and the dorsal part of the abdominal segments of the female *C. sesamiae* were teased out. The ovipositor was grasped and pulled free to remove the reproductive system. Upon each single dissection, the ovaries were placed in an Eppendorf tube containing 100 μ l protease inhibitor cocktail (Sigma P2714)

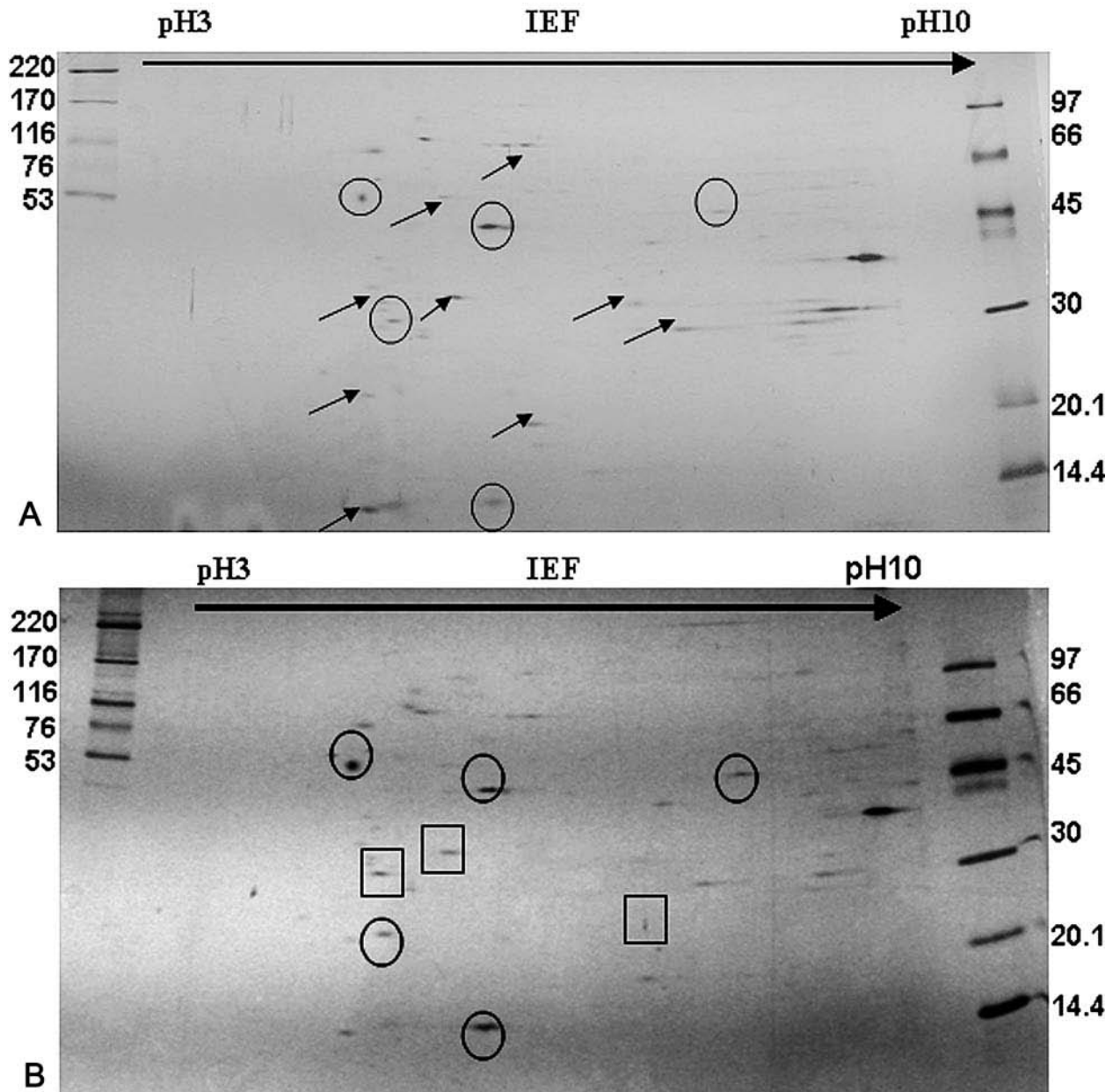


Figure 2

IEF 2-D gel migration of calyx fluid from virulent (A) and avirulent (B) biotypes of *C. sesamiae*. First dimension: pH 3-10; second dimension 4-20 % acrylamide. Open circles: protein spots present in both virulent and avirulent line calyx fluids. Arrows: protein spots unique to the virulent line calyx fluid. Open squares: protein spots unique to the avirulent line calyx fluid. Only the prominent spots were considered.

in PBS. The samples of the ovaries from each location were pooled together and maintained on ice until all dissections were completed. To shear the ovaries and release the calyx fluid, the ovaries were drawn in and out into a 17 G syringe followed by a 23 G syringe for 5 minutes each syringe. The contents were then centrifuged for 4 minutes at 3000 g at 4 °C. The supernatant was collected and transferred into a clean-labelled centrifuge tube and placed on ice.

2-D gel electrophoresis on calyx fluid proteins of *Cotesia sesamiae* Kitale and Mombasa strains

2D-PAGE was performed as described by O'Farrell (1975). For each sample, 60 µl calyx fluid supernatant was added to 60 µl of IEF sample buffer and 60 mg of urea. Urea was mixed by gently tapping the sample until all the particles dissolved. For the first dimension (isoelectric focusing, IEF) we used gradient gels covering the range pH3 to pH10 (ampholines, Millipore Inc). The IEF gels were run at 160 V for 16 hrs and then 320 Volts for 1 hr. We used 4-20% acrylamide gels for the second dimension. The 2-D gels were silver stained as described by Morrissey (1981).

Protein profiles for haemolymph and fat bodies of larvae parasitized at different time points

Cotesia sesamiae were allowed to oviposit on *B. fusca* and *S. calamistis* larvae using the hand-sting method (Overholt *et al.* 1994). Larvae were placed in artificial diet until dissections 6, 12 or 24 hours post oviposition. The larvae were washed with 70% ethanol and rinsed in distilled water before collection of fat bodies and haemolymph. The abdominal proleg was snipped to release haemolymph into an Eppendorf tube containing 100 µl protease inhibitor. Care was taken not to rupture the gut and any samples that were contaminated were discarded. Fat bodies were thereafter dissected and other tissues carefully removed and discarded. The fat body tissues were washed in PBS five times to remove haemolymph residues and thereafter placed in 100 µl protease inhibitor.

Proteins in both fat body and haemolymph samples were purified under denaturing conditions using Urea, Tris-Cl and Sodium monophosphate. The samples were prepared for Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) by boiling 100 µl of sample with equal amounts of disruption mix (Glycerol, SDS, β mercaptoethanol and Tris-HCL and bromophenol) blue for five minutes. Fat body

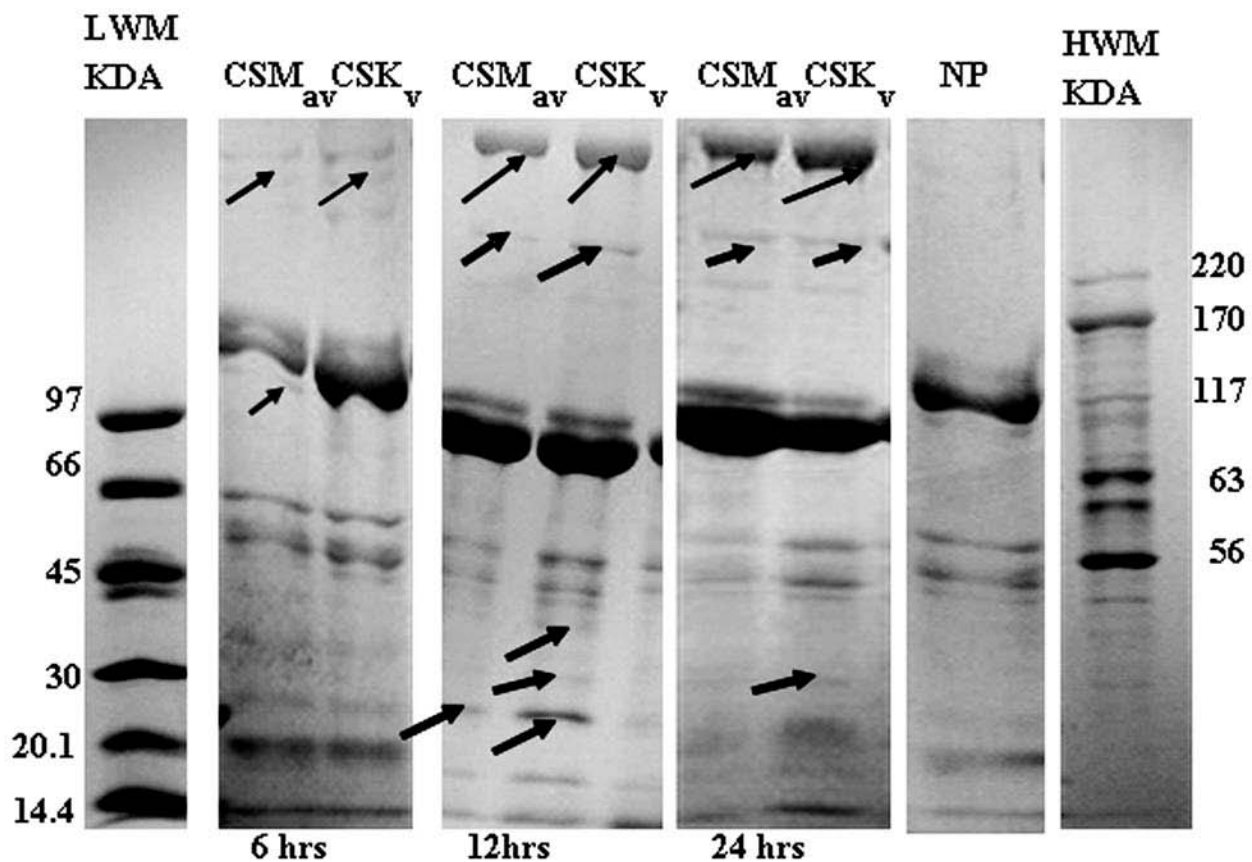


Figure 3

SDS-PAGE migration (4-20%) of proteins from *Busseola fusca* tissues dissected from larvae parasitized by virulent and avirulent lines of *Cotesia sesamiae* at different time points post-infestation. The bands were compared with haemolymph from non-parasitized larvae at 12 hours post infestation. Samples of 30µl were loaded in each lane (A: fat body samples; B: haemolymph samples; Bf: *Busseola fusca*, /Av: parasitized by avirulent line of *C. sesamiae* from Mombasa; /V: parasitized by virulent line of *C. sesamiae* from Kitale; /NP: non parasitized larvae; LWM: low weight molecular standard; HWM: high weight molecular standard).

tissues were homogenised with a tissue grinder before they were prepared for loading. SDS-PAGE was carried out according to the method of Laemmli (1970) using 4-20 % (w/v) acrylamide gels which were run by loading 30 μ l of each sample alongside 30 μ l high and low molecular weight standards (Amersham Biosciences). A negative control was run on samples from *B. fusca* larvae that were not parasitized while a positive control was run on samples that were derived from the permissive host, *S. calamistis*. Gels were stained by the Coomassie Brilliant R250 and destained at room temperature.

Data analysis and interpretation

Silver stained 2-D gels spots were compared by superimposing the corresponding Kitale and Mombasa gels on a light box. We considered differences in spots either present or absent in gels of samples run on the 2-D gel electrophoresis. Only distinct spots were considered. For the haemolymph and fat body samples, specific bands on the coomassie stained SDS gels were

compared with the control and bands by the permissive host *S. calamistis*. Scoring was done qualitatively for bands that were present in the test samples but not in the control. Both the 2-D gels and the SDS gels were repeated at least 3 times. Only repeatable qualitative differences were considered.

Results

2-D gels protein migration patterns for calyx fluid samples extracted from the virulent Kitale and avirulent Mombasa *Cotesia sesamiae* are shown in fig. 2. Sustainable differences were observed between the two biotypes. There were more protein spots in protein gels with calyx fluid samples from virulent *C. sesamiae* biotype compared to the avirulent biotype (Chi sq = 7.00; df = 1; P = 0.0082). There were nine virulent specific spots present in Kitale calyx-fluid gels,

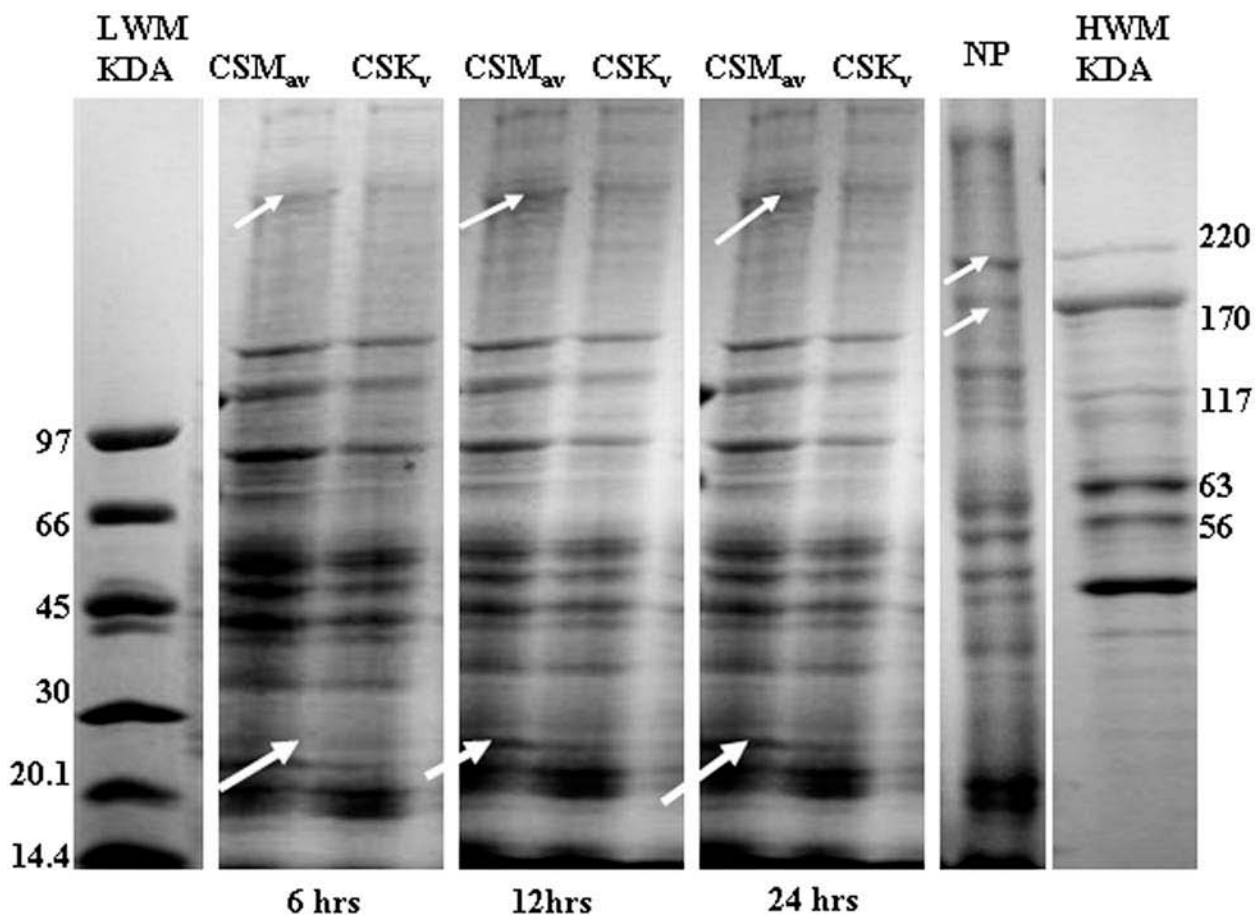


Figure 4

SDS-PAGE migration (4-20% gradient) of proteins from *Sesamia calamistis* and *Busseola fusca* tissues dissected from larvae parasitized by virulent (V) and avirulent (Av) lines of *C. sesamiae* at 12 hours post-infestation. 30 μ l of samples were loaded in each lane. Bf : *B. fusca*, Sc: *S. calamistis*, /Av: parasitized by avirulent line of *C. sesamiae* from Mombasa, /V: parasitized by virulent line of *C. sesamiae* from Kitale, /NP: nonparasitized. LWM: low weight molecular standard; HWM: high weight molecular standard in kilodaltons (A: fat body samples; B: haemolymph samples; Bf: *Busseola fusca*; /Av: parasitized by avirulent line of *C. sesamiae* from Mombasa; /V: parasitized by virulent line of *C. sesamiae* from Kitale; /NP: non parasitized larvae; LWM: low weight molecular standard; HWM: high weight molecular standard in Kilodaltons).

5 avirulent-specific spots present in Mombasa calyx-fluid gels, and five spots were present in both gels.

SDS-PAGE gels were compared for haemolymph and fat body (fig. 3, 4). The bands scored are shown in tab. 1 and tab. 2. The intensity of bands increased with time post oviposition for the bands scored for tissues parasitized by the two *C. sesamiae* strains. Bands were always more intense for fat bodies from larvae parasitized by virulent *C. sesamiae* females than for fat bodies from larvae parasitized by avirulent *C. sesamiae* females. There were more bands on fat bodies compared to haemolymph samples signifying more complex expression in the fat bodies than in the haemolymph. Only two protein bands, at >220 and 28 Kb were scored for haemolymph from larvae parasitized by avirulent *C. sesamiae*. These bands were absent in the control samples signifying that these proteins were induced by parasitism by the avirulent *C. sesamiae* strain.

Protein bands compared between permissive (*S. calamistis*) and non-permissive (*B. fusca*) host species from the fat body and haemolymph samples parasitized with the virulent and avirulent *C. sesamiae* strains (figs. 5 and 6 respectively). The marked bands present on the permissive host were similar for larvae parasitized by both strains, for both fat body and haemolymph samples. This is consistent with the hypothesis that the proteins induced by parasitism in permissive hosts do not depend on the virulence to non permissive host of the parasitoid strain.

Table 1. Analysis of fat body samples at different time points compared with the control samples for selected bands obtained with SDS-PAGE. CSM=*Cotesia sesamiae* from Mombasa, CSK=*Cotesia sesamiae* from Kitale. av and v=avirulent and virulent respectively.

Analyzed bands Molecular weight in KDA	NP	CSM _{av} 6 h	CSK _v 6 h	CSM _{av} 12h	CSK _v 12 h	CSM _{av} 24 h	CSK _v 24 h
>>220	0	*	**	*	**	**	***
>220	0	0	*	*	**	*	*
40	0	0	0	0	*	0	0
32	0	0	0	0	*	0	*
28	*	*	*	**	0	0	0

Amplification of the band pattern by SDS-PAGE on the gel. *** = Strong; ** = Moderate; * = Slight; 0 = No band; av=avirulent; v=virulent.

Discussion

In host insects with non-cellular defence capacities, additional strategies are required to completely protect the parasitoid against the host defence reactions. As bracoviruses are released from calyx cells by a lysis process, it is possible that non-assembled virus proteins are present in the calyx fluid. Eggs that pass through the calyx gland are exposed to components from the fluid and some of the proteins might become attached to the eggs surface offering it protection before PDV expression begins. Studies with *Cotesia rubecula* show that dissected eggs from the ovaries get encapsulated whereas eggs from the calyx gland and in the oviduct are protected (Asgari & Schmidt 1994b). This indicates that the protective layer is acquired within the calyx gland as the egg passes from the ovary to the oviduct. It is speculated that the proteins also protect the intact virus from recognition by the host.

Virulent *Cotesia sesamiae* populations showed marked differences in the proteins present in the calyx fluid compared to avirulent *C. sesamiae* population. The avirulent strain is lacking two particular spots present in the Kitale *C. sesamiae* strain, one of 40 kDa, another of 32 kDa. It can be speculated that these different spots may play a role in immune suppression since they are absent in the avirulent strain. Common protein spots present in the two strains can exhibit amino acid substitution, leading to a non functional protein in avirulent line in *Busseola fusca* host. Alternatively, common proteins are not involved in the variations between the strains.

Table 2. Analysis of haemolymph at different time points compared with the control samples for selected bands obtained with SDS-PAGE. CSM=*Cotesia sesamiae* from Mombasa, CSK=*Cotesia sesamiae* from Kitale. av and v=avirulent and virulent respectively.

Analyzed bands Molecular weight in KDA	NP	CSM _{av} 6 h	CSK _v 6 h	CSM _{av} 12 h	CSK _v 12 h	CSM _{av} 24 h	CSK _v 24 h
>220	0	0	*	*	**	*	*
28	0	**	*	**	*	**	*

Amplification of the band pattern by SDS-PAGE on the gel. *** =

Strong; ** = Moderate; * = Slight; 0 = No band; av=avirulent; v=virulent.

A 32-kDa protein (Crp32) and a heat-shock proteins CrHs70 and calreticulin CrCRT have been implicated in the prevention of cellular encapsulation of *C. rubecula* eggs in *Pieris rapae* (Asgari *et al.* 2003). Beckage *et al.* (1986) found that *Manduca sexta* (L. 1763) (Lepidoptera: Sphingidae) larvae naturally parasitized by *Cotesia congregata* (Say 1836) produced proteins of 56-kDa and 60-kDa whereas larvae injected with calyx cells (cells where PDV's are produced) extract, produced a 33-kDa polypeptide. The authors concluded that this polypeptide results from a viral gene expression or is a protein induced by presence of the virus. In *C. rubecula*, a non PolyDNA virus 65-kDa protein reacted with specific antibodies similar in size to protein known to be involved in host immune suppression. In the present study, a 32-kDa and a 40-kDa protein were observed in fat body samples parasitized by the virulent strain at 12 and 24 hours but not on fat body samples parasitized by the avirulent strain. Proteins size greater than 220-kDa was also observed in the haemolymph and fat bodies larval samples parasitized by both the virulent and avirulent *C. sesamiae* strains. Grossniklaus-Burgin *et al.* (1998) observed a 212-kDa protein band in haemolymph of *Spodoptera littoralis* (Boisduval 1833) (Lepidoptera: Noctuidae) parasitized by *Chelonus inanitus* L. 1767 (Hymenoptera: Braconidae). This band was absent in the non-parasitized larvae.

To examine possible changes in the host organism after parasitization, protein extracts from haemolymph and fat bodies were analysed and compared to non-parasitized larvae. Results from this study provide some evidence that calyx fluid proteins or proteins expressed by polydnavirus may be capable of inducing significant physiological alterations, in *B. fusca* larvae as well as in the permissive host *Sesamia calamistis*. Compared to the control, some proteins seem to be inhibited when avirulent *C. sesamiae* Mombasa parasitizes *B. fusca* while others are enhanced in the fat body when the virulent *C. sesamiae* Kitale parasitizes the hosts. These alterations may be associated with suppression of host defence mechanism as suggested by other authors (Beckage *et al.* 1986; Stolz & Guzo 1986; Beckage & Kanost 1993; Strand & Noda 1991).

Fat bodies of larvae infested by the virulent strain had four different protein bands that were absent in the non-parasitized control. Fat bodies are likely to be the tissues in which the proteins are expressed, or changes within the host tissues are most noticeable. Obviously, parasitism has drastic physiological effects on this tissue which gradually atrophies after parasitoid eggs hatch and the host stops feedings. Although the fat body is not directly consumed by the wasp, it apparently

experiences severe metabolic stress during the early and final stages of association of the endoparasitoids and their hosts. Encapsulated eggs can be observed mostly in the fat bodies 6 hours post parasitism (Ngi-Song *et al.* 1998; Gitau unpublished).

Host immune evasion in PDV containing wasps is likely mediated by expression of viral genes or host genes under the regulation of the PDV, calyx proteins or venom which may differ depending on the host. This is the first study that compared virulent and avirulent lines of parasitoids associated with PDVs for their calyx protein contents. The identification of differences opens new lines of researches. Two main issues deserve investigation. Are the proteins in the calyx fluid of *C. sesamiae* females related to viral proteins or do they get attached to the egg surfaces or the virus particles on their passage from the ovaries into the oviduct.

Conclusion

The differences in protein spots and bands show that there is variation in the two *Cotesia sesamiae* biotypes at the protein level. The virulent strain would be the best host to release or redistribute in areas where *Busseola fusca* is the dominant species. The avirulent strain would be best when released in areas where other stem borers are abundant. Studies to compare the fecundity of the virulent and avirulent strain are currently going on (Gitau *et al.* unpublished). Information gathered from both these studies will shed light into whether the avirulent strain is inferior to the virulent one and if it can be used for biological control where *B. fusca* is absent.

The present study indicates that the calyx proteins of virulent and avirulent strains are different, and that larvae parasitized by either the *C. sesamiae* strain elicits production of different proteins in the host which may affect development of the parasitoids larvae. Clarification of the origin of these proteins *i.e.* hosts versus parasitoid tissues and characterization of their biological role during parasitism remains a big challenge. Whether the differences in the protein patterns are responsible for the variation in the ability of *C. sesamiae* from Mombasa to develop in *B. fusca* still remain to be examined. Several proteins differ between virulent and avirulent parasitoid calyx fluids, suggesting virulence variations are governed by several factors, likely with epistatic interactions especially between viral and parasitoid proteins. Sequencing the bands, common or specific to each biotype would elucidate the role of calyx fluid proteins in host immune evasion. Eluting and injecting them in *B. fusca* would allow us to determine their respective function and potential

epistatic interactions. Ultimately, developing markers for all these factors would allow the survey of genetic adaptation of this parasitoid to its hosts in endemic regions, or in exotic areas, following its introduction for biological control.

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