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Evolution of a Polydnavirus Gene in Relation to Parasitoid–Host Species Immune Resistance

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Abstract

CrV1, a polydisperse DNA virus (polydnavirus or PDV) gene contributes to the suppression of host immunity in *Cotesia* genus parasitoids. Its molecular evolution was analyzed in relation to levels of resistance in the sympatric host species. Natural selection for nonsynonymous substitutions (positive Darwinian selection) was observed at specific amino acid sites among *CrV1* variants; particularly, between parasitoid strains immune suppressive and nonimmune suppressive to the main resistant stem borer host, *Busseola fusca*. In *Cotesia sesamiae*, geographic distribution of *CrV1* alleles in Kenya was correlated to the relative abundance of *B. fusca*. These results suggest that PDV genes evolve through natural selection and are genetically linked to factors of suppression of local host resistance. We discuss the forces driving the evolution of *CrV1* and its use as a marker to understand parasitoid adaptation to host resistance in biological control.

Parasitoids have developed a wide array of mechanisms to overcome their host immunity (Salt 1968). One of the most original mechanisms is the unique association with endosymbiotic viruses termed polydisperse DNA virus (polydnavirus or PDV), observed in some Braconid and Ichneumonid parasitoid wasps (Webb and Strand 2005). The viruses are injected into the host along with the parasitoid's eggs and are expressed in various tissues. PDVs contribute to the active regulation of the nutritional physiology, development, and immunity of the host. They have been extensively studied as model systems to investigate parasitoid–host immune interactions (Glatz et al. 2004) and have been considered unique tools to study the evolution of parasitoid–host range (Whitfield 1994).

To our knowledge, molecular variations of PDV immune suppressive genes have never been investigated within species or among closely related species especially in relation to variation in the parasitoid–host range. Parasitoid–host immune interactions do vary geographically. In some localities, the parasitoid larvae successfully develop and the host dies and in others the host successfully resists parasitoid infestation, killing the infesting parasitoid larvae (Kraaijeveld and Godfray 1999; Dupas et al. 2003). Understanding the geographic patterns of resistance–virulence interactions is economically important for parasitoid–host interactions associated with biological control. Insects associated with PDVs may be good models because PDV harbor genes necessary for parasitoid success which can be used as potential markers. PDVs have been the subject of molecular investigations since the early 1970s, and some genomes have been completely sequenced (Espagne et al. 2004; Glatz et al. 2004; Webb and Strand 2005). The best-characterized genome, the CcBV, is integrated as a macrolocus in the *Cotesia congregata* (Braconidae) parasitoid genome (Belle et al. 2001). The virulence factors exhibit genetic linkage in this macrolocus, which should facilitate the future development of molecular markers of virulence.

Cotesia sesamiae Cameron (Hymenoptera: Braconidae) is a parasitoid wasp associated with the PDV attacking stem borer larvae on maize (*Zea mays* (L.) and sorghum (*Sorghum bicolor* (L.) Moench) in sub-Saharan Africa. The main endemic stem borer pest on maize and sorghum is *Busseola fusca* Fuller (Lepidoptera: Noctuidae) (Schulthess et al. 1997). *Cotesia sesamiae* varies in its developmental success on one of

its major hosts, *B. fusca*. *Cotesia sesamiae* exists as 2 biotypes: one virulent and able to develop successfully and the other avirulent, encapsulated by the *B. fusca* immune reaction (Ngi-Song et al. 1995; Mochiah et al. 2001; Mochiah, Ngi-Song, Overholt, and Stouthamer 2002; Gitau 2006; Gitau et al. 2006). *Busseola fusca* is the only resistant host observed so far. Other known maize and sorghum stem borer pest species in East Africa, namely, *Sesamia calamistis* Hampson, *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae), and *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) are unable to develop an immune response to *C. sesamiae* (Schulthess et al. 1997). In the field, *B. fusca* dominates stem borer communities in the highlands and is rare in the lowlands (Ong'amo et al. 2006). As expected the virulent parasitoid strain was collected in the highlands and the nonvirulent parasitoid strain was collected in lowlands (Ngi-Song et al. 1998; Gitau 2006). This is consistent with the hypothesis of local adaptation to host community structure. This hypothesis assumes that trade-offs are responsible for counter selection of virulence to *B. fusca* in localities where *B. fusca* is absent or rare. Trade-offs for virulence have been suggested in other described parasitoid–host systems (Dupas and Boscaro 1999; Kraaijeveld et al. 2001). Infestation experiments of different geographic strains of *B. fusca* by different populations of *C. sesamiae* confirm this general trend (Gitau et al. 2007). Variation in virulence is an on/off switch and is correlated with the average stem borer community composition (Gitau 2006). Strong selective pressures are expected which may lead to rapid coevolutionary cycles of host resistance and parasitoid virulence (Thompson 2005). In addition, the seasonal changes in stem borer species composition, especially in mid-altitude areas (Ong'amo et al. 2006), may permanently modify selection mosaics and lead to transient local maladaptations. It would be easier to unravel the dynamics of coevolution in a changing geographic mosaic of selective pressures by developing a molecular marker for virulence.

Injection of virulent calyx fluid into larvae infested by avirulent wasps demonstrated that the factors responsible for the variation in *C. sesamiae* ability to suppress *B. fusca* immune reactions were present in the calyx fluid of the wasp (Mochiah, Ngi-Song, Overholt, and Botchey 2002). Calyx fluid contains PDVs and other factors, such as venom, but PDV certainly have major effects (Asgari et al. 1996). The PDV genome is segmented with different segments containing numerous genes expressed during parasitic processes that are involved at different steps in immune suppression and host regulation (Espagne et al. 2004). It is likely that all genes are colocalized and cosegregate in the genome. In *C. congregata*, results obtained from in situ DNA hybridization experiments suggest that the PDV segments are integrated at the same macrolocus in the wasp chromosomes (Belle et al. 2001). In *Cotesia rubecula*, the only gene expressed in the host hemocyte is the gene *CrV1*. The *CrV1* gene is secreted into the hemolymph causing the destabilization of the cytoskeleton; disabling the capacity of hemocytes to spread onto foreign surfaces (Asgari et al. 1996). Binding and incorporation to the host hemocyte is caused by a coiled-coil region at the end of the gene (Asgari and Schmidt 2002). The gene has a homolog in *C. congregata*, which is expressed in host hemocytes at different time points of immune disruption (Amaya et al. 2005). It is likely to function as an immune suppressor in several *Cotesia* species. In *C. sesamiae*, Gitau et al. (2007) showed that the *CrV1* gene was expressed in *B. fusca* parasitized by the virulent strain, in both fat body and hemolymph but not or only faintly by the avirulent strain, suggesting a possible link between *CrV1* and virulence to *B. fusca*.

This work aimed to examine selection at the PDV *CrV1* locus in relation to the evolution of *Cotesia* host range, especially to its ability to attack the host *B. fusca*. In order to detect selection at the molecular level, ω ratios or the ratios of nonsynonymous to synonymous distances were analyzed among sites and branches of *CrV1* variants within and between species. An ω ratio above 1 suggests that nonsynonymous mutations are positively selected for due to natural selection (positive Darwinian selection). Positive Darwinian selection was detected at the *CrV1* locus among populations and species related to *C. sesamiae*. To detect selection at the population level in Kenya, the distribution of *CrV1* alleles in *C. sesamiae* was compared with the distribution of *B. fusca*. The correlated distribution observed suggests that *CrV1* locus variation is related to selection by *B. fusca*. Based on these results, we discuss the use of *CrV1* as a marker to understand the evolution of parasitoid success in biological control.

Material and Methods

Parasitoid PDV CrV1 Genotyping

Stem borer infested maize and sorghum stems were collected, and larvae were reared on artificial diet in the laboratory until parasitoid emergence. Cocoon masses were identified as *C. sesamiae* or *Cotesia flavipes* morphologically (Kimani-Njogu et al. 1997) and by PCR test (Dupas et al. 2006). *Cotesia sesamiae* individuals selected for genotyping were from different cocoon masses originating from 55 localities in Kenya (93 individuals, 37 females and 56 males), 20 localities within an area of 15 km^2 in French Congo (8 females and 20 males), and 3 localities in Tanzania (4 female and 3 males). *Cotesia plutellae* Kurdjumov was obtained from T. Guilloux. Sequences from other species have been published by Whitfield (2000) and Dupas et al. (2006).

CrV1 fragment genotyping was performed by sequencing or with PCR tests. All site positions refer to *C. rubecula* original sequence (GenBank AF359344, Asgari et al. 1996). Each of 25 individuals covering the geographic range sampled were sequenced. Each individual was from a different locality in French Congo (3 localities), Tanzania (3 localities), and Kenya (19 localities). Whole insect bodies were frozen in liquid nitrogen for 1 min and ground by mortar and pestle. Total DNA was extracted, using a DNEasy tissue Kit (Qiagen, Hilden, Germany). *CrV1* was amplified and sequenced using primers CrV1087F (5′ ATGTCACTCGTCAAAAGTGC 3′) and CrV2107R (5′ AAAGTTTGCGATGGGGTTGT 3′). Another set of

primers CsfV1125F (5′ TCTCCTGTGTCAATCATGTA-AGTT 3′) and CsfV1944R (5′ACTCCTTCAACGCTGG-GTTCCTTG 3′) was designed from *C. sesamiae* PDV sequences obtained in the present study and *C. flavipes* PDV sequences (Dupas et al. 2006). PCR cycling conditions were as follows: initial denaturation 5 min at 94 °C, 40 cycles of 50 s at 94 °C, 80 s at 51 °C (52 °C for CsfV1125F–CsfV1955R primer couple), and 80 s at 72 °C. Final extension was 10 min at 72 °C. The reaction mix contained 0.4 μM primers, 400 μM dNTP, and 1 μl DNA plus 1 μl Promega (Madison, WI) *Taq* DNA polymerase per 25 μl of reaction. $MgCl_2$ concentration was 1.5 mM for CrV1087–CrV2109 primer pair and 2 mM MgCl2 for CsfV1125F–CsfV1944R primer couple. PCR products were sequenced in both directions using the amplification primers on an automated sequencer (ABI Prism Avant Genetic Analyser 3130, PerkinElmer, Wellesley, MA).

Another set of individuals was genotyped using multiplex PCR and PCR-RFLP tests. DNA was extracted in 100 μl 5% Chelex (BioRad, Hercules, CA) resin. The ground insect was mixed with the Chelex with mortar and pestle and the mix was heated twice for 10 min at 99 °C separated by 3 tube inversions to mix at room temperature. The first PCR test was a primer-specific multiplex PCR with 1 reverse and 2 forward primers. The reverse primer CrV1955R annealed to both variants. The forward primers annealed specifically to coast and inland alleles at different positions. Two different multiplex PCR tests using different primer sets were performed to confirm the results. The first set annealed to inland and coast alleles at positions 1345 and 1458, respectively (CrVcoast1345F (5′ TCGTTCCTCTCC-AAAATCAGATTTCTCAGA 3′) and CrVinland1458F (5′ CTGAAGCACCTAGAAAGCAGTCTAATTTTG 3′)), producing a large amplimer for the coast clade and a small amplimer for the inland. The second set did the opposite (CrVinland1345F (5′ TCGTTCCTCTCCAAAATCAGAT-TTCTCAGC 3′) and CrVcoast1458F (5′ CTGAAGCACC-TAGAAAGCAGTCTAATTTTA 3′)) producing a small amplimer for the coast clade and a large for the inland. The reaction mix contained 3.5 mM $MgCl_2$, 0.4 μM primers, 400 μM dNTP, and 0.5 μl DNA plus 0.5u Promega *Taq* DNA polymerase per 12.5 μl of reaction. PCR cycles were the same as above, but with the annealing temperature at 56 °C. The second PCR test was carried out using the PCR-RFLP. It was used to confirm heterozygosity of individuals when multiplex PCR produced 2 bands. The CrV1 gene fragment was amplified using the CsfV1125F–CsfV1944R primer pair. Restriction digests were performed with *Tfi*I and *Dra*I, cutting specifically inland and coast allele amplimers at positions 1503 and 1552, respectively.

Wolbachia Status

We tested for the presence of *Wolbachia* in each individual genotyped for CrV1 using the *wsp* PCR test (Zhou et al. 1998). Two primer pairs were used separately: 81F-691R for nonspecific amplification and 81F-522R for confirmation of presence of clade B *Wolbachia* sp.

Sequence Analyses

The translated sequences were aligned using Multalin (Corpet 1998) in order to maintain coding frames and then checked visually at the nucleotide level. Patterns of nucleotide substitutions in *CrV1* exon 2 were analyzed between *C. sesamiae* and *C. plutellae* Kurdjumov alleles sequenced in this study and the published *CrV1* sequences from *C. rubecula* (GenBank AF283297, Asgari et al. 1996), *C. flavipes* (Dupas et al. 2006), and CrV1-like sequences from *C. congregata* (Amaya et al. 2005). Phylogenetic relationships between sequences were estimated with MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Model settings were obtained from Modeltest version 3.10 (Posada and Crandall 1998) and used in MrBayes and PAML. Akaike information criterion (AIC) selected the TrN model (Tamura and Nei 1993) and the likelihood ratio test (LRT) selected the HKY model (Hasegawa and Kishino 1985). These models are not tested or implemented in MrBayes. The GTR model which corresponds to the closest generalization of TrN and HKY was used in MrBayes (Lavane et al. 1984). Maximum parsimony (MP) bootstrap support for the Bayesian tree topology was assessed by MP bootstrap procedures in PAUP 4.0b 10 (Swofford 2002) with 1000 replicates (full heuristic search) of 10 simple addition replicates each. The result from MrBayes was used as a phylogenetic hypothesis for the estimation of nonsynonymous to synonymous substitution rate ratio ($\omega = dN/dS$) models in PAML 3.14 (Yang 1997). Eight different models of site- and/or branch-specific ω ratios were compared using AIC and LRT (Wong et al. 2004; Yang et al. 2005).

Proportion of *B. fusca* in Stem Borer Community on Cultivated Cereals

Stem borers were surveyed in 2002–2003 in 67 localities in Kenya to estimate occurrence of each species. They were collected from maize and sorghum 2–5 times a year per locality and reared to the imago stage in the laboratory for identification (Ong'amo et al. 2006).

Statistical Analyses

Environmental Regression

The statistical relationships between *CrV1* clade distribution and percentage of *B. fusca* in the stem borer community on cultivated cereals were estimated using logistic regression taking into account other environmental variables as cofactors. *P* values are not valid due to spatial autocorrelation, but the rank order of importance of each factor is informative. The *CrV1* clade status was considered as a binary response. For each locality, climatic cofactors were maximum and minimum temperature (°C), precipitation (mm), evapotranspiration, and ecological cofactors were expressed as the percentage of maize plants infested by stem borers and percentage of *C. sesamiae* infested by *Wolbachia*. Presence of *Wolbachia* was considered because recent studies have linked a Hymenoptera parasitoid success to the presence of *Wolbachia* in the wasp (Fytrou et al. 2006). All

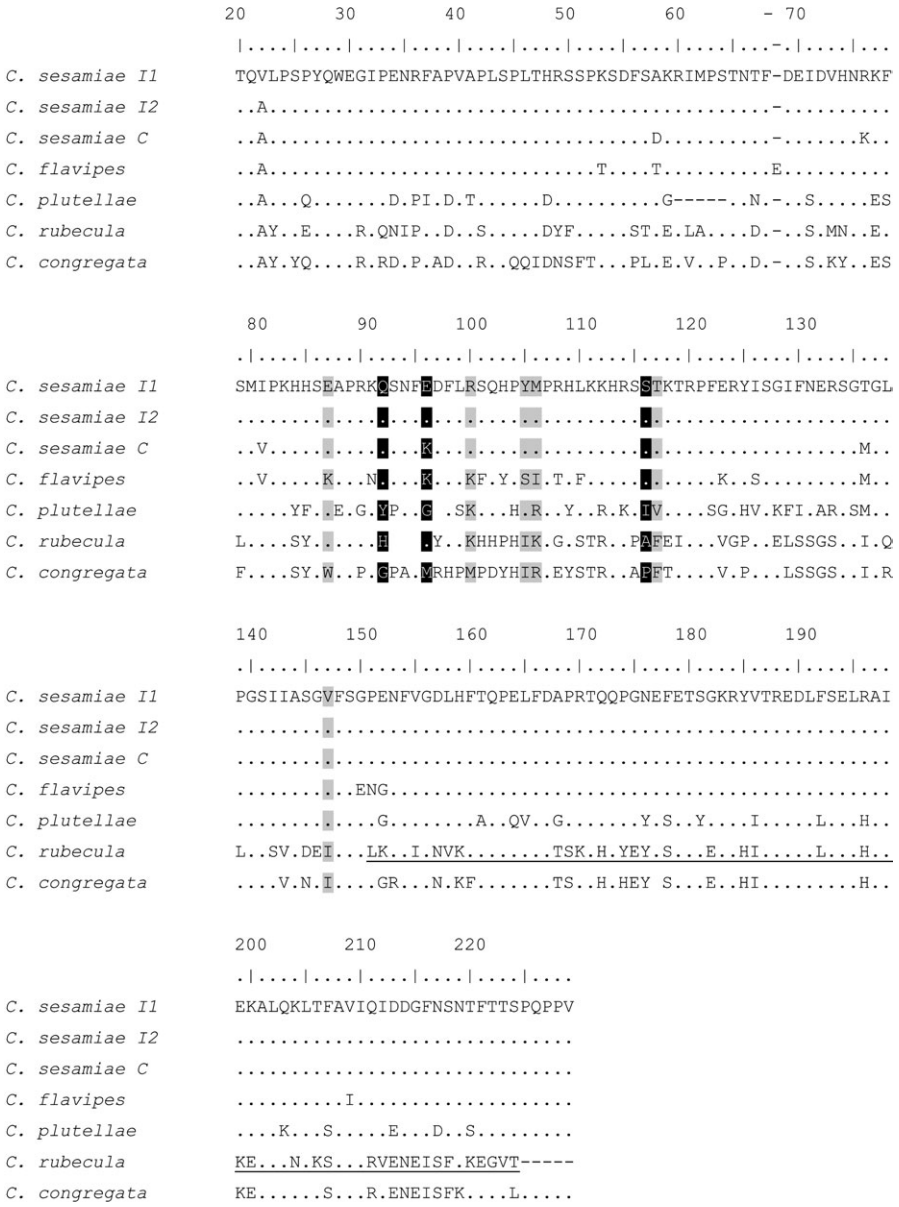

Figure 1. CrV1 protein alignment and location of selected sites. Sites shaded in gray and black have Bayes empirical Bayes posterior probabilities belonging to the selected class of site ($\omega = 2.83$, model M8) superior to 0.90 and 0.95, respectively. Sites belonging to the coil-coiled region necessary for hemocyte binding and uptake and for hemolymph protein formation are underlined. Nucleotide position 1 corresponds to position 1259 in GenBank CrV1 AF359344.

percentages were arcsine square root transformed to increase variance at the interval limits. Mean annual climatic variables were extracted from Kenya AWhere ACT database (Mudsprings Geographers Inc., 2002).

Results

A 695-bp fragment of the PDV CrV1 gene was sequenced or obtained from the literature for different *Cotesia* species and populations (Figure 1). The phylogenetic relationships obtained by using Bayesian inference and MP are described in Figure 2. The 2 avirulent strains of stem borer parasitoid (*C. sesamiae* "Coast" and *C. flavipes*) in one case and the 2 virulent strains of stem borer parasitoid (*C. sesamiae* "Inland1" or I_1, and "Inland2" or I_2) in the opposing case belong to separate clades. Both virulent and avirulent clades have Bayesian posterior probabilities greater than 83% (Figure 2).

The patterns of substitution along the tree was analyzed in the program PAML 3.14 to obtain maximum likelihood estimates of the occurrence of positive Darwinian selection among branches and sites under different models of codon evolution (M0, M0B, M1, M2A, M7, M8A, and M8). The M1, M2A, M7, M8A, and M8 models allow site-specific

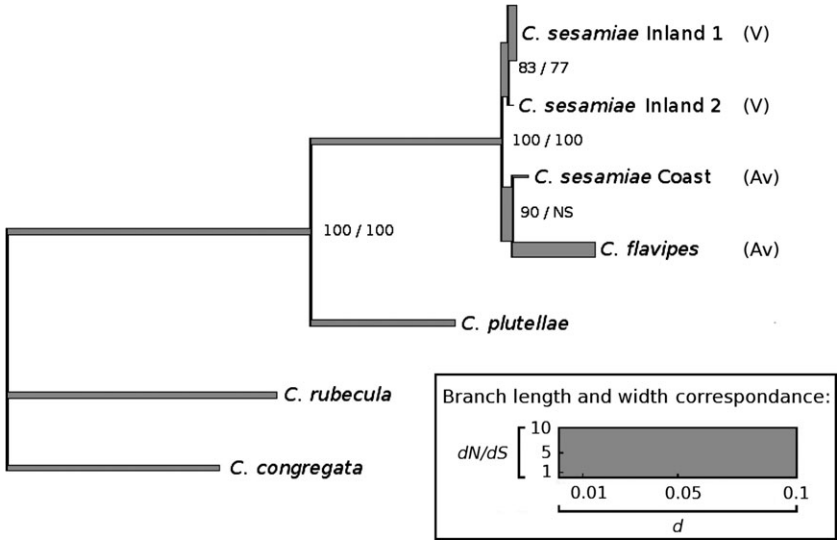

Figure 2. CrV1 PDV phylogenetic tree with branch width representing positive Darwinian selection. Branch widths are proportional to ω ratio estimated under the branch-specific model in PAML. Branch lengths are estimated from the Bayesian inference analysis under the GTR model. X/Y, Bayesian posterior probabilities/MP bootstrap values; NS, nonsignificant at 50% level.

variation in ω. M0B includes branch-specific variation of ω, but the others do not. The M2 and M8 models include positively selected sites class $\omega > 1$. The results are summarized in Table 1. The lowest AIC, model M8, was selected as providing the best fit to the data. Positive Darwinian selection was significant at level $P < 10^{-3}$ in LRT for M8/M8a and M8/M7 comparison (Swanson et al. 2003; Wong et al. 2004). Tree length for the M8 model was 1.80 substitutions per codon. This value is not too different from the optimal value of 1 for accuracy of LRT tests (Swanson et al. 2003). Although the lower number of species analyzed may reduce the accuracy of the tests (Swanson et al. 2003), the high level of significance observed suggests positive Darwinian selection is acting on this gene. The optimal ω ratio of positively selected sites in model M8 was 2.83 (Table 1). Figure 1 shows distribution of selected sites along the sequence. The amino acid site positions refer to the *C. rubecula* Marshall protein (Asgari and Schmidt 2002).

Among the 25 populations of *C. sesamiae* analyzed on maize and sorghum throughout Kenya, Tanzania, Congo (a large part of its geographic range), and on *Panicum* sp. in Congo (3 individuals), only 3 haplotypes were observed. Two haplotypes (I_1 and I_2) were separated by one nonsynonymous substitution at position 22. These were found in inland Kenya. One haplotype was found in coastal Kenya (called allele *C*). It differed from its closest haplotype, allele I_2, by 5 nonsynonymous substitutions at site positions 58, 76, 81, 96, and 136 and one synonymous at position 187 (Figure 1). No single amino acid site was significantly conserved (all belonged to class of sites with ω ratio above 0.61). Variation of ω ratios between branches of the *CrV1* phylogenetic tree estimated in the branch-specific model

Table 1. Codon substitution model selection and parameter estimates in PAML 3.14

Model	Parameter estimates	AIC	LTR	
M0: one rate	ω = 1.3	3393.73		
M0b: branch specific, one rate	ω = 1.14	4000.46	NS	
M1: nearly neutral	$p0 = 0.11$, $p1 = 0.89$	3992.45		
M2A: selected	$p0 = 0.61$, ω0 = 0.67, $p1 = 0$, ω1 = 1, $p2 = 0.39$, ω2 = 2.82	3992.45		**
M7: beta	ωbeta = 0.91 ± 0.04	3992.52		
M8A: beta + ω's = 1	ωbeta = 0.01 ± 0.00	3993.45		
M8: beta + ω's > 1	ωbeta = 0.68 ± 0.29	3980.50	***	***

ω: nonsynonymous/synonymous distance ratio (ω ratio). ω0, ω1, and ω2: ω ratios of site class 0, 1, and 2, respectively. $p0$, $p1$, and $p2$: probabilities of site class 0, 1, and 2, respectively. Models descriptions: M0, one class of ω ratio (all sites have the same optimized ω); M1a, nearly Neutral (2 classes of ω: ω0 < 1, ω1 = 1); M2a, positive selection (3 classes of ω: ω0 < 1, ω1 = 1, ω2 > 1); M7, beta (one class with beta distribution of ω, optimized); M8, beta + ω > 1 (one class with beta distribution of ω and one class ω2 > 1 optimized); M8A, beta + ω = 1 (one class with beta distribution of ω, optimized and one class ω2 = 1, fixed).

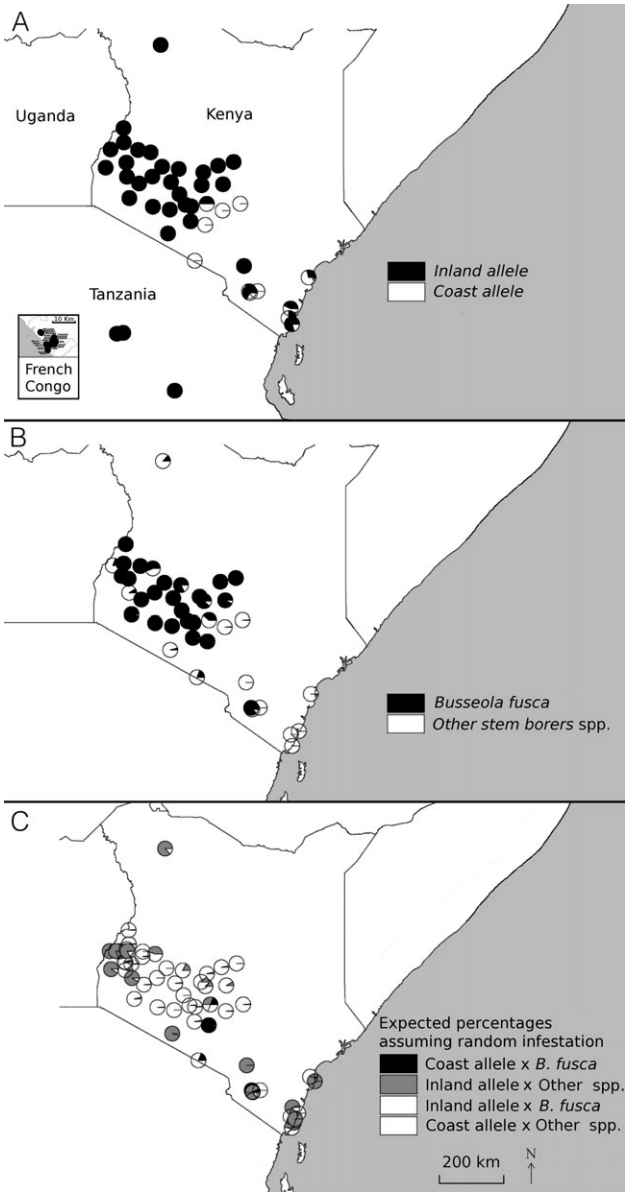

Figure 3. Distributions of *Cotesia sesamiae CrV1* alleles, *Busseola fusca* percentage, and their correlation. (**A**) *CrV1* alleles distribution in Kenya. Black pie portion, inland allele; white pie portion, coast allele. Geographically close and climatically similar localities were merged together in order to make the figure more readable and compare with *B. fusca* percentage distribution. (**B**) *Busseola fusca* percentage in cereal stem borer community distribution. Black pie portion, *B. fusca*, white pie portion, other stem borer species (*Chilo partellus* (Lepidoptera: Crambidae), *Sesamia calamistis* (Lepidoptera: Noctuidae), and *Chilo orichalcociliellus* (Lepidoptera: Crambidae)). (**C**) Probabilities of *CrV1* allele × Host species occurrences, assuming random association for each locality. Coast allele × *B. fusca* = percentage of CrV1 coast allele × percentage of *B. fusca*. Inland allele × other spp. = percentage of inland allele × percentage of other cereal stem borer species. Adapted = percentage of *CrV1* coast allele × percentage of other cereal stem borer species + percentage of inland allele × percentage of *B. fusca*.

M0B is represented by branch thickness in Figure 2. Stronger positive Darwinian selection was observed in branches between populations and species attacking stem borers, especially between strains immune suppressive (*C. sesamiae* I) and not immune suppressive (*C. sesamiae* and *C. flavipes*) to *B. fusca* (Figure 2).

The geographic distribution of *CrV1 I* and *C* alleles in *C. sesamiae* was estimated by genotyping 119 individuals throughout Africa with PCR tests (Figure 3A). All individuals from French Congo were homozygous *II*. In Kenya, haploid males were 10.7% *C* and 89.3% *I*, diploid females were 25.0% *CC*, 5.6% *CI*, and 69.4% *II*. In Kenya, the distribution of *CrV1* (Figure 3A) was analyzed in relation to the percentage of *B. fusca* among maize stem borers (Figure 3B). The statistical relationship between *CrV1* clade status and occurrence of *B. fusca* was analyzed with a logistic regression taking into account other co-variables, including climatic (evapotranspiration, precipitation/rainfall, and minimum and maximum temperature) or ecological (average percentage of stem borer infestation and *Wolbachia* prevalence in the *C. sesamiae* population) for each locality. Results of logistic regression do not depend on the order of the variables included in the model. Table 2 shows that *B. fusca* is the factor having the most significant effect on CrV1 distribution ($P < 0.001$). The only other significant effect was due to precipitation ($P < 0.01$). *Busseola fusca* is the environmental variable accounting for most of the total deviance when tested alone (29.8%). Removing this factor from the full model (including all the factors) increases residual deviance from 57.5 to 71.9 (20.1% increase).

Discussion

The phylogenetic relationships observed with the PDV gene CrV1 are congruent with that reported for parasitoid nuclear genes (Michel-Salzat and Whitfield 2004). These data confirm the results and hypothesis of Whitfield (2000) that no horizontal transfer of PDV occurred during *Cotesia* spp. evolution. The significant grouping of avirulent *C. sesamiae* and *C. flavipes CrV1* variants may suggest that the virulent strain of *C. sesamiae* is another species that anciently diverged from the avirulent *C. sesamiae–C. flavipes* complex. However, crossing experiments showed that virulent and avirulent *C. sesamiae* interbreed when cured from *Wolbachia* (Mochiah, Ngi-Song, Overholt, and Stouthamer 2002), whereas *C. sesamiae* and *C. flavipes* do not interbreed (Kimani and Overholt 1995). The best interpretation of the grouping of avirulent strains from the *C. sesamiae* and *C. flavipes* at the CrV1 locus is therefore that avirulence is ancestral and virulence was acquired once in *C. sesamiae*.

Eight of the 9 sites of the *CrV1* protein positively selected with a Bayes empirical Bayes posterior probability above 0.90 were located in a 30–amino acid fragment between positions 87 and 117 of the 211 amino acids sites in the sequenced protein fragment. The role of this region in host hemocyte disruption is unknown. Bacterial constructs

Table 2. Logistic regression between CrV1 allele frequencies and ecological or climatic factors in *Cotesia sesamiae* in Kenyan localities

	Models with one factor		Model with all factors	
Parameter	Deviance	% Dev. Expl.	*z* value	*P* value Pr(>|z|)
Intercept	113.35		−2.94**	0.0033
Bf	79.60	29.8%	3.31***	0.0009
Inf	106.95	5.6%	−0.39	0.7002
*E*0	112.60	0.7%	1.59	0.1114
Pmm	103.82	8.4%	2.51*	0.0119
% *Wb*	113.07	0.2%	−0.6	0.5500
*T*max	104.74	7.6%	1.31	0.1919
*T*min	91.28	19.5%	−1.13	0.2594

Bf, percentage of *Busseola fusca* among stem borer hosts; *Inf*, percentage of host plant attacked by stem borers; *Wb*, percentage of *C. sesamiae* infested by *Wolbachia*. *Bf*, *Inf*, and *Wb* were arcsine square root transformed. *Pmm*, Annual precipitation; *E*0, annual evapotranspiration; *T*min, annual average of daily minimum temperature. *T*max, annual average of daily maximum temperature. Deviance: residual deviance of the model. %Dev. Expl., percentage of null model residual deviance explained. *z* value: Standardized parameter estimated value. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; $0.05 < P < 0.1$. Note that the results do not depend on the order the variables are included in the model.

with this region deleted were still able to bind to insect hemocytes (Asgari and Schmidt 2002). None of the selected sites and none of nonsynonymous changes between virulent and avirulent lines of *C. sesamiae* were in the coil-coiled leucine zipper region involved in hemocyte binding in *C. rubecula* (Asgari and Schmidt 2002) (positions 151–220, Figure 1). However, the stronger positive Darwinian selection among branches of the *CrV1* (Figure 1) tree was observed in branches between populations and species attacking stem borers, especially between strains immune suppressive (*C. sesamiae* I) and nonimmune suppressive (*C. sesamiae* and *C. flavipes*) to *B. fusca* (Figure 2). This shows that the variation is not linked to hemocyte-binding ability but may be related to immune suppressive variation. Only 2 protein sequence variants are observed among 25 localities in a large part of *C. sesamiae*'s geographic range. This suggests that only 2 discrete adaptive molecular niche are present in cultivated crops, one for the development on *B. fusca* and the other for the development on the other hosts. No molecular diversity is observed within each niche. This contrasts with the diversifying selection observed at many loci involved in other host–parasite interactions. If antagonistic coevolution is responsible for the positive selection observed at virulence locus, it did not lead to important diversification of virulence factors in pairwise interactions with *B. fusca* (there is only one CrV1 variant associated to the resistant host *B. fusca*). Mendelian genetic analyses also lead to similar conclusions in the *Drosophila* parasitoid wasp *Leptopilina boulardi*, with one gene with 2 alleles governing virulence variation against *Drosophila melanogaster* and one gene with 2 alleles governing virulence variation against *Drosophila yakuba* (Dupas et al. 2003). This parasitoid gene for host–species relationship may be a human feature of parasitoid–host immune interactions.

There was a significant association between *CrV1* variants and *B. fusca* percentage in host community. The significance may be overestimated because the data are spatially correlated, but it is the factor explaining most of the variance among different ecological factors. The same geographic pattern was observed in Kenya for parasitism success of *C. sesamiae* on *B. fusca*. Eastern populations were avirulent and Western virulent (Gitau et al. 2007). Altogether, this suggests that the geographic distribution of the *CrV1* alleles in *C. sesamiae* are genetically linked to virulence to *B. fusca* and were affected by natural selection. Genes involved in host immune suppression may be in genetic linkage, constituting few segregating units. For the PDV genes, the genome is segmented, but the 3 PDVs circles analyzed in *C. congregata* with in situ hybridization were found colocalized in the wasp genome (Belle et al. 2001). In *C. congregata*, as well, genetic crosses between CcPDV molecular variants showed only 2 classes of restriction patterns in backcross progeny (Stoltz 1990). For the nonviral genes, the venom protein gene Vn4.2 and ovarian protein gene CrP32 involved in host immune suppression in *C. rubecula* were found to be genetically linked and colocalized physically in the same parasitoid genome fragment (12.5 Kb), but the orientation is in the opposite direction (Asgari et al. 2003). Altogether, these data suggest that selection could occur at other virulence loci in genetic linkage with *CrV1*. What could be the evolutionary forces maintaining *CrV1* inland/coast polymorphisms? It is expected that the CrV1 inland allele–segregating factor increases developmental success on *B. fusca* and that this success has a cost on other hosts. Such cost for virulence was observed in other parasitoid–host immune interactions (Kraaijeveld et al. 2001, Dupas et al. 2003).

What could be the consequences for biological control? Maladapted infestations may occur at places where *B. fusca* occur at intermediate percentage. Figure 3C presents the average match between *B. fusca* and *CrV1* allele percentages. Assuming infestations were random (absence of assortative host choice), this figure represents the proportion of each infestation. *Cotesia sesamiae* infesting *B. fusca* larvae should be at 94% of inland allele and at 6% of coast allele. Infestations of *B. fusca* by coast allele are expected to occur only in the area of transition between highland and lowland on the eastern side of Mount Kenya or in Taita Hills (an isolated highland in eastern coastal Kenya). Reciprocally, *C. sesamiae* infesting other hosts than *B. fusca* should be at 65% of coast

allele and at 35% of inland allele. There is therefore a potential for maladaptation at the CrV1 PDV locus, which is consistent with predictions of the geographic mosaic theory of coevolution (Thompson 2005). Because the stem borer pest species composition varies greatly between seasons (Ong'amo et al. 2006), especially in mid-altitude areas, the system is probably highly dynamic leading to several local maladaptations. The possibility of assortative host choice, not considered in the random infestation assumptions of Figure 3C, may reduce the number of maladapted infestations.

PDVs have been studied intensively for their role in immune suppression and developmental regulation of parasitoid's host for more than 30 years. They are considered model systems for evolutionary adaptation causing immune suppression (Glatz et al. 2004). But their molecular evolution in relation to the host immunity is little studied. Positive Darwinian selection was observed at the *CrV1 Cotesia* PDV locus in this study. The distribution of *CrV1* alleles in Kenyan *C. sesamiae* suggests that *CrV1* variation is associated (directly or indirectly through genetic linkage with other PDV selected gene) to parasitoids' adaptation to geographic differences in immune resistance of a local host community. One possible application in biological control is the use of *CrV1* gene as a virulence marker to monitor parasitoid introduction in a way adapted to local host community composition. Another issue of this work is to document the ability of biological control agents to adapt naturally to their hosts. It was observed that biological control is less prone than chemical control to evolutionary decay due to the evolution of resistance in the pest (Holt and Hochberg 1997). This work documents the hypothesis that this longer sustainability is partly due to the ability of biological control to coevolve with host resistance through a process of natural selection.

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