

Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa

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Abstract

The population genetics and phylogeography of African phytophagous insects have received little attention. Some, such as the maize stalk borer *Busseola fusca*, display significant geographic differences in ecological preferences that may be congruent with patterns of molecular variation. To test this, we collected 307 individuals of this species from maize and cultivated sorghum at 52 localities in West, Central and East Africa during the growing season. For all collected individuals, we sequenced a fragment of the mitochondrial cytochrome *b*. We tested hypotheses concerning the history and demographic structure of this species. Phylogenetic analyses and nested clade phylogeographic analyses (NCPA) separated the populations into three mitochondrial clades, one from West Africa, and two – Kenya I and Kenya II – from East and Central Africa. The similar nucleotide divergence between clades and nucleotide diversity within clades suggest that they became isolated at about the same time in three different refuges in sub-Saharan Africa and have similar demographic histories. The results of mismatch distribution analyses were consistent with the demographic expansion of these clades. Analysis of molecular variance (AMOVA) indicated a high level of geographic differentiation at different hierarchical levels. NCPA suggested that the observed distribution of haplotypes at several hierarchical levels within the three major clades is best accounted for by restricted gene flow with isolation by distance. The domestication of sorghum and the introduction of maize in Africa had no visible effect on the geographic patterns observed in the *B. fusca* mitochondrial genome.

Keywords: African biogeography, *Busseola fusca*, phylogeography, Pleistocene, population genetics

Received 14 February 2005; revision received 17 June 2005; accepted 1 September 2005

Introduction

Several species of noctuid moths behave as larval stem borers of cultivated plants. *Busseola fusca*, *Sesamia calamistis*, and *Sesamia nonagrioides* are major pests on maize (*Zea mays* L.) and cultivated sorghum [*Sorghum bicolor* (L.) Moench] throughout sub-Saharan Africa (reviewed in Kfir *et al.* 2002). Their larvae damage cereal crops, reducing yields. Some of these species, such as *B. fusca*, are endemic to sub-Saharan Africa. *B. fusca* known as the maize stalk borer displays ecological preferences that differ between regions. This pest

is more adapted to middle- and high-altitude conditions in East Africa, where it is abundant in the highlands (above 1500 m) and is rarely reported in the lowlands (Kfir *et al.* 2002). In Cameroon (Central Africa), it is abundant from high- (above 1000 m) to mid-altitude (700 m) regions (Ndemah *et al.* 2001). However, in West Africa, it is generally more abundant at lower altitudes than in other regions. Moyal (1998) reported that this insect was more frequent in Guineo-Congolian rainforest (dry type) (White 1983) areas than in savannah, whereas other studies reported that it is more frequent in dry savannah than in forests (Kfir *et al.* 2002). The pest status of *B. fusca* on cereal crops also varies between regions. It is the most important pest in the highlands of East and Southern Africa (Nye 1960; Sithole

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1989; Ndemah *et al.* 2001; Kfir *et al.* 2002) whereas in West Africa, it generally has a lower economic impact (Schultess *et al.* 1997) except in sorghum-growing areas where it is the most important stem borer (Dakouo & Ratnadass 1997; Ajayi 1998). In Cameroon, it was shown to be the main pest across years, seasons and ecological zones (Ndemah *et al.* 2001). Locally, for each region, the population density of *B. fusca* varies according to season and year. Climatic factors, and biotic factors such as human activity, availability of wild and cultivated plants and natural enemies, are responsible for the population fluctuations from year to year (Dakouo & Ratnadass 1997; Moyal 1998; Ndemah *et al.* 2000). *B. fusca* has been reported to attack a wide range of host plants from three monocotyledonous families: Cyperaceae, Poaceae and Typhaceae (Khan *et al.* 1997; Polaszek & Khan 1998; Haile & Hofsvang 2001). However, a 2-year exhaustive survey carried out across East Africa (Kenya, Tanzania, Uganda) indicates that *B. fusca* is uncommon in the wild habitat, showing on the contrary that it is specifically adapted to maize and sorghum crops (Le Rü, unpublished). The domestication of sorghum probably began some 5000 years ago in northeast Africa (Doggett 1988; Murty & Renard 2001) whereas maize was introduced more recently, at the end of the 16th century (Chastanet 1998; Madeira Santos & Ferraz Torrao 1998). Since its rapid expansion at the beginning of the 20th century, maize has become one of the most important crops in sub-Saharan Africa (Ristanovic 2001). Several insect species switched from wild plants to cultivated crops, and became pests. The timing of these events, and whether there were multiple or independent host switches is unknown. Molecular phylogeographic analysis can help to understand the possible effects of sorghum domestication and maize introduction on *B. fusca* genetic structure and can provide insights on the ecological and historical conditions of the switch.

Apart from domestication, the genetic structure of *B. fusca* is also likely to be influenced by its evolutionary history on wild host plants before domestication. Very little is known about the population genetics and phylogeography of African insects, despite their diversity and, for some of them, their economic importance (Kuchta & Meyer 2001). For example, only 10 of the 246 articles published in *Molecular Ecology*, in the 'Phylogeography, Speciation and Hybridization' section between January 2001 and June 2004 dealt with African animal species – vertebrates in each case. The ecological and economic importance of African phytophagous insects makes them particularly suitable models for studies of the biogeography and demographic history of African insects. Ndemah *et al.* (2001) suggested that geographic barriers, such as mountains or forests, might facilitate the development of specific races differing in climatic requirements and host-plant specificities. If this is the case, then it should be possible to identify these natural barriers by analysing the genetic structure of molecular markers. An

understanding of the ecological and genetic diversity of insect pests would make it possible to develop and to improve monitoring and biological control strategies. Two main questions need to be answered: Do the different ecological preferences of *B. fusca* from different regions correspond to molecular genetic differences? Has the ecological differentiation of populations of *B. fusca* from different regions depended on geography (West vs. East Africa), palaeogeographic history, or biotopes occupied (high- or low-altitude biotopes, wet or dry biotopes, savannah or forests)? The aims of this study were to (i) analyse the genetic structure and phylogeography of the *B. fusca* populations present on maize and cultivated sorghum in various countries and biotopes from West, Central and East Africa, and (ii) determine the historical, demographic and ecological factors shaping the genetic diversity of this species. We studied the mitochondrial cytochrome *b* gene, because it is informative at the intraspecific level in Lepidoptera (Simmons & Weller 2001).

Several approaches must be combined to infer the demographic history of a species from sequence data. Some of these approaches are used to describe the genetic structure, whereas others such as nested clade phylogeographic analyses (NCPA) are used to develop and test hypotheses concerning the evolutionary history responsible for generating the observed structure (Bernatchez 2001). This latter methodological approach is widely used in phylogeography (Althoff & Pellmyr 2002; Duran *et al.* 2004). Knowles & Maddison (2002) pointed out the lack of statistical tests to distinguish between inferences. However, Templeton (2004) re-estimated the reliability and the validity of this method by applying the NCPA methodology to cases in which there were clear a priori expectations. He concluded that NCPA makes the correct inferences in the vast majority of cases. We therefore combined descriptive methods, statistical methods, and NCPA to investigate the historical factors of genetic differentiation of *B. fusca* populations.

Materials and methods

Insect DNA sampling

Busseola fusca larvae were collected from maize and sorghum plants during the wet seasons of 2001–2003. The larvae and pupae sampled were reared to adult stage in the laboratory on host plant stems. Moths were preserved in absolute alcohol immediately after emergence. Three hundred seven individuals from sub-Saharan Africa were analysed: 85 individuals from 13 localities in Benin, Togo, Ghana and Mali in West Africa; 19 individuals from three localities in Cameroon in Central Africa; and 203 individuals from 36 localities in five different agroecological zones in Kenya, East Africa (Hassan *et al.* 1998) (Fig. 1a–d). Table 1 provides information about the localities, latitude and longitude and the individuals of *B. fusca* analysed for each locality.

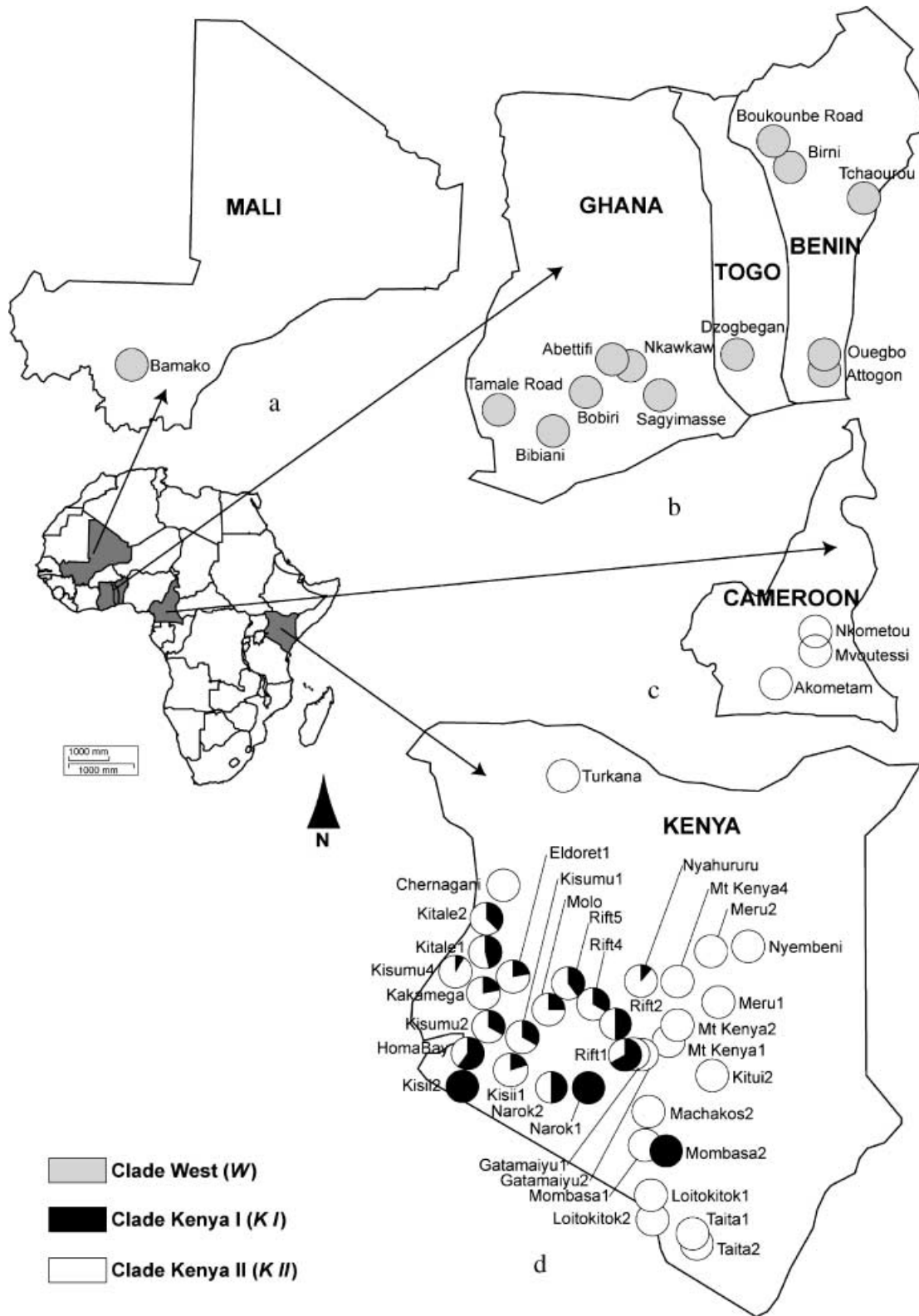


Fig. 1 (a) Geographic distribution of localities where *Busseola fusca* was sampled and the percentage of each clade in Mali (West Africa). (b) Geographic distribution of localities where *B. fusca* was sampled and the percentage of each clade in Benin, Togo and Ghana (West Africa). (c) Geographic distribution of localities where *B. fusca* was sampled and the percentage of each clade in Cameroon (Central Africa). (d) Geographic distribution of localities where *B. fusca* was sampled and the percentage of each clade in Kenya (East Africa).

Country/ Locality sampled	Latitude	Longitude	Individuals sequenced
Benin			
Attogon	06°42'13" N	02°09'59" E	9
Ouegbo	06°50'34" N	02°10'13" E	5
Tchaourou	09°05'34" N	02°33'29" E	9
Birni	09°56'47" N	01°32'53" E	3
Boukounbe Road	10°18'29" N	01°21'04" E	12
Togo			
Dzogbegan	07°14'24" N	00°41'38" E	13
Ghana			
Sagyimasse	06°14'09" N	00°31'20" W	11
Nkawkaw	06°40'21" N	00°45'08" W	7
Abettifi	06°48'36" N	00°52'40" W	3
Bobiri	06°42'12" N	01°22'00" W	1
Bibiani	06°18'00" N	02°14'33" W	8
Tamale Road	06°27'40" N	02°54'01" W	1
Mali			
Bamako	13°20'10" N	08°14'30" W	3
Cameroon			
Nkometou	04°10'50" N	11°50'01" E	12
Mvoutessi	03°40'03" N	11°53'40" E	4
Akometou	02°55'40" N	11°10'15" E	3
Kenya			
Cherangani	01°20'48" N	35°15'14" E	2
Eldoret 1	00°34'53" N	35°13'33" E	9
Gatamaiyu 1	01°00'03" S	36°43'01" E	9
Gatamaiyu 2	01°05'05" S	36°51'38" E	1
HomaBay	00°27'51" S	34°33'06" E	10
Kakamega	00°13'31" N	34°53'48" E	23
Kisii 1	00°46'13" S	34°58'47" E	5
Kisii 2	00°54'47" S	34°31'44" E	2
Kisumu 1	00°21'40" S	35°15'12" E	3
Kisumu 2	00°10'21" S	34°54'48" E	6
Kisumu 4	00°35'46" N	34°27'09" E	12
Kitale 1	00°47'27" N	34°49'30" E	8
Kitale 2	01°11'44" N	34°49'06" E	13
Kitui 2	01°19'29" S	38°03'41" E	5
Loitokitok 1	02°43'06" S	37°31'10" E	2
Loitokitok 2	02°55'46" S	37°30'59" E	9
Machakos 2	01°29'21" S	37°16'36" E	10
Meru 1	00°23'02" S	37°36'38" E	3
Meru 2	00°08'20" N	37°28'17" E	6
Molo	00°10'09" S	35°37'38" E	4
Mombasa 1	01°50'09" S	37°15'20" E	5
Mombasa 2	02°05'27" S	37°29'23" E	1
Mt Kenya 1	00°55'47" S	37°09'20" E	6
Mt Kenya 2	00°43'02" S	37°16'02" E	5
Mt Kenya 4	00°02'50" S	37°03'22" E	2
Narok 1	01°05'32" S	36°07'01" E	2
Narok 2	01°00'38" S	35°38'28" E	6
Nyahururu	00°04'44" S	36°34'16" E	9
Nyembeni	00°14'39" N	37°54'25" E	5
Rift 1	00°57'26" S	36°33'28" E	3
Rift 2	00°39'53" S	36°23'09" E	2
Rift 4	00°19'24" S	36°09'02" E	3
Rift 5	00°06'33" N	35°54'01" E	5
Taita 1	03°23'37" S	38°20'20" E	3
Taita 2	03°26'17" S	38°21'57" E	3
Turkana	03°32'17" N	35°51'38" E	1

Table 1 List of sampled localities with geographic coordinates and number of *Busseola fusca* individuals sequenced

Busseola phaia (Lepidoptera, Noctuidae) has been chosen as the outgroup based on recent unpublished work. Although the systematics of African lepidopteran stem borers is still rather confused (Holloway 1998), our studies (Moyal *et al.*, unpublished) show that *B. phaia*, collected from various regions of East Africa, is the sister species of *B. fusca*.

Molecular analyses

Total DNA was extracted from insect thoraxes, using the DNeasy Tissue Kit (QIAGEN GmbH). A 1000-bp fragment of the gene encoding cytochrome *b* was amplified with the primers CP1 (modified from Harry *et al.* 1998): 5'-GATGAT-GAAATTTTGGATC-3' and TRs (Simon *et al.* 1994): 5'-TATTCCTTATTATGTTTTCAAAC-3'. Polymerase chain reaction (PCR) cycling conditions were as follows: initial denaturation for 5 min at 94 °C; 40 cycles of 94 °C for 1 min, 46 °C for 1 min 30 s, 72 °C for 1 min 30 s and a final extension period of 10 min at 72 °C. The reaction mixture contained 3 mM MgCl₂, 0.4 μM primers, 0.24 μM dNTPs, and 100 ng of DNA plus 2 U of Promega *Taq* polymerase per 50 μL of reaction mixture. Amplified products were purified with the QIAquick purification kit (QIAGEN) and sequenced on an automated sequencer using the amplification primers in both directions. The consensus sequences obtained were aligned manually, using MACCLADE 4.05 (Maddison & Maddison 2002).

Haplotype phylogeny

Phylogenetic relationships were estimated by means of maximum parsimony (MP) and neighbour joining (NJ) using maximum-likelihood distances. MP analyses were performed using heuristic search strategy starting with stepwise addition trees replicated 10 times, using a random input order of sequences to get the initial tree for each replicate. Robustness of MP topologies was assessed by bootstrap with 1000 replicates (full heuristic search) of 10 random stepwise addition replicates each, for all analyses. MODELTEST version 3.07 (Posada & Crandall 1998) was used to select the substitution model that best describes the data. An NJ analysis of the ML distances obtained using the parameter estimates derived from MODELTEST was performed. Bootstrap values for this analysis were obtained from 1000 replications.

In order to estimate divergence time between clades, corrected pairwise genetic distances between haplotypes were calculated using the parameters inferred from the nucleotide substitution model selected by MODELTEST. All phylogenetic analyses were performed with PAUP* version 4.0b10 (Swofford 2002).

Population genetic structure and diversity indices

An analysis of molecular variance (AMOVA) was performed as described by Excoffier *et al.* (1992). The population

structure of *B. fusca* mitochondrial DNA was explored by calculating the different fixation indices (Φ_{CT} and Φ_{SC}). At the regional scale, Central and East Africa were grouped and separated from West Africa according to biogeographic relationships highlighted by studies on the African herbivore species that are associated with gramineous plant communities (Arcander *et al.* 1999; Flagstad *et al.* 2001; Alpers *et al.* 2004). Within each region, localities were grouped based on hypothetical biogeographic barriers (the effect of East Africa Rift Valley in Kenya) or according to ecological categories. In West Africa, for which ecological categories were defined based on White (1983) phytogeographic map, three zones concerned our study area: Sudanian woodland with abundant *Isobertinia*, mosaic of Guineo-Congolian lowland rainforest and secondary grassland, and Guineo-Congolian rainforest (drier types). In East Africa, ecological categories were based on Hassan *et al.* (1998) agro-ecological zones. Five zones were used for our study, Dry-mid-altitude, Dry-transitional, Moist-mid-altitude, Moist-transitional, Highland-tropical. All possible regrouping of these five zones were tested for their effect on Φ_{SC} and Φ_{CT} values.

Haplotypic (*h*) and nucleotide (π) diversities have been used to estimate the level of polymorphism and to infer the demographic history of each clade (Grant & Bowen 1998) independently of fragment length and sample size (Nei & Li 1979; Nei 1987).

AMOVA and diversity index calculations were performed with the ARLEQUIN 2.000 software (Schneider *et al.* 2000).

Demographic history of populations

We investigated the demographic history of the clades identified in the phylogenetic analyses. The distribution of pairwise differences between individual sequences was analysed by means of mismatch distribution analysis (Slatkin & Hudson 1991; Schneider & Excoffier 1999). A unimodal distribution would be expected for populations in expansion and a multimodal distribution for populations at equilibrium (Slatkin & Hudson 1991). The raggedness index of the observed distribution (*r*) representing the modality of the distribution, and the sum of square deviation from the mismatch expected from a model of sudden population expansion (SSD) were calculated. Since the nucleotide substitution model selected by hierarchical likelihood-ratio tests (hLRTs) and Akaike information criterion (AIC) (HKY + I + G) was not available in the ARLEQUIN 2.000 software, the *r* and SSD indices were calculated by using pairwise difference. The significance of these statistics was tested as implemented in ARLEQUIN. We calculated Tajima's *D* index. This index can provide information about demographic history, with demographic expansion leading to negative values, and subdivided populations at equilibrium leading to positive values (Tajima

1989a, b). All demographic parameters were calculated with the ARLEQUIN 2.000 software (Schneider *et al.* 2000).

Nested clade phylogeographic analysis

NCPA was performed as described by Templeton (1998, 2004). The genealogic relationships were represented through a haplotype parsimony network to define a series of nested clades. The hypothesis of random geographic distributions was tested through permutation tests for each clade and subclade components and the causes of the significant geographic patterns observed were inferred by means of an inference key. The probabilities of haplotype connections were calculated using TCS 1.17 software (Clement *et al.* 2000) and the network with probabilities above the parsimony limit (0.95) was selected. Statistical analyses of geographic distances were carried out with GEODIS 2.1 (Posada *et al.* 2000). GPS coordinates of all the sampling localities were used for the analyses. The geographic distances between centres of distributions of clades were tested for significance in permutation tests, within clade (D_c), with nested clade centre (D_n) or between interior and tip [(I-T) D_c and (I-T) D_n] at each level. Significant values were interpreted in terms of population history, using the latest inference key from Templeton (2004).

Results

Phylogenetic reconstruction

A fragment of 965 bp encoding cytochrome *b* was sequenced from 307 individuals of *Busseola fusca* from West, Central and East Africa. We observed 70 different haplotypes (GenBank Accession nos AY769536–AY769605). One hundred eleven nucleotide sites were variable (11.50%) and 50 were informative in parsimony analysis (5.18%). According to both hLRTs and AIC, the HKY + I + G model of evolution (Hasegawa *et al.* 1985; Yang 1993; Gu *et al.* 1995) was selected by MODELTEST version 3.07. The parameters estimated from this model were: transition/transversion (ti/tv) ratio = 13.21; Pinvar = 0.6981; gamma shape parameter = 0.9657; nucleotide base frequencies: A = 0.34; C = 0.14; G = 0.10; T = 0.42. As commonly observed in insect mitochondria, the nucleotide sequenced had a high A + T content (A + T = 76%).

Parsimony analyses generated six equiparsimonious trees (length = 162, CI = 0.698, RI = 0.931). Each of these trees was divided into same three clades: a clade grouping sequences from the West African region only (*W*), a Kenya I clade (*KI*) and a Kenya II clade (*KII*), which also contained sequences from Cameroon. Discrepancies between these six equiparsimonious topologies concerned only the apical nodes. It was therefore possible to construct a strict consensus of the most parsimonious trees (Fig. 2). NJ tree obtained using ML distances was similar to topology derived from MP analyses.

The three conspicuous clades of individuals and haplotypes are supported by bootstrap values exceeding 50% both for MP and NJ analyses. The smallest clade *KI* comprised 8 haplotypes and 43 individuals, all of which came from Kenya. The clade *KII* comprised 39 haplotypes and 179 individuals and had the largest distribution from East to Central Africa. Finally, the clade *W* comprised 23 haplotypes and 85 individuals and was found only in West Africa. No haplotype was shared between West African populations and East–Central African populations. Both in MP and NJ analyses, *W* and *KI* were supported by high bootstrap values whereas *KII* was supported by lower bootstrap values. The phylogenetic relationships between these three major clades remain unclear. The sister group status of clades *W* and *KI* was observed only in NJ analysis and supported by low bootstrap values.

The corrected mean pairwise genetic distances between haplotypes within and between clades are shown in Table 3.

Genetic structure and diversity indices of the B. fusca populations

Significant genetic structure was observed at various hierarchical levels (Table 2). Most of the molecular variation was accounted for by the differentiation between large population units corresponding to biogeographic units, West Africa on one hand and Eastern Africa + Cameroon on the other hand ($\Phi_{CT} = 0.708$, $P < 10^{-3}$). Significant genetic differentiation was observed among the populations within these two biogeographic units ($\Phi_{CS} = 0.360$, $P < 10^{-3}$). Within Kenya, most of the variation was accounted for by the Rift Valley ($\Phi_{CT} = 0.397$, $P < 10^{-3}$), suggesting that this geographic feature constitutes a strong natural barrier to gene flow. Other arrangements based on Hassan's agroclimatic zones in Kenya were tested. The differentiation among these agroclimatic zones was significant ($\Phi_{CT} = 0.106$, $P = 0.026 \pm 0.005$) but this value remained low compared to effect of the East Africa Rift Valley. The highest Φ_{CT} value among combinations of agroclimatic zones (0.197 , $P = 0.005 \pm 0.002$) was obtained by grouping dry vs. others agro-ecological zones. In West Africa, no genetic structure was observed between White's (1983) phytogeographic zones. For all hierarchical structures tested in West and East Africa, the genetic differentiation between localities (Φ_{SC}) was significant within group (Table 2).

The three conspicuous clades are separated by similar mean distances and displayed similar nucleotide and haplotype diversity indexes (Table 3).

Demographic history of B. fusca

In the absence of a suitable intrinsic calibration we can apply the approximate 2% per million years divergence rate for insect mtDNA of Brower (1994). While this provides

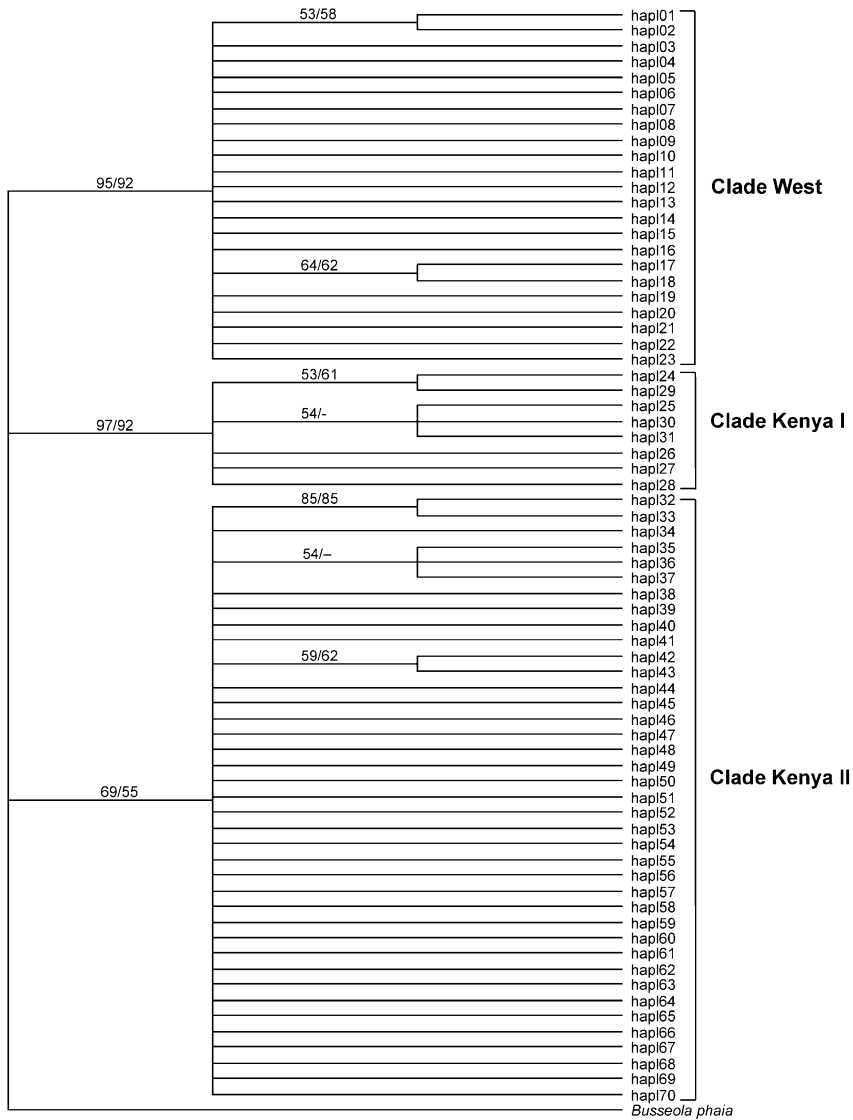


Fig. 2 Strict consensus of the most parsimonious trees. Bootstrap support of > 50% in both MP (first number) and NJ (second number) searches in 1000 replicates are given for the relevant nodes. *Busseola phaia* was used as the outgroup taxon.

Table 2 Values of Φ_{SC} and Φ_{CT} for different hierarchical levels and associated probabilities

Hierarchical level	Φ_{SC}	Φ_{CT}
➤ Major biogeographic zones (East + Central Africa) vs. (West Africa)	0.360 ($P < 10^{-3}$)	0.708 ($P < 10^{-3}$)
➤ East Africa:		
– Rift Valley Barrier	0.104 ($P < 10^{-3}$)	0.397 ($P < 10^{-3}$)
– Hassan’s Agroclimatic zones (1, 2, 3, 4, 5)*	0.250 ($P < 10^{-3}$)	0.106 ($P = 0.026 \pm 0.005$)
– Hassan’s Agroclimatic zones grouped Dry vs. Others (1 + 2),(3 + 4 + 5)*	0.264 ($P < 10^{-3}$)	0.197 ($P = 0.005 \pm 0.002$)
➤ West Africa:		
– White’s phytogeographic regions†	0.443 ($P < 10^{-3}$)	0.000 ($P = 0.332 \pm 0.013$)

Legend: values of Φ_{SC} (localities within groups) and Φ_{CT} (groups) for different hierarchical levels, and associated probabilities.

*Hassan’s agroclimatic zones in Kenya; 1: Dry-mid-altitude; 2: Dry-transitional; 3: Moist-mid-altitude; 4: Moist-transitional; 5: Highland-tropical.

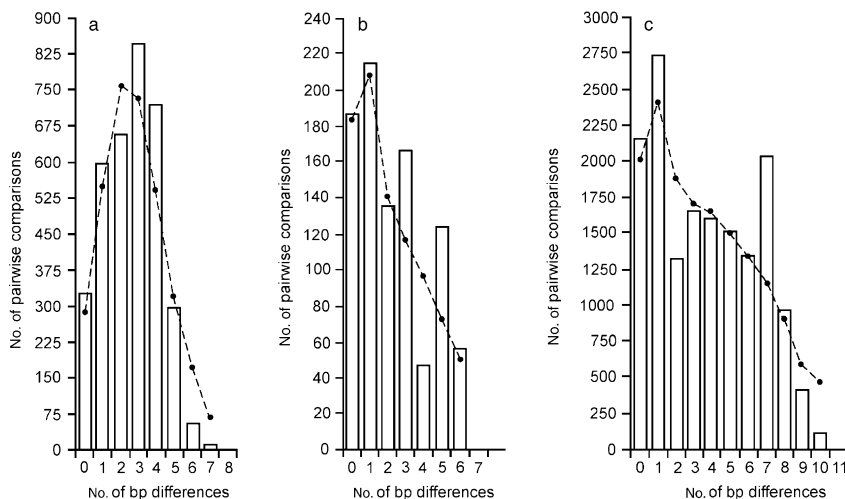
†White’s phytogeographic regions in West Africa (numbers are from White’s map). #27: Sudanian woodland with abundant *Isobertinia*;

#11a: Mosaic of Guineo-Congolian lowland rainforest and secondary grassland; #2: Guineo-Congolian rainforest (drier types).

Table 3 Corrected mean pairwise genetic distance between haplotypes (p dist.), haplotype diversity (h) and nucleotide diversity (π)

Clades	p dist. (%)	Haplotype diversity	Nucleotide diversity (%)
W	0.3775 \pm 0.1253	0.9062 \pm 0.0166	0.2714 \pm 0.1624
KI	0.3228 \pm 0.1195	0.8106 \pm 0.0426	0.2111 \pm 0.1343
KII	0.4888 \pm 0.2243	0.8276 \pm 0.0260	0.3838 \pm 0.2156
W – KI	2.7957 \pm 0.2223		
W – KII	3.1235 \pm 0.2387		
KI – KII	2.5660 \pm 0.2466		

estimates for the placing of divergence dates amongst our clades, it must be recognized that the error associated with these is unknown. The percentages of nucleotide divergence observed between the three clades (2.5–3.1%) suggest they have been separated for about a million years (Myr). Within each of the three clades, the mean distance between haplotypes suggests that their mean divergence time is around 200 000 years. The three clades display strong haplotypic diversity (between 0.81 and 0.91) associated with low levels of nucleotide diversity (between 0.21 and 0.381). This accumulation of haplotypes suggests that the clades experienced bottlenecks at their origins, followed by major population demographic expansion (Grant & Bowen 1998; Avise 2000). Both the variance (SSD) and raggedness index (r) tests suggested that the curves (Fig. 3a–c) do not significantly differ from the distribution under a model of population expansion ($P_{SSD} = 0.15$ and $P_r = 0.41$ for W; $P_{SSD} = 0.69$ and $P_r = 0.75$ for KI; $P_{SSD} = 0.81$ and $P_r = 0.88$ for KII). Similarly, the negative values obtained for Tajima's D index for each clade (–1.30536; –0.9992; –0.84178 for clades W, KI and KII, respectively) are all consistent with this hypothesis of population expansion since the origin of the clades.

**Fig. 3** Mismatch distribution analysis showing histogram of observed mismatch frequencies and best-fit curve of the sudden expansion model. (a) West Africa population; (b) Kenya I population; (c) Kenya II population.

Nested clade phylogeographic analysis

The NCPA network calculation defined the same three clades revealed by MP and NJ analyses. With the parsimony threshold of 0.95, these three clades were not linked (Fig. 4a–c). The West African clade contained the 23 haplotypes observed exclusively in this region and five hypothetical intermediate haplotypes, hierarchically grouped into 10 'one-step clades', 4 'two-step clades', and 2 'three-step clades'. The Kenya I clade contained the eight observed haplotypes and three hypothetical haplotypes: 4 'one-step clades' and 2 'two-step clades'. Finally, the Kenya II clade, with 39 observed haplotypes and only four hypothetical is organized into 11 'one-step' clades and 4 'two-step clades'.

Nested contingency analysis on the haplotype network revealed significant geographic associations in the three major networks at all clade levels. These significant values were interpreted using Templeton's (2004) interpretation key (Table 4). Seven of the eight clades displaying geographic associations were interpreted by restricted gene flow with isolation by distance, and one by contiguous range expansion.

Discussion

All three phylogenetic analyses separate *Busseola fusca* into three major clades corresponding to three geographic units: one originating from the West African region (W), one restricted to East Africa (KI), and one found from Central to East Africa (KII). Geographic overlap was observed only between the two East African clades. Applying a molecular clock of 2% substitution per Myr for insects, these different clades would have expanded from populations that experienced bottlenecks around 1 million years ago (Ma). This is long before sorghum domestication and maize introduction to Africa. These bottlenecks occurred in ancient periods

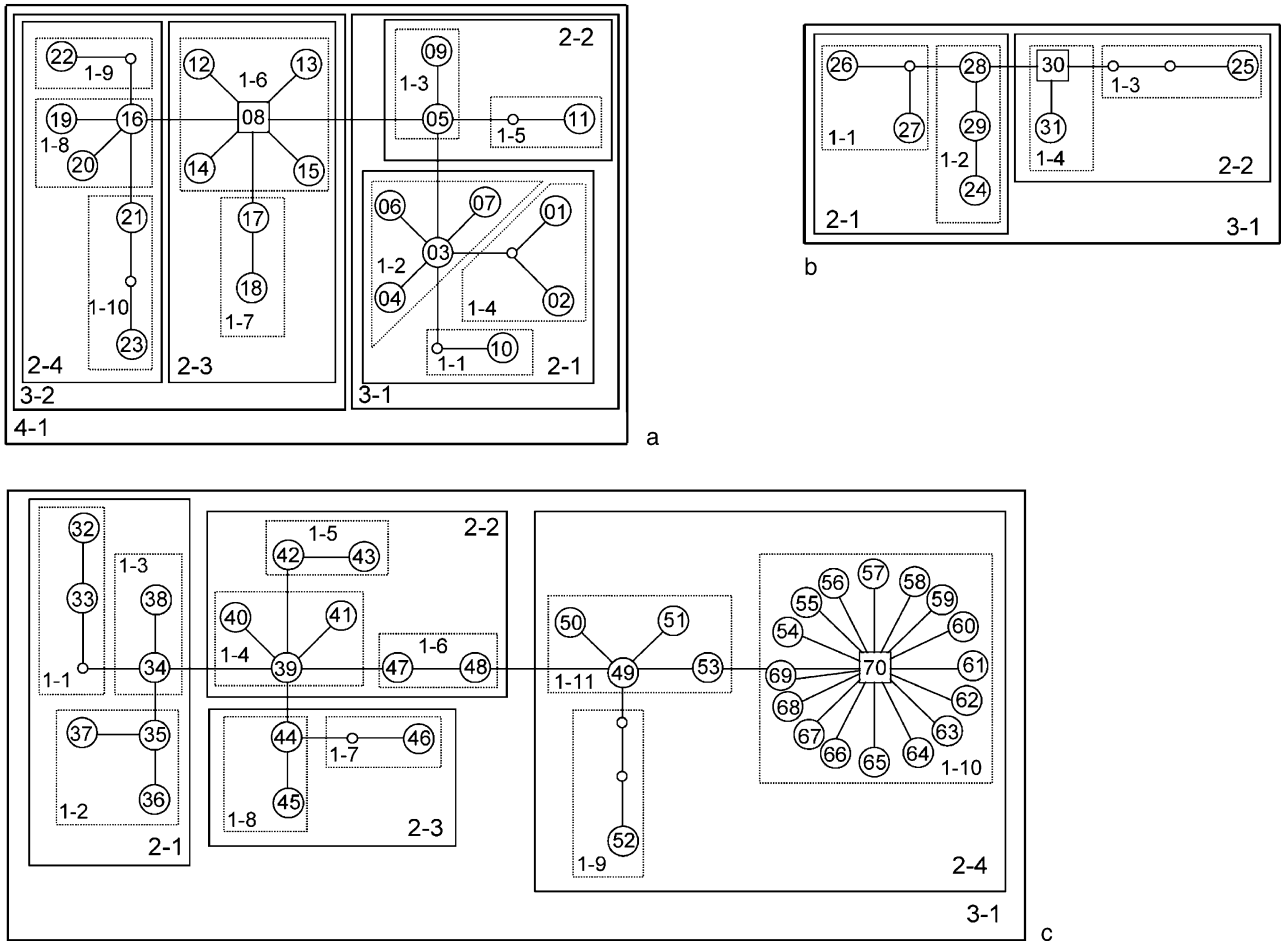


Fig. 4 Haplotype network of all haplotypes detected for *Busseola fusca*. Each haplotype is labelled by its number. Haplotypes not sampled or extinct are designated by small circles. (a) West Africa population; (b) Kenya I population; (c) Kenya II population.

probably due to climatic and/or geological events. Major climatic changes occurred in sub-Saharan Africa during the Pleistocene. The period of climatic instability started 3.3–2.45 Ma, oscillating between hot/humid and cooler, drier periods (Wagner 2002). A shift to arid, open conditions occurred in near 2.8 Ma, 1.7 Ma, and 1.0 Ma (deMenocal 1995). deMenocal (1995) concluded that this alternation of cold, dry periods and warmer, wetter periods led to oscillations in savannah biotope expansion. Considering the approximate nature of molecular clock in insects, and the absence of fossil data, the three major clades may have started their divergence in three bottlenecked populations isolated at one period in the Pleistocene. In comparison to other lepidopteran stem borer pest species, *B. fusca* is less abundant at lower altitude and warmer conditions (Kfir *et al.* 2002). This is consistent with a hypothesis of reduction of its geographic range, isolation of small populations and differentiation in high altitude refuges during a period of warmer conditions. Such climatic changes may also have provided opportunities for some populations of *B. fusca*, as

clade *W*, to adapt to warmer conditions. The genetic structure of the *B. fusca* populations resembles the patterns observed for some mammalian herbivores (Alpers *et al.* 2004). For example, the phylogeographic patterns of African mammals are due to alternation between periods of population isolation and genetic differentiation in one or several refuge areas during warmer climatic conditions and periods of population expansion and genetic exchange during colder and drier climatic conditions (Arctander *et al.* 1999; Flagstad *et al.* 2001; Van Hooft *et al.* 2002). Mean divergence age observed for rodent populations from the African genus *Sylvioorex* are also consistent with early Pleistocene differentiation. Increased aridity during this period was regarded as the main factor responsible for population divergence of this group (Quérrouil *et al.* 2003). The East Africa region has been described as a mosaic of secondary refuge zones for herbivorous mammals, with periodic exchanges through temporary contact bridges in the East Africa Rift Valley (Livingstone 1982; Arctander *et al.* 1999). Although the East African populations of *B. fusca* are now overlapping, the

Table 4 Result of nested clade analysis for clades in which a significant geographic association was detected. Haplotypes/clades shaded in grey are interior. RGF-IBD: restricted gene flow with isolation by distance, CRE: contiguous range expansion. D_c , average distance of individuals from the clade's geographic centre. D_n , the average distance of individuals from the geographic centre of all members of the nested clade. $(I-T)_c$, the average distance between interior and tip clades within a given clade, and $(I-T)_n$, the average distance between interior and tip clades in the nested clade. Superscript L or S denotes significantly large distances and significantly small distances for $P < 0.05$, respectively.

Clade 1-2 (W)					Clade 1-6 (W)					Clade 3-2 (W)							
Haplotypes	3	4	6	7	Haplotypes	8	12	13	14	15	Clades	2-3	2-4				
D_c	136.7	0	191.3	36.5	D_c	181.8	0	5.2	7.7 ^S	0	D_c	192.8	113.2 ^S				
D_n	356.8 ^L	273.4	298.2	111.0 ^S	D_n	243.6 ^L	309.6	124.8 ^S	128.6 ^S	162.5	D_n	206.4 ^L	148.1 ^S				
$(I-T)_c$			64.5		$(I-T)_c$			175.9 ^L			$(I-T)_c$		79.5 ^L				
$(I-T)_n$			185.5 ^L		$(I-T)_n$			99.2 ^L			$(I-T)_n$		58.2 ^L				
Steps in inference key 1, 2, 11, 17, 4 NO : RGF-IBD					Steps in inference key 1, 2, 3, 4 NO : RGF-IBD					Steps in inference key 1, 2, 3, 4 NO : RGF-IBD							
Clade 2-1 (KI)					Clade 1-3 (KII)												
Clades	1-1	1-2				Haplotypes	34	38									
D_c	35.1 ^S	108.3				D_c	115.7	36.1 ^S									
D_n	147.2	107.8				D_n	231.9 ^L	51.2									
$(I-T)_c$		73.2 ^L				$(I-T)_c$	79.7										
$(I-T)_n$		-37.4				$(I-T)_n$	180.6 ^L										
Steps in inference key 1, 2, 3, 4 NO : RGF-IBD						Steps in inference key 1, 2, 3, 4 NO : RGF-IBD											
Clade 1-10 (KII)																	
Haplotypes	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
D_c	0 ^S	0	0	149.2	149.5	66.9	0	45.2	0	0	0	0	0 ^S	0	0	0	1063.4 ^L
D_n	686.7	407.4	472.2	520.2	401.9 ^S	424.1 ^S	424.7	496.5	424.7	233.1 ^S	503.3	472.2	482.7	559.3	2398.2	503.3	976.2 ^L
$(I-T)_c$								1022.1 ^L									
$(I-T)_n$								419.2 ^L									
Steps in inference key 1, 2, 3, 4 NO : RGF-IBD																	
Clade 2-1 (KII)					Clade 2-4 (KII)												
Clades	1-1	1-2	1-3				Clades	1-9	1-10	1-11							
D_c	33	124.8	80.7				D_c	0	846.2	120.6							
D_n	113.7 ^S	152.8	104.6				D_n	222.9	825.6 ^L	273.2 ^S							
$(I-T)_c$		-3.2					$(I-T)_c$		-716.7 ^S								
$(I-T)_n$		-30.8 ^L					$(I-T)_n$		-546 ^S								
Steps in inference key 1, 2, 11, 17, 4 NO : RGF-IBD					Steps in inference key 1, 2, 11, 12 NO : CRE												

Rift Valley explains most of the molecular variation in Kenya and seems to have worked as an important natural barrier to maintain population structure. The Rift Valley have arisen and expanded from 2.9 to 1.6 Ma (Zeitoun 2000). It works as a natural barrier reinforcing population structure in many species of vertebrates (Arctander *et al.* 1999; Pitra *et al.* 2002). Our results on *B. fusca* suggest this natural barrier plays a similar role on reinforcement of population structure within this species, which in our case were probably initially isolated in climatic refuges. In addition, biogeographic distinction has been observed between the vertebrate faunas of West Africa and Cameroon/East Africa (Alpers *et al.* 2004) and this pattern is what we found for *B. fusca* populations. In summary, the major effect of Rift Valley, the possible importance of Pleistocene climatic cycles and the strong differentiation between Central + East Africa and West Africa suggest that similar climatic and geological processes drove the genetic differentiation of *B. fusca* and herbivorous vertebrate populations. Various demographic indices suggest that each of the three clades of *B. fusca* experienced population expansion after this period of bottlenecks. Within each clade, genetic diversity is now geographically distributed in a pattern suggestive of restricted gene flow with isolation by distance.

The presence of a large population unit, clade *KII*, from Cameroon to Kenya is consistent with the hypothesis of a faunistic link between these two regions (Carcasson 1964; De Jong & Congdon 1993; Bruhl 1997), which are separated by a distance of 3000 km. An eastern origin is a possibility as it was suggested for a butterfly species in Cameroon (De Jong & Congdon 1993). De Jong & Congdon (1993) argue that the low animal species diversity in highland forests of Cameroon suggests that these species originated from long-distance migration from East Africa. However, the nature of this faunistic link was not elucidated by our study and remains unknown for *B. fusca*.

In West Africa, only a few haplotypes were shared between localities. The same was true, but to a lesser extent, in East Africa. Significant fine-scale structure was observed within each biogeographic region, and each geological and ecological category. This fine-scale structure suggests that *B. fusca* is a fairly sedentary species. However, field experiments are required to determine whether this behavioural pattern is the rule in all ecological situations. Kfir *et al.* (2002) have shown that, during the dry season, crop residues contain a large number of *B. fusca* larvae. The authors concluded that the insects present in the crops came from crop residues at the same site rather than migrating from other areas. Moyal (1998) reported large differences in larval population densities between two sites less than 50 km apart, which might be due to demographic isolation and low dispersal capacities. Nevertheless, our recent spurious observations of *B. fusca* in remote poorly suitable sites in northern Kenya, suggest that within this region, *B. fusca*

population density is low or that long-distance migration may occur sporadically. The population from western Kenya (*KI*) seems to be more restricted geographically than the other two clades. Future collections of moths in Central African states (such as Uganda and Congo) will be required to improve our estimates of the geographic range of this clade.

The original wild host plant of *B. fusca* has not been identified in the field since this maize stalk borer is uncommon in wild habitats. However, the preference of this species for cultivated sorghum (Khan *et al.* 1997; Haile & Hofsvang 2002) suggests that it was originally associated with a particular species of wild sorghum. Experimental laboratory studies of oviposition choice among a large number of wild and cultivated Poaceae, Cyperaceae and Typhaceae demonstrated that both adults (for oviposition) and larvae (for their development) display a preference for a particular wild sorghum variety (*Sorghum vulgare* pres. var. *sudanense*) (Khan *et al.* 2000; Haile & Hofsvang 2002). Haile & Hofsvang (2002) also showed that *S. vulgare* was the only wild host plant allowing complete development of this insect. The simplest hypothesis is that *B. fusca* switched from wild to cultivated sorghum during the process of domestication.

The domestication of wild sorghum began in northeast Africa, but investigations have shown that genetic exchange occurred locally between this common genetic stock and wild sorghum in all regions during the expansion of agriculture in Africa. Ollitrault *et al.* (1989) revealed that wild sorghum is geographically structured into three main groups, one from West Africa, one from southern Africa and one group associating East and Central Africa. The genetic structure of populations of cultivated sorghum has therefore, to some extent, been influenced by regional hybridization/introgression events with the wild sorghum species and this may have resulted in various regionally distinct genetic groups within cultivated sorghum (Deu & Hamon 1994). For insects associated with wild or cultivated sorghum, the same regional influence seems to be true. *B. fusca* displays a similar geographic pattern, with clades specific to West Africa and clades from East and Central Africa. Further analyses on southern African populations will show us whether they belong to yet another clade. Although we have found a similar pattern, we cannot conclude that the same events structured the insect and its putative wild host plant. We infer the existence of palaeo-refuges for *B. fusca* based on phylogeographic analyses and present ecological preferences of this species. In contrast, the ecological preferences of wild sorghum strains are highly variable (De Wet 1978). This suggests that the refuges and/or fragmentation barriers that have shaped the population structure of the insect may be different for the plant. However, for both the insect and the plant it is likely that ancient events shaped the observed genetic

structure. The influence of ancient genetic events may be even more important for the insect than for the plant: the genetic structure of *B. fusca* populations clearly does not derive from a recent invasion of a common genetic pool originating from the region of sorghum domestication (northeast Africa), but it is instead exclusively inherited from patterns determined by the ancient history of the insect on wild Poaceae in a palaeoclimatic context that favoured the geographic isolation of three different populations in refuge zones corresponding to *B. fusca* ecological preferences. This suggests that the switch from wild to cultivated sorghum occurred several times from local populations of *B. fusca*. This hypothesis of the stability of genetic structure from ancient wild *B. fusca* to actual populations associated to cultivated plants should be tested for other regions of the genome, in particular nuclear genes. This will increase our understanding on the signature of plant domestication on pest population genetic structure. For instance, in the pyralid *Ostrinia nubilalis*, the European corn borer, no genetic differentiation was observed between insects on maize and wild hosts for mitochondrial markers whereas strong differentiation was observed for nuclear allozymes (Thomas *et al.* 2003). Thus, only part of the genome may be affected by domestication and involved in the adaptation to cultivated plants.

In conclusion, based on mitochondrial DNA, three major groups of populations can be distinguished in *B. fusca*: a homogeneous and geographically isolated population from West Africa, and two populations from East and Central Africa with overlapping distributions. Phylogenetic relationships suggest that the three populations differentiated in different regions of Africa during the same period, before human-mediated ecological changes. Analysis of molecular variance and NCPA interpretations suggest that local population differentiation was shaped by restricted gene flow with isolation by distance. The strong genetic structure observed suggests that *B. fusca* has in general limited dispersal capacities.

Although little is known about the biogeography of African phytophagous insects, the factors responsible for the patterns observed in *B. fusca* can be compared with those identified in studies on other herbivores, particularly mammals. The East Africa Rift Valley was also identified as a major factor for genetic structure for several groups of vertebrates in Africa (Arctander *et al.* 1999; Pitra *et al.* 2002). More generally, the study on *B. fusca* suggests that climatic fluctuations in the Pleistocene generated isolated populations and shaped the genetic structures of various groups of herbivores associated with gramineous plant communities in Africa, both for mammals (Arctander *et al.* 1999; Flagstad *et al.* 2001; Alpers *et al.* 2004) and insects. These results for *B. fusca* generate new hypotheses for future investigations of African insect phylogeography.

Acknowledgements

This study was supported by IRD UR072. We would like to thank D. Baille for technical assistance in the development of molecular markers and preliminary phylogeographic analyses, and the members of the ICIPE Noctuid Stem Borer Biodiversity Project for technical assistance during insect collection and insect rearing in the laboratory in Kenya. We are grateful to C. Wanjiru Gitau and L. Corley for their help to the English correction of the manuscript. We are indebted to G. Goergen for his support and Z. Djihou for technical assistance at IITA-Cotonou in Benin. B. Emerson and two anonymous referees also helped greatly in improving this manuscript.

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