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ORIGINAL PAPER

Insights into the genetic structure of the cowpea pest *Callosobruchus maculatus* in Africa

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Abstract The bean beetle *Callosobruchus maculatus* is a worldwide generalist legume seed pest, present in the tropics and subtropics. Despite its economic importance, little is known about the population structure and migration patterns of this bruchid beetle in its continent of origin, both at local and regional scales. Gaining knowledge in the demographic processes experienced by *C. maculatus*, in particular in its native area, might give clues to understand evolutionary forces that have driven its expansion in traditional agroecosystems. Here, we analyze the genetic structure of 23 African populations of *C. maculatus* located in eleven different countries, using nuclear (28S) and mitochondrial (CytB) markers, and further apply phylogenetic and demographic analyses. The 28S sequences showed low genetic variability. In contrast, the analysis of CytB indicated significant genetic differentiation between most populations, suggesting low levels of gene flow, even at a reduced spatial scale. The results obtained from the estimation of various demographic parameters suggest evidence of recent population expansion, in agreement with a scenario of long-distance dispersal through trade of legume seeds for human consumption and population growth related to its pest nature. Overall, our results show that biogeography, isolation processes and human-mediated dispersal events drive the genetic structure of this pest in Africa.

Keywords Bruchinae · *Callosobruchus maculatus* · Cytochrome B · Demographic history · Legume pest · Population structure

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Key message

- This study was conducted to examine the population structure and migration patterns of *Callosobruchus maculatus* in Africa, its continent of origin.
- The combination of biogeographic processes, isolation by distance and human-mediated dispersal events explain the genetic structuring of *C. maculatus* in Africa.
- Our findings provide important baseline information for new approaches of biological control against this pest.

Introduction

The Southern cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchinae) is one of the most widely distributed legume pests in the world (Wightman and

Southgate 1982). It develops on ca. ten genera of legumes (Anton et al. 1997; Tuda et al. 2005), among which soybean (*Glycine max* L.), lentils (*Lens culinaris* Medikus), fava beans (*Vicia faba* L.), peas (*Pisum* spp.), chickpea (*Cicer arietinum* L.), pigeon pea [*Cajanus cajan* (L.) Millsp.], and, in particular, the African cowpea, *Vigna unguiculata* L. (Jackai and Daoust 1986). The latter is suspected to be its preferred host plant (Tuda et al. 2006), on which it causes large cultivation yield losses, especially in West Africa (Appleby and Credland 2003). Adults of *C. maculatus* lay their eggs on the surface of pods or dehisced seeds in the field before or around the time of harvest (Caswell 1968; Germain et al. 1987). These eggs are later brought unnoticed into seed stores, where the protected environment results in rapid insect development and population growth. If unchecked, storage populations of *C. maculatus* can grow exponentially causing significant losses in seed weight, germination viability, and marketability (Caswell 1968; Southgate 1979). The ability of *C. maculatus* to multiply fast under storage conditions results in rapid population build-up and deterioration of pulse seeds in storage (Adugna 2006). According to Tuda et al. (2006), the Southern cowpea weevil forms a monophyletic group with *C. analis* (F.), *C. rhodesianus* (Pic), and *C. subinnotatus* (Pic), all using dry beans from the cowpea genus *Vigna* (Savi) as their natural hosts.

Similarly to several other insect pests, *C. maculatus* shows great intra-specific variation in a large number of bionomic traits, what is accompanied by a tremendous ability to adapt to local environmental conditions and/or hosts (Appleby and Credland 2004). Indeed, different populations have been found to exhibit significant differences in life-history traits such as female fecundity, development time, survival, adult weight (Credland et al. 1986), oviposition behavior (Messina and Mitchell 1989), and larval competition strategies (Mitchell 1990), suggesting local adaptation. The occurrence of such an intra-specific variation may have a significant impact on the development and implementation of effective, long-term, and sustainable control methods against this and, potentially, other bruchids species.

Phylogeography was originally conceived to explore the processes underlying the spatial distribution of genetic lineages within and among species (Avise et al. 1987; Avise 2009), and interpret their spatial history (Hickerson et al. 2010). Although initial studies focused on wild species (see e.g., Hewitt 2001), it quickly became obvious that inferring the phylogeography of pest species was important from an evolutionary point of view. Indeed, understanding the genetic spatial structure of an insect pest can also provide important biological information for deploying pest-resistant cultivars and control strategies since the spatial genetic structure could indicate the potential for evolution of local adaptation.

Spatial isolation is one major factor leading to genetic divergence among populations. In the case of *C. maculatus*, only one study has investigated its spatial genetic structure at the global scale; Tuda et al. (2014) evaluated global sequence variation, showing that the species demonstrates a pronounced genetic differentiation both globally and within Africa, but not within Asia. Getting a better resolution of the African spatial genetic structure and demographic history is important to understand its initial expansion, as this continent is viewed as its center of origin (van Alebeek 1996; Stolk et al. 2001). When evaluating the spatial genetic structure of pest species, it is sought to identify the processes that are dominating the establishment and maintenance of the observed genetic structure. This is important for better informing control strategies, since it provides a more realistic sight on the demographic and evolutionary dynamics of the species (e.g., Hampton et al. 2004; Porretta et al. 2007). For instance, in the case of *C. maculatus*, the spatial genetic structure of this species in Africa (i) can be driven purely by biogeographic and isolation processes (fitting isolation by distance scenarios and the main African biogeographic areas); (ii) can be shaped by human-mediated dispersal events (HMDE) and linked to human networks of seed exchange; and (iii) can be driven by a combination of both processes [structure can be partially explained by (i) and (ii)]. From an applied perspective, while scenario (i) could indicate a low probability of long-distance dispersal of pesticide resistant lineages, scenario and (ii) could indicate the opposite, what would lead to radically opposite control strategies.

To test these hypotheses and to advance in the understanding of this pest species in Africa, we sampled *C. maculatus* specimens in 11 countries in Africa, and we used both mitochondrial (mtDNA) and nuclear DNA to investigate the genetic structure in *C. maculatus* at the continental and local scales and identified the drivers of such spatial genetic structure.

Materials and methods

Sampling

Callosobruchus maculatus individuals were sampled in 23 locations in Africa. Cowpea seeds were first collected during the dry period (after the rainy season) of years 2009, 2010, and 2012. Adults of *C. maculatus* were obtained by rearing the larvae found infesting seeds, identified following Beck and Blumer (2007) and preserved in 100 % ethanol until DNA extraction (see below). Samples were in total, 156 specimens from 23 localities were used (Fig. 1 and Supporting Information). Based on Tuda et al. (2006), the closely related species *C. subinnotatus, C. chinensis*

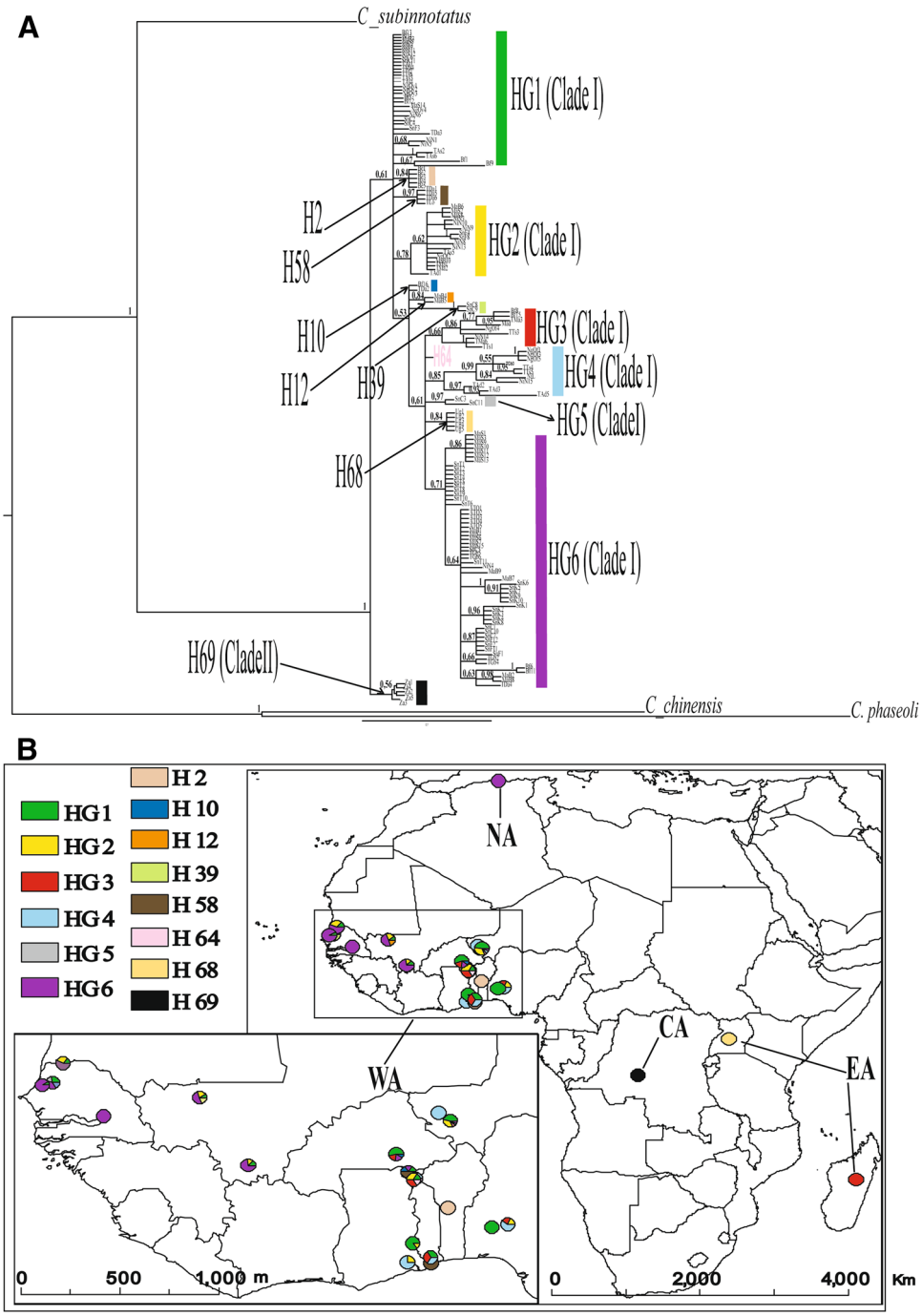

Fig. 1 Phylogenetic relationships and spatial distribution of CytB haplogroups/haplotypes found in *C. maculatus*. (**a**) Bayesian phylogenetic relationships of *C. maculatus* samples. *Values* on branches represent node supports derived from Bayesian posterior probability (only values above 0.5 are shown). (**b**) Spatial distribution of haplogroup/haplotypes with pie-chart proportions corresponding to the frequency of haplogroup/haplotypes

and *C. phaseoli* were used as outgroups. Samples of these species were obtained from laboratory-kept colonies.

DNA extraction, gene amplification, sequencing, and alignment

DNA was extracted from individual insects following the standard QIAGEN DNeasy tissue kit (Qiagen GmbH, Germany) protocol. Because we aimed to construct a dataset containing both nuclear and cytoplasmic markers, we decided to amplify the nuclear region 28S and the mitochondrial region cytochrome B, which had been shown to be informative in a preliminary reduced sampling of the species. All specimens were individually sequenced for the mitochondrial cytochrome b (CytB), while 93 of them were selected for the 28S ribosomal RNA gene. This difference in the sample sizes for each marker is due to the low genetic diversity observed in the latter (see Results).

The primers used were D4-5F (5′-CCCGTCTTGAAACACGGACCAAGG-3′) and D4-5R (5′-GTTACACACTCCTTAGCGGA-3′) (Belshaw and Quicke 2002) for 28S and CB-J-10933 (5′-TATGTACTACCATGAGGACAAATATC-3′) and CB-N-11367 (5′-ATTACACCTCCTAATTTATTAGGAAT-3′) (Simon et al. 1994) for CytB. PCRs were performed in 50 µl, containing 1 U Taq-polymerase and 2 µl of genomic DNA at 2.5 mM $MgCl_2$, primers at 10 µM each and 0.1 mM dNTP. After an initial denaturation at 94 °C for 3 min, conditions for 28S consisted of 30 cycles of a denaturation phase of 30 s at 92 °C, an annealing of 1 min at 47 °C and an extension of 1 min at 72 °C. For CytB, conditions included 35 cycles of a denaturation phase of 1 min at 94 °C, an annealing of 1 min at 47 °C and 1 min of extension at 72 °C. In both cases, the program finished with a final extension of 10 min at 72 °C.

After visualizing the amplifications on a gel, both DNA strands were sequenced by Macrogen Inc., Seoul, South Korea. Alignments were performed using the ClustalW (Thompson et al. 1997) algorithm, as implemented in BioEdit v.5.0.6 (Hall 2001), and followed by minor manual adjustments.

Genetic diversity

We calculated the number of variable and parsimony informative sites, the transitions/transversions rate bias (R) and the frequency of nucleotides, using MEGA v. 4.0 (Tamura et al. 2007). To determine levels of genetic variability, the number of unique mtDNA haplotypes and nDNA alleles, as well as haplotype and nucleotide diversity were calculated using DnaSP version 5.10.01 (Librado and Rozas 2009). CytB codon frames were checked using MEGA v.4.0 (Tamura et al. 2007) and revealed no evidence of putative nuclear pseudogenes (NUMTS; Lopez et al. 1994). Finally, we searched for evidence of recombination in the region 28S, using the software Recombination Detection Program (RDP) version 4.5 Beta (Martin et al. 2010).

Analysis of genetic structure

Because of the low genetic variation displayed by the nuclear marker, genetic population structure was investigated for the CytB sequences only. Because we seek to identify if simple natural or human-driven processes shape the genetic structure of the pest, we used hierarchical analysis of molecular variance (AMOVA) using two different hierarchies. In the first hierarchy, groups were defined according to geographic regions: Western Africa (WA), Central Africa (CA), Eastern Africa (EA), and Northern Africa (NA). In the second, we wanted to test whether or not economic exchanges could explain the genetic structure of the pest. For this reason, groups were defined according to the countries of origin of the samples. Populations corresponded to sampling localities and we only used populations with more than three individuals, (Mad, NiL, and TLo excluded). All AMOVAs, as well as analyses of genetic differentiation between population pairs were performed in ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010), by calculating pairwise *Fst* statistics. A permutation test (1000 bootsraps) for evaluating the level of significance in differentiation of pairs of localities was applied following the approach described in Excoffier et al. (1992). The genetic distance (*d*) between pairs of populations was computed in MEGA v.4.0 (Tamura et al. 2007) using the model Kimura (1980) 2-parameter (K2P). We tested for isolation by distance (IBD) with a Mantel test using XLSTAT 2012 (Addinsoft, Paris, France) and using the Kendall correlation coefficient. For this, we calculated the correlation between the genetic distance (*d*) and the Euclidian geographic distance (in km) generated in Franson CoordTrans 2.3 (GpsGate AB, Johanneshov, Sweden) from the geographic coordinates of each collected locality. Significance was determined based on 50,000 randomized permutations.

Phylogenetic analysis

Because of the low variability of the 28S phylogenetic relationships at the intra-specific level (see below), all phylogenies were inferred based on CytB sequences only. For the phylogenetic reconstructions, we used MrBayes v. 3.1 (Huelsenbeck and Ronquist 2001) and performed four MCMC runs, each of them with four chains. We used the model GTR+I+G, which was identified as the best-fit model using MrModeltest (Nylander 2004). Phylogenetic searches were run for 5,000,000 generations, sampling every 1000th. After checking for run convergence on Tracer v. 1.3 (Rambaut and Drummond 2007), we applied a burn-in of 2500 (what left a total 5000 trees from both runs to estimate posterior probabilities).

Demographic history of the population

Due to lack of variation in 28S, only CytB data was used to study the demographic history of the species. To test the null model of neutral mutation and constant population size, we calculated Tajima's *D* (Tajima 1989), Fu's *Fs* test (Fu 1997), *R2* (Ramos-Onsins and Rozas 2002), and mismatch distributions. Tajima's *D* and Fu's *Fs* are known to be sensitive to departures from mutation-drift equilibrium due to population size changes (i.e., expansion, bottlenecks) and selection (Ramirez-Soriano et al. 2008). The *Fs* and *R2* tests are the most robust analyses for detecting population growth, with *Fs* being better adapted for large

sample sizes and *R2* for small sample size (Ramos-Onsins and Rozas 2002). These statistics were calculated using DnaSP, with significance assessed with 10,000 coalescent simulations. Under constant population size conditions, Tajima's *D* and Fu's *Fs* are expected to approach zero, while significant negative or positive values suggest sudden population expansions or recent population bottlenecks, respectively. Significant negative *Fs* and non-significative *D* values may indicate expanding populations, while the opposite suggests selection. The parameters of a demographic expansion model, including the expansion factor (*tau*), initial (*h0*) and final (*h1*) thetas of the populations were estimated using ARLEQUIN. These estimators were used to plot the distribution of the number of pairwise differences between sequences ('mismatch distributions') assuming a population Growth-Decline model. Rapid recent demographic growth is characterized by unimodal distributions, while multimodal distributions are observed for populations at demographic equilibrium (Rogers and Harpending 1992). The sum of squared deviations (SSD) between observed and expected distributions, as well as the raggedness index *rg* of the observed distribution of the mismatch classes was computed as test statistics under the hypothesis of population growth using ARLEQUIN.

Results

Genetic diversity

The length of the 28S alignment was 520 base-pairs (bp) and recombination was not detected. Sequences displayed limited polymorphism, with only one parsimony informative site, yielding two alleles (Table 1). The dominant allele, represented in ca. 90 % of the dataset, is widely distributed and is present at all sampled sites. The second allele is found in nine individuals from Togo. The overall haplotype and nucleotide diversities were 0.18 ± 0.050 and 0.0003 ± 0.0001, respectively. The haplotype diversities of the different populations varied from zero to 0.60 ± 0.175, while nucleotide diversity varied from zero to 0.0012 ± 0.00034 (Table 2).

The length of CytB was 480 bp. As expected for protein coding genes, no insertions, deletions, or stop codons were present in CytB sequences, providing evidence that those sequences were not nuclear pseudogenes. Seventy-six variable sites were detected, of which 45 were parsimony informative, yielding 69 different haplotypes among which 42 were restricted to single individuals (Table 1). Values of haplotype and nucleotide diversity (0.97 ± 0.005 and 0.0121 ± 0.00053, respectively) were higher than in 28S. Haplotype diversity in the different populations varied from zero to 1.00 ± 0.177, while nucleotide diversity varied from zero to 0.0170 ± 0.0033 (Table 2). The haplotype diversities were high in all the West African locations except in Benin and some locations in Senegal, while all other locations contained only one haplotype. Populations with the highest CytB nucleotide diversities were identified in Burkina Faso (Bf, 0.017), Togo (TAd and TTs, 0.015), and Nigeria (NgOf, 0.014).

Spatial genetic structure

Our AMOVAs indicated that 19.31 and 9.72 % of the genetic variance could be significantly explained at, respectively, the inter-region and inter-country levels. The remainder of the genetic variance could be significantly explained at the population and within population levels (Table 3).

The *Fst* pairwise values among the 20 populations ranged from −0.02156 to 1.00000 (Table S2). Twenty nine population pairs (28 of them in West Africa) showed no significant genetic differentiation ($P > 0.05$), suggesting that most populations are highly divergent. Based on results from the Mantel test, we could reject the null hypothesis of the absence of correlation between genetic and geographic distances in our dataset ($r = 0.36$, $P < 0.0001$ for CytB), what indicates the presence of IBD among the sampled populations.

Table 1 Summary statistics for 28S and CytB sequences of *C. maculatus*

	28S	CytB
Sample size	93	156
Sequence length	520	480
Number of variables sites	1 (0.20 %)	76 (15.83 %)
Number of parsimony informative sites	1 (100 %)	45 (59.21 %)
Trs/Trv rate bias (R)	na	12.274
Haplotype diversity (Hd)	0.18 ± 0.055	0.97 ± 0.005
Nucleotide diversity (π)	0.0003 ± 0.0001	0.0121 ± 0.0005
Number of alleles/haplotypes	2	69

Table 2 Genetic diversity in the different sampled populations of *C. maculatus* for 28S and CytB

Country	Locality	28S				CytB			
		Sample size	Number of alleles	Haplotype diversity (±SD)	Nucleotide diversity (±SD)	Sample size	Number of haplotypes	Haplotype diversity (±SD)	Nucleotide diversity (±SD)
Burkina Faso	Tenkodogo	11	1	0.00 ± 0.000	0.0000 ± 0.0000	11	8	0.94 ± 0.054	0.0170 ± 0.0033
Mali	Bougoudere	10	1	0.00 ± 0.000	0.0000 ± 0.0000	10	8	0.96 ± 0.059	0.0124 ± 0.0073
	Sikasso	10	1	0.00 ± 0.000	0.0000 ± 0.0000	15	5	0.73 ± 0.089	0.0073 ± 0.0013
Niger	Niamey	4	1	0.00 ± 0.000	0.0000 ± 0.0000	15	11	0.93 ± 0.054	0.0110 ± 0.0018
Senegal	Coki	9	1	0.00 ± 0.000	0.0000 ± 0.0000	11	7	0.91 ± 0.066	0.0120 ± 0.0013
	Fouta	7	1	0.00 ± 0.000	0.0000 ± 0.0000	7	4	0.81 ± 0.130	0.0120 ± 0.0025
	Kebemer	9	1	0.00 ± 0.000	0.0000 ± 0.0000	11	5	0.78 ± 0.093	0.0086 ± 0.0015
	Tambacounda	9	1	0.00 ± 0.000	0.0000 ± 0.0000	11	3	0.34 ± 0.172	0.0011 ± 0.0006
Togo	Adidogome	4	1	0.00 ± 0.000	0.0000 ± 0.0000	4	4	1.00 ± 0.177	0.0150 ± 0.0050
	Assigame	5	2	0.60 ± 0.175	0.0012 ± 0.0003	6	4	0.80 ± 0.172	0.0050 ± 0.0015
	Dapaong	5	2	0.60 ± 0.175	0.0012 ± 0.0003	6	4	0.80 ± 0.172	0.0103 ± 0.0027
	Gbinga	5	2	0.50 ± 0.265	0.0001 ± 0.0005	5	3	0.80 ± 0.164	0.0100 ± 0.0040
	Mango	5	2	0.40 ± 0.237	0.0001 ± 0.0004	6	5	0.93 ± 0.122	0.0100 ± 0.0023
	Tsevie	5	1	0.50 ± 0.265	0.0001 ± 0.000	6	4	0.87 ± 0.129	0.0151 ± 0.0032
Algeria	Tizi-Ouzou	n/a	n/a	n/a	n/a	5	1	0.00 ± 0.000	0.0000 ± 0.0000
Benin		n/a	n/a	n/a	n/a	5	1	0.00 ± 0.000	0.0000 ± 0.0000
Nigeria	Offa	n/a	n/a	n/a	n/a	5	3	0.70 ± 0.220	0.0140 ± 0.0043
	Oyo	n/a	n/a	n/a	n/a	4	2	0.50 ± 0.265	0.0010 ± 0.0005
Uganda		n/a	n/a	n/a	n/a	5	1	0.00 ± 0.000	0.0000 ± 0.0000
Democratic Republic of Congo		n/a	n/a	n/a	n/a	5	1	0.00 ± 0.000	0.0000 ± 0.0000

Table 3 AMOVA results for each level of variation in the *C. maculatus* samples, based on the analysis of CytB

Source of variation	Sum of squares	Variance (%)	*F* (*P* value)
Among subregions	37.77	19.31	$Fct = 0.200$ ($P = 0.01$)
Among populations within subregions	134.56	22.09	$Fst = 0.41$ ($P < 0.0001$)
Within populations	278	58.60	$Fsc = 0.27$ ($P < 0.001$)
Among countries	99.5	9.72	$Fct = 0.100$ ($P = 0.02$)
Among populations within countries	67.26	22.03	$Fst = 0.32$ ($P < 0.0001$)
Within populations	265.13	68.24	$Fsc = 0.24$ ($P < 0.001$)

Phylogenetic analyses

The monophyly of *C. maculatus* was strongly supported (PP = 1.00) in the CytB phylogenetic reconstruction, which retrieved two weakly supported clades (Fig. 1). One of them (clade I) is widely distributed and composed of haplotypes 1–68, whereas the second one (clade II) is restricted to the Democratic Republic of the Congo and contains only one haplotype (H69). In clade I, many haplotypes were grouped to form six haplogroups (HG) which overall agree with their geographic origin (see Table S3), while six haplotypes were associated with one to five individuals and were specific to single sampling localities. Globally, our phylogenetic analysis confirms the strong structuration of the populations of *C. maculatus* in Africa.

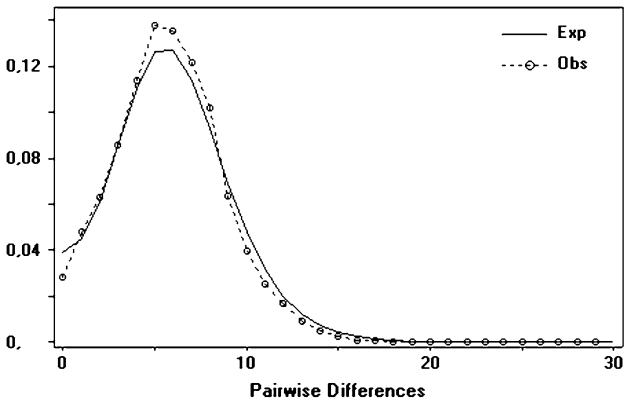

Fig. 2 Mismatch distribution analysis for the African specimens of *Callosobruchus maculatus* with the expected and observed frequencies (as *full* and *dotted lines*, respectively) of ranked pairwise distances among specimens

Demographic expansion

The value of the neutrality test statistics Tajima's *D* was −1.845 ($P = 0.0062$), while that of Fu's *Fs* was −61.59 ($P < 0.0001$) and the Ramos-Osins and Rozas' *R*2 (0.036) was significant ($P = 0.014$). These results reject the null hypothesis of constant population size, suggesting a rapid population expansion. This pattern was corroborated by the mismatch analyses (Fig. 2), which revealed no significant deviation from the null hypothesis of population expansion in the two genes (PSSD = 1), and the raggedness index *rg* confirmed the unimodal distribution characteristic of population expansion ($P = 0.95$).

Discussion

Molecular markers

In this study, we sought to depict the spatial genetic structure and demographic history of *C. maculatus* in Africa. Our analyses show low levels of genetic diversity in the nuclear marker we used, which is in agreement with the low intra-specific variability usually shown by this rDNA region (e.g., Vink et al. 2003; Kavar et al. 2006) and recent results at the global scale (Tuda et al. 2014). However, unlike to Tuda et al. (2014) this marker could sort out some individuals from Togo, suggesting genetic differentiation between these and all other locations. In contrast, our analysis of CytB revealed a high level of genetic variation in *C. maculatus* in Africa, as already shown in this species at the global scale (Tuda et al. 2014) and in other insects (e.g., Sezonlin et al. 2006; Torres-Leguizamòn et al. 2011; Yang et al. 2011). It is important to note that because of the lack of variability in the nuclear marker, our conclusions are based on the analysis of a single-gene (CytB). Although this is not ideal, the gene appeared to harbor enough variability and the results it provides make biological sense. More importantly, a reduced number of studies have investigated the genetic diversity and structure of *C. maculatus* in Africa, although that information is urgently needed for pest management. Keeping this in mind, our results should be considered with the necessary caution, and we strongly recommend that future research on this species focus on other more variable nuclear markers, on microsatellites and/or genome-wide approaches.

Biogeography, isolation processes and HMDE drive the genetic structure of *C. maculatus* in Africa

Until now, little was known on the population genetic structure of *C. maculatus* in Africa. Our AMOVA results

show that similar percentages of mtDNA genetic variance are explained at the inter- and intra-region levels, but that most genetic variance is explained at the within-population level (Table 3). In accordance with this, 161 of the 190 pairwise estimated *Fst* values were significantly different from zero, indicating limited gene flow between populations (Table S2). Such a result is similar to those obtained for other insect pests, such as *Acanthoscelides obtectus* say (Alvarez et al. 2005) or *Tecia solanivora* povolny (Torres-Leguizamòn et al. 2011), but contrasting with those obtained for the aphid *Sitobion avenae* (Xu et al. 2011), the beetle *Phyllodecta vulgatissima* L. (Batley et al. 2004), and the diamondback *P. vitellinae* L. (Endersby et al. 2006).

The genetic differentiation among geographic regions was significant (Table 3). This agrees and further expands Tuda et al.'s (2014) observation, indicating that regional differentiation is significant in Africa (Table 3). This pattern of high geographic genetic differentiation also agrees with our Mantel tests. Indeed, our analyses identified IBD, suggesting that one major factor limiting gene flow between populations is geographic isolation. Our phylogenetic results also agree with these observations. On this, while one of the two clades was composed of samples from EA, NA, and WA; the other was exclusively composed of samples from CA (the Democratic Republic of the Congo, DRC; Fig. 1). Furthermore, this geographic structure was also carried to the within-Clade I level. Remarkably, samples from EA (Uganda) formed a monophyletic group with strong support and were highly differentiated from their WA counterparts. Because of the strong geographic signal identified in our analyses, it is very likely that the split between CA, EA, and the other African regions reflects the biogeographic history of Africa.

Although the spatial genetic structure was strong at the continent level, we also identified signals of human-mediated events. First, our AMOVAs based on country of origin also identified a non-negligible percentage of variance explained by the country (Table 3). Because it is expected that human-mediated processes such as seed-trading networks and pest control strategies are strongly country based, such a result is a strong indication of human-mediated processes shaping the genetic structure of this pest. Second, although our phylogeny was strongly spatially structured, we also identified some outliers, such as the Algerian samples grouping with the WA HG_6 or the Madagascar sample clustering with WA samples in HG_3 (Fig. 1). Such observations can suggest the signature of long-distance dispersion associated to seed marketing and exchange, what also agrees with the observations of Tuda et al. (2014) at the global scale and with the presence of international trading agreements including at least some of these countries (e.g., ECOWAS/UEMOA regions, the "Permanent Interstate Committee for drought control in the Sahel"). Third, our historical demography analyses indicate recent historical population expansion in Africa, indicated by the obtained negative *D* and *Fs* values and the mismatch distribution analyses (Fig. 2). These results agree with Tuda et al.'s (2014) observations at the global scale, and would support a scenario of population size growth associated with increases in seed trading. Fourth, contrary to what we might expect under a strict IBD scenario, gene flow was extremely restricted between two population pairs distant by less than 90 km (SnC and SnK; TDa and TMa; TDa and TGb; TGb and TMa, Table S2) and not isolated by any geographic barrier, a pattern that can be explained by processes of local adaptation. Indeed, they can indicate selection acting strongly at the local level, a scenario that would agree with the wide use of insecticides (Ajayi and Wintola 2006; Yusuf et al. 2011), natural products such as diatomaceous earths (Badii et al. 2014) or the growth of potentially radically different cowpea varieties in the sampled populations, both of which can and do lead to local adaptation (Rousselet et al. 2010; Kébé and Sembène 2011). In relation to this, Tuda et al. (2014) documented no significant among-host differentiation in Africa, contrasting to both global and within-Asia differentiation. A larger and more localized sampling with information on the host plants would be needed to test the hypotheses of adaptation to host plants and insecticides in Africa.

Conclusion

Our results show that there is high genetic divergence ($Fst = 0.41$) among populations of *C. maculatus* in Africa, what can be explained by a combination of biogeographic processes and IBD, but also by human-mediated events such as insecticide or host-plant selection, or dispersion mediated by international seed exchanges. Our discovery of possible widespread gene flow for this pest in West Africa has implications on the control methods used in these countries. The genetic homogeneity across geographical sites in West Africa bodes well for regional control strategies, although such a strategy should take into account the suggested high levels of genetic diversity and gene flow. Indeed, high levels of diversity indicate that the genetic reservoir would be rich enough to allow natural selection for insecticide-resistant specimens, while high gene flow could facilitate a fast spread of insecticide-resistant specimens throughout the West African region.

Author contribution statement

K.K, M.S, and I.O conceived the study; K.K and M.S collected most of the material; K.K and F.J conducted the DNA analyses; K.K, N.A, and A.E analyzed the data and

wrote the paper. All authors read, commented on and approved the final manuscript.

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