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Original article

Multi-locus genotyping reveals absence of genetic structure in field populations of the brown ear tick (*Rhipicephalus appendiculatus*) in Kenya

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ABSTRACT

Rhipicephalus appendiculatus is an important tick vector of several pathogens and parasitizes domestic and wild animals across eastern and southern Africa. However, its inherent genetic variation and population structure is poorly understood. To investigate whether mammalian host species, geographic separation and resulting reproductive isolation, or a combination of these, define the genetic structure of *R. appendiculatus*, we analyzed multi-locus genotype data from 392 individuals from 10 geographic locations in Kenya generated in an earlier study. These ticks were associated with three types of mammalian host situations; (1) cattle grazing systems, (2) cattle and wildlife co-grazing systems (3) wildlife grazing systems without livestock. We also analyzed data from 460 individuals from 10 populations maintained as closed laboratory stocks and 117 individuals from five other species in the genus *Rhipicephalus.* The pattern of genotypes observed indicated low levels of genetic differentiation between the ten field populations ($F_{ST} = 0.014 \pm 0.002$) and a lack of genetic divergence corresponding to the degree of separation of the geographic sampling locations. There was also no clear association of particular tick genotypes with specific host species. This is consistent with tick dispersal over large geographic ranges and lack of host specificity. In contrast, the 10 laboratory populations ($F_{ST} = 0.248 \pm 0.015$) and the five other species of *Rhipicephalus* ($F_{ST} = 0.368 \pm 0.032$) were strongly differentiated into distinct genetic groups. Some laboratory bred populations diverged markedly from their field counterparts in spite of originally being sampled from the same geographic locations. Our results demonstrate a lack of defined population genetic differentiation in field populations of the generalist *R. appendiculatus* in Kenya, which may be a result of the frequent anthropogenic movement of livestock and mobility of its several wildlife hosts between different locations.

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1. Introduction

Rhipicephalus appendiculatus (brown ear tick; Acari, Ixodidae) is the most important vector of *Theileria parva*, which causes East Coast fever (ECF) in cattle in eastern, central and southern Africa (Perry et al., 1990). Adults of the tick mainly infest large ungulates, both domestic – particularly cattle – and wild species such as Cape buffalo (*Syncerus caffer*), and various other bovids including waterbuck (*Kobus ellipsiprymnus*) and blue wildebeest (*Connochaetes taurinus*) (Walker et al., 2000). The nymphal and larval stages often infest smaller mammals, particularly hares (*Lepus* sp.). *R. appendiculatus* is an important vector of various pathogens of economic and veterinary significance, particularly the protozoan *T. parva* but also Nairobi sheep disease virus, the Thogoto virus and *Rickettsia conorii* (Perry et al., 2002; Minjauw and McLeod, 2003). The

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geographic distribution and population dynamics of *R. appendiculatus* are influenced to a great extent by eco-climatic and biophysical factors (Lessard et al., 1988, 1990; Perry et al., 1990; Norval et al., 1992; Estrada-Peña, 2001). Differences in diapause (Madder et al., 2002), body size (Norval et al., 1992; Shaw and Young, 1994), vector competence (Ochanda et al., 1998) and differential response to acaricides (Chigagure et al., 2000) are some of the biological and morphological differences reported in *R. appendiculatus*, which suggest the existence of genetically diverse tick populations.

Several stocks of *R. appendiculatus* have been bred and maintained in the laboratory as resource populations for experimental use, and to provide representative examples of field populations. The standard laboratory strain, *R. appendiculatus* Muguga, has been used to produce trivalent stabilates of the 'Muguga cocktail' vaccine for live infection and treatment immunization (ITM) for control of East Coast fever in cattle (Di Giulio et al., 2009). ITM is based on the inoculation of live sporozoites (produced using a pool of three different *T. parva* stocks from thousands of infected *R. appendiculatus* ticks (reviewed by Radley, 1981) together with a long-acting formulation of antibiotic. Previous assessments of the biology of laboratory stocks of *R. appendiculatus* revealed differences in infection rates (Young et al., 1995) suggesting differences in susceptibility and ability to transmit *T. parva* (Odongo et al., 2009; Ochanda et al., 1998). The extent to which these laboratory strains have diverged genetically and remain representative of field populations has not been investigated due to lack of appropriate tools. Monitoring the genetics of laboratory colonies of vectors may reveal if they have diverged from their parental field populations. For instance, DNA polymorphism and heterozygosity were shown to be drastically reduced among laboratory colonies of the mosquito *Aedes aegypti* compared to field populations (Norris et al., 2001). Therefore, knowledge of the genetic diversity of laboratory tick colonies and how they relate to the field populations will enhance their application in laboratory-based experiments. In addition, baseline genotyping of laboratory tick stocks that are used in the production of live vaccines is important for vaccine standardization and quality control.

To develop effective control strategies for vector-borne diseases either through vector control and/or anti-vector/anti-pathogen vaccination (Dai et al., 2009; Gillet et al., 2009), understanding the genetics of vector populations is important. Host population dynamics can significantly influence the dynamics and population structure of *R. appendiculatus*, which can affect the co-evolutionary interaction between mammalian host, vectors and pathogens. Factors such as high host mobility, low host specificity, and frequent metapopulation extinction and recolonization, can reduce the within- and between-population genetic variation. By contrast reproductive isolation driven by sedentary hosts, host specialization and patchiness in space and time favour strong genetic structure and divergence (Nadler, 1995). Whilst several studies have investigated the population dynamics, ecology and biology of *R. appendiculatus* (Norval and Perry, 1990; Norval et al., 1992; Randolph, 1994, 2004), there is still a paucity of information on the within- and between-population genetic variability and structure of *R. appendiculatus*.

In this study, we used allelic data generated from 979 individual ticks using the twenty nine polymorphic EST-based markers that were described previously (Kanduma et al., 2012) to analyze and evaluate the genetic diversity and population structure of *R. appendiculatus* from different host species and geographic areas in Kenya. Data from 10 stocks bred and maintained in the laboratory were also included. Whereas the earlier study primarily focused on the value of the set of 29 VNTR markers for differentiation of *R. appendiculatus* genotypes, in this study we re-analyzed the data using additional algorithms to provide a more in depth resolution of the levels of population differentiation in the field ticks. The aim of the current study was to investigate whether the genetic diversity of the species is spatially structured across its geographic range and between different host species and also whether the genotypic composition of the laboratory bred strains is representative of their field counterparts.

2. Materials and methods

2.1. Tick samples

A total of 979 individuals comprising of 862 *R. appendiculatus* ticks and 117 of other rhipicephaline species had been genotyped in an earlier study (Kanduma et al., 2012). Adult ticks were collected from animal hosts (cattle and buffaloes) and from pastures/vegetation grazed by cattle, wildlife, or mixtures of wildlife and cattle by dragging. Ticks were plucked from ears of cattle and sedated buffaloes using good quality steel forceps. All ticks were preserved in 70% ethanol and stored at 4 °C. Species identification was done at ILRI's Tick Unit by examining morphological features using a stereoscopic light microscope. Taxonomic descriptions and illustrations (Hoogstraal, 1956; Walker, 1960; Walker et al., 2000, 2003) were used as aids towards identification. Ticks whose identity could not be determined were sent to the Onderstepoort Veterinary Institute (OVI), Pretoria, South Africa for identification. Details of the area of origin of the ticks, population and sampling site characteristics and code used were as previously described by Kanduma et al. (2012).

2.2. Processing of samples

Genomic DNA was extracted using the DNeasy® Blood and Tissue Kit (Qiagen GmbH, Germany) with minor modifications (Kanduma et al., 2012). Genotyping had been performed using 29 micro- and mini-satellite markers as described by Kanduma et al. (2012).

2.3. Statistical analysis

Allele data generated from 979 individual ticks using twenty nine polymorphic EST-based markers (Kanduma et al., 2012) was further analyzed using additional algorithms. For each population, allelic (total number of alleles (TNA), mean number of alleles (MNA), private alleles (PA) and genetic diversity (expected (H_e) and observed (H_o) heterozygosity) were estimated from allele frequencies using the Microsatellite toolkit (Park, 2001). For each locus-population combination we used Fisher's exact test with the Bonferroni correction to test for deviations from Hardy-Weinberg equilibrium (HWE) using GENEPOP 3.4 (Raymond and Rousset, 1995).

We investigated the underlying population genetic stratification using a Bayesian clustering algorithm implemented in STRUCTURE (Pritchard et al., 2000; Falush et al., 2003), incorporating the admixture and correlated allele frequency model (Falush et al., 2003). Assuming that the genotypic data could be partitioned into K genetic clusters independent of prior population information, we performed ten runs for each K with 200,000 iterations with a "burn in" of 100,000 generations. The analysis was performed on (i) the global dataset incorporating 27 populations ($1 \leq K \leq 27$) and (ii) field and laboratory stocks of *R. appendiculatus* incorporating 22 populations ($1 \leq K \leq 25$). The upper value of K was set much higher than the total number of populations in each dataset to allow detection of possible sub-structures within populations. The optimal number of genetic clusters was inferred from the mean estimated log probability of the data (Pritchard et al., 2000) and its second-order rate of change (ΔK) (Evanno et al., 2005) as calculated in

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