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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.

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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STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Cluster matching and permutation was performed using CLUMPP (Jakobsson and Rosenberg, 2007) and a graphical display of the results was generated with DISTRUCT (Rosenberg, 2004).

The Bayesian algorithm implemented in STRUCTURE relies on a genetic model, which requires that populations satisfy Hardy–Weinberg equilibrium (HWE) expectations and that there is linkage equilibrium between loci. Such assumptions are often violated in natural and inbred populations. Various multivariate analyses that circumvent such assumptions have been proposed (Jombart et al., 2010). Here, we used the discriminant analysis of principal components (DAPC) (Jombart et al., 2010) to further investigate the genetic stratification of the two groups of ticks analyzed with STRUCTURE. DAPC describes global diversity by optimizing the variance between groups while minimizing the variance within groups. To identify the optimal number of genetic clusters that explain the data, we ran *K*-means clustering sequentially with increasing values of *K*, and compared different clustering solutions using the Bayesian Information Criterion (BIC). We performed DAPC using the ADEGENET v1.3-9.2 package (Jombart, 2008) in the R environment v2.15.3 (R Core Team, 2013).

To investigate whether the ten field populations of *R. appendiculatus* were at migration-drift equilibrium, we regressed geographic distance (km) between populations against population pairwise estimates of F_{ST} following 10,000 pseudorandom permutations performed in IBDWS 3.05 (http://ibdws.sdsu.edu). Geographic distances were estimated using the MapCrow Travel Distance Calculator (http://www.mapcrow.info/) as the distances between the central most towns in each sampling location.

To infer demographic history and dynamics, we assessed whether the study populations were at mutation-drift equilibrium using the intra-locus kurtosis test (*k*-test) and the inter-locus variance test (*g*-test) (Reich and Goldstein, 1998; Reich et al., 1999). Allele distribution patterns differ between expanding and demographically stable populations. In expanding populations, the kurtosis (*k*), or the combination of the variance and kurtosis (Reich et al., 1999) of the distribution of allele sizes is negative. The *k*-test uses a binomial approach to test whether the number of positive *k*-values differ from the expected one of an almost equal probability ($P = 0.515$) of negative and positive *k*-values. The *g*-test compares the observed and estimated values of the variance in allele sizes across loci. In stable populations the variance is highly variable among loci, whereas in expanding populations it is much more even. For this test, low variance in allele sizes provides evidence of expansion. For inference purposes, we used the cut-off values given in Table 1 (page 455) of Reich et al. (1999). Both *k*- and *g*-tests were performed using the macro programme "kgtests" (Bilgin, 2007) implemented in Microsoft Excel®.

To assess the magnitude of genetic variation present within and between populations and groups of populations, we performed an analysis of molecular variance (AMOVA) as a weighted average over loci in Arlequin v3.5 (Excoffier and Lischer, 2010) applied to seven population groupings. Four different groupings had one hierarchical cluster, two groupings had two clusters and one had three hierarchical clusters as follows (i) the global dataset (one cluster); (ii) all field *R. appendiculatus* (one cluster); (iii) all laboratory stocks of *R. appendiculatus* (one cluster); (iv) other species of *Rhipicephalus* (one cluster); (v) all populations of *R. appendiculatus* when compared to the five other species of *Rhipicephalus* (two clusters); (vi) all ticks sampled in the grazing fields (cattle, cattle-wildlife and wildlife) verses laboratory bred stocks (two groups); (vii) field ticks analyzed as cattle stocks, cattle-wildlife stocks and wildlife stocks (three groups). Levels of significance for the covariance components associated with the different hierarchical clusters (one, two and three) tested were determined with 1000 non-parametric permutations.

3. Results

A total of 291 alleles were observed at the 29 loci that had been genotyped in 979 individuals from 27 populations, representing an average of 10.03 ± 4.24 alleles per population (Table 1). The overall average expected and observed heterozygosities were 0.633 ± 0.032 and 0.442 ± 0.003 respectively (Table 1), and laboratory bred stocks had the lowest values of genetic and allelic diversity. Although the five additional species of *Rhipicephalus* had a higher level of expected heterozygosity, the 11 field populations of *R. appendiculatus* had a higher level of observed heterozygosity and allelic diversity (MNA and TNA; Table 1). Several population-specific alleles were observed, with the number of these ranging from zero in KF, SN, MU, ZE, ZM, SL and RZ to eleven in RL (Table 1). The highest overall number of private alleles was observed in the field populations of *R. appendiculatus* (37) and in the five additional species of *Rhipicephalus* (34). Except in the laboratory stock KU (Kiambu unselected line), which had a negative F_{IS} value, indicating an excess of heterozygotes relative to expectation, and the laboratory stock ML (Muguga low line) with a zero F_{IS} value, indicating an equal number of homo- and heterozygotes, the other populations had positive F_{IS} values indicating deficiencies in heterozygotes (Table 1). KU is the only population where the observed heterozygosity exceeded the expected, while, ML is the only population that had the same value for observed and expected heterozygosity.

A Mantel test was performed to investigate whether the ten field populations of *R. appendiculatus* sampled in Kenya were at migration-drift equilibrium. A negative but non-significant correlation ($r = -0.1636$; $P = 0.7396$) between geographic (km) and genetic distances (population pairwise F_{ST}) was observed (Fig. 1).

We performed "kg-tests" to investigate population demographic dynamics (Table 2). In expanding populations, the kurtosis (*k*) value is always negative (Reich et al., 1999). When analyzed as one group the 27 populations were found to contain 26 loci with a significant negative kurtosis ($P \leq 0.05$). Similarly, the 22 populations of field and laboratory bred populations of *R. appendiculatus* when analyzed as one group had 22 loci with significant negative kurtosis ($P \leq 0.05$). However, when we analyzed each group of ticks separately, the results differed. The eleven field populations of *R. appendiculatus* had 27 loci with significant negative kurtosis ($P \leq 0.05$) supporting population expansion while, the eleven laboratory bred populations of *R. appendiculatus* had 17 loci and the other five *Rhipicephalus* species had 18 loci with non-significant ($P \geq 0.05$) negative kurtosis providing no statistical support for expansion (Table 2).

We investigated the level of genetic variation present between different groups of ticks using AMOVA (Table 3). Among the different hierarchical clusters tested, the highest level of genetic variation occurred between individuals and ranged between 45.08% in the five other species of *Rhipicephalus* to 80.56% in the eleven field populations of *R. appendiculatus*. The lowest level of genetic variation (0.12%) occurred among the eleven field populations of *R. appendiculatus* grouped on the basis of their host species i.e. cattle, cattle-wildlife, and wildlife respectively. When these were analyzed as a single group, the level of genetic variation present between the eleven populations rose to only 1.78%. This demonstrates low genetic differentiation between populations of *R. appendiculatus* from different locations and from the three host systems. The highest overall level of genetic variation occurs between the five other species of *Rhipicephalus* (38.62%) while the second highest level (25.14%) was observed between the eleven populations of laboratory bred *R. appendiculatus*. Similar trends were observed with respect to the global F_{ST} values that represent genetic differentiation within and between different groups of ticks (Table 4).

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Table 1
Genetic and allelic diversity of the 27 tick populations analyzed in the present study.

Population	Sample size	Loci typed	Genetic diversity		Allelic diversity		F_{IS}	Private alleles	HWE ($P<0.01$)
			H_e (SD)	H_o (SD)	MNA (SD)	TNA			
Field populations									
KF	25	29	0.588 (0.040)	0.514 (0.021)	4.38 (1.88)	127	0.137	–	–
MK	46	29	0.613 (0.037)	0.501 (0.014)	5.93 (2.88)	172	0.183	1	7
KT	25	29	0.642 (0.029)	0.523 (0.020)	5.59 (2.85)	162	0.189	5	3
RU	36	29	0.606 (0.035)	0.518 (0.017)	5.31 (2.02)	154	0.148	1	4
BU	48	29	0.616 (0.034)	0.526 (0.014)	5.83 (2.11)	169	0.148	2	3
BO	34	29	0.624 (0.035)	0.488 (0.017)	5.10 (2.40)	148	0.221	1	4
FP	46	29	0.629 (0.037)	0.504 (0.014)	5.76 (2.37)	167	0.200	3	6
NB	36	29	0.608 (0.040)	0.506 (0.016)	5.90 (2.47)	171	0.170	3	2
MA	48	29	0.614 (0.037)	0.473 (0.014)	5.86 (2.37)	170	0.230	1	8
BF	48	26	0.541 (0.046)	0.454 (0.016)	5.38 (2.42)	140	0.168	1	2
SN	5	29	0.527 (0.054)	0.316 (0.040)	2.86 (1.30)	83	0.446	–	–
Overall	397	29	0.623 (0.035)	0.505 (0.005)	8.28 (3.88)	240	0.189	37	14
Laboratory populations									
LP	48	29	0.441 (0.046)	0.427 (0.014)	3.48 (1.72)	101	0.030	4	6
KH	48	29	0.472 (0.043)	0.388 (0.014)	3.69 (1.75)	107	0.186	2	6
KU	48	29	0.449 (0.042)	0.470 (0.014)	3.07 (1.33)	89	−0.044	1	7
MU	48	29	0.579 (0.032)	0.475 (0.014)	4.62 (1.95)	134	0.181	–	7
MF	48	29	0.566 (0.030)	0.430 (0.013)	4.38 (2.03)	127	0.243	1	8
ML	48	29	0.384 (0.048)	0.384 (0.013)	2.62 (1.15)	76	0.000	2	4
UG	48	29	0.442 (0.037)	0.387 (0.014)	2.97 (1.15)	86	0.126	1	4
ZS	48	29	0.364 (0.043)	0.334 (0.012)	2.83 (1.17)	82	0.082	1	5
ZE	48	29	0.409 (0.041)	0.391 (0.014)	3.10 (1.32)	90	0.042	–	2
ZM	28	29	0.491 (0.043)	0.474 (0.018)	3.45 (1.45)	100	0.035	–	2
SL	5	29	0.344 (0.051)	0.291 (0.040)	1.79 (0.56)	52	0.205	–	–
Overall	465	29	0.592 (0.034)	0.411 (0.004)	6.59 (3.03)	191	**0.305**	14	26
Other *Rhipicephalus* species									
RE	15	28	0.474 (0.057)	0.391 (0.031)	3.11 (1.93)	87	0.206	3	–
RP	24	26	0.515 (0.063)	0.261 (0.030)	3.12 (1.61)	81	0.611	10	3
RV	12	28	0.587 (0.042)	0.370 (0.038)	3.36 (1.70)	94	0.410	2	–
RL	18	27	0.454 (0.045)	0.310 (0.024)	3.22 (1.31)	87	0.339	11	2
RZ	48	29	0.490 (0.040)	0.350 (0.013)	3.31 (1.17)	96	0.288	–	6
Overall	117	29	0.680 (0.022)	0.343 (0.010)	7.10 (2.93)	206	0.497	34	28
Total (all ticks)	**979**	**29**	**0.633 (0.032)**	**0.442 (0.003)**	**10.03 (4.24)**	**291**	**0.301**		

Note: KF = Kilifi County, MK = Makuyu, KT = Kitale, RU = Rusinga Island, BU = Busia County, BO = Bomet County, FP = Field stock Ol Pejeta Conservancy, Nanyuki, NB = Nairobi National Park, MA = Maasai Mara National Park, BF = Buffaloes, Maasai Mara National Park, SN = Natal Province, South Africa.
LP = Laboratory stock from Ol Pejeta ranch, KH = Kiambu highline (shows high *T. parva* infectivity), KU = Kiambu unselected line, MU = Muguga unselected line, MF = Muguga infected line, ML = Muguga low line (shows low *T. parva* infectivity), UG = Uganda stock, ZS = Zambia (Southern province), ZE = Zambia (Eastern province), ZM = Zimbabwe (West Mashonaland), SL = Laboratory stock.
RE = *Rhipicephalus evertsi*, RP = *Rhipicephalus praetextatus*, RV = *Rhipicephalus pravus*, RL = *Rhipicephalus pulchellus*, RZ = *Rhipicephalus zambeziensis*.
He = expected heterozygosity, Ho = observed heterozygosity, MNA = mean number of alleles, SD = standard deviation, TNA = total number of alleles, F_{IS} = coefficient of inbreeding, HWE = Hardy–Weinberg equilibrium.

STRUCTURE and DAPC partition genetic diversity at the individual and population level based on multi-locus genotypes independent of prior population information. For the overall dataset (27 populations), the ΔK approach shows $K=20$ as the most probable number of genetic clusters present in the dataset (Fig. 2a). None of the eleven field populations exhibit clear genetic clusters (Fig. 2b). Four laboratory-bred stocks (KU, MU, MF, ZM) show high levels of admixture while, six (LP, KH, ML, UG, ZS, ZE) appear to be genetically distinct. Although the genetic makeup of the five additional *Rhipicephalus* species were made up of a single predominant genetic background which was different in the RZ population, they all show varying levels of admixture. Of the two *R. appendiculatus* populations sampled from South Africa, SL (a laboratory-bred stock) is distinct while SN (a field population) has an admixed genotype.

The regression of Bayesian Information criterion (BIC) values against K following DAPC analysis shows $K=23$ to be the highest optimal number of genetic clusters explaining the variation present in the overall dataset (Fig. 3 inset). This value is higher than that revealed by the ΔK approach following STRUCTURE analysis (Fig. 2a). However, as with STRUCTURE, the eleven field populations of *R. appendiculatus* are assigned to multiple clusters as are

Table 2
Expansion parameters for different stocks of ticks determined using "kg-tests".

Type of tick stock	Number of populations	Loci with negative *kurtosis*	*P*-values	
			k-test	*g*-test
All ticks (*R. appendiculatus* and other *Rhipicephalus* species)	27	26	3.75E−06	6.2563
R. appendiculatus (field and laboratory stocks)	22	22	0.002504	5.2550
R. appendiculatus (field ticks only)	11	27	3.77E−07	6.2668
R. appendiculatus (Laboratory stocks only)	11	17	0.182863	4.7296
R. appendiculatus (wildlife ticks only)	3	27	3.77E−07	7.0837
R. appendiculatus (cattle ticks only)	5	28	2.45E−08	5.8338
Other species of *Rhipicephalus* species	5	18	0.100746	9.1686

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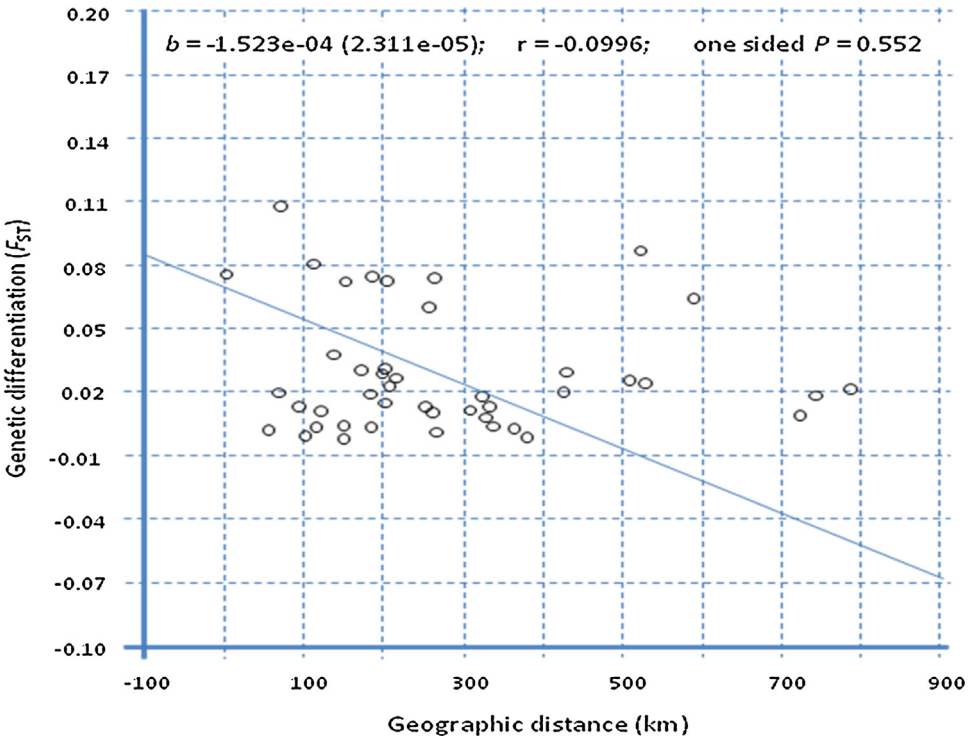

Fig. 1. Analysis of isolation-by-distance showing correlation between geographic (in km) and genetic distances between ten field populations of *R. appendiculatus* sampled in Kenya.

Table 3
Analysis of molecular variance (AMOVA) as a weighted average across loci for different groups of ticks and hierarchical levels.

Source of variation	Hierarchical clusters	Variance components	% of variation
Global dataset (all 27 tick populations)	1	Within individuals	69.63
		Among individuals within populations	11.74
		Among populations	18.63
R. appendiculatus (11 field populations only)	1	Within individuals	80.15
		Among individuals within populations	18.08
		Among populations	1.78
R. appendiculatus (11 laboratory bred stocks only)	1	Within individuals	67.76
		Among individuals within populations	7.11
		Among populations	25.14
Other *Rhipicephalus* species	1	Within individuals	45.08
		Among individuals within populations	16.30
		Among populations	38.62
All *R. appendiculatus* vs. other *Rhipicephalus* species	2	Within individuals	66.15
		Among individuals within populations	11.16
		Among populations within groups	16.64
		Among groups	6.06
R. appendiculatus (field populations verse Laboratory bred stocks)	2	Within individuals	71.49
		Among individuals within populations	11.83
		Among populations within groups	14.34
		Among groups	2.33
R. appendiculatus field populations (cattle, cattle/wildlife, wildlife stocks)	3	Within individuals	80.56
		Among individuals within populations	17.85
		Among populations within groups	1.47
		Among groups	0.12

Table 4
Estimates of global F-statistics (Cockerham and Weir, 1987) for different groups of ticks.

Group of ticks	$F_{IT} \pm$ se	$F_{ST} \pm$ se	$F_{IS} \pm$ se
All ticks (*R. appendiculatus* and other species of *Rhipicephalus*)	0.307 ± 0.039	0.184 ± 0.010	0.151 ± 0.045
R. appendiculatus (field and laboratory stocks)	0.273 ± 0.042	0.157 ± 0.010	0.137 ± 0.046
R. appendiculatus (field populations)	0.186 ± 0.042	0.014 ± 0.002	0.174 ± 0.041
R. appendiculatus (laboratory stocks)	0.318 ± 0.044	0.248 ± 0.015	0.094 ± 0.054
Other species of *Rhipicephalus*	0.569 ± 0.042	0.368 ± 0.032	0.317 ± 0.053

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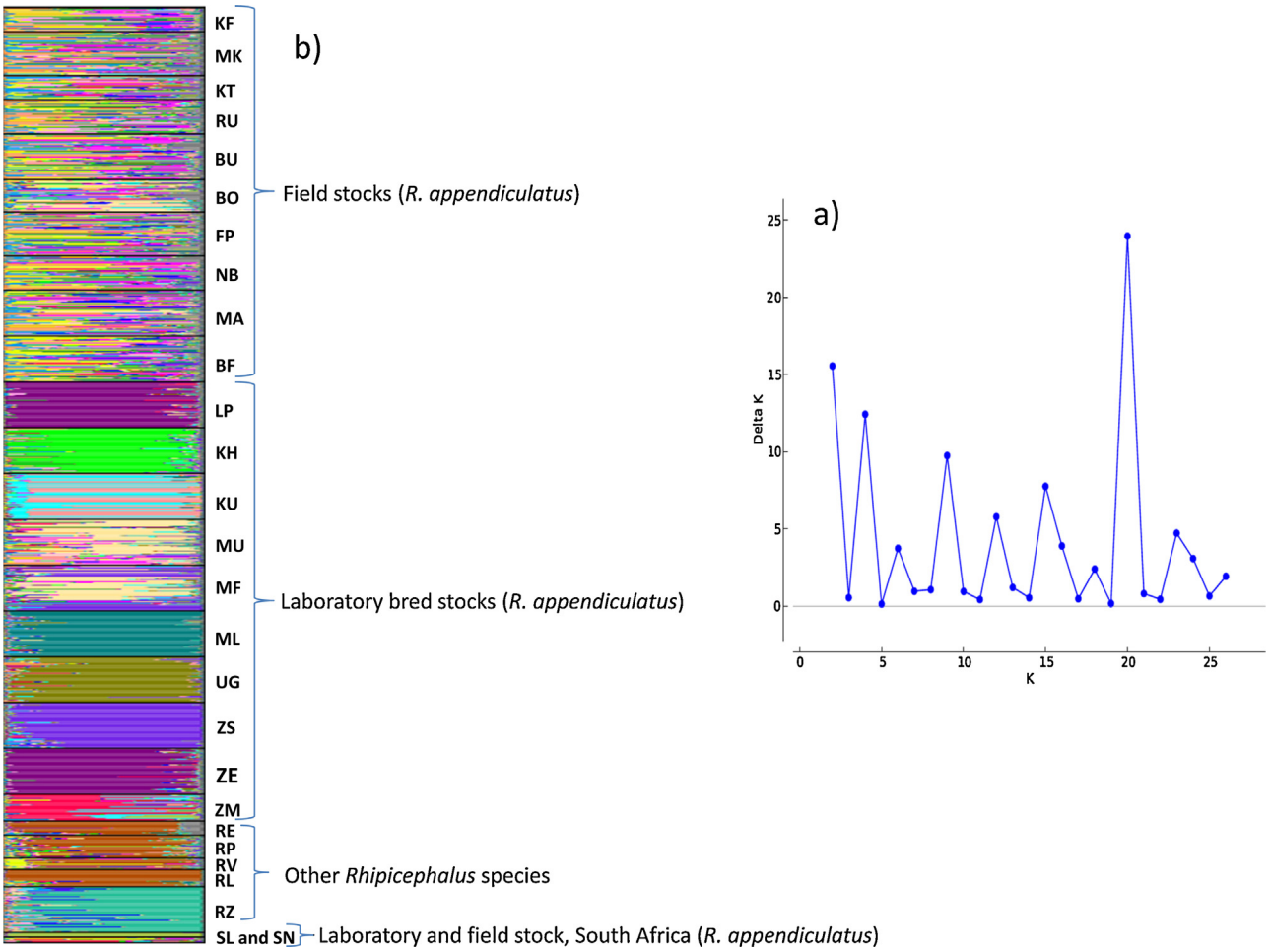

Fig. 2. Assignment of the 979 individuals of ticks to different clusters using STRUCTURE (inset: ΔK values plotted against K following Evanno et al., 2005 approach).

two populations of laboratory bred stocks (MU and MF) (Fig. 3). LP and ZE are assigned to three clusters (2, 4 and 20); KU to two clusters (21 and 22) and ZM to four clusters (1, 7, 8, and 21) with the highest proportion of individuals being allocated to clusters 7 and 21. Both KU and ZM share cluster 21 with less than five individuals of MF, MK and NB. The highest proportion of individuals (≥45) of KH, ML, UG and ZS are assigned to clusters 18, 14, 15 and 12 respectively (Fig. 3). In the case of the five additional *Rhipicephalus* species, RE and RZ are assigned exclusively to clusters 5 and 6, respectively, while majority of the individuals of RP are assigned to cluster 19 which, they share with about 15 individuals of RV and RL (Fig. 3). The two populations of *R. appendiculatus* from South Africa (SL and SN) are assigned to cluster 1 and 23 respectively, together with their field counterparts from Kenya.

Both STRUCTURE and DAPC reveal that most of the laboratory-bred stocks are genetically distinct from each other and their field counterparts; the exception is MU and MF which have an admixed genotype that is similar to that of field populations. For instance, LP, a laboratory bred population collected in 1987 from Ol Pejeta ranch in Laikipia County, Kenya, does not share any genetic background with FP, a field population sampled recently from the same ranch (Figs. 2b and 3).

To visualize the genetic divergence between field populations and laboratory bred stocks of *R. appendiculatus* in greater detail, STRUCTURE and DAPC were run on these two groups together, but excluding the two South African (SL and SN) populations. The ΔK approach revealed the highest optimal number of clusters to be 12 (Supplementary Figure S1 inset) while BIC gave a value of 18 (Supplementary Figure S2 inset). From the results of STRUCTURE, it can be observed that KU, MU and MF, show some degree of admixture, the seven other laboratory bred populations were predominantly distinct while the ten field populations had an admixed genotype (Supplementary Figure S1). This result was replicated by DAPC, which assigned the ten field populations, including MU and MF, to multiple clusters, while the remainder of the laboratory bred stocks were genetically distinct (Supplementary Figure S2). Furthermore, STRUCTURE assigned LP and ZE to the same cluster (Supplementary Figure S1) while DAPC assigned them to two clusters, 1 and 13, in almost equal proportions (Supplementary Figure S2). While majority of the individuals of KU are assigned to a single cluster by STRUCTURE with a small degree of admixture (Supplementary Figure S1), DAPC assigns this population to two clusters, 2 and 5 (Supplementary Figure S2). Although ZS appears to almost genetically distinct by STRUCTURE (Supplementary Figure S1), it shares its genetic makeup with some individuals of MF (Supplementary Figure S2).

4. Discussion

The identification and characterization of a panel of 29 polymorphic EST-based micro and minisatellite markers of *R. appendiculatus* (Kanduma et al., 2012) provided data that was re-analyzed in the current study to assess the genetic diversity and population structure within and between field ticks from different animal host species and geographic areas in Kenya as well as laboratory-bred tick populations from the genus *Rhipicephalus*.

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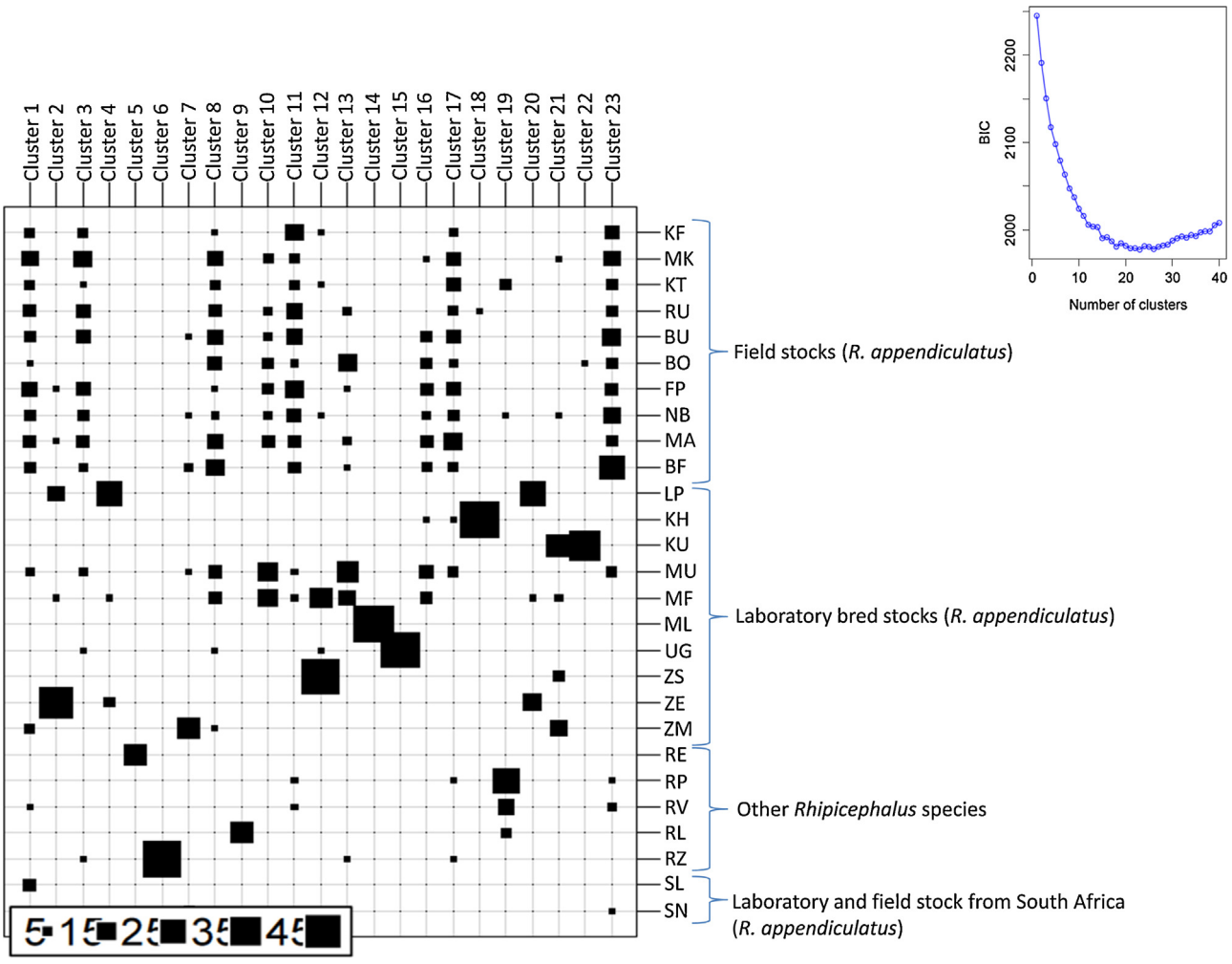

Fig. 3. Assignment of the 979 individual ticks to different clusters following a discriminant analysis principal component approach (inset: the BIC values plotted against *K*).

The earlier analysis of the 29 loci had demonstrated considerable variability across the study populations with some markers having high and others low levels of variability, on average, but there were also differences in the value of the individual markers for differentiating between specific populations (Kanduma et al., 2012). In order to achieve a balanced assessment of the levels of genetic diversity and divergence within and between *R. appendiculatus* populations, we used all the 29 markers that had been characterized previously. The markers had separated *R. appendiculatus* populations into two groups but failed to discriminate between certain field and laboratory tick stocks, thus it was necessary to apply additional algorithms to define the genetic relationships between the field and laboratory populations at higher resolution. Though in the earlier study (Kanduma et al., 2012), the Principle Component Analysis (PCA) and Multiple Co-inertia Analysis (MCoA) had indicated that five markers contributed a significant percentage of the total overall genetic variance, the population structure and the genetic relationships within- and between-populations within *R. appendiculatus* were not investigated in detail. Data from the current study demonstrates the application of the multiple satellite markers in discriminating tick populations at a higher resolution within *R. appendiculatus* (intra-population variation). Since the EST-based satellite markers were derived from transcribed and potentially functional sequences, some of which contained protein coding DNA, an additional justification for using all 29 markers in the genetic diversity studies was to minimize the potential bias contributed by variation at loci that are under selection in deriving estimates of population genetic parameters (Ellis and Burke, 2007).

Understanding genetic variation among vector populations is important for designing effective strategies to control vector-borne diseases via integrated vector control and/or anti-vector vaccination strategies (Dai et al., 2009; Gillet et al., 2009). *R. appendiculatus* has a wide distribution across eastern, central and southern Africa where it is a vector of several pathogens of veterinary and economic importance (De Vos, 1981; Perry et al., 1990; Walker et al., 2003). In Kenya, the tick occurs in areas with a mean annual rainfall above 500 mm and a mean annual maximum temperature below 35 °C. Genetic analysis of different populations of *R. apppendiculatus* can help to establish whether agro-climatic conditions, type of host, livestock farming system and tick control strategies affect population genetic structure. Such information can be used to reveal genetic changes that may be associated with acaricide resistance, as well as areas where transmission cycles between domestic and maintenance (wild) hosts overlap. This is important in evaluating risks of tick reinvasion and allows the development of better targeted integrated tick control strategies. For example, designing effective control strategies for ECF depends on understanding whether recurrent infestations are due to residual domestic tick populations that have survived acaricide control, or to reinvasion by ticks from alternative hosts.

We examined the genetic and phylogeographic structure of *R. appendiculatus* across its geographic range in Kenya by addressing two questions: (i) is the genetic diversity in *R. appendiculatus*

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spatially structured or homogeneously distributed geographically and across a range of host systems; and (ii) do tick colonies maintained as laboratory stocks for over three decades retain the same level of genetic diversity and variation present among field populations. Under field conditions, several ticks often occur in a single herd or an individual animal. We observed a higher level of genetic and allelic diversity among field populations of ticks suggesting that these populations most likely maintain equilibrium genotype frequencies within – and across – individual host animals, herds and species, therefore weakening the effects of genetic drift due to reproductive isolation and inbreeding. This is supported by the mantel test, which revealed that the ten populations of field ticks sampled in Kenya were at migration-drift equilibrium. By contrast laboratory-bred stocks had comparatively lower levels of allelic and genetic diversity possibly due to higher levels of inbreeding (overall $F_{IS} = 0.305$) and genetic drift, both of which are accentuated by small effective population sizes.

The genetic data revealed positive F_{IS} values for all the populations analyzed except for KU and ML (laboratory bred stocks) which had negative and zero values respectively. The significant deficiency of heterozygotes revealed by positive F_{IS} values, suggests that inbreeding is common in both field (overall $F_{IS} = 0.189$) and laboratory stocks (overall $F_{IS} = 0.305$) but is less prevalent in the former. The positive F_{IS} values can also arise at least partly as a result of Wahlund's effect (Wahlund, 1928), that is, co-sampling of genetically differentiated groups, in which case if each group exhibits different allele frequencies, then the overall heterozygosity within the pooled sample is reduced. This may explain the high overall F_{IS} value that we observe for the five additional species of *Rhipicephalus*. The excess of heterozygote genotypes in KU ($F_{IS} = -0.044$) may be explained by a mating strategy that deliberately avoids inbreeding. ML ($F_{IS} = 0.000$) on the other hand appears to represent a normal outbred population where mating between unrelated individuals occur at random.

The host species is a key component of an ecto-parasite's environment and is important in its evolution (Combes, 2001). In host-parasite associations with strong linkages, co-speciation may lead to host specificity while for parasites with free-living stages, mobile hosts and hence the capacity for population intermixing, environmental adaptation may impact on host specificity (Gandon and Michalakis, 2002; Greischar and Koskella, 2007). The evolution of host-specificity has been studied in the tick *Ixodes uriae* (McCoy et al., 2001, 2005), and experiments have revealed an adaptive basis for such specificity (Dietrich et al., 2011). We found no evidence of host-associated population genetic structure and specificity in our field populations of *R. appendiculatus*. Individuals sampled from isolated cattle herds, mixed cattle-wildlife populations and wildlife populations shared the same genetic background with no evidence of genetic sub-structuring. Furthermore, the level of genetic variation present between ticks sampled from these three host groups was the lowest among all the clusters tested with AMOVA (Table 3). This contrasted markedly with the pattern observed among the five additional species of *Rhipicephalus* and the laboratory maintained stocks which aggregated into distinct genetic clusters. AMOVA also showed the highest levels of genetic variation to be in these two categories (Table 3). Our results suggest a lack of host-associated adaptation/specialization within the species *R. appendiculatus* and we hypothesize that this provides the species with micro-niches, which allows it to flourish in different eco-climatic environments.

Both STRUCTURE and DAPC revealed weak genetic structure among the eleven populations of field ticks. All the sampled individuals had admixed genetic backgrounds, with no clear genetic distinction that correlates with the type of host system (cattle, cattle-wildlife, and wildlife). Both approaches therefore support the fact that all field populations are genetically admixed, share a significant proportion of their genetic makeup and have a low or very weak level of genetic subdivision and differentiation over a wide geographical range characterized by different climatic parameters, including mean annual rainfall and mean maximum temperature. This is supported by the lack of isolation by distance as revealed by the mantel test, and a low level of genetic differentiation ($F_{ST} = 0.014$). These results suggest that tick mobility over large geographic scales is not uncommon. Indeed, a weak population genetic structure has also been reported among indigenous small East African shorthorn zebu cattle in Kenya (Rege, 2001), the predominant type of cattle across eastern and southern Africa. Rege (2001) attributed this weak structure to extensive movement of cattle for trading, socio-cultural exchange and breeding purposes. Such movements may facilitate high indirect dispersal of *R. appendiculatus* across geographic regions.

High within-population genetic variation and weak genetic structure over a large geographic range has been reported for various species of ixodid ticks. Delaye et al. (1997) suggested that populations of *Ixodes ricinus* across Switzerland were panmictic because they exploited a large number of host species. McCoy et al. (2003) found a higher level of genetic substructure among *I. uriae* parasitizing black-legged Kittiwakes than those parasitizing Atlantic puffins, with estimates of population differentiation in the former being almost twice as large as those in the latter. Kittiwake ticks also showed evidence of isolation by distance suggesting that opportunities for dispersal of puffin ticks are greater due to differences in the patterns of movement and social behaviour of host species. Such observations are not limited to ticks, and have been reported for other parasites as well. For instance, in the host-parasite system involving *Geomydoecus actuosi* and its host *Thomomys bottae*, low genetic variation within parasite populations and strong population structure, has been directly linked to the social behaviour and movement of the host (Nadler et al., 1990). In a study that examined the genetic structure of five nematode parasites from three host species, Blouin et al. (1995) observed a clear relationship between parasite population structure and the host species exploited. Nematodes parasitizing livestock transported across distant locations had weak genetic structure compared to those parasitizing wild populations of white-tailed deer. It is evident from these studies that the genetic structure of parasites with low dispersal rates (ticks, nematodes etc.) is shaped by the dynamics of transmission and dispersal of their hosts. Where there are high dispersal rates among hosts, genotype intermixing and panmixia is much more likely. Thus, improved tick control can be enhanced by a better understanding of tick population dynamics (Walker, 2011).

STRUCTURE and DAPC showed that the laboratory maintained stocks have diverged from their field counterparts; only two laboratory-bred stocks, MU and MF, had a mixed genetic makeup similar to the eleven populations of field ticks. Furthermore, apart from LP and ZE, the laboratory bred stocks appear to have diverged from each other, each being characterized by a distinct genetic background. KH and ML which are bred to be of high and low susceptibility to *T. parva* infections respectively (Young et al., 1995; Odongo et al., 2009) appear to have completely diverged from their original parental stocks, KU and MU, respectively. Selection, prolonged reproductive isolation and inbreeding have probably led to the differentiation of the two laboratory stocks (KH and ML) into genetically distinct populations given that the original stocks originated from the same geographic region in Central Kenya. Though both strains (KH and ML) are capable of transmitting tick-borne diseases in an experimental context, it remains to be seen if similar biological and genetic differences occur in field populations and whether they influence the transmission of such infections. Two laboratory stocks from Zambia (ZE from

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eastern province and ZS from southern province) are genetically distinct. Biological, morphological and genetic differences have been reported between populations of *R. appendiculatus* sampled from southern and eastern Zambia (Speybroeck et al., 2002; Mtambo et al., 2007a,b; Ochanda et al., 1998). Our results confirm that at the nuclear chromosomal level these two populations are also differentiated. The LP population shares a similar genetic background with ZE but has completely diverged from FP, a field population sampled recently from the same ranch where LP was originally sampled in 1987, while FP has no genetic similarity with ZE.

The 29 polymorphic EST markers used in this study defined nuclear loci within *R. appendiculatus* that were informative in revealing population genetic diversity between individual ticks. The optimal number of population clusters was twenty for the 27 study populations, two for Kenyan field *R. appendiculatus* ticks, twelve for the combined field and laboratory *R. appendiculatus* ticks and three for the laboratory stocks. Studies of the genetic diversity and phylogeography of *R. appendiculatus* in East and Southern Africa based on the mitochondrial genes cytochrome c oxidase subunit I (COI) and 12S rDNA gene have reported the existence of two geographically, genetically and phenotypically differentiated maternal lineages (Mtambo et al., 2007a,b). Ticks found in the Southern African region (South Africa, Southern Zambia and Zimbabwe) and those distributed mainly in Eastern Africa (parts of Kenya, Tanzania and Uganda, Burundi and Rwanda) appeared to constitute two geographically separated groups that display major morphological, ecological and epidemiological differences (Mtambo et al., 2007b). While the satellite markers used in this current study reveal the occurrence of two genetically admixed populations in Kenya, the previous COI and 12S data suggested that ticks in East Africa including Kenya constitute a genetically similar maternal lineage. However, mitochondrial COI and 12S data (Kanduma et al. manuscript in preparation) indicate that the two genetically differentiated maternal groups of *R. appendiculatus* both occur in Kenya. The differentiation of the two genetic groups observed in Zambia was thought to be significantly driven by agro-ecological and climatic factors (Madder et al., 2002; Speybroeck et al., 2004; Mtambo et al., 2007a,b). The mitochondrial COI and 12S data from Kenya are similar to the nuclear satellite data, in that although there is considerable genetic variation as indicated by the identification of multiple haplotypes, this does not segregate geographically among field populations with most variation occurring within- rather than between-populations. Inter-specific competition in immature stages of the two host tick *Hyalomma rufipes* with a second *Hyalomma* species *H. truncatum* has been shown to influence population genetic structure by disrupting gene flow (Cangi et al., 2013). We do not currently have any evidence for this in *Rhipicephalus* in East Africa. However multiple *Rhipicephalus* species can be observed feeding on cattle and buffalo in Kenya (Norval et al., 1992) and it would therefore be interesting to examine whether this occurs particularly in the two hosts of *Rhipicephalus* species.

In conclusion, we have shown that *R. apppendiculatus* sampled from different geographic locations and three different host systems in Kenya are characterized by a clear lack of genetic structure. The genotypes of the individuals are admixed indicating extensive gene flow across the geographic range of the species. However, there is a clear genetic divergence between field ticks and most laboratory bred stocks and also among the latter. Our results have direct implications for the co-evolutionary interactions of *R. appendiculatus* and its hosts which has relevance to the epidemiology of ECF. More broadly, the results confirm that for most generalist ticks, a major determinant of genetic structure is host distribution and mobility combined with low host specificity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2015.08.001

References

Bilgin, R., 2007. Kg tests: a simple Excel Macro program to detect signatures of population expansion using microsatellites. Mol. Ecol. Notes 7 (3), 416–417.
Blouin, M.S., Yowell, C.A., Courtney, C.H., Dame, J.B., 1995. Host movement and the genetic structure of populations of parasitic nematodes. Genetics 141, 1007–1014.
Cangi, N., Horak, I.G., Apanaskevich, A.D., Matthee, S., das Neves, L.C.B.G., Estrada-Peña, A., Matthee, C.A., 2013. The influence of interspecific competition and host preference on the phylogeography of two African ixodid tick species. PLOS ONE 8 (10), e76930.
Chigagure, N.N., Baxter, G.D., Barker, S.C., 2000. Microsatellite loci of the cattle tick *Boophilus microplus* (Acari: Ixodidae). Exp. Appl. Acarol. 24, 951–956.
Cockerham, C.C., Weir, B.S., 1987. Correlations, descent measures: drift with migration and mutation. Proc. Natl. Acad. Sci. 84 (23), 8512–8514.
Combes, C., 2001. Parasitism: The Ecology and Evolution of Intimate Interactions. University of Chicago Press.
Dai, J., Wang, P., Adusumilli, S., Booth, C.J., Narasimhan, S., Anguita, J., Fikrig, E., 2009. Antibodies against a tick protein, Salp15, protect mice from the Lyme disease agent. Cell Host Microbe 6 (5), 482–492.
De Vos, A.J., 1981. *Rhipicephalus appendiculatus*: cause and vector of diseases in Africa. J. S. Afr. Vet. Assoc. 52 (4), 315–322.
Delaye, C., Beati, L., Aeschlimann, A.A., Renaud, F., De Meeuis, T., 1997. Population genetic structure of *Ixodes ricinus* in Switzerland from allozymic data: no evidence of divergence between nearby sites. Int. J. Parasitol. 27, 769–773.
Di Giulio, G., Lynen, G., Morzaria, S., Oura, C., Bishop, R., 2009. Live immunization against East Coast fever–current status. Trends Parasitol. 25 (2), 85–92.
Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. Vector-borne Zoonotic Dis. 11 (5), 453–470.
Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4 (2), 359–361.
Ellis, J.R., Burke, J.M., 2007. EST-SSRs as a resource for population genetic analyses. Heredity 99, 125–132.
Estrada-Peña, A., 2001. Forecasting habitat suitability for ticks and prevention of tick-borne diseases. Vet. Parasitol. 98 (1–3), 111–132.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 14, 2611–2620.
Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10 (3), 564–567.
Falush, D., Stephens, M., Pritchard, J., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.
Gandon, S., Michalakis, Y., 2002. Local adaptation, evolutionary potential and host–parasite coevolution: interactions between migration, mutation, population size and generation time. J. Evol. Biol. 15 (3), 451–462.

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Gillet, P., Mori, M., Van Esbroeck, M., Van den Ende, J., Jacobs, J., 2009. Assessment of the prozone effect in malaria rapid diagnostic tests. Malar. J. 8, 271.
Greischar, M.A., Koskella, B., 2007. A synthesis of experimental work on parasite local adaptation. Ecol. Lett. 10 (5), 418–434.
Hoogstraal, H., 1956. African Ixodoidea. (vol. I). Ticks of the Sudan (with Special Reference to Equatoria Province and with Preliminary Reviews of the Genera *Boophilus*, *Margaropus*, and *Hyalomma*). US Government Printing Office.
Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23 (14), 1801–1806.
Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405.
Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11 (1), 94.
Kanduma, E.G., Mwacharo, J.M., Sunter, J.D., Nzuki, I., Mwaura, S., Kinyanjui, P.W., Kibe, M., Heyne, H., Hanotte, O., Skilton, R.A., Bishop, R.P., 2012. Micro- and minisatellite-expressed sequence tag (EST) markers discriminate between populations of *Rhipicephalus appendiculatus*. Ticks Tick-borne Dis. 3 (3), 128–136.
Lessard, P., L'Eplattenier, R., Norval, R.A.I., Perry, B.D., Dolan, T.T., Burrill, A., Croze, H., Sorensen, M., Grootenhuis, J.G., Irvin, A.D., 1988. The use of geographical information systems in estimating East Coast fever risk to African livestock. Acta Vet. Scand. Suppl. 84, 234–236.
Lessard, P., L'Eplattenier, R., Norval, R.A.I., Perry, T.T., Dolan, Burrill, A., Croze, H., Sorensen, M., Grootenhuis, J.G., Irvin, A.D., 1990. Geographical information systems for studying the epidemiology of cattle diseases caused by *Theileria parva*. Vet. Rec. 126, 255–262.
Madder, M., Speybroeck, N., Brandt, J., Tirry, L., Hodek, I., Berkvens, D., 2002. Geographic variation in diapause response of adult *Rhipicephalus appendiculatus* ticks. Exp. Appl. Acarol. 27 (3), 209–221.
McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. J. Evol. Biol. 14 (3), 395–405.
McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003. Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. Evolution 57 (2), 288–296.
McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Le Bohec, C., Yvon Le Maho, Gauthier-Clerc, M., 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. Proc. R. Soc. B: Biol. Sci. 272 (1579), 2389–2395.
Minjauw, B., McLeod, A., 2003. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small scale and marginal livestock owners in India, eastern and southern Africa. In: Animal Health Program Report. Centre for Tropical Veterinary Medicine, University of Edinburg, UK.
Mtambo, J., Madder, M., Van Bortel, W., Geysen, D., Berkvens, D., Backeljau, T., 2007a. Genetic variation in *Rhipicephalus appendiculatus* (Acari: Ixodidae) from Zambia: correlating genetic and ecological variation with *Rhipicephalus appendiculatus* from eastern and southern Africa. J. Vector Ecol. 32 (2), 168–175.
Mtambo, J., Madder, M., Van Bortel, W., Berkvens, D., Chaka, G., Backeljau, T., 2007b. Further evidence for geographic differentiation in *R. appendiculatus* (Acari: Ixodidae) from Eastern and Southern provinces of Zambia. Exp. Appl. Acarol. 41, 129–138.
Nadler, S.A., Hafner, M.S., Hafner, J.C., Hafner, D.J., 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomyidae). Evolution, 942–951.
Nadler, S.A., 1995. Microevolution and the genetic structure of parasite populations. J. Parasitol., 395–403.
Norris, D.E., Shurtleff, A.C., Touré, Y.T., Lanzaro, G.C., 2001. Microsatellite DNA polymorphism and heterozygosity among field and laboratory populations of *Anopheles gambiae* ss (Diptera: Culicidae). J. Med. Entomol. 38 (2), 336–340.
Norval, R.A.I., Perry, B.D., 1990. The introduction and subsequent disappearance of the brown ear-tick *Rhipicephalus appendiculatus*, from the southern lowveld of Zimbabwe. Exp. Appl. Acarol. 9, 103–111.
Norval, R.A.I., Perry, B.D., Young, A.S., 1992. The Epidemiology of Theileriosis in Africa. Academic Press, London.
Ochanda, H., Young, A.S., Medley, G.F., Perry, B.D., 1998. Vector competence of 7 rhipicephalid tick stocks in transmitting 2 *Theileria parva* parasite stocks from Kenya and Zimbabwe. Parasitology 116, 539–545.
Odongo, D.O., Ueti, M.W., Mwaura, S.N., Knowles, D.P., Bishop, R.P., Scoles, G.A., 2009. Quantification of *Theileria parva* in *Rhipicephalus appendiculatus* (Acari: Ixodidae) confirms differences in infection between selected tick strains. J. Med. Entomol. 46 (4), 888–894.
Park, S.D.E., 2001. The Excel Microsatellite Toolkit, http://oscar.gen.tcd.ie/(sdepark/mstoolkit/index.php
Perry, B.D., Lessard, P., Norval, R.A.I., Kundert, K., Kruska, R., 1990. Climate, vegetation and the distribution of *Rhipicephalus appendiculatus* in Africa. Parasitol. Today 6, 100–104.
Perry, B.D., Randolph, T.F., Mcdermott, J.J., Sones, K.R., Thornton, P.K., 2002. Investing in Animal Health Research to Alleviate Poverty. International Livestock Research Institute Report, Nairobi, Kenya.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria.
Radley, D.E., 1981. Infection and treatment method of immunization against Theileriosis. In: Irvin, A.D., Cunningham, M.P., Young, A.S. (Eds.), Advances in the Control of Theileriosis: Proceedings of an International Conference Held at ILRAD, Nairobi, 9–13 February. Martinus NijhoV Publishers, The Hague, pp. 227–236.
Randolph, S.E., 1994. Population dynamics and density-dependent seasonal mortality indices of the tick *Rhipicephalus appendiculatus* in eastern and southern Africa. Med. Vet. Entomol. 8 (4), 351–368.
Randolph, S.E., 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129, S37.
Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86 (3), 248–249.
Rege, J.E.O., 2001. Zebu Cattle of Kenya: Uses, Performance, Farmer Preferences, Measures of Genetic Diversity and Options for Improved Use (Animal Genetic Resources Research 1, International Livestock Research Institute, Nairobi).
Reich, D.E., Goldstein, D.B., 1998. Genetic evidence for a Paleolithic human population expansion in Africa. Proc. Natl. Acad. Sci. 95 (14), 8119–8123.
Reich, D.E., Wayne, R.K., Goldstein, D.B., 1999. Genetic evidence for a recent origin by hybridization of red wolves. Mol. Ecol. 8 (1), 139–144.
Rosenberg, N.A., 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4 (1), 137–138.
Shaw, M.K., Young, A.S., 1994. The biology of Theileria species in ixodid ticks in relation to parasite transmission. Adv. Dis. Vector Res. 10, 23–63.
Speybroeck, N., Madder, M., Van Den Bossche, P., Mtambo, J., Berkvens, N., Chaka, G., Mulumba, M., Brandt, J., Tirry, L., Berkvens, D., 2002. Distribution and phenology of ixodid ticks in southern Zambia. Med. Vet. Entomol. 16 (4), 430–441.
Speybroeck, N., Madder, M., Thulke, H.H., Mtambo, J., Tirry, L., Chaka, G., Marcotty, T., Berkvens, D., 2004. Variation in body size in the tick complex *Rhipicephalus appendiculatus*/*Rhipicephalus zambeziensis*. J. Vector Ecol. 29, 347–354.
Wahlund, S., 1928. The combination of populations and the appearance of correlation examined from the standpoint of the study of heredity. Hereditas 11, 65–106.
Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I., Latif, A., Pegram, R., Preston, P., 2003. Ticks of Domestic Animals in Africa. A Guide to Identification of Species. University of Edinburgh, Scotland.
Walker, A.R., 2011. Eradication and control of livestock ticks: biological, economic and social perspectives. Parasitology 138 (08), 945–959.
Walker, J.B., 1960. Notes on the Common Tick Species of East Africa. Cooper McDougal & Robertson, Nairobi.
Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The Genus Rhipicephalus (Acari, Ixodidae): A Guide to the Brown Ticks of the World. Cambridge University Press, Cambridge.
Young, A.S., Dolan, T.T., Mwakima, F.N., Ochanda, H., Mwaura, S.N., Njihia, G.M., Muthoni, M.W., Dolan, R.B., 1995. Estimation of heritability of susceptibility to infection with *Theileria parva* in the tick *Rhipicephalus appendiculatus*. Parasitology 111, 31–38.

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