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Population structure and genetic diversity of *Rhipicephalus microplus* in Zimbabwe

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ABSTRACT

Recently there was an expansion in the geographic range of *Rhipicephalus microplus* in Zimbabwe. In order to understand gene flow patterns and population structure in this highly invasive and adaptable cattle tick, a population genetics study was carried out. Eighty-seven *R. microplus* tick samples drawn from 5 distinct populations were genotyped using eight polymorphic microsatellite loci. Genetic diversity (H_e) was high (0.755–0.802) in all the populations, suggesting high levels of gene flow with 97% of genetic variation found within populations and 3% amongst populations. No isolation by distance was observed with low but significant genetic differentiation amongst the populations (0–0.076). Most of the sampled individuals had admixed genetic backgrounds, except for those from Matabeleland North whose genetic makeup appeared different from the rest. *Rhipicephalus microplus* was recently recorded in this area and the environmental conditions do not support survival of the tick there. These results confirm recent range expansion of the tick and the lowest genetic diversity recorded in the Matabeleland North population is suggestive of a founder effect, which may lead to genetic drift. Generally, the very low levels of genetic differentiation amongst the populations could be a result of the frequent movement of livestock from one area to another, which will have implications for disease control. This study offers further opportunities to study evolutionary adaptation of *R. microplus* in Zimbabwe and southern Africa.

1. Introduction

The use of molecular markers in the study of ticks provides new insights into their population structure and taxonomic relationships (Paulauskas et al., 2006). Investigating the genetic structure of tick populations allows acarologists to answer crucial questions about their biology. This is important because the control of tick-borne diseases (TBDs) is primarily focused on the vector ticks (Giles et al., 2014). Among the factors under investigation are tick dispersal mechanisms, mating patterns and evolutionary adaptations to the environment (McCoy, 2008). It is important to note that such factors will have important implications on the transmission dynamics of pathogens that these ticks carry as vectors, as well as resistance to the acaricide chemicals used to control the ticks (Chevillon et al., 2013).

Sungirai et al. (2017) showed that the distribution of the one-host tick *R. microplus* in Zimbabwe has expanded, and this was supported by collections from previously unrecorded and ecologically different areas. Of particular concern are the low-lying areas, where temperatures and humidity levels are not favourable for the proliferation of this tick species. The expansion of the geographic range of *R. microplus* could be attributed to cattle movement within and between the different provinces of Zimbabwe. Sungirai et al. (2016) noted the bi-directional movement of cattle between the Masvingo and Manicaland provinces of Zimbabwe, since farmers in the former area trade their small framed cattle for the heavy framed cattle in the latter area.

Due to the absence of strict movement controls of livestock, cattle may move from one province to another without being inspected for the presence of diseases or vectors such as ticks. Therefore, cattle carrying

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ticks or other parasites can move between areas, leading to parasite invasion in previously unoccupied areas. This movement can be in one direction, or it can be bi-directional. One-way movement of cattle together with ticks might result in geographic or genetic isolation of ticks, leading to founder effects that may result in genetic drift. In contrast, bi-directional movement of ticks will result in panmixia, which is characterised by high levels of genetic exchange between populations. All of these scenarios might influence the transmission dynamics of vector pathogens, as well as resistance of vector ticks to acaricides (Chevillon et al., 2013).

An investigation of gene flow between tick populations in Zimbabwe may be helpful to infer cattle movement patterns, which in turn might have led to tick migrations. Additionally, the evolutionary adaptations to different ecological environments of the cattle tick can be investigated. Therefore, the aims of the current study were to investigate genetic differentiation and gene flow patterns in *R. microplus* sub-populations of Zimbabwe. The null hypothesis was that there would be little to no genetic differentiation between geographically close sub-populations, and that differentiation would increase as a function of distance and decreased gene flow.

2. Materials and methods

2.1. Study area and sample preparation

Rhipicephalus microplus tick samples were obtained from a nationwide survey conducted as described by Sungirai et al. (2017). Each province was represented by thirty tick samples, forming provincial populations. Provinces included in the study were Manicaland, Mashonaland Central, Masvingo, Matabeleland North, and Midlands. Total genomic DNA was extracted from *R. microplus* ticks using the QIAamp genomic DNA kit (Qiagen, Hilden, Germany).

2.2. Microsatellite selection and data analysis

A total of 27 microsatellite loci were evaluated for their utility to study the population genetics of *R. microplus* in Zimbabwe. Fifteen of these were obtained from the University of Pretoria, Department of Genetics, Ticks and Tick-Borne Disease Research Unit (unpublished), four were described by Chigagure et al. (2000), five by Cutullé et al. (2009), and three by Busch et al. (2014).

Thirteen microsatellite loci were chosen based on their PCR efficiency (> 75% amplification success), type of repeats, and the presence of polymorphism in a test sample of 11 ticks. Fluorescently labelled forward primers were used to amplify each locus from each tick sample, and the fragment sizes were determined by the VIB genetic service facility, University of Antwerp. Genotyping was performed using Geneious software (Kearse et al., 2012).

Eight loci with average estimates of gene diversity (H_s) greater than 0.6 (Koffi et al., 2006) were selected for further analysis (Table 1). Population genetic analyses in ixodid ticks should be based on a minimum of five to seven markers (Chevillon et al., 2013), thus eight loci were considered enough in this study. These loci were PCR amplified in single-plex for 150 *R. microplus* DNA samples, and analysed in three panels using an ABI 3730 Genetic Analyser (VIB Genetic Service Facility, University of Antwerp).

The genotyped samples were tested for the presence of null alleles, scoring errors and large allele dropout using the software MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004). Linkage disequilibrium (LD) amongst pairs of loci was tested using FSTAT software (Goudet, 2001) based on the log-likelihood ratio G statistic. The same software was used to estimate allelic richness and the average genetic diversity (H_s). Estimations of the mean number of alleles, number of private alleles and Analysis of Molecular Variance (AMOVA) were performed using GenAlex (Peakall and Smouse, 2012). Pairwise F_{ST} (θ) values corrected for sample size (Weir and Cockerham, 1984) were computed using Genodive v2.0b14 (Meirmans, 2009) to compare genetic differentiation amongst populations.

To visualise the geographic clustering of different populations, Principal Co-ordinate Analysis (PCoA) was done using GenAlex. Additionally, a Mantel test was done in GenAlex to show the correlation between genetic and geographic distances. The genetic structure of the population and the likely number of clusters (K) was explored using STRUCTURE v.2.3.4 (Pritchard et al., 2000). All of the genotyped tick samples were included, and the number of potential clusters was set from 1 to 5, with 10 independent runs and a burn-in period of 50 000 iterations followed by 150 000 Markov Chain Monte Carlo (MCMC) iterations. An admixture model with correlated allelic frequencies was used together with a LOCPRIOR model, which takes into account the original population of each tick individual. The most likely number of clusters (K) was inferred by assessing ΔK (Evanno et al., 2005) using STRUCTURE HARVESTER v0.6.94 (Earl, 2012).

3. Results

3.1. Microsatellite selection and data analysis

Only 87 of the 150 samples (58%) resulted in positive amplification for the eight loci (Table 2). The number of alleles ranged from 5 to 25 per locus, with an allelic richness of 4–13. The levels of genetic diversity amongst the loci (He) were relatively high (0.6–0.9), while the F_{IS} values were relatively low for all the loci except locus C39A. The MICRO-CHECKER results showed homozygous excesses in all the loci except P801G, while no evidence of scoring errors and large allele dropouts was seen in all the loci except locus C39A, which showed potential scoring errors due to stuttering. Upon further analysis of the peak sizes at this locus, stuttering appeared unimportant, hence this locus was retained. No LD was observed ($P < 0.001$) amongst pairs of loci, signifying that they are statistically independent, and thus these loci were considered suitable for further population genetic analyses.

3.2. Genetic differentiation and population structure

The median genetic diversity (He) was 0.763 (0.755–0.802) with Matabeleland North having the lowest genetic diversity (Fig. 1). Manicaland had the highest number of private alleles as compared to other populations (Fig. 1). The AMOVA analysis revealed that 97% of the genetic variation existed within populations, while 2% of the genetic variation existed between populations ($F_{ST} = 0.023$, $P < 0.001$, Table 3).

There was little to no genetic differentiation amongst the populations. However, the pairwise F_{ST} (θ) values were significant at the 5% level amongst all pairs of populations, except between Masvingo and Manicaland, and between Masvingo and Midlands (Table 4). This observation was supported by the PCoA analysis (Fig. 2), which did not show an obvious clustering of populations, except for partial clustering of samples from Matabeleland North. These results were further corroborated by the Mantel test, which did not show significant patterns of isolation by distance (IBD) among the different populations ($P = 1.000$). The correlation between geographic and genetic distance was very low ($r = 0.078$) (Fig. 3).

STRUCTURE analysis suggested that the probable number of *R. microplus* populations in Zimbabwe was K = 2, with ΔK = 32.8. No ΔK values were reported for K = 1 and K = 5, while for K = 3 and K = 4 the ΔK values were 1.7 and 0.02 respectively, confirming that there were two genetically distinct clusters (Fig. 4).

4. Discussion

The study revealed high levels of genetic diversity within *R. microplus* populations and little genetic differentiation amongst them. There was high allelic diversity amongst the loci, despite an excess of

Table 1
Final list of selected microsatellite loci and their reaction conditions.

Locus	Primer Sequences (5′- > 3′)	Dye	Annealing Temperature/°C	Size range (bp)	Panel
C39A	F:ATAGAAACACTTAAATCGCATAAC R: GTCCCTTTGTTGCCGTTTAG	VIC	53	332(310–342)	1
P804J	F:TTAACTGGCTGAACATAGGAGGAG R: CGTGATTTTCCCGAGTTGAT	6FAM	54	318 (315–342)	1
P801L	F: AACATCACAGAGGCGGTAATC R: TTCGCTCCTCTTTCCTCATTACT	PET	55	339 (275–355)	1
P801G	F: AACTGCCTTTCCTGTGAGTTCAA R: CCCGATTCTTGGCCGATCTC	6FAM	58	300 (272–305)	2
P804A	F: CCAAGCGATAACACATGTATAGG R: GACAGCAAAATCCCGAAGAT	VIC	55	332 (199–343)	2
P804G	F: CTCTATTTTCCCTTAGTGCTCAA R: TCAGAAAGAAGCCTACTGATG	NED	54	345 (295–363)	2
P807F	F: GCCACAAAGCTCGACCTAACTA R: GACTGGGTTAACTGGCGGAACAA	VIC	58	322 (315–333)	3
C27A	F: TCTGACGATACCCCGAACTACAT R: TACTACCGCGACAAGCACAATGA	NED	55	344 (320–348)	3

Table 2
Summary statistics of loci used in this study across all populations.

Locus	Na	Ne	AR	Ho	Hs	F_{IS}
P804J	5	2.57	4.57	0.35	0.62	0.37
C39A	9	3.38	5.57	0.35	0.73	0.49
P801L	14	6.78	10.11	0.59	0.83	0.30
P801G	10	5.82	8.32	0.78	0.85	0.05
P804A	25	7.48	13.06	0.57	0.90	0.33
P804G	17	8.23	11.20	0.76	0.91	0.14
P807F	7	4.47	6.44	0.67	0.79	0.12
C27A	8	3.71	5.96	0.60	0.76	0.18

Na = No. of Alleles, Ne = No. of Effective Alleles, AR = Allelic Richness, Ho = Observed Heterozygosity, Hs = Average estimate of within sample gene diversity, F_{IS} = Fixation Index.

homozygotic loci. The source population of *R. microplus* in Zimbabwe, namely the Manicaland province, had the largest number of private alleles and genetic diversity. The results of the study suggest an infinite island population structure model for *R. microplus* in Zimbabwe, which is in migration-drift equilibrium. This was supported by population structure analysis, which showed admixture in all the sub-populations, although the recent range expansion in Matabeleland North was suggestive of founder effects.

Studies on the population structure of *R. microplus* so far have been limited to South America, Australia and New Caledonia (Busch et al., 2014; Chevillon et al., 2013; Cutullé et al., 2009; Giles et al., 2014). This study is the first to report on the population structure of *R. microplus* in Zimbabwe and Africa in general. The high levels of genetic diversity observed in this study indicate increased gene flow within and among populations. High levels of within-population genetic variation and weak genetic structure between populations has been observed in other ixodid tick species (Delaye et al., 1997; Kanduma et al., 2015; McCoy et al., 2012) as well as studies carried out on *R. microplus* in South America (Busch et al., 2014) and *R. asutralis* (formerly *microplus*) in Australia and New Caledonia (Chevillon et al., 2013; Cutullé et al., 2009). This was attributed to high dispersal rates amongst host species, resulting in genotype mixing and panmixia. These movements will indirectly facilitate dispersal of ticks. In Kenya, a weak genetic structure in cattle amongst different populations was observed and this was attributed to extensive movement of cattle for socio-cultural exchange and trading purposes (Rege, 2001) and subsequently explained the

Table 3
AMOVA for the different populations (df = degrees of freedom, SS = sum of squares, MS = mean square).

Source	df	SS	MS	Variance Component	% of Total Variance/%
Among Populations	4	27.161	6.790	0.107	3%
Within Populations	169	537.667	3.158	3.158	97%
Total	173	560.828		3.265	100%

Table 4
Pairwise F_{st} (θ) amongst different populations calculated according to Weir and Cockerham (1984) and adjusted for sample size. F_{ST} (θ) values in bold are significant at the 5% level.

	Manicaland	Mashonaland Central	Masvingo	Matabeleland North
Mashonaland Central	0.015			
Masvingo	0.000	**0.028**		
Matabeleland North	**0.059**	**0.076**	**0.060**	
Midlands	**0.023**	**0.025**	0.011	**0.041**

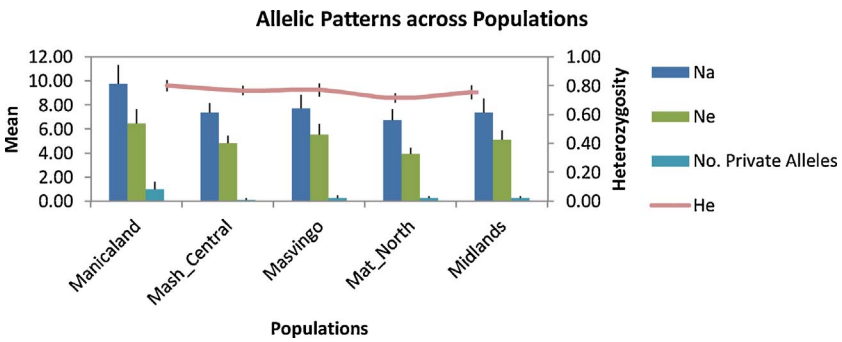

Fig. 1. Distribution of allelic patterns between populations (Na = No. of Different Alleles, Ne = No. of Effective Alleles, No. Private Alleles = No. of alleles unique to a single population, He = Expected Heterozygosity).

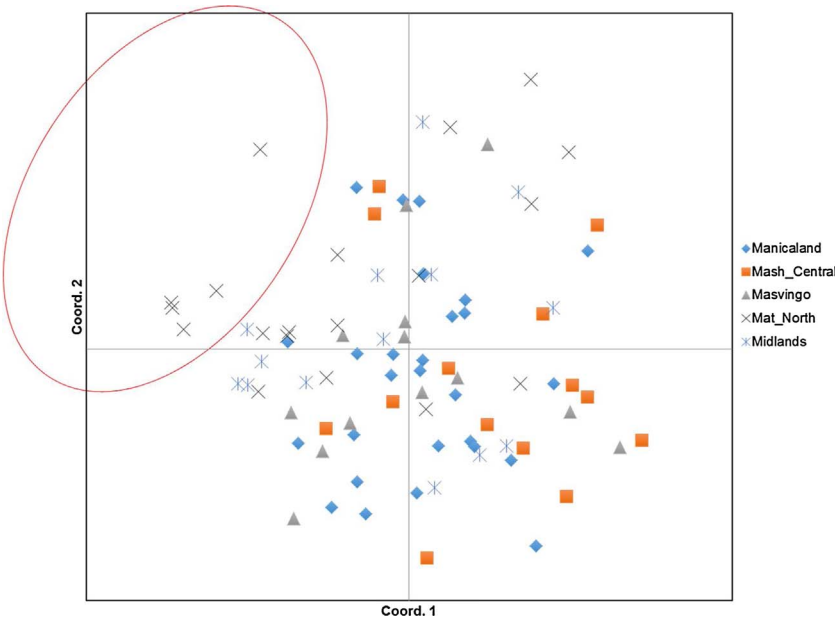

Fig. 2. Principal Co-ordinate Analysis (PCoA) of genotypes of samples originating from the five provinces where *R. microplus* was found.

weak genetic structure observed amongst *Rhipicephalus appendiculatus* tick populations (Kanduma et al., 2015). Increased gene flow amongst populations may lead to a spread of acaricide resistant alleles (Beesley et al., 2017), but the lack of genetic differentiation means that there will be no genetic barriers to tick control programmes (Gooding, 1996).

Alleles may be lost as parasites move between populations, resulting in founder effects (Balloux and Lugon, 2002). This phenomenon was observed in the current study, since the source population in Manicaland had the highest number of private alleles as compared to the other populations. This could further result in genetic drift (Roderick and Navajas, 2003), which can be an indicator for local adaptation (Gandon and Michalakis, 2002). This could explain why the population in Matabeleland North appeared to be partially clustered, as seen by the results of the Principal Co-ordinate and STRUCTURE analyses.

Although our results indicated that there was no IBD, thus suggesting migration-drift equilibrium (Kanduma et al., 2015), the low levels of genetic differentiation amongst the populations were significant. Apart from frequent dispersal between established populations, this could also suggest recent population expansion (*cf.* McCoy et al., 2003). However, for the sub-populations that share borders such as Manicaland and Masvingo, and Masvingo and Midlands, the differentiation was insignificant. This tended to partially support the null hypotheses, which was that differentiation would increase as a function of distance and decreased gene flow. The total absence of genetic differentiation between Masvingo and Manicaland is a result of the bi-directional movement of cattle as noted by Sungirai et al. (2016).

The excess homoyzgotes observed in the microsatellite loci were not attributed to the presence of null alleles, since only positive samples were sent for fragment size analysis after PCR amplification in singleplex. The occurrence of these homozygote excesses could rather be attributed to the Wahlund effect as a result of the inadvertent pooling of individuals from different populations (Dharmarajan et al., 2011). Alternatively, this could be attributed to the biology of the tick at the infra-population level, where development occurs simultaneously within large brotherhoods of individuals, which go on to seek hosts as a group, develop to adults simultaneously, and mate with each other (Koffi et al., 2006). This results in inbreeding and increased homozygosity (*cf.* Dharmarajan et al., 2011).

Unrestricted cattle movement may be responsible for the frequent gene flow amongst the different *R. microplus* tick populations leading to

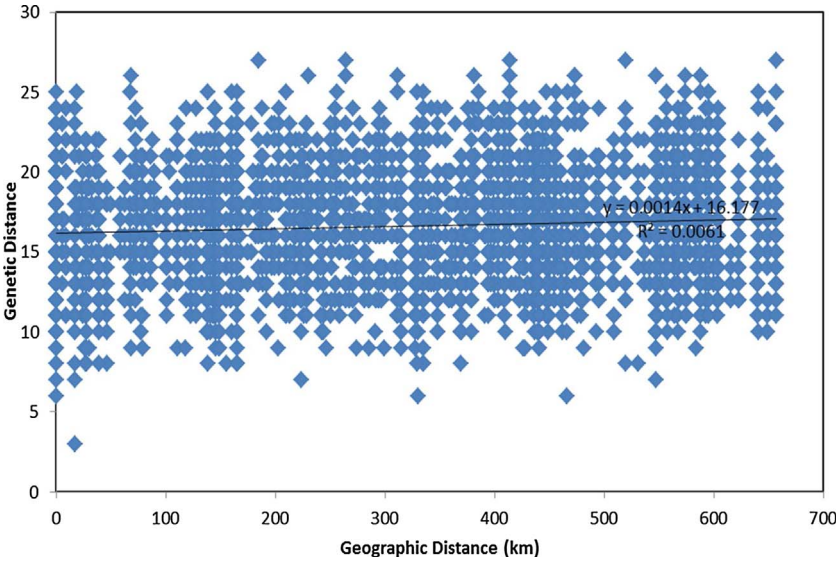

Fig. 3. Analysis of Isolation by Distance (IBD) showing correlation between geographic distance and the genetic distance between the *R. microplus* samples.

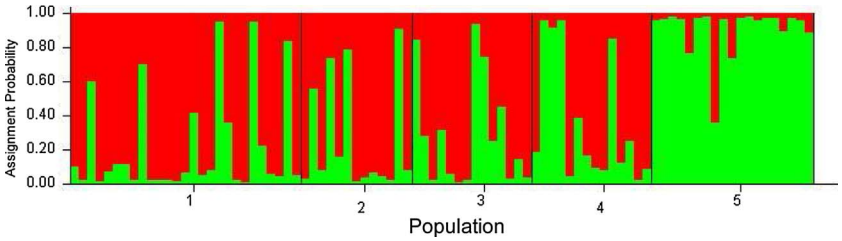

Fig. 4. *Rhipicephalus microplus* tick population structure (1 – Manicaland, 2 – Masvingo, 3 – Mashonaland Central, 4 – Midlands, 5– Matabeleland North).

weak population structure. However it is observed that alleles are lost as ticks migrate from the source population. The consequences of such allele losses have not been clearly observed in the current study. Thus, it will be important to study the phenology of *R. microplus* in these ecologically different habitats, and compare those results with the genetic diversity in order to understand local adaptation.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

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