Low effective population sizes in *Amblyomma variegatum,* the tropical bont tick

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ABSTRACT

Effective population sizes have rarely been estimated in ticks despite the importance of this parameter for evaluating the evolutionary and adaptive potential of tick populations. The present study was aimed at evaluating the effective population sizes of *Amblyomma variegatum*, the tropical bont tick, in three villages in Burkina Faso. For this purpose, microsatellites markers were developed. Eight out of 19 assessed markers provided good amplification results with 4 to 24 alleles recorded per marker on 216 genotyped ticks. The within-samples polymorphism was congruent with Hardy-Weinberg expectations at four markers while sex linkage and/or null alleles were observed at the others. As sampling involved two tick generations, effective population sizes were independently estimated by two methods insensitive to heterozygosity: the first one is based on linkage disequilibrium analysis within a single cohort while the second uses the changes in allele frequencies across generations. Both methods estimated the number of reproducing ticks ranging from two to a few tens reproductive adults per village and cohort. Such small estimates are congruent with the rarity of records of acaricide resistance in *A. variegatum*.

1. Introduction

While the past decades have seen the multiplication of successful applications of phylogenetic and population genetics analyses to many ticks for tracking their historical changes in distribution, identifying cryptic species as well as their mating patterns, evaluating their dispersal ranges and/or understanding how biotic and/or abiotic parameters jointly define their population genetic structure (see for review Araya-Anchetta et al., 2015), evaluation of effective population sizes (N_e) in ticks had so far been restricted to the *Rhipicephalus microplus* complex (Koffi et al., 2006a; De Meeûs et al., 2010; Biguezoton, 2016). This is curious given, on the one hand, the amount of the tick literature related to the efficacy of tick-control programs and/or acaricide resistance, and on the other hand, the key importance of Ne in the

evolutionary potential of the studied populations. Indeed, the acquisition of new alleles (via migration or mutation) is proportional to N_e, the polymorphism lost by a population at each generation via genetic drift is proportional to $1/Ne$ and thus only the selection pressures s verifying $s > > 1/Ne$ overcome the effect of drift to actually impact the frequency of selected alleles (e.g. Hartl and Clark, 1997; Hedrick, 2000).

The present study was aimed at evaluating the effective population sizes in Burkina Faso of a three-host tick species of major veterinary importance in West Africa: the tropical bont tick, *Amblyomma variegatum*. There, *A. variegatum* used to be the most abundant tick species found on livestock (Kabore et al., 1998; Farougou et al., 2006, 2007) until the last decade when *R. microplus* started to invade this area (Madder et al., 2011). Today, it remains the second (after *R. microplus*) most abundant tick on cattle throughout Benin and Burkina Faso

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(Biguezoton et al., 2016). Originated from sub-Saharan areas, *A. variegatum* has been reported in more than 30 African countries (Walker et al., 2003) and got out of Africa via the cattle trade to reach Indian Ocean islands by the 7th century (Stachurski et al., 2013) and the Caribbean's in mid-18th century (Maillard and Maillard, 1998). The veterinary importance of *A. variegatum* arises from a combination of factors. Firstly, *A. variegatum* directly impairs animal growth and tend to heavily reduce milk production due to its preference as adults to attach on cattle udder (Stachurski, 2000a). Secondly, its saliva favors the development of severe acute dermatophilosis, a bacterial skin disease of animals (Barré et al., 1988). Thirdly and finally, *A. variegatum* vectors *Ehrlichia ruminantium*, the agent of heartwater (cowdriosis), that induces up to 80% mortality in susceptible sheep and goats (Uilenberg, 1983) and induces morbidity in the cattle breeds imported from free-heartwater areas (Uilenberg and Camus, 1993). *E. ruminantium* infection risks are quite high in West Africa: 10–16% of *A. variegatum* adult and nymphal stages are infected in Benin (Farougou et al., 2012) as well as 39–61% cattle and at least 51% sheep and goats in Ghana (Farougou et al., 2013; Koney et al., 2004). The only commercial vaccine available to protect against heartwater is based on the infectious challenge using a virulent *E. ruminantium* strain (Bezuidenhout, 1989) followed by antibiotic treatments. Meanwhile, selecting West African cattle breeds for poor attractiveness to *A. variegatum* was shown to be difficult (Stachurski, 2007). As a result, the usage of acaricide footbaths still seems as the most accurate way to protect cattle from all the deleterious effects of *A. variegatum* (Stachurski and Lancelot, 2006). As the durability of such protection would depend on the potential of *A. variegatum* to evolve acaricide resistance, we estimated the effective population sizes of *A. variegatum* in three neighbor villages from Burkina Faso. To this purpose, we sampled two successive tick generations within each village and characterized the polymorphism of newly developed microsatellite markers per village and tick-generation.

2. Material and methods

2.1. Tick sampling

Amblyomma variegatum ticks were collected in 2007 on herds merging cattle, sheep and goats which foraged communal pastures from either one of three neighbor villages (pairwise distances ~ 10 km) from Burkina Faso: Bekuy (3.91 °W, 11.60 °N), Sara (3.83 °W, 11.71 °N) and Lamba (4.05 °W, 11.66 °N). There, *A. variegatum* reproduces once a year with adults attaching on hosts in the early rainy season (mid-May to July), freshly hatched larvae in late rainy season (September) and nymphs in the early dry season (November) (Stachurski, 2006). Three sampling campaigns were performed in May, July and November with the purpose to genotype ~ 25 ticks per village and campaign. In May and July, the only available ticks were adults feeding on cattle. The next tick generation was collected as nymphs on sheep and goats in November. As nymphs cannot be sexed, they were allowed to molt under laboratory controlled conditions, so that sexing and genotyping were performed on adult stages.

2.2. Identification of microsatellite markers

Microsatellite loci were isolated from two microsatellite enriched libraries according to Billotte et al. (1999).Total genomic DNA was extracted from a mix of adult males and females *A. variegatum* with the Qiamp DNA mini kit (Qiagen) following manufacturer's instructions. DNA was restricted by *HaeIII* and fragments were ligated to Rsa21 and Rsa25 self-complementary primers (5′-CTCTTGCTTACGCGTGGA CTA-3′ and 5′-TAGTCCACGCGTAAGCAAGAGCACA-3′) and amplified by polymerase chain reaction (PCR). Products were hybridized to biotin-labelled $I_5(GA)_8$ and $I_5(GT)_8$ probes and fragments containing microsatellite sequences were captured using Streptavidin Magne-Sphere Paramagnetic Particles (Promega). Enriched fragments were

PCR amplified, cloned in pGEM-T (Promega) and transformed in XL1-Blue competent cells (Stratagene). Randomly picked recombinant colonies were PCR amplified with Rsa21 primer. PCR products were run on a 1.2% agarose gel and transferred onto a Hybond N + membrane (Amersham) which were hybridized with $[\gamma^{32}P]dATP$ end-labelled $(GA)_{15}$ and $(GT)_{15}$ probes to improve fragment selection. Among these clones 55% gave a satisfactory positive signal. A total of 192 positive clones were sent for sequencing (Genome Express). Sequences were analyzed and primers were designed using the PRIMER 3 program (Rozen and Skaletsky, 2000).

2.3. Genotyping

Nineteen loci were preliminary tested on DNA extracts from individual ticks obtained using the QIAamp DNA Mini Kit (Qiagen). PCR amplifications were performed following a M13 protocol where each forward primer is 5′-tagged with the M13-tail sequence (5′- CACGAC GTTGTAAAACGAC-3′) and a 5′-dye (IR700 or IR800) labeled M13 is added to the reaction mix.

PCR amplifications were run in a final volume of 20 μl containing 10-to-50 ng of genomic DNA, PCR buffer (Qiagen), 60 μM dNTPs (Invitrogen), 1.5–3.5 mM $MgCl_2$, 0.2 μM of primer, 0.16 μM of M13 tailed primer, 0.2 μM M13 dye and 0.5 U Taq DNA polymerase (Qiagen). PCR amplifications were performed using a "touch down" procedure defined as followed: a denaturing step at 95 °C for 5 min; 10 cycles including a denaturing step at 95 °C for 1 min, a hybridization step at (Ta + 7) °C for 1 min with Ta decreasing by 0.7 °C at each cycle, and an elongation step at 72 °C for 1 min; 25 cycles including a denaturing step at 95 °C for 1 min, a hybridization step at Ta for 1 min, and elongation at 72 °C for 1 min; the final elongation step was operated for 5 min at 72 °C. An automated infrared fluorescence technology (4300; LI-COR Biosciences, www.licor.com) was used to detect each PCR product sample.

2.4. Data analyses

The possible occurrence of genotyping errors due to the presence of null alleles, stutter bands and/or large-alleles dropout was assessed with MICROCHECKER 2.2.3 (van Oosterhout et al, 2004). Genotypic data were analyzed using GENEPOP 4.2 (Raymond and Rousset, 1995; Rousset, 2008) and FSTAT 2.9.4 (Goudet, 1995). Estimates of F_{IS} were computed according to Weir and Cockerham (1984). Deviations from Hardy–Weinberg equilibrium (HWE) were tested using F_{IS} statistic and performing 10 000 within-samples permutations of alleles among individuals. Assuming null alleles as the primary cause of heterozygote deficits, we estimated their frequency per sample using Brookfield's (1996) method. We then tested, by an exact binomial test, the goodness of fit of the observed frequencies of blank genotypes (putative null homozygotes) to those expected under the hypothesis of panmixia (unilateral tests). Genotypic equilibrium among pairs of loci was tested over all samples with the randomization log-likelihood G-based test that is implemented in FSTAT software, and by performing 10 000 within samples permutations of bi-loci genotypes. This generates a distribution in P-values that was compared to an exact binomial test undertaken with R 2.15.2 (R Development Core Team, 2018) in order to detect cases where the multiplicity of tests resulted in a false rejection of the null hypothesis tested (de Meeûs et al., 2009).

We tested whether the genotypic composition of tick samples clustered accordingly to the sampling geography and/or sampling dates using the principal component analyses adapted to genotypic data that is implemented in PCA-GEN v.1.2 (http://www2.unil.ch/popgen/softwares/pcagen.htm). Permutation tests included in the program enabled testing the significance of individual axes inertia (10 000 genotype randomizations).

Estimation of the effective population sizes (N_e) was performed using the two methods implemented in NEESTIMATOR v1.3 software

Table 1

Characterization of eight microsatellite markers in *Amblyomma variegatum*: description of marker (name, GenBank access to reference clone, primers' sequence, structure of repeated array), thermocycling conditions (annealing temperature, Ta; $MgCl_2$ concentration) and genetic variation (alleles number, Na; allele size range; allelic richness, A_R).

Locus name	GenBank Acession no.	Primers (5'- > 3')	Repeat motif	$MgCl_2$ (mM)	Ta (°C)	Na	Size range (pb)	A_R
AvE04	KR534760	F: AGACGGGCCATACCTAGA R: GATTTCTGTTGCCACTGC	$(CA)_6$	3.5	55	8	102-113	3.53
AvCl6	KR534759	F: GAGGGGCAACCTGTAAAC R: TGCACTCTAACGAACTGG	$(TAAC)_3$	3.5	55	5	196-211	2.40
AvG02	KR534761	F: ACTTCGCAGCAAAACTCT R: ATGCTTTCGCATTAAACC	$(TG)_7$	2.7	57	6	205-214	3.64
AvA05	KR534755	F: TCCCTACTGCTCATTGGA R: AAGCGAAACCAGGTAAGG	$(TG)_7$	2.7	56	5	205-211	2.09
AvA02	KR534754	F: ATAGGGATTTGCCCTCAC R: CTTGGGTAGCACAACCTG	$(CA)_6(AATA)_3$	3.5	55	8	209-230	3.78
AvB03	KR534756	F: TACGTGTCCCCTGTGCAT R: CCCATTCCTGTCCAGTTG	$(AC)_7$	1.5	56	24	148-178	11.67
AvC01	KR534757	F: TTCCAGGGTGCTACATGA R: CTTCACGGCGCTAGTAAT	$(TG)_7(CGTG)_4$	3.5	59	4	257-269	2.22
AvC08	KR534758	F: GGTGTCACGGTTTTGGTA R: GCGATACATGCTCAATGC	$(CA)_7$	2.7	59	14	234-251	8.45

A_R is based on the minimum sample size of 12 diploid individuals.

(Peel et al., 2004), both insensitive to heterozygosity. The first is a temporal method based on sequential sampling of different populations (Waples, 1989) while the second is based on linkage disequilibrium (Bartley et al., 1992). The temporal method was run considering the tick samples collected in November 2007 and their parental generation sampled either in May or July. Complementarily, we also applied Balloux (2004)'s method, a method based on heterozygote excess; it is worthy to note that this last estimate Ne is sensitive to deviations from HWE since it is directly driven from the Weir and Cockerham (1984)'s F_{IS} estimator so that $Ne = 1/(-2F_{IS}) + 1/2(1-F_{IS})$.

3. Results

Eight out of 19 developed markers were retained since they provided reproducible amplification results that were congruent with the expectations under the hypothesis of absence of duplication of the targeted loci. They displayed from 4 to 24 alleles each (Table 1) without any effect of large-alleles dropout. Possible occurrences of stutter bands were nonetheless detected at AvA02 and AvC08 markers.

Only one pair (AvCl6; AvE04) out of the 28 possible pairs of markers showed linkage disequilibrium- i.e., non-random association of genotypes significant at 5% risk (P = 0.008). This was not an observation significantly different from that expected by chance under the null hypothesis of statistical independence of the genotypic composition across eight markers (Exact binomial test defined by k' success = 1, k = 28 attempts and mean α = 0.05, unilateral test, P-value = 0.77). Therefore, the eight markers can be reasonably assumed to provide independent information on tick population structure.

No deviation from Hardy Weinberg expectations (HWE) was detected at four markers (AvE04, AvG02, AvA05 and AvC01, Table 2). Significant heterozygote deficits were detected at the other four (AvA02, AvB03, AvCl6 and AvC08; P < 0.05, Table 2). The AvB03 marker appeared to be X-linked since restricting data to the males resulted in $F_{IS} \approx 1$ while the F_{IS} average drawn from females was $F_{IS} = 0.65$ (range across samples: 0.5 to 1). Therefore, in male ticks, the genotype observed at AvB03 marker was coded either as homozygous when computing statistics insensitive to the correlation between alleles within individuals (i.e., effective population sizes) or as missing data otherwise (i.e., F-statistics). Assuming the presence of null alleles was enough to explain the observed heterozygote deficits at three markers (AvA02: P = 0.13; AvB03: P = 0.15; AvCl6: P = 0.12) but not at AvC08 (P = 0.004).

The principle component analysis of multi-locus genotypes supported the existence of two distinct clusters (Fig. 1). The first axis captured a significant part (32.7%, P = 0.038) of the total genetic variance; this axis discriminated the samples from the parental generation collected in May and July (on the right in Fig. 1) from those of the offspring generation (collected in November; on the left in Fig. 1).

The estimates in effective population sizes computed from the examination of two successive generations at the eight microsatellite markers ranged from 11 to 23 reproducing ticks per village and generation (see 'temporal method' in Table 3). The 95% confidence intervals (95% CI) of such estimates remained bounded by the extreme of six and 127 reproducing ticks per village (respectively observed at Lamba and Sara; Table 3). Congruent estimates in effective population sizes were provided by the linkage disequilibrium method. For seven of the nine samples, the average estimate in effective population size ranged from 2 to 17 reproducing adults per generation and village and the associated 95% CI remained bounded by finite estimates ($2 \leq minimum \leq 11$ and $4 \leq maximum \leq 29$; Table 3). Two samples were different. The samples collected either in May at Lamba or in July at Sara provided average estimates in effective population sizes about 70 reproducing ticks per village and generation (without a finite upper bound for the 95% CI; Table 3). We tested the sensitivity of such estimates to the set of markers used by re-estimating the effective population sizes with the linkage disequilibrium method after having removed one pair of markers (8 tests performed with the following pair of markers successively removed: AvA02/AvC08, AvB03/AvC08, AvA05/AvG02, AvA02/AvCl6, AvC01/AvE04, AvA02/AvB03, AvC08/AvCl6, AvB03/AvCl6). No changes in the magnitude order of the estimates in effective population sizes was ever noticed (Table 3). Complementarily, the hypothesis of tick effective populations sizes below 20 reproducing adults per village was also supported by Balloux (2004)'s method: the estimates driven on the parental generation collected in either May or July were Ne = 5.4 and 13.4 in Bekuy, 3.0 and 16.9 in Lamba, and 3.5 and 3.6 in Sara; those driven on the offspring generation were Ne = 3.2, 7.3 and 7.4 in Bekuy, Lamba and Sara, respectively.

4. Discussion

Despite a relative abundance of microsatellite markers in *A. variegatum*, the main difficulty encountered was to identify loci that could be amplified specifically (i.e. without any suspicion of locus duplication) and unambiguously genotyped. Eight polymorphic microsatellite markers were characterized in the tropical bont tick *A. variegatum*. Half of them displayed, within cohorts and villages, polymorphism patterns

Table 2
Testing Hardy-Weinberg expectations. Are detailed for each marker: the number of ticks with non-blank genotypes (N) out of all tick samples genotyped (N_{all}), the observed and expected heterozygosity (H_o and H_e, respectively), F_{is} estimates (per sample and averaged across the nine samples) and the P-values of the exact probability test of Hardy-Weinberg equilibrium. Significant P-values appear in bold; they are underlined when remaining significant after sequential Bonferronni correction (Rice, 1989).

Marker:		May			July			November			all samples
		Bekuy	Lamba	Sara	Bekuy	Lamba	Sara	Bekuy	Lamba	Sara	
AvE04	N	24	23	25	23	19	23	19	25	19	
	H_o	0.25	0.39	0.20	0.48	0.37	0.30	0.32	0.32	0.26	
	H_e	0.30	0.34	0.19	0.50	0.33	0.35	0.29	0.22	0.33	
	F_{is}	0.166	−0.172	−0.053	0.042	−0.120	0.123	−0.108	−0.116	0.214	0.008
	P	0.3198	0.3864	0.8042	0.7160	0.5150	0.3972	0.6238	0.7180	0.1909	0.48
AvCl6	N	24	25	24	25	18	23	22	22	21	
	H_o	0.25	0	0.125	0.04	0.06	0.04	0.05	0	0	
	H_e	0.30	0.08	0.20	0.12	0.26	0.04	0.13	0.17	0.33	
	F_{is}	NA	1	0.37	0.657	0.79	0	0.656	1	1	0.761
	P	NA	**0.0218**	0.0680	0.0631	**0.0004**	1.0000	0.0676	**0.0006**	**0.0001**	**0.0001**
AvG02	N	22	24	25	22	18	19	16	24	23	
	H_o	0.59	0.71	0.72	0.45	0.56	0.47	0.56	0.08	0.22	
	H_e	0.48	0.54	0.53	0.45	0.46	0.40	0.46	0.08	0.20	
	F_{is}	−0.238	−0.325	−0.378	−0.01	−0.219	−0.178	−0.222	−0.022	−0.068	−0.222
	P	0.076	**0.0095**	**0.0072**	0.9830	0.1776	0.2946	0.2030	0.9784	0.7844	1
AvA05	N	24	25	25	21	16	20	20	25	23	
	H_o	0.08	0.16	0.12	0.19	0.25	0.25	0.05	0.04	0.04	
	H_e	0.08	0.15	0.12	0.18	0.33	0.22	0.05	0.04	0.04	
	F_{is}	−0.022	−0.049	−0.043	−0.081	0.25	−0.118	0	0	0	0.004
	P	0.9814	0.8764	0.9380	0.8560	0.2558	1	1	1	1	0.58
AvA02	N	24	24	23	24	19	23	19	22	23	
	H_o	0.21	0.29	0.35	0.08	0.26	0.17	0.26	0.41	0.57	
	H_e	0.57	0.30	0.57	0.47	0.57	0.08	0.69	0.58	0.65	
	F_{is}	0.637	0.036	0.396	0.827	0.545	0.669	0.625	0.295	0.133	0.473
	P	**0.0002**	0.7058	**0.0114**	**0.0001**	**0.0007**	**0.0001**	**0.0001**	0.0902	0.3904	**0.0001**
AvB03	N	16	17	18	18	14	12	13	17	18	
	H_o	0.13	0	0.11	0.22	0.21	0.42	0.15	0.18	0.22	
	H_e	0.92	0.86	0.89	0.89	0.91	0.90	0.77	0.87	0.92	
	F_{is}	0.867	1	0.879	0.756	0.771	0.547	0.821	0.801	0.764	0.808
	P	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
AvC01	N	22	15	22	25	19	23	20	24	24	
	H_o	0.14	0	0.27	0.08	0.21	0.09	0	0.16	0.04	
	H_e	0.13	0	0.24	0.08	0.36	0.16	0	0.15	0.04	
	F_{is}	−0.024	NA	−0.135	−0.021	0.426	0.47	NA	−0.07	0	0.142
	P	0.9275	NA	1	0.9785	**0.0421**	0.1372	NA	0.8699	1	0.071
AvC08	N	24	25	25	20	16	17	20	25	22	
	H_o	0.33	0.44	0.36	0.30	0.38	0.35	0.10	0.36	0.41	
	H_e	0.80	0.88	0.87	0.89	0.86	0.63	0.85	0.84	0.89	
	F_{is}	0.587	0.507	0.593	0.67	0.57	0.458	0.886	0.577	0.545	0.598
	P	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
All loci	N_{tot}	24	25	25	25	19	25	24	25	24	
	Fis	0.479	0.375	0.38	0.489	0.446	0.365	0.557	0.506	0.49	
	P	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	

congruent with Hardy-Weinberg expectations (HWE). A fifth marker was sex-linked while three others displayed heterozygote deficits relatively to HWE that looked mainly caused by the presence of null alleles. The presence of null alleles at microsatellite markers is common in Ixodidae (De Meeûs et al., 2004; Koffi et al., 2006b; Noel et al., 2012; Van Houtte et al., 2013).

Relatively to the parental generation, the offspring generation collected displays a reduced genotypic diversity with corresponding samples apparently clustering together in the principle component analysis (PCA). Given the short distances between the villages involved in study (~10 km), it is possible that domestic hosts from different villages shared some grazing areas at a time when tick larvae searched hosts to attach on. This would promote *A. variegatum* dispersal across villages, reducing thus the among-villages differences in tick genotypic compositions. As signals of host-associated genetic differentiation were reported in other tick-species (De Meeûs et al., 2010; Kempf et al., 2011; McCoy et al., 2005), one may wonder whether or not this could be

involved in the apparent contrast along the PCA first axis between the genotypic composition of *A. variegatum* nymphs (collected on sheep and goats) and that of adults (collected on cattle). This hypothesis can easily be ruled out. For a signal of host-associated genetic divergence to emerge and persist, differences in host-preferences among adults and assortative mating with respect to the host-preferences are minimal requirements which are not fulfilled in *A. variegatum*. Indeed, *A. variegatum* adults usually avoid blood-feeding on sheep and goats. This avoidance is even more pronounced around Bobo-Dioulasso, Burkina Faso, where the local sheep race (Djallonké race) can host hundreds of *A. variegatum* nymphs without being infested by a single *A. variegatum* adult (Petney et al., 1987). Therefore, the most likely explanation to the contrast in genotypic composition defining the PCA first axis relies on the occurrence of a strong impact of genetic drift from parental to offspring generations (i.e., small effective population sizes). This conclusion was strongly supported by the congruency in the estimates of effective population sizes for *A. variegatum* driven by three estimation
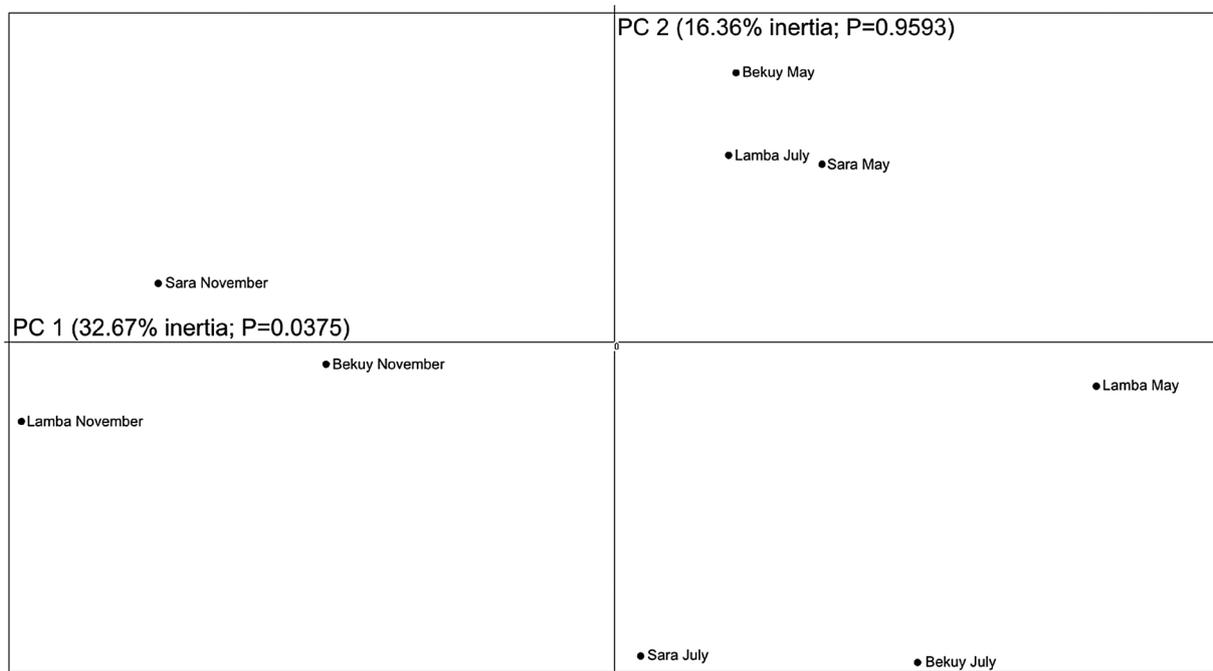
Fig. 1. Principal component analysis (PCA) of multilocus genotypic composition. The first axis explains 32.67% of the total variation (p = 0.038).

methods: in all cases, the estimates driven ranged from a few units to a few tens reproducing adults per village and generation.

The Ne estimates presently driven for *A. variegatum* in Burkina Faso ($2 \leq Ne \leq 90$ per village) are much lower than those driven for the *R. microplus* ticks feeding on cattle in West Africa (Ne ~150 per cattle herd; Biguezoton, 2016) or in the Pacific island of New Caledonia (Ne ~1000 per cattle herd; Koffi et al., 2006a; De Meeûs et al., 2010). Let us investigate how the biology of *A. variegatum* can explain such a low estimate in effective population sizes. Could mating patterns provide an explanation? Indeed, one may *a priori* expect uneven reproduction success among *A. variegatum* males despite the field observation of a 1:1 sex-ratio among adults (Stachurski, 2000b). *A. variegatum* females do not attach on host until attached males have started to produce attachment pheromone, so that mating clusters could form around the

most attractive males (Norval and Rechav, 1979). Moreover, as 50% males remain attached for at least 80 days (Barré, 1989), some males could easily mate with many more females than others (Norval et al., 1992). However, the absence of deviations from HWE within *A. variegatum* populations observed at four statistically independent markers does not support the hypothesis of a skewed distribution in male reproductive success. Low effective population sizes are not either due to low fertility since *A. variegatum* females lay up to 20 000 eggs (Barré, 1989; Nuttall, 1915). From then, the only explanation for these small effective population sizes has to be sought on weak survival rates somewhere along the *A. variegatum* life cycle. Such a life-cycle starts with mated and fully-engorged *A. variegatum* females dropping off their host to lay eggs on the ground. After egg-hatching, larvae wait for hosts to attach on by clustering in tight clumps at the tips of grass stems, so

Table 3

Effective population sizes estimates. Estimation of effective population sizes with the linkage disequilibrium method and the temporal based method as described in the text. 95% confidence intervals are presented in brackets. For each method, minimum and maximum values of Ne obtained on the 8 tests performed by removing 2 markers are presented.

Location	Collection period	8 markers	AvB03 and AvCl6 removed	AvA02 and AvC08 removed	Ne_{min}	Ne_{max}
Linkage disequilibrium method						
Bekuy	May	14 [11 ; 26]	67 [25 ; ∝]	6 [4 ; 9]	6	78
	July	8 [6 ;10]	7 [5 ; 10]	5 [4 ; 7]	4	15
	November	4 [3 ; 5]	2 [2 ; 3]	5 [3 ; 10]	1	18
Lamba	May	78 [21 ; ∝]	280 [33 ; ∝]	102 [18 ; ∝]	26	322
	July	17 [11 ; 29]	24 [12 ; 110]	13 [8 ; 25]	11	24
	November	2 [2 ; 3]	22 [11 ; 88]	1 [1 ; 1]	1	22
Sara	May	4 [3 ; 4]	14 [9 ; 24]	2 [1 ; 2]	2	33
	July	67 [23 ; ∝]	8 [5 ; 13]	∝ [53 ; ∝]	2	67
	November	8 [6 ; 10]	6 [4 ; 8]	5 [4 ; 7]	4	13
Temporal based method						
Bekuy	May-November	11 [5 ; 32]	16 [5 ; 177]	12 [4 ; 60]	9	22
	July-November	8 [4 ; 19]	11 [4 ; 51]	7 [3 ; 18]	7	11
	Average	14 [7 ; 35]	19 [7 ; 118]	13 [5 ; 42]	12	21
Lamba	May-November	7 [3 ; 14]	8 [3 ; 23]	5 [2 ; 11]	4	12
	July-November	13 [5 ; 52]	16 [5 ; 830]	9 [3 ; 31]	8	19
	Average	11 [6 ; 25]	13 [5 ; 39]	9 [4 ;21]	8	18
Sara	May-November	20 [8 ; 189]	18 [5 ; ∝]	16 [6 ; 137]	9	29
	July-November	14 [6 ; 64]	13 [4 ; 93]	15 [5 ; 147]	8	21
	Average	23 [9 ; 127]	17 [6 ; 92]	20 [7 ; 133]	9	39

that many sibs could attach on the same individual-host. From then, the heterogeneous drop-off rhythm of ticks at nymphal and adult stages (Stachurski and Adakal, 2010) coupled with hosts movements ensure two successive rounds of sib re-distributions among the individual-hosts grazing on the same pastures i.e., among infrapopulations (Bush et al., 1997). According to this cycle, low survival rates of any stage present on vegetation (eggs, larvae, nymphs, adults) look as the most parsimonious explanation that will efficiently result in low effective population sizes. In addition, it is noteworthy that extinctions of some *A. variegatum* infrapopulations would also reduce the effective population sizes within communal herds (Criscione and Blouin, 2005), and so, without promoting deviations to HWE given the two rounds of ticks re-distributions among individual-hosts occurring at both nymphal and adult stages.

What is the information provided by such low estimates in effective population sizes in *A. variegatum* regarding the sustainability of tick-control programs in Burkina Faso? The first information concerns the probability of apparition by mutation of a new allele conferring resistance to acaricides. The lowest the effective population size, the lowest is such a probability since the polymorphism resulting from mutation/drift balance is proportional to $Ne.\mu$ with μ the mutation rate (e.g. Hartl and Clark, 1997; Hedrick, 2000). The second information relies to the probability that a new allele conferring acaricide resistance may disappear from the tick population before having a chance to be selected for by tick-control programs. The lowest the effective population sizes, the highest the risk that the effect of genetic drift overcomes that of the positive selection resulting from acaricides use (Hartl and Clark, 1997; Hedrick, 2000). Today, the selection pressures caused by acaricide chemical remain rather low in Burkina Faso. Indeed, tick-control programs follow variable strategies (including manual removal and/or application of oil or dung on cattle) in the traditional and low input systems dominating the livestock production there, and, where occurring, chemical-based strategies are mainly achieved through empirical recycling of agricultural pesticides (Adakal et al., 2013). The low Ne estimates presently driven for the *A. variegatum* effective population sizes in Burkina Faso enhances thus the high probability for the genetic drift to actually overcome the effect of acaricide usages onto the fate of a newly arisen mutant conferring acaricide resistance.

Regarding the evolution of acaricide situation, the situation of *A. variegatum* in Burkina Faso (with Ne ranging from a few unities to a few tens and s being weak when non-null) dramatically contrasts with that of the southern cattle tick *R. microplus* in the Pacific island of New Caledonia (with $Ne \sim 1\,000$ reproducers per cattle herd and s intense everywhere along the Western Coast; Koffi et al., 2006a). In the later case, such a high Ne estimate coupled with low gene flow across cattle herds and homogeneously high selection pressures applied along the West Coast were interpreted as favoring an evolutionary scenario of acaricide resistance due to recurrent events of local positive selection of diverse newly-arisen mutants conferring acaricide resistance rather than an alternative scenario involving the migration among tick populations of a very few resistance mutants (Koffi et al., 2006a). The characterization of the diversity of physiological changes involved in acaricide resistance in these populations allowed testing and confirming this prediction (Chevillon et al., 2007, 2013). The same rationale would indicate a very low probability of both apparition and selection for acaricide resistance mutants in *A. variegatum,* at least in West Africa. Interestingly enough, the absence of reports of acaricide resistance in the *A. variegatum* populations from Guadeloupe or Puerto Rico despite intensive eradication campaigns (Garris and Barré, 1991) may provide some support to such a prediction, even if population genetics studies performed in these New World areas are still required to settle this point.

5. Conclusions

We characterized eight microsatellite markers from the tropical

bont tick *A. variegatum*. These markers allowed highlighting very low effective population sizes of the tick populations from Burkina-Faso; a result suggesting that the evolution of acaricide resistance in *A. variegatum* is expected to be much slower than observed in its invasive competitor, *R. microplus*.

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