

# Founder effects, inbreeding and effective sizes in the Southern cattle tick: the effect of transmission dynamics and implications for pest management

BROU BASILE KOFFI,\* THIERRY DE MEEÛS,\* NICOLAS BARRÉ,† PATRICK DURAND,\*  
CÉLINE ARNATHAU\* and CHRISTINE CHEVILLON\*

\*Génétique et Evolution des Maladies Infectieuses (G.E.M.I.), UMR CNRS-IRD 2724, Centre IRD, 911 avenue Agropolis, BP 64501, 34 394 Montpellier Cedex 5, France, †IAC/CIRAD UR 22, BP 73, 98890 Païta, Nouvelle Calédonie, France

## Abstract

Since its immigration in the Pacific island of New Caledonia in 1942 (i.e. about 240 tick-generations ago), the cattle tick *Boophilus microplus* has experienced a remarkable adaptive diversification there. In order to better understand the population factors involved, we have investigated the *B. microplus* population structure on that main host-species, *Bos taurus*. This study was based microsatellite loci and confirmed that the island colonization came along with a significant bottleneck. Knowledge on *B. microplus* biology led us to expect *B. microplus* populations to be composed of highly inbred lineages irregularly dispatched among the individual hosts belonging to the same herds. Instead, this study evidenced a weak inbreeding level and an absence of genetic differentiation within herds. Complementarily, a significant signal of isolation by distance exhibited that human-traffic of cattle does not promote high tick dispersal within the island. Finally, the tick density was found to be about a few hundreds of reproducing adults per squared kilometre, for a gene dispersal range of about a few hundred metres per tick generation. Results are discussed with regard to the evolution of new adaptive changes.

*Keywords:* *Boophilus microplus*, bottleneck, effective population size, inbreeding, isolation by distance, parasite

Received 9 April 2006; revision accepted 10 July 2006

Mating pattern, population subdivision and extinction are complementary determinants of the probability to select for newly arisen adaptive mutations. The effect of population subdivision has to be determined on a case-per-case basis, as it results from the interplay between migration and the potential variation in the sense of selection among subpopulations (e.g. Nagylaki 1975; Lenormand & Raymond 1998; Vacher *et al.* 2003). By contrast, extinction always decreases the fixation probability of advantageous mutations irrespectively of the re-colonization pattern (Cherry 2004). Inbreeding has a similar effect through a reduction in population effective size, i.e. in the genetic diversity available for recruiting newly advantageous mutations (Charlesworth 2003). Once adaptive mutations have been retained within populations, high inbreeding will speed up

their fixation. For parasites, the population effective sizes and the local fixation of adaptive mutations also strongly depend on the variation among individual hosts in the genetics of parasite recruitment, and especially on whether or not the offspring produced on a given host are transmitted in clump (Criscione & Blouin 2005). We investigated the complementary determinants of inbreeding, putative bottlenecks and extinction events, and transmission patterns among individual hosts for an invasive parasite with high adaptive diversification potentials, *Boophilus microplus*.

*Boophilus microplus*, also called *Rhipicephalus microplus* (Murrel & Barker 2003) and probably of Southeast Asian origin, has become a major pest in tropical and subtropical agrosystems (Frisch 1999). This is partly due to the recurrent introductions of *Bos taurus* European breeds in the tropical belt (Frisch 1999). Indeed, contrarily to the tropical host *B. indicus*, these introduced hosts are unable to mount efficient immune responses to *B. microplus* infestation (Frisch 1999).

Correspondence: Christine Chevillon, Fax +33 (0)467 41 62 99; E-mail: christine.chevillon@mpl.ird.fr

Tick-burdens on *B. taurus* are thus controlled through intensive pesticide programs. The situation is worsened by the capacity of *B. microplus* to quickly cope with new environments and new environmental heterogeneity. Invasive in many areas (McCoster 1979), *B. microplus* has recurrently succeeded to exploit non-Bovidae hosts (Barré *et al.* 2001; Labruna *et al.* 2001; Battsetseg *et al.* 2002) and to develop resistances to diverse pesticides (He *et al.* 1999; Miller *et al.* 1999; Aguirre *et al.* 2000; Hernandez *et al.* 2000; Jamroz *et al.* 2000; Bianchi *et al.* 2003; Foil *et al.* 2004; Li *et al.* 2004; Ducornez *et al.* 2005; and references therein). Moreover, *B. microplus* has quickly achieved adaptive diversification within isolated metapopulations, as in the Pacific island of New Caledonia. *Boophilus microplus* immigrated there in 1942 (Rageau 1966), a few years before drastic quarantine started to protect the island from further tick immigration. In 200–250 generations, the cattle tick has colonized all *B. taurus* herds, has developed resistance to any pesticide used in tick-controls (Bianchi *et al.* 2003; Ducornez *et al.* 2005), and has even acquired the capacity to exploit a new and invasive host (*Cervus timorensis russa*, Barré *et al.* 2001).

Interestingly, these adaptive diversifications took place in presence of several factors expected to reduce the genetic diversity within tick populations. Indeed, intensive pesticide-controls are aimed at promoting extinctions or at least large shrinks in population sizes. Complementarily, the colonization of a new area is likely to come along with an original bottleneck. Furthermore, the life cycle of that dioecious parasite is expected to result in high inbreeding. If eggs and young larvae are free stages, juveniles and adults are thought to spend their entire lifespan on the same individual host. Fully engorged females drop on the soil and die when laying eggs, so that large tick brotherhoods ( $N \approx 1.800$ , Barré *et al.* 2001) are present as clusters in the environment. This characteristic of brotherhood clusters is likely to last along subsequent life stages, as development looks synchronous within brotherhoods, and as neighbour larvae regroup themselves to seek a host to develop and mate. In addition, mate guarding apparently prevent females to mate more than once. Therefore, mating between relatives seems difficult to avoid, unless mechanisms such as sex-biased dispersal (Prugnolle *et al.* 2003) and/or adjustment in the choice among related or nonrelated mates (Trouvé *et al.* 1999; Lücher & Millinski 2003) have evolved in that species. Alternatively, clump transmission of offspring may counterbalance the inbreeding effect if it induces high genetic differentiation among nearby infrapopulations (i.e. the within-individual-hosts collections of ticks, see Criscione & Blouin 2005). To the best of our knowledge, no data were available to evaluate these alternative possibilities.

We thus undertook the first analysis of *B. microplus* population structure using microsatellite polymorphism and the field situation of New Caledonia. *Boophilus microplus* does not vector any micropathogen there. This is an advant-

age for a first case study, as vector–pathogen interactions can affect the migration/drift balance of the tick vector (e.g. de Meeûs *et al.* 2004b). Focusing on one adult cohort, we evaluated inbreeding and addressed the determination of mating pattern. This latter investigation was performed by testing the possibilities of sex-biased dispersal and of genetic differentiation among individual hosts within herds. We also searched for possible bottlenecks' signature in this recently founded metapopulation, and for the possible constraints on tick density and gene dispersal range.

## Materials and methods

### *Boophilus microplus* ecology in New Caledonia

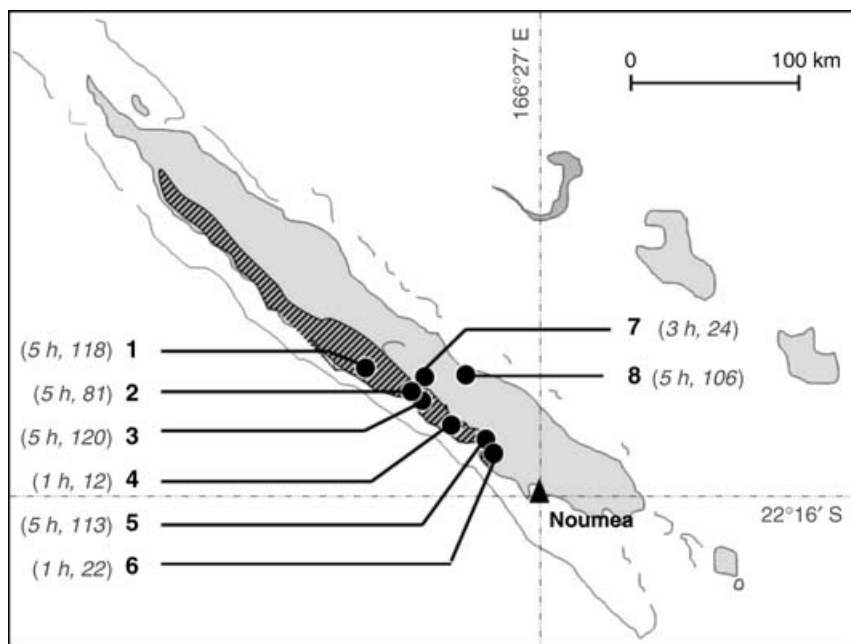
Predominant hosts are European *Bos taurus* breeds (Charolais, Limousin and their intercrosses) that are highly susceptible to *Boophilus microplus* infestations. For a main part (60%), cattle are concentrated in a western plane where herds are continuously distributed, and where an average herd regroups 80 steers per km<sup>2</sup> and forages on about 2.5 km<sup>2</sup>. In that environment, *B. microplus* faces weak competition from other ticks (*Haemaphysalis longicornis*), but intense intraspecific competition within and among cohorts (Barré *et al.* 2001).

### Sampling

An adult tick cohort ( $N = 596$ ) was collected between 8 April and 5 May 2003. Ticks were collected onto 30 individual hosts belonging to eight herds. The sampling geography follows two traffic axes with different cattle distribution (Fig. 1). Six herds (n°1–6) are dispatched within the midwestern plane where cattle are continuously distributed. Two herds (n°7 and n°8) are located in a transversal valley where cattle are more patchily distributed. Sex ratio was balanced within infrapopulation samples, with 'infrapopulation' referring to the parasites harboured by one individual host. Sampling sizes per infrapopulations were  $20 \leq N \leq 24$  except in 10 cases. Exceptions concerned four infrapopulations from herd n°2 ( $N = 12; 15; 15; 17$ ), the infrapopulation from herd n°4 ( $N = 12$ ), one infrapopulation from herd n°5 ( $N = 15$ ), the infrapopulations from herd n°7 ( $N = 12; 8; 4$ ), and one infrapopulation from herd n°8 ( $N = 12$ ).

### Genotyping

Alcohol-conserved ticks were sexed and washed in water for 20 min. Female bloodmeal content was removed to avoid blood polymerase chain reaction (PCR)-inhibitory effects (Wilson 1997). DNA extraction was performed with DNeasy Tissue Kit (QIAGEN). Genotyping was processed as described in Koffi *et al.* (2006), i.e. at microsatellite loci with GenBank Accession nos DQ001903–DQ001904 and



**Fig. 1** Sampling geography. Nouméa is a capital of New Caledonia. Bovine cattle and cattle ticks are omnipresent in the main island, except south of Nouméa and in central mountains. They are mainly concentrated in midwestern plane (stripped area). The location of each herd is indicated with, in parentheses, the associated number of sampled hosts (h) and the total number of collected ticks.

DQ001906–DQ001911. PCRs were performed in 25  $\mu$ L with 10 pmol of each primer, 20 pmol of dNTPs (Invitrogen), 2.5  $\mu$ L  $MgCl_2$  (25 mM, Promega), 2.5 U *Taq* polymerase (Promega), and 2–5  $\mu$ L of DNA extracts. Thermocycling consisted in a denaturation step (94  $^{\circ}C$  for 3 min) and 28 to 30 cycles (30 s at 94  $^{\circ}C$ ; 30 s at annealing temperature; 30 s at 72  $^{\circ}C$ ). For a given DNA template, automatic electrophoresis on ABI PRISM 310 sequencer was performed by pooling 0.5  $\mu$ L of size standard (GeneScan-500 LIZ, Applied), Hi-Di formamide (20  $\mu$ L QSP), and 1  $\mu$ L of the PCR product of each multiplexed locus. Multiplex M1 regrouped the loci BmA12, BmC04 and BmC07; multiplex M2 regrouped the loci BmD12, BmB12 and BmC03; and multiplex M3 regrouped the loci BmA05 and BmD10. Allele lengths were measured with GENESCAN (Applied Biosystems).

#### Evaluation of genotyping errors

Genotyping was replicated through independent PCRs and electrophoresis runs for a subset of templates. Within infrapopulations, the genotype frequency distribution was analysed with regard to within-genotypes differences in allele sizes using MICRO-CHECKER software (van Oosterhout *et al.* 2004). This allows detecting stuttering errors and/or possible cases of large allele dropout (i.e. short allele dominance, van Oosterhout *et al.* 2004). These sources of genotyping errors would induce an artificial deficit in the heterozygous classes defined by allele-size difference of either one or large numbers of base pairs, respectively (van Oosterhout *et al.* 2004). Short allele dominance also changes allele frequency estimates (de Meeùs *et al.* 2004a).

#### F-statistics

Ignoring the nested sampling design, *F*-statistics analyses among infrapopulations were performed with *FSTAT* software version 2.9.3.2 (Goudet 1995). Estimates of  $F_{IS}$  and  $F_{ST}$  were computed according to Weir & Cockerham (1984). For per-locus estimates, means and standard errors were computed by jackknifing over populations. Global  $F_{IS}$  and  $F_{ST}$  estimates were computed by only considering the loci that had separately brought congruent results. Means and standard errors were computed by jackknifing over loci and confidence intervals by bootstrapping over loci. 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 50 000 within-samples permutations of bi-loci genotypes. Deviations from Hardy–Weinberg equilibrium were tested with  $F_{IS}$  statistic and by performing 50 000 within-samples permutations of alleles among individuals. Population differentiation was tested by using the log-likelihood ratio G described in Goudet *et al.* (1996), and by performing 50 000 among-samples permutations of individual genotypes. For clarity's sake,  $F_{IS}$  parameters are indexed by 'infrapopulations' or 'herds' when evaluated at infrapopulation or herd scales, respectively.

The relative importance of herds and infrapopulations in differentiation was assessed with the multilocus hierarchical *F*-statistics  $F_{\text{cow-herd}}$  and  $F_{\text{herd-total}}$  respectively. This analysis was performed with *HIERFSTAT* package (Goudet 2005). These tests were based on 10 000 permutations of either tick genotypes among infrapopulations and within herds ( $H_0: 'F_{\text{cow-herd}} = 0'$ ), or infrapopulations among herds ( $H_0: 'F_{\text{herd-total}} = 0'$ ).

### Sex-biased dispersal

The two smallest infrapopulations were removed to investigate the possibility of sex-biased dispersal with  $F_{ST}$  (Goudet 1995). We used the parameters most sensitive to sex-biased dispersal (Goudet *et al.* 2002):  $F_{ST}$  and the mean mAlc of the assignment index described in Favre *et al.* (1997). The sex that disperses the most is expected to be associated with lower mAlc estimate (because immigrants have lower Alc values than residents), and lower  $F_{ST}$  estimates (because that sex regroups within a given sample both residents and immigrants of various origins, while the other sex only regroups residents). Using bilateral testing and 1000 permutations, statistical analyses were performed as in Goudet *et al.* (2002). For parameter  $X$ , the within-sex estimates  $X_r$  and  $X_d$  were computed, with  $r$  and  $d$  subscripts referring to residents and migrants, respectively. Between-sexes differences were then computed as  $(X_r - X_d)$  for  $X = F_{ST}$ , and as a  $t$ -statistics for  $X = \text{mAlc}$  (Goudet *et al.* 2002). Finally, within- and between-sexes estimates were re-computed after permuting the sex-assignment of individuals. For mAlc, the  $P$  value associated with absence of sex-biased dispersal is that of the  $t$ -statistic used for the randomization test. For  $F_{ST}$ , the  $P$  value associated with absence of sex-biased dispersal is obtained by comparing the observed between-sexes difference to the distribution obtained by permutations.

### Mutation/drift balance

This analysis was performed with BOTTLENECK software (Piry *et al.* 1999). Given  $H_E$  the expected heterozygosity of a population, let  $H_{EQ}$  be the heterozygosity that would be expected for a population at mutation/drift equilibrium with the same sampling size and allele number. As allele number decreases faster than heterozygosity when populations shrink in size, bottlenecks are signed by  $H_E > H_{EQ}$  in subsequent generations (Cornuet & Luikart 1996). As most of the assessed microsatellite loci harboured interrupted repetitions,  $H_{EQ}$  was determined by assuming that mutation pattern followed an infinite allele model (Cornuet & Luikart 1996; Piry *et al.* 1999). Deviation from mutation/drift equilibrium ( $H_0$  vs. ' $H_E > H_{EQ}$ ') was tested by a sign test (Test 1 in Cornuet & Luikart 1996).

### Isolation by distance

This analysis was performed with GENEPOP software version 3.4 (Raymond & Rousset 1995). Regression analyses between the ratio  $[F_{ST}/(1 - F_{ST})]$  and the logarithm of genetic distance were performed by ignoring the pairs of infrapopulations collected in the same herd. The geographical distances that separated infrapopulations of different herd origin were evaluated as between-farms distances. Testing whether

the regression slope  $b$  was null or strictly positive was performed with Mantel tests based on 10 000 permutations.

Given  $\sigma$  be the average distance separating parents' and offspring birthplaces, and  $D$  the local density in reproducing ticks, the parameters  $b$ ,  $\sigma$  and  $D$  are linked by  $b = [4\pi D\sigma^2]^{-1}$  (Rousset 1997). Now, the signal of isolation by distance (hence the value taken by  $D \cdot \sigma^2$ ) responds quickly to demographic variations (Leblois *et al.* 2004). We wondered hence whether this signal varies with the connectivity among pastures foraged by distinct herds, and how the local density in reproducing ticks connects with local tick burdens. We thus investigated the variation in  $D \cdot \sigma^2$  among data sets corresponding to different environments. Data set-1 considered the entire sampling. Herd n°8 was excluded from data set n°2 to investigate the influence of the low cattle density encountered in the transversal valley. Data set-3 was restricted to four nearby herds from midwestern plane where the connectivity among pastures foraged by distinct herds is maximal (herds n°1-4, separated from one another by either 6 km, 10-11 km, or 29-40 km, Fig. 1).

## Results

### Potential genotyping errors

Independent replicates led to consistency in genotyping results per DNA template. Six cases of three-allelic patterns were recorded at locus BmC04, and were coded as missing data. Null genotypes were recorded at each of the eight loci. A mutation step of a single base pair was observed at loci BmA12, BmC04, and BmD10 within each of the 30 infrapopulations. Among the 90 combinations, the possibility of stuttering errors was only suspected once (for BmD10 in the fifth infrapopulation from herd n°3). Large allele differences (from 20 bp to 32 bp) were regularly observed at BmC07, BmD12 and BmC03 loci. However, no case of large allele dropout had ever been detected. Overall, the risk of strong bias resulting from genotyping errors looked limited.

### Congruency among loci

Each locus brought independent information, as no genotypic linkage had ever been detected ( $P > 0.1$  for all loci pairs).  $F$ -statistics analysis was performed for each locus among all 30 infrapopulations. This analysis was performed within sexes. The resulting per-locus  $F_{IS-infrapopulation}$  and  $F_{ST-infrapopulation}$  estimates were congruent among all but two loci (Table 1). At BmA05,  $F_{IS-infrapopulation}$  differed between sexes. Noting that males are heterogametic in *Boophilus microplus* (Crampton *et al.* 1998), this observation reveals that BmA05 is located on chromosome X. Only two of the 287 males genotyped were heterozygous indeed. As

**Table 1** Comparison among loci. The repeated arrays present in reference clones are recalled under locus names.  $N_{\text{ticks}}$  and  $N_{\text{alleles}}$  refer to sample sizes and allele numbers, respectively. The means and standard errors of  $F_{\text{IS}}$  and  $F_{\text{ST}}$  estimates were computed on the entire data set and within sexes. Significant results appear in bold characters

	BmA05 (CA) <sub>5+10</sub>	BmA12 (CA) <sub>3+7</sub> (CG) <sub>4</sub>	BmB12 (TA) <sub>4</sub> (TG) <sub>9</sub>	BmC03 (CA) <sub>10+9</sub>	BmC04 (TG) <sub>8</sub>	BmC07 (GT) <sub>17</sub>	BmD10 (GT) <sub>13</sub>	BmD12 (CA) <sub>10+5</sub>
<b>Overall</b>								
$N_{\text{ticks}}$	575	586	582	507	571	551	576	585
$N_{\text{alleles}}$	4	5	5	7	3	6	8	11
Mutations steps (bp)	2	1; 2; 4	2; 4	2; 4; 10	1	5; 6; 7; 12; 18	1; 2; 3	2; 4; 6
<b>Within infrapopulations</b>								
$N_{\text{ticks}}$	19.4 ± 5.7	19.5 ± 5.7	19.4 ± 5.7	19.9 ± 5.8	19.0 ± 5.8	18.4 ± 5.4	19.2 ± 5.7	19.5 ± 6.2
$F_{\text{IS}}$	<b>0.591 ± 0.039</b>	0.029 ± 0.032	0.039 ± 0.027	0.023 ± 0.024	0.039 ± 0.041	0.036 ± 0.026	<b>0.099 ± 0.035</b>	<b>0.075 ± 0.034</b>
<i>P</i> value	< 2 · 10 <sup>-5</sup>	0.13	0.08	0.15	0.10	0.07	0.00018	0.0010
$F_{\text{IS-female}}$	<b>0.182 ± 0.062</b>	0.034 ± 0.046	<b>0.068 ± 0.043</b>	0.038 ± 0.033	<b>-0.156 ± 0.055</b>	0.055 ± 0.038	0.063 ± 0.051	<b>0.090 ± 0.047</b>
$F_{\text{IS-male}}$	<b>0.982 ± 0.013</b>	0.015 ± 0.032	0.010 ± 0.028	0.010 ± 0.034	<b>0.224 ± 0.068</b>	0.015 ± 0.032	<b>0.107 ± 0.044</b>	<b>0.073 ± 0.042</b>
<b>Among infrapopulations</b>								
$F_{\text{ST}}$	<b>0.065 ± 0.023</b>	<b>0.008 ± 0.006</b>	<b>0.014 ± 0.007</b>	<b>0.013 ± 0.007</b>	<b>0.014 ± 0.009</b>	<b>0.014 ± 0.006</b>	<b>0.010 ± 0.005</b>	<b>0.022 ± 0.009</b>
<i>P</i> value	< 2 · 10 <sup>-5</sup>	0.034	0.0027	< 2 · 10 <sup>-5</sup>	0.023	2 · 10 <sup>-4</sup>	3 · 10 <sup>-4</sup>	< 2 · 10 <sup>-5</sup>
$F_{\text{ST-female}}$	<b>0.065 ± 0.031</b>	0 ± 0.007	0.010 ± 0.009	0.012 ± 0.011	0.035 ± 0.017	0.017 ± 0.011	0.028 ± 0.014	0.017 ± 0.011
$F_{\text{ST-male}}$	0.017 ± 0.029	0.011 ± 0.010	0.004 ± 0.011	0.010 ± 0.008	0.004 ± 0.011	0.005 ± 0.008	0.019 ± 0.012	0.017 ± 0.011

these two individuals may have been inaccurately sexed [or may have experienced duplicated loci, e.g. de Meeûs *et al.* (2004a)], they were removed from male data sets. Within the female sex, BmA05 still provided large  $F_{\text{IS-infrapopulation}}$  and  $F_{\text{ST-infrapopulation}}$  estimates relatively to other loci (Table 1). Sex difference in  $F_{\text{IS-infrapopulation}}$  discriminated BmC04 from all other loci (Table 1). Overall, loci BmA05 and BmC04 looked inaccurate for present investigations and were no longer considered. Meanwhile, congruency in  $F_{\text{IS-infrapopulation}}$  estimates among other loci (Table 1) indicated that null alleles were unlikely to be the main cause of heterozygote deficits.

*Population structure*

A low but significant heterozygote deficit was observed within infrapopulations ( $0.030 \leq F_{\text{IS-infrapopulation}} \leq 0.071$ ;  $P < 0.001$ ), and a significant differentiation was observed among the 30 infrapopulations ( $0.010 \leq F_{\text{ST-infrapopulation}} \leq 0.017$ ;  $P < 0.01$ ).

Considering the hierarchy of sampling, significant differentiation was detected between herds ( $F_{\text{herd-total}} = 0.014$ ;  $P < 0.01$ ) but not within herds ( $F_{\text{cow-herd}} = 0.001$ ;  $P > 0.1$ ). Ignoring the subdivision of herds into infrapopulations actually provided a new  $F_{\text{IS-herds}}$  confidence interval ( $0.028 \leq F_{\text{IS-herds}} \leq 0.074$ ) that was strictly overlapping that of  $F_{\text{IS-infrapopulation}}$ . Assuming brother-sister mating as the only cause involved in the positive  $F_{\text{IS-herds}}$  estimate, the frequency  $f_{\text{bs}}$  of brother-sister mating would be given by  $f_{\text{bs}} = 4 \cdot F_{\text{IS}} / (1 + 3 \cdot F_{\text{IS}})$  (Hartl & Clark 1989). Here,  $f_{\text{bs}}$  would thus range from 0.10 to 0.24.

*Sex-biased dispersal*

Sex-biased dispersal was detected neither in overall data set ( $P > 0.5$ ), nor within the herds represented by five infrapopulations ( $P > 0.10$  in all but two cases; the mAIC-test performed in herd n°2 and the  $F_{\text{ST}}$ -test performed in herd n°8 led to  $P = 0.04$  and  $P = 0.052$ , respectively). Moreover, the sex that tended to be the most dispersing one varied among herds (details not shown).

*Mutation/drift balance*

Clear bottleneck signatures were detected within and among herds (Table 2).

**Table 2** Mutation/drift balance within herds.  $N_{\text{ticks}}$  refers to the sample sizes. The per-herd *P* values correspond to the test 1 of Cornuet & Luikart (1996). The overall *P* value is the binomial probability to observed six significant *P* values among eight trials at a risk of 5% (e.g. Prugnolle *et al.* 2002a)

Herds ( $N_{\text{ticks}}$ )	Number of loci with		<i>P</i> value (bilateral testing)
	$H_E < H_{\text{EQ}}$	$H_E > H_{\text{EQ}}$	
n°1 (118)	0	6	0.036
n°2 (81)	0	6	0.036
n°3 (120)	0	6	0.036
n°4 (12)	1	5	0.20
n°5 (113)	0	6	0.036
n°6 (22)	0	6	0.036
n°7 (24)	1	5	0.20
n°8 (106)	0	6	0.036
Overall	—	—	0.0001

**Table 3** Covariation in tick density and gene dispersal per generation.  $D$  values were arbitrarily chosen to compute the corresponding  $\sigma$  estimates (see text for further explanations)

$D$ (number of reproducing adults per km <sup>2</sup> )	$\sigma$ (km)		
	data set-1	data set-2	data set-3
10	3.84	2.10	2.89
100	1.21	0.66	0.92
250	0.76	0.42	0.58
500	0.54	0.30	0.41
750	0.44	0.24	0.33
1000	0.38	0.21	0.29
2500	0.24	0.13	0.18
5000	0.17	0.094	0.13
7500	0.14	0.077	0.11
10 000	0.12	0.066	0.092
100 000	0.038	0.021	0.028

### Isolation by distance

A significant signal of isolation by distance was detected (data set-1,  $P = 0.00020$ ). Nevertheless, the increase in  $F_{ST}/(1 - F_{ST})$  with the logarithm of the geographical distance was weak (slope  $b_1 = 0.00054$ ). Removing the impact of the low host-density in transversal valley and/or restricting the sampling geography to the midwestern plane where cattle are continuously distributed did not alter this signal (data set-2 and -3;  $P < 0.001$ ;  $b_2 = 0.0014$  and  $b_3 = 0.00095$ ). This robustness is particularly evident when looking at ( $D$ ;  $\sigma$ ) covariation patterns (Table 3).

## Discussion

### Determination in mating and local genetic diversity

The relevance of the infrapopulation as a unit of parasite evolution has been a debated issue, yet too rarely tested (Criscione *et al.* 2005). The present study clearly indicated that it is not the relevant unit for *Boophilus microplus*, at least among highly susceptible and genetically similar hosts. In that case, mating pattern is determined at herd scale after a sex-independent re-distribution among individual hosts. This is enough for *B. microplus* to avoid high inbreeding. Interestingly, Criscione & Blouin (2006) reported inbreeding avoidance associated with well-mixed infrapopulations in a salmon parasite, with an apparent enhancement of such traits in aquatic relatively to terrestrial indirectly transmitted parasites. If data remain too scarce and incomplete for testing the role of environment on directly transmitted parasites, the present study and those performed on whale and salmon lice (Todd *et al.* 2000, 2004, 2005; Kaliszewska *et al.* 2005) represent an interesting start.

In *B. microplus*, the within-herd absence of genetic differentiation among infrapopulations has four main consequences. First, this result modifies our understanding of *B. microplus* transmission by indicating imperfect clump-transmission of the offspring-larvae seeking a host and/or among-hosts movements of the *B. microplus* parasitic life stages. Second, spatial Wahlund effect can be rejected as an explanation for the weak but significant heterozygote deficit observed. More investigation is required to decipher whether this deficit results from weak inbreeding or from temporal Wahlund effect (Vilas & Panaguia 2004). Taking advantage of *B. microplus* mate guarding behaviour to directly test pangamy (i.e. whether or not mating pairs are randomly formed with respect to genetic relatedness) looks as the most fruitful way to settle that point (e.g. Prugnolle *et al.* 2002b). Third, in absence of pesticide control, the migration of a few steers is likely to promote the dispersal of most (if not all) the within-herd *B. microplus* genetic diversity. Complementarily, the potential of pesticide-resistance for inducing severe bottlenecks would be greatly reduced if a few steers escape treatment, as most of the within-herd tick genetic diversity is harboured by any steer. In other words, the tick brotherhood's re-distribution among infrapopulations somehow protects newly arisen mutations from disappearing through drift.

### Bottleneck and postcolonization population size

Bottlenecks may be associated with the island colonization and/or current pesticide usage, but these two types of bottlenecks may differ in their probability to be detected by Cornuet & Luikart (1996)'s method. These authors showed that, with less than 10 loci and given  $N_e$  the local effective population size after bottleneck, their methods can only detect bottlenecks that had between  $\tau = (2N_e)/10$  and  $\tau = 2N_e$  generations ago indeed. Assuming four *B. microplus* generations per year and a bottleneck resulting from a tick immigration in 1942 (Rageau 1966), the effective size of the first naturalized populations would be  $120 \leq N_e \leq 1200$ . Alternatively, if the detected bottleneck resulted from current pesticide controls, the recovering populations would have much lower effective sizes (for instance  $1 \leq N_e \leq 10$  for  $\tau = 4$  generations  $\equiv$  1 year). Complementarily, given the weak inbreeding observed and the absence of within-herds genetic differentiation, the current tick populations are expected to display similar effective sizes and adult census sizes.

Isolation by distance offers an independent estimate of the present number of reproducing adults per squared kilometre ( $D$ ), providing a range distance of gene dispersal per generation ( $\sigma$ , Table 3). Meanwhile, independent biological data constraint  $D$  upper-bound independently from  $\sigma$ . First, maximal adult tick burden in a standard western farm is likely to correspond to that described by

Bianchi & Barré (2003) where 3 months of tick control interruption resulted in a mean density of 100 fully engorged females per steer. Second, a herd regroups about 80 steers per km<sup>2</sup> in western coast (Bianchi and Barré, unpublished data). Thus, a crude maximum in  $D$  would be of the order of 8000 adults per km<sup>2</sup>. Complementarily, independent biological data constraint  $\sigma$  lower-bound independently from  $D$ . Indeed, cattle from typical western herds rotate among two or five pastures with a mean pasture surface about 2.5 km<sup>2</sup> (Bianchi and Barré, unpublished data). Thus, two subsequent tick generations are unlikely to be systematically born on the same pasture, and hence  $\sigma$  values much lower than 0.5 km per tick generation look unlikely. Altogether, when combining the covariation constraint on  $D$ ,  $\sigma^2$  (Table 3) with those separately affecting  $D$  and  $\sigma$ , the most likely estimates would be, for  $D$ , a few hundreds reproducing adults per km<sup>2</sup>, and, for  $\sigma$ , a few hundred metres per tick generation. In turn, with herds foraging in average on 2.5 km<sup>2</sup> pasture, the order of the census size  $N$  of adult reproducing ticks per herd would be of the order of a few hundreds to a few thousands.

Altogether, the assumption of currently low tick effective population sizes ( $N_e \ll 100$  with  $100 < N < 10\,000$ ) look thus too unlikely for the detected bottleneck referring to current pesticide usage. Therefore, the bottleneck detected by Cornuet & Luikart (1996)'s method more certainly refers to the founding colonization event than to current pesticide controls. Under that assumption, it is worthy of note that the first naturalized tick populations had similar effective sizes ( $120 \leq N_e \leq 1200$ ) than the present pesticide-controlled populations.

#### Human impact on *B. microplus* populations

Pesticide control programs are expected to represent the main human impact on *B. microplus* populations. Yet, the average density in reproducing adult ticks was estimated of a few hundreds per squared kilometre. Does this mean that pesticide control programs are inefficient in New Caledonia? The answer is partly given from an experiment that was performed in absence of pesticide control (Barré *et al.* 2001). In that optimal environment, the manipulation of tick larval survival to either 10<sup>8</sup> or 3.10<sup>9</sup> larvae per km<sup>2</sup> consistently resulted in a density of adults reaching the reproductive stage of about 10<sup>7</sup> individuals per km<sup>2</sup>. This indicates thus a carrying capacity of *B. microplus* in New Caledonia of about 10<sup>7</sup> reproducing adults per km<sup>2</sup> (Barré *et al.* 2001). In turn, this indicates that pesticide control programs are consistently maintaining the density of reproducing ticks four to five magnitude orders below the carrying capacity.

Comparisons in *B. microplus* population structure among areas differing in their agriculture rules are required to

settle whether human activities might influence tick migration range. Present data however, attests that human activities have no impact on tick migration patterns in New Caledonia. Indeed, bovine exchanges are not determined by geographical proximity in New Caledonia where cattle markets are mostly centralized in a single town (Bourail nearby herd n°1, Fig. 1) and where farmers have relatives in every part of island. Therefore, no isolation by distance would have been expected if tick migration resulted from cattle exchange. By contrast, the clear pattern of isolation by distance presently evidenced indicates that tick dispersal is mainly determined by geographical proximity. The continuous distribution of cattle along western coast (see Fig. 1) and the within-herd cattle rotation among pastures are likely to be sufficient to allow the low dispersal ranges observed (see above). The apparent adaptation of *B. microplus* to infest the invasive host *Cerous timorensis russa* (Barré *et al.* 2001) may also induce tick dispersals. Interestingly, the removal of the transversal valley where the presence of cattle is scarce but *C. timorensis russa* exists did not affect  $D$ ,  $\sigma^2$  estimation. This is certainly due to the robustness of the method (Leblois *et al.* 2004). The precise role of *C. timorensis russa* in the genetic differentiation of *B. microplus* in New Caledonia is under current investigation.

#### Consequences on the evolution of new adaptive changes

The present analysis has evidenced that *B. microplus* forms large and weakly inbred populations in areas where European breeds of *Bos taurus* cattle predominate. It is noteworthy that these tick populations are large even in presence of intensive pesticide controls, since pesticides are usually applied every 4 or 6 weeks in New Caledonia (i.e. more than once per tick generation). Complementarily, the unexpected regular re-distribution of tick brotherhoods among hosts provides some protection of newly arisen mutations from drift disappearance. Meanwhile, the gene dispersal range per tick generation looks rather limited among bovine herds. Altogether, this leads to expect new adaptive mutations to be likely to arise in parallel in different tick populations that are facing the same ecological challenge. This expectation could be directly tested in near future by investigating the genetic diversity involved in the presently evolving resistances toward deltamethrine and amitraz pesticides (Bianchi *et al.* 2003; Ducornez *et al.* 2005).

#### Acknowledgements

We thank J.M. Delathière for providing data on New Caledonian herds. We are grateful to M.M. Guillermet, Marcel, Martin, Moglia, Ouetchou, Orezza and the IAC experimental farm to have allowed tick sampling on their cattle. This work was funded by the program 'INVABIO2' (MEDD, contract n°CV02000234). B.B. Koffi has been financed by a PhD-fellowship from Ivory Coast (contract n°501/MESRS/DBP/SD-BHCI/ACA-SD), C. Arnathau

by a French fellowship (CNRS-contract n°04-772), and N. Barré is a CIRAD researcher financed by Institut Agronomique néo-Calédonien. T. de Meeûs, P. Durand and C. Chevillon are co-financed by the French Research Institutions CNRS and IRD.

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B. B. Koffi has performed his PhD investigating, with population genetics analyses, the characteristics and consequences of the invasion of New Caledonia Island by *B. microplus*. N. Barré is a veterinary researcher interested in understanding the biological traits of *B. microplus* invasion in order to design optimal pest management program. T. de Meeûs, P. Durand and C. Chevillon are population geneticists. C. Chevillon is mainly interested in understanding how species adapt to new environmental conditions and the consequences of such adaptations, with models ranging from pesticide resistance in vectors to vector-changes in micro-pathogens. T. de Meeûs and P. Durand have centred their researches on parasite models for long investigating host-parasite co-evolution as well as hosts' constraints on parasite population genetics. In parallel, T. de Meeûs has started to decipher the evolutionary consequences of clonality, i.e. a reproduction mode common in parasites. P. Durand and C. Arnathau are presently focusing on tick-borne diseases and *Anopheles-Plasmodium* interactions.

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