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# Demographic scenario inferred from genetic data in leatherback turtles nesting in French Guiana and Suriname

P. Rivalan<sup>a,\*</sup>, P.H. Dutton<sup>b</sup>, E. Baudry<sup>a</sup>, S.E. Roden<sup>b</sup>, M. Girondot<sup>a</sup>

<sup>a</sup>Laboratoire d'Ecologie, Systématique et Evolution, UMR 8079 CNRS, ENGREF et Université Paris-Sud XI, 91405 Orsay Cedex, France

<sup>b</sup>NOAA-Fisheries, Southwest Fisheries Sciences Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037, USA

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## ABSTRACT

Nesting beaches around the Maroni estuary, at the border between French Guiana and Suriname (South America), currently host about 40% of the world's population of breeding female leatherback turtles. Although the population size has been variable but always large since the 1980s, observations of leatherbacks in the region before 1950 were sporadic or absent and the number of nesting females was extremely low. These observations motivated us to use molecular markers to investigate whether the appearance of leatherbacks in this region was the result of natural long-term population cycles or of immigration. Analysis of 12 microsatellite loci failed to detect the signature of a recent demographic perturbation in the Maroni population of leatherbacks. This result suggests that the marked increase in population size can neither be explained by natural long-term cycles, nor by a founder effect. We conclude that the current population of the Maroni region likely resulted from extensive migrations of animals from elsewhere. Consequently, the Maroni population probably belongs to a metapopulation whose limits remain currently unknown. This metapopulation dynamic suggested by our results is a major feature that should be taken into account in future conservation strategies. We also estimate the effective population size at 90–220 individuals, which is lower than the target effective population size for conservation purposes. This relatively low effective population size indicates vulnerability, despite the large number of nesting turtles currently observed annually.

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## 1. Introduction

The leatherback turtle (*Dermochelys coriacea*), the largest of the seven species of sea turtles, is a worldwide distributed pelagic reptile that migrates to tropical or subtropical beaches to nest (Miller, 1996; Spotila et al., 1996). The leatherback turtle is classified as "Critically Endangered" by the Species Survival Commission of the International Union for the Conservation of Nature (Hilton-Taylor et al., 2002): major threats to leatherback turtles include incidental catches by industrial fisheries (also called "bycatch" Lewison et al., 2004) and destruction of

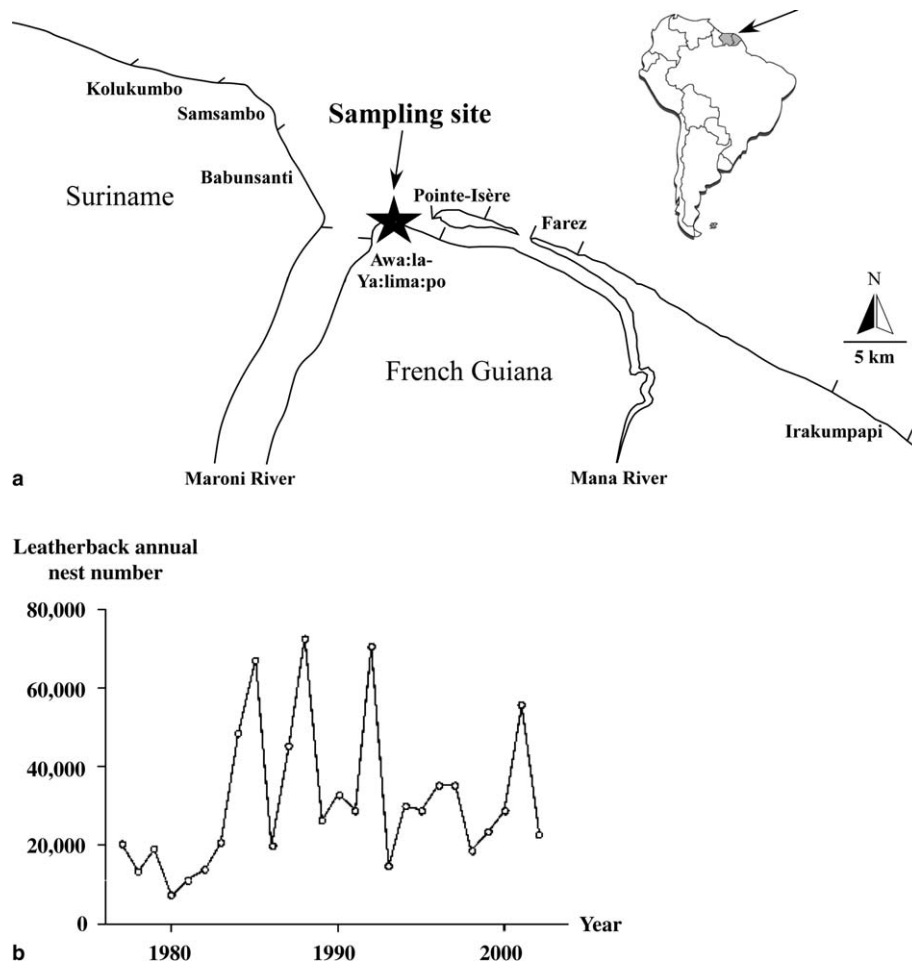
coastal nesting habitats (Pritchard, 1996). Spotila et al. (1996) pointed out that most of the Pacific nesting populations have undergone a severe decline since the early 1980s. For instance, the extinction of the largest Pacific nesting colony (Playa Grande, Costa Rica) is predicted within 20 years (Spotila et al., 2000). The two largest remaining nesting populations in the world are located in the Atlantic, around the estuary of the Maroni River (Fig. 1), which forms the boundaries of French Guiana and Suriname (South America; Girondot and Fretey, 1996), and on the coast of Gabon (Western Africa; Fretey and Billes, 2000). Because the nesting beaches of Gabon

\* Corresponding author. Present address: Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada E3B6E1. Tel.: +1 506 458 7253; fax: +1 506 453 3583.

E-mail addresses: [privalan@unb.ca](mailto:privalan@unb.ca), [philippe.rivalan@ese.u-psud.fr](mailto:philippe.rivalan@ese.u-psud.fr) (P. Rivalan).

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**Fig. 1 – (a) Major nesting beaches around the estuary of the Maroni River and (b) annual number of nests recorded in this region (from Girondot et al., 2002).**

have only been recently discovered, the demographic history in this area remains unclear (Fretey and Billes, 2000). On the contrary, the demographic past of the population nesting in the Maroni estuary, which hosts about 40% of the world's population of breeding females (Spotila et al., 1996), has been monitored since the early 1970s (Girondot and Fretey, 1996).

In French Guiana and Suriname, the nesting season occurs from early-March to mid-August (Girondot and Fretey, 1996). Each female nests every two to three years and lays on average eight clutches per season (Rivalan, 2004; Rivalan et al., 2005). The total number of nests laid annually in this region has fluctuated dramatically in the last 80 years. Before 1950, observations of leatherbacks in the region were sporadic (Barrière, 1741; Debien, 1965; Kappler, 1881). Considering the 'spectacular' size, distinguishing anatomy, the length of the nesting season of this species, it is highly improbable that observers would have missed leatherbacks if they were present in the region. Although the historic literature is far from complete, the lack of references to leatherbacks suggests that they may indeed have been periodically rare over the last few centuries. For instance, Stedman (1796) reported the presence of the green and olive ridley turtles but did not mention the presence of leatherbacks. Since the 1980s, the population size has been variable but always large (Fig. 1), with for instance 70,000 nests in 1992 (Girondot et al., 2002). Two main biological

hypotheses could explain the low number of leatherbacks observed before 1970 followed by variable but consistently high numbers. Pritchard (1996) hypothesized that leatherback populations may go through long term natural cycles of considerable amplitude. During these cycles, population size of leatherbacks may drop dramatically and later recover to large numbers (Pritchard, 1996). Natural factors, such as density-dependence in hatching success (Girondot et al., 2002), predator concentrations or food competition, could induce such cycles by limiting recruitment success of high density populations. The alternative hypothesis considers that fluctuations in population size result from colonization of new beaches by migrants from populations outside of French Guiana and Suriname (Pritchard, 1996).

Demographic events are expected to leave a temporary genetic signature (Cornuet and Luikart, 1996b). In a population subjected to a demographic reduction or an expansion, the allelic distribution will differ from the distribution expected under equilibrium between genetic drift and mutation (Cornuet and Luikart, 1996b). A population that has experienced a bottleneck will show an excess of gene diversity relatively to the diversity estimated from the observed allele number under the assumption of mutation-drift equilibrium (Nei et al., 1975). Likewise, a population that has expanded recently will show a gene diversity deficiency because it contains too many

rare alleles. Genetic tools can therefore be used to distinguish between two kinds of demographic scenarios: natural long-term cycles versus foundation by immigration. Mitochondrial DNA (mtDNA) has been frequently used in marine turtles to make inference about phylogeography and population structure (Bowen et al., 1996; Bowen et al., 1994; Dutton et al., 1996; FitzSimmons et al., 1997). However, studies performed on leatherbacks failed to reveal polymorphism among the nesting females of the Maroni population (Dutton et al., 1999). This lack of variation did not allow us to use mtDNA data to test the two demographic hypotheses above. In contrast, microsatellites are well suited for addressing questions about past demography (Jarne and Lagoda, 1996; Kichler et al., 1998; Luikart and Cornuet, 1998) or conservation genetics (Beaumont and Buford, 1998) because of their expected neutrality and their relatively high mutation rate that leads to high polymorphisms, even in bottlenecked populations (Ernest et al., 2000; Maudet et al., 2002).

We used 12 polymorphic microsatellite loci screened on 147 female leatherbacks from the Maroni population to investigate whether this population could be considered at equilibrium or whether it had recently undergone a demographic perturbation (e.g., bottleneck or demographic expansion). We also estimated the effective population size, a parameter often used as a criterion for the determination of conservation status (Franklin and Frankham, 1998; Mace and Lande, 1991). Many studies have shown that populations with small effective population size or bottlenecked populations are more vulnerable to genetic drift, inbreeding, loss of genetic variability and evolutionary potential (Allendorf and Ryman, 2002; Couvet, 2000; Frankham, 1995). In the context of an endangered species such as the leatherback sea turtle, population dynamics and effective population size are critical issues for relevant conservation policies.

## 2. Materials and methods

### 2.1. Sampling and DNA extraction

Samples were obtained from female leatherbacks nesting in the Maroni region, on Awa:la-Ya:lima:po beach (French Guiana, Fig. 1). During the nesting season, females come ashore at night to lay eggs on average 8 times over the 5 month period (Girondot and Fretey, 1996; Rivalan et al., 2005). Blood samples were collected by venipuncture from 147 nesting females from early-June to late-July 2002. Each nester was identified by a PIT tag (McDonald and Dutton, 1996), to ensure that the same individual was only sampled once. Five milliliters of blood were collected from the dorsal surface of the rear flipper while the turtle was laying eggs (Dutton et al., 1999) and preserved in a lysis buffer prior to processing according to the specifications of Bahl and Pfenninger (1996). Genomic DNA was extracted from blood using Dneasy™ Kit (QIAGEN GmbH, Germany) following the manufacturer's protocol.

### 2.2. Microsatellite markers

DNA samples were analyzed at 12 microsatellite loci developed for marine turtles: one was previously developed for

green turtles (CM3; FitzSimmons et al., 1995) and two for leatherbacks (N32 and DC99 Dutton, 1995). The other nine loci (14-5; DC106; DC110; DC128; DC133; DC141; DC142; DC143; DC145) were recently developed for leatherbacks (Dutton, unpublished results; sequences and annealing temperature of each primer are available from the authors upon request). PCR was performed in a 25- $\mu$ l total reaction volume containing 1.5 units of *Taq* polymerase, 2.5- $\mu$ l of buffer (5 mM KCl, 1 mM Tris-HCl (pH 8.3), 0.2 mM MgCl<sub>2</sub>), 0.6 mM of dNTP, and 0.3  $\mu$ M each of reverse and forward primers. After an initial denaturation step of 3 min at 94 °C, 30 cycles of PCR were performed, each cycle consisting of a 10-s denaturation at 94 °C, 10-s annealing temperature optimized for each primer, and primer extension for 10-s at 73 °C. Five extra minutes at 72 °C allowed the completion of the extension. PCR products were screened on an ABI Prism 3100 Genetic Analyzer (Perkin-Elmer).

### 2.3. Genetic diversity analysis

Exact tests for deviation from Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP v.1.2 (Raymond and Rousset, 1995). Departures from HWE were calculated per locus and across all loci. Linkage disequilibrium was estimated between all loci pairs using GENEPOP. The observed heterozygosity ( $H_o$ ) and the unbiased expected heterozygosity under HWE ( $H_e$ ) were computed using GENETIX software (Belkhir et al., 1996).

### 2.4. Test of demographic history

In order to determine the demographic history of the Maroni population, we compared the observed allelic distribution with the distribution expected under equilibrium between genetic drift and mutation, assuming neutral variation. A population that has experienced a bottleneck will show an excess of gene diversity relative to the diversity estimated from the observed allele number under the assumption of mutation-drift equilibrium. Likewise, a population that has expanded recently will show a gene diversity deficiency because it contains too many rare alleles. We used the 'sign test' (Cornuet and Luikart, 1996b) and the 'Wilcoxon signed test' (Luikart and Cornuet, 1998) implemented in BOTTLENECK (<http://www.ensam.inra.fr/URDC>) to determine whether the Maroni population exhibits a significant number of loci with gene diversity excess or deficit. To test the occurrence of a bottleneck, we assumed that the population does not present hidden substructure or immigration. In addition, we assumed that there is no linkage disequilibrium between loci and that no loci present null-allele (Cornuet and Luikart, 1996b). Because most mutations in microsatellites are expected to generate previously existing allele sizes and to be dominated by additions or deletions of one copy of the tandem repeat (single stepwise mutation model, SMM), it has been recognized that the infinite allele mutation model (IAM) that underlies most population genetic models is probably inappropriate (Jarne and Lagoda, 1996; Slatkin, 1995). Moreover, mutations that involve more than one repeat unit are also observed, although less frequently. These features can be approximated by the two-phase mutation model (TPM), where a mutation

leads to the addition of one repetition unit with a probability  $p_1$  and the addition of several repeat units with the probability  $p_2$  (Di Rienzo et al., 1994). The occurrence of TPM has been recently confirmed in marine turtles (Hoekert et al., 2002). We therefore performed our analyses using the TPM model. In the latter mutation model, single step probability ( $p_1$ ) and multiple step probability ( $p_2$ ) were first, respectively, fixed to 0.95 and 0.05. These values, suggested by Piry et al. (1999), are consistent with the ones estimated for microsatellites of marine turtles (Hoekert et al., 2002). To determine whether our conclusions depended on these parameter values, we also used two alternative probability sets ( $p_1 = 0.98/p_2 = 0.02$  and  $p_1 = 0.90/p_2 = 0.10$ ).

### 2.5. Estimation of effective population size

To our knowledge, there is no simple formula to compute the effective population size,  $N_e$ , under the TPM model. However, the TPM is essentially the intermediate model between the IAM and the SMM. Therefore, we estimated  $N_e$  for SMM and IAM models, considering that the actual  $N_e$  is likely to fall within the range of those two estimates.  $N_e$  computations were performed from estimates of expected heterozygosity using the following formulas:

$$\text{IAM } N_e = H/4\mu(1 - H) \quad (\text{Nei, 1987}),$$

$$\text{SMM } N_e = [1/(1 - H)^2 - 1]/8\mu \quad (\text{Ohta and Kimura, 1973}),$$

where  $N_e$  is the effective population size,  $H$  is the mean expected heterozygosity and  $\mu$  is the mutation rate per locus and per generation. Mutation rates of microsatellites usually range between  $10^{-3}$  and  $10^{-5}$  mutation per locus and per generation (Buford and Wayne, 1993; Schlötterer and Pemberton, 1994). In leatherbacks, mutation rates have been estimated for three microsatellite loci to be  $2.8 \times 10^{-3}$  (DC99 and DC14-5; Dutton, 1998) and  $5.6 \times 10^{-3}$  (DC2-95; Dutton, 1998). Using these two mutation rates and the two mutation models, we calculated estimates of  $N_e$ .

## 3. Results

### 3.1. Genetic analyses

All 12 microsatellite loci were polymorphic (Fig. 2). The number of alleles per locus varied from 3 to 11 (Table 1) with a mean of 5.83. The expected heterozygosity ( $H_e$ ) ranged from 0.10 to 0.79 (Table 1) and the mean expected heterozygosity over all loci was 0.59 (Hardy–Weinberg test  $P < 10^{-3}$ ). Ten loci were in agreement with the Hardy–Weinberg equilibrium. One locus, DC143, showed a significant heterozygote deficit ( $P = 0.038$ ) but this deficit was no longer significant after Bonferroni correction. The last locus, CM3, showed a highly significant heterozygote deficit ( $P < 10^{-4}$ ). This deficit may result from the presence of a non-amplifying allele (“null allele” Jarne and Lagoda, 1996). Considering that CM3 was developed for green turtles whose divergence with leatherbacks is estimated to be more than 100 millions years old (Zangerl, 1980) whereas the other primers were developed for leatherbacks, the presence of a null allele at this locus is not unexpected. Since we suspected the presence of a null allele at CM3, we excluded this locus from all subsequent

analyses. Without locus CM3, the mean expected heterozygosity over all loci was 0.57 (Hardy–Weinberg test  $P = 0.50$ ). Linkage disequilibrium between loci was significant ( $P < 0.05$ ) in three pair wise comparisons among a total of 66, which is exactly the number expected by chance alone. No comparisons were significant after Bonferroni correction. All the loci can therefore be considered as genetically independent.

### 3.2. Testing putative demographic perturbations

A population whose effective size has remained constant in the recent past is expected to show an approximately equal probability of excess or deficit in the gene diversity of a locus. To determine whether the Maroni population exhibits a significant number of loci with excess and deficit in gene diversity, we used the method of Cornuet and Luikart (1996a). We found that under a TPM mutation model with a 5% probability of multiple steps, 4 loci exhibited a heterozygosity deficiency and 7 loci a heterozygosity excess (Table 2). A sign test did not reject demographic equilibrium ( $P = 0.46$ ; Table 3). Since this test suffers from low statistical power we also performed the Wilcoxon signed rank test that provides relatively high power (Cornuet and Luikart, 1996b). No significant departure from equilibrium was detected (two-tailed test for  $H$  excess or deficiency;  $P = 0.83$ ; Table 3). Similar results were observed with probabilities of multiple steps ranging from 2% to 10% (Table 3).

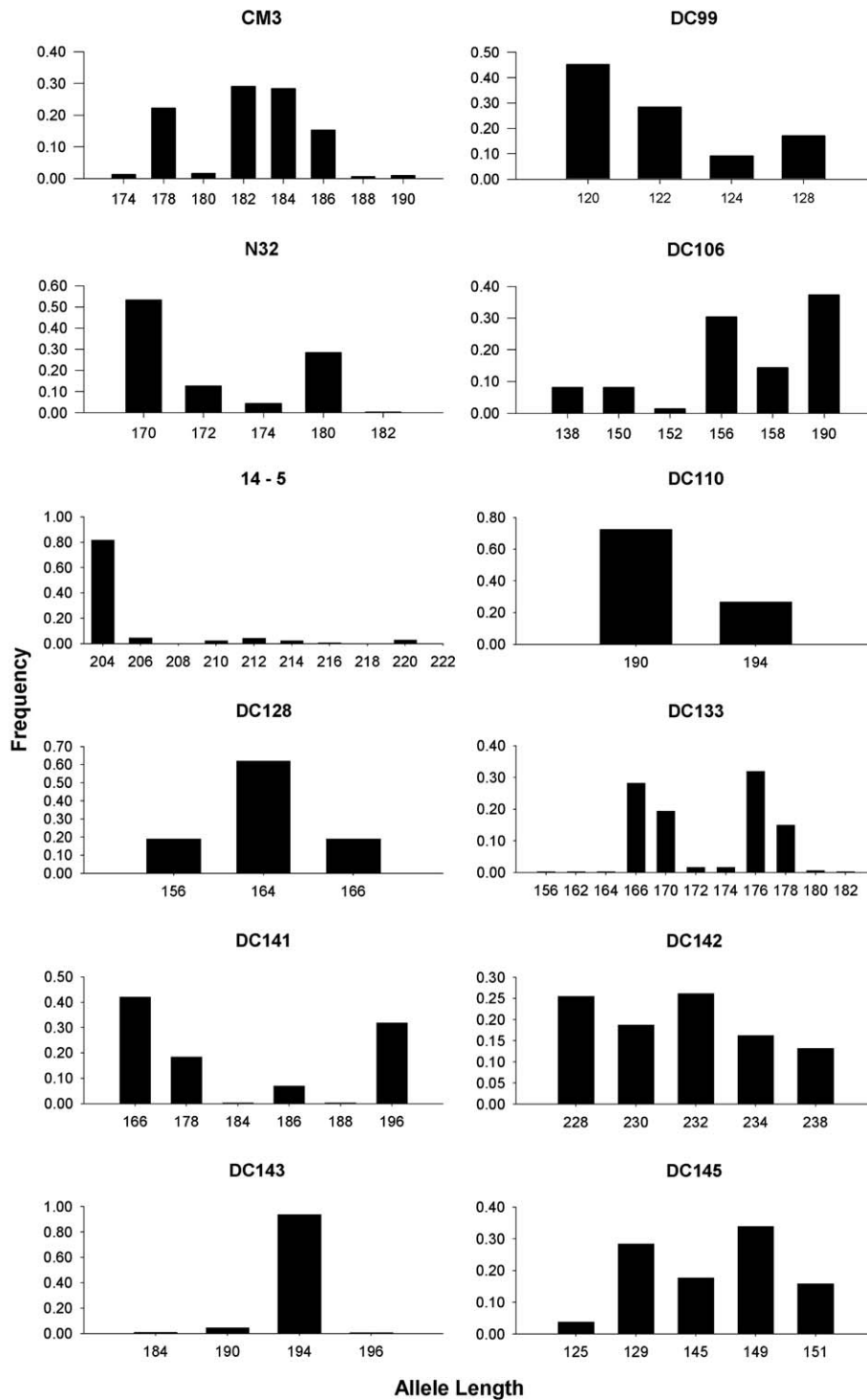
### 3.3. Effective and census population size

Based on mean expected heterozygosity, effective population size was estimated at 110–220 individuals under SMM vs. 60–130 under IAM, using the range of mutation rates observed on three leatherbacks microsatellites. Note that these estimates are highly sensitive to the actual values of the microsatellite mutation rates. We also estimated  $N_e$  with the lowest mutation rate estimated in microsatellites in sea turtles ( $\mu = 5.7 \times 10^{-4}$ ; FitzSimmons, 1998). With this value, the effective population size increased by one order of magnitude and reached 700 under IAM and 1200 under TPM.

## 4. Discussion

### 4.1. Demographic scenario

Our data did not enable us to detect a population size reduction or expansion of a magnitude such that it would have influenced allelic diversity. The probability of detecting a heterozygote excess when a recent bottleneck has occurred is a complex function of four parameters: the time elapsed since the beginning of the bottleneck ( $\tau$ ), the effective population size ratio before and after the bottleneck ( $\alpha$ ), the mutation rate ( $\mu$ ), the number of polymorphic loci scored ( $n$ ) and the sample size of genes ( $N$ ) (Cornuet and Luikart, 1996b). Given the parameters of our study (i.e.,  $\tau < 100$  years,  $\alpha = 100$ –1000,  $\mu = 10^{-3}$ – $10^{-4}$ ,  $n = 12$ ,  $N = 147$  diploid individuals), simulations showed that the probability of detecting a bottleneck under a single-step mutation model reached 80% (Cornuet and



**Fig. 2 – Allele frequency at 12 microsatellite loci in 147 female leatherbacks nesting in the Maroni region.**

Luikart, 1996b). Note, however, that we cannot rule out the possibility that small demographic fluctuations remained undetected by the test. The low number of leatherbacks before 1950 followed by a marked increase since the 1980s can thus not be explained by natural long-term cycles, as proposed by Pritchard (1996). Therefore, the current population of the estuary of the Maroni River most likely resulted from migration of animals from elsewhere. This migration may theoretically involve a few (i.e., a founder effect) or a large

number of individuals. However, a founder effect would have left the genetic signature of a bottleneck, which was not observed in our data. Therefore, the Maroni population has probably arisen from massive immigration.

Previous studies on leatherbacks using mtDNA have indicated population structuring that suggests philopatry and natal homing in females (i.e., female return on their natal beach, Dutton et al., 1999). Unlike other sea turtles, which can exhibit very accurate natal homing (Allard et al., 1994),

**Table 1 – Summary statistics for 12 microsatellite loci from Maroni leatherbacks**

Locus	Sample size	Number of alleles	$H_O^a$	$H_E^b$	HWE probability test
CM3	146	8	0.308	0.763	0.000
N32	145	5	0.600	0.613	0.938
14-5	146	8	0.323	0.325	0.626
DC99	146	4	0.685	0.679	0.998
DC106	146	6	0.747	0.736	0.553
DC110	131	4	0.428	0.397	0.661
DC128	146	3	0.582	0.545	0.142
DC133	147	11	0.789	0.760	0.234
DC141	143	6	0.641	0.641	0.814
DC142	147	5	0.796	0.790	0.094
DC143	143	4	0.077	0.101	0.038
DC145	143	6	0.720	0.744	0.114

a  $H_O$ : Observed heterozygosity.

b  $H_E$ : Expected heterozygosity under Hardy–Weinberg equilibrium.

**Table 2 – Statistics for detecting departure from mutation-drift equilibrium under two-phase mutation models (TPM) for 11 microsatellite loci from Maroni leatherbacks (locus CM3 excluded)**

Locus	$H_E^a$	$H_E^b$ (SD)	DH/SD <sup>c</sup>
N32	0.613	0.621 (0.105)	–0.078
14-5	0.325	0.760 (0.058)	–7.515
DC99	0.679	0.536 (0.133)	1.082
DC106	0.736	0.673 (0.090)	0.699
DC110	0.397	0.179 (0.164)	1.329
DC128	0.545	0.404 (0.155)	0.909
DC133	0.760	0.823 (0.044)	–1.441
DC141	0.641	0.622 (0.103)	0.186
DC142	0.790	0.619 (0.103)	1.668
DC143	0.101	0.415 (0.151)	–2.087
DC145	0.744	0.628 (0.105)	1.103

Single step mutation probabilities  $p_1 = 0.95$  and multiple step mutation probabilities  $p_2 = 0.05$ .

a  $H_E$ : Expected heterozygosity under Hardy–Weinberg equilibrium.

b  $H_E$ : Expected heterozygosity under mutation-drift equilibrium and SD the associated standard deviation.

c DH: Difference in heterozygosity,  $DH = H_E - H_O$ .

**Table 3 – Statistics for detecting departure from mutation-drift equilibrium under three two-phase mutation models (TPM) for 11 microsatellite loci from Maroni leatherbacks (locus CM3 excluded)**

$p_1$	$p_2$	Sign test probability	Wilcoxon rank test
0.98	0.02	$P = 0.466$	$P = 0.966$
0.95	0.05	$P = 0.464$	$P = 0.831$
0.90	0.01	$P = 0.229$	$P = 0.356$

The three TPM models differed in the single and multiple step mutation probabilities (respectively,  $p_1$  and  $p_2$ ). The occurrence of a demographic perturbation is rejected if  $P > 0.05$  (Cornuet and Luikart, 1996).

field observations collected on leatherbacks attested that natal homing could be relaxed in this species, allowing occasional migrations between distant beaches. For instance, tag recoveries pointed out that two females tagged in Trinidad have nested in Suriname, about 1500 km away, in 2002 (Hilterman and Goverse, 2003). Merging mtDNA data (Dutton et al., 1999) and tagging data, one might hypothesize that population spatial dynamics in the leatherback turtle can be approximated by an island population model, where a population is divided into structured local subpopulations connected by migrating individuals. However, our results suggest that migrations can sometimes involve a large number of animals over a short time scale (e.g., one or a few decades) and lead to the settlement of a new population on nesting grounds previously unoccupied. Therefore, we propose that the leatherback turtle might follow a meta-population model where a population is divided into subpopulations which go extinct locally and are subsequently recolonized by immigration, rather than an island population model.

#### 4.2. Putative origin of the Maroni population

The Maroni population shows only one mtDNA haplotype. Most other Atlantic populations, including nearby Trinidad and the US Virgin Islands populations, contain the mtDNA haplotype found in the Maroni population but also one or two additional haplotypes at high frequency (Dutton et al., 1999). These populations are unlikely to be the origin of the Maroni nesting population if we rule out a recent bottleneck in this population. In contrast, nesting leatherback populations from Florida and South Africa are the only known populations that exclusively share the mtDNA haplotype found in the Maroni population (Dutton et al., 1999). These two distant populations may therefore be the origin of the animals currently observed in French Guiana and Suriname. However, because most evidence to date suggests only occasional long-distance mistakes in natal homing, massive immigration from such distant beach is unlikely. The highly dynamic nature of the coastline (Augustinus, 2004; Fromard et al., 2004) suggests that Maroni population might originate from nearby beach subsequently to destruction of their original beach. Large scale genetic sampling, partially conducted at Western Atlantic scale (Dutton et al., 2003), might enable us to infer the location of this “parental” population.

#### 4.3. Effective population size

Awise (1994) found that in nearly all cases, the effective population size was 10–1000 times less than the census population size. Based on a sex-ratio of 0.52 (Godfrey et al., 1996) and an average value of 8 nests per female (Rivalan, 2004; Rivalan et al., 2005), the census population size  $N$  of the leatherbacks from Maroni is roughly estimated at 2750–20,000 individuals. The  $N_e$  values estimated from genetic variability in leatherbacks (i.e., from 60 to 220 depending on the mutation rate and the mutation model) are thus apparently in agreement with Awise’s statement (1994). Observed discrepancies between  $N_e$  and census population size may result from high

variability in reproductive success between individuals: leatherbacks exhibit a size-dependent fecundity (Tucker and Frazer, 1991), and thus bigger (i.e., older) adults can have a higher contribution to the following generation. Low  $N_e/N$  ratio is also found in highly fecund species with high juvenile mortality. Although this statement is generally proposed for fish (Hauser et al., 2002), it also fits leatherbacks which may lay up to 13 clutches per season, each with 85 eggs (Fretey and Girondot, 1988; Girondot and Fretey, 1996) and with a first year survival rate estimated at only 0.0625 (Spotila et al., 1996). Finally, in species with temperature dependent sex determination such as the leatherback (Bull and Charnov, 1989), temporal fluctuations in sex ratio could also reduce  $N_e$  (Crow and Kimura, 1970).

#### 4.4. Conservation implications

The minimum effective population size to ensure long-term integrity is still a matter of debate. An effective population of 500 would be, in theory, needed to maintain equilibrium between loss of adaptive genetic variation due to genetic drift and its replacement by mutation (Franklin, 1980; Franklin and Frankham, 1998). However, for several reasons Lynch and Lande (1998) have argued that the target effective population size for conservation program for an endangered species should range from 500 to 1000. Although these values likely depend on the species considered, our estimate of the effective population size from data collected in the Maroni region is below the lower value proposed by Lynch and Lande (1998). However, even if genetic considerations are important in the management of endangered species because they allow a better understanding of population dynamics, they affect populations on a longer time-scale than ecological and other impacts that degrade survival rate and/or habitat. Human induced perturbations present the greatest challenge to most endangered and threatened species (Caro and Laurenson, 1994). Intensive conservation efforts are underway in French Guiana and Suriname to increase the number of nesting females in the major nesting sites around the Maroni estuary (i.e., Awa:la-Ya:lima:po, Samsambo, Babunsanti, Kolokumbo). Our results suggest that the Maroni nesting population may be part of a large metapopulation, whose boundaries remain currently unknown. By focusing conservation effort on maintaining a few of the large populations, there is a danger of overlooking the relatively smaller ones, which might be involved in replenishing locally depleted populations. Therefore, we emphasize the need for international conservation strategies in conservation policies for leatherback turtles rather than regional ones.

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13461D/1B/ENV) and imported under CITES permit CITES No. FR-02-091-08148-R.

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