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Genetic Diversity of Translocated Populations of *Iguana delicatissima* in Saint Barthélemy, Lesser Antilles

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***Abstract.*—The island of St Barthélemy, part of the Anguilla Bank in the northern Lesser Antilles is one of the last strongholds for the Critically Endangered Lesser Antillean Iguana (*Iguana delicatissima*). The arrival of invasive alien iguanas (*Iguana rhinolophus*, *I. iguana*) has resulted in hybridization, threatening the persistence of the endemic species. We present a long-term assessment of two *I. delicatissima* populations (consisting of 14 individuals: 5 males, 9 females for each population) translocated in 2011 from St Barthélemy to the satellite islets of Fourchue and Frégate, 10–11 y post-translocation. We analyzed partial sequences of the nuclear *Cmos* gene, the mitochondrial *ND4* gene, and 10 microsatellite loci to evaluate genetic diversity and founder effects. Our results demonstrate that each founder population is genetically distinct and lacks the full genetic variability of the source population. Genetic drift in the two descendant populations likely precipitated the loss of specific *Cmos* and *ND4* haplotypes and reduced microsatellite polymorphism. The relict population on îlet Fourchue retained a unique polymorphism, and one individual captured in 2022 was homozygous for a *Cmos* haplotype characteristic of *I. rhinolophus*. Furthermore, iguanas on Frégate, which was unoccupied prior to translocation, exhibited *Cmos* and *ND4* haplotypes absent from the original founders, indicating potential inter-islet dispersal. Across the three populations (St Barthélemy, Fourchue, Frégate), four novel *Cmos* and 11 novel *ND4* haplotypes were identified. We recommend continued genetic monitoring, prevention of invasive alien iguana arrivals, removal of hybrids, and management of these small, interconnected populations as a metapopulation.**

Key Words.—DNA analysis; founder effect; genetic drift; introgression; Lesser Antillean Iguana; translocations

Introduction

The conservation of endangered species often necessitates management plans incorporating restocking operations in historical territories (Sarrazin and Barbault 1996; Knapp 2001; Wilson et al. 2016; Daltry 2006a,b) or population reinforcements to increase the number of populations and their population sizes (Carstairs et al. 2019; Furlan et al. 2020; Oppel et al. 2021; Ramade 2020). Translocation is frequently employed to increase genetic diversity within and among populations of threatened species where genetic drift predominates (Ramade 2020). Random genetic drift disproportionately impacts small populations, reducing adaptive potential to environmental change (Kimura and Ohta 1969; Furlan et al. 2020; Hvilsom et al. 2022). Conversely, the influx of novel alleles from populations with different evolutionary histories may cause fitness declines through outbreeding depression, potentially leading to the eradication of the threatened population (Rhymer and Simberloff 1996; Verhoeven et al. 2011).

This general problem of translocation provides the context for our study on the Lesser Antilles Iguana (*Iguana delicatissima*) on St Barthélemy in the Caribbean. While translocation holds great promise for re-establishing endangered species and restoring degraded ecosystems, its success is highly variable (Ramade 2020) and depends on the biology,

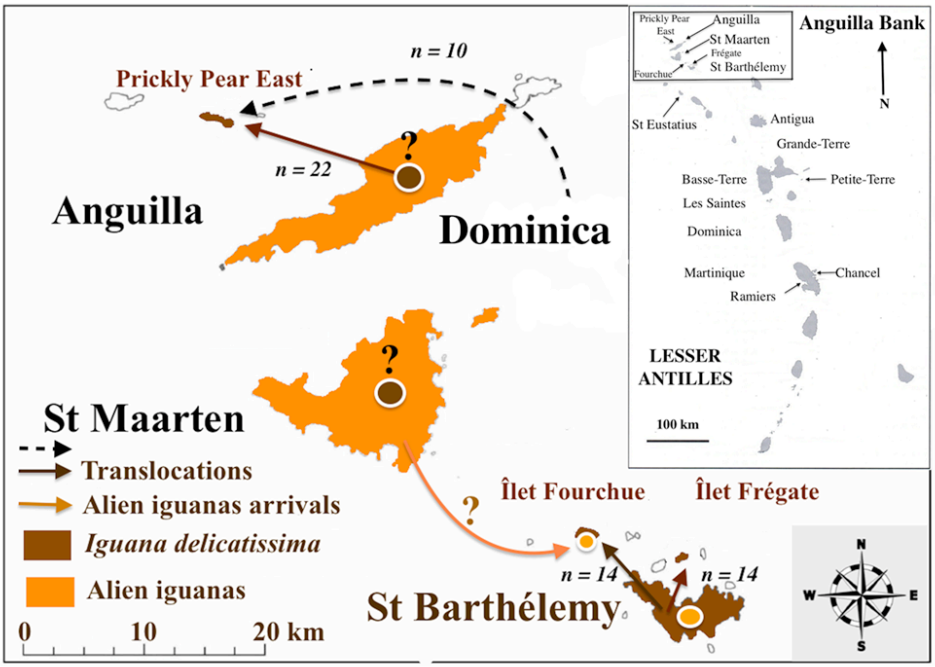

Figure 1. Study areas on the three main islands of the Anguilla Bank and the three translocations on satellite islands. Inset is the location of the study areas in the Lesser Antilles indicates the possible presence of some relictual Lesser Antilles Iguanas (*Iguana delicatissima*) on Anguilla and St Maarten and the putative origin of hybrids on îlet Fourchue (this study) and Pounder et al. (2020) as well as the genetic rescue of 10 *I. delicatissima* from Dominica (Farah Mukhida et al., unpubl. data), and the number of iguanas involved in these different translocation programs.

genetic diversity, habitat quality at release sites, and management strategies of a species. We aimed to examine the interaction between the genetic diversity of translocated *I. delicatissima* and establishment success, long-term viability, and genetic diversity of the descendants of these founder populations, thereby contributing to a more nuanced understanding of this critical conservation practice.

Iguana delicatissima is an emblematic endemic species in the Caribbean threatened by anthropogenic factors including hunting, habitat destruction, predation, roadkill, and hybridization with the invasive common Green Iguanas (*Iguana iguana*) from French Guiana and from Central America (Breuil et al. 1994; Day et al. 2000; Breuil 2002, 2022; Vuillaume et al. 2015). Initial hybridization was identified on morphology (sample MCZ R 10969 collected by Noble in 1914) in Les Saintes (Guadeloupian archipelago) in the early 20th Century though it likely began earlier (Breuil 2013). Breuil (2002, 2013) and Vuillaume et al. (2015) showed that hybridizations have occurred in St Barthélemy, Basse-Terre, Grande-Terre, and Martinique (îlet Ramiers, pers. obs). Hybridization is now known on St Eustatius (van den Burg et al. 2018a), Dominica (van den Burg et al. 2020), Anguilla (Pounder et al. 2020) and Antigua (van den Burg et al. 2024). The International Union for Conservation of Nature (IUCN) considers this species as Critically Endangered (Knapp et al. 2014; van den Burg et al. 2018b).

The Anguilla Bank, which is the frame of our study, located at the northern end of the Lesser Antilles, comprises three primary islands: Anguilla, St Martin/Sint Maarten, St Barthélemy (St Barts), and their satellites (Fig. 1). These islands were part of a contiguous landmass during the last Glacial Maximum and share common biodiversity (Biknevicius et al. 1993; Thorpe 2022). Due to their proximity and substantial trade and tourism, they face comparable conservation challenges. On Anguilla, invasive alien iguanas first arrived via vegetation rafts during hurricanes Luis and Marilyn in 1995 (Censky et al. 1998; Hodge et al. 2003). These iguanas originated from the Guadeloupian archipelago (Breuil 1999) and thus are from French Guiana (Breuil 2013; Breuil et al. 2019, 2020), as are the ones found on Antigua by van den Burg et al. (2024). Subsequently, a population of Central American Green Iguanas (*I. rhinolophus*), dispersed into southwestern Anguilla, likely via the pet trade on St Maarten. Both invasive alien species have

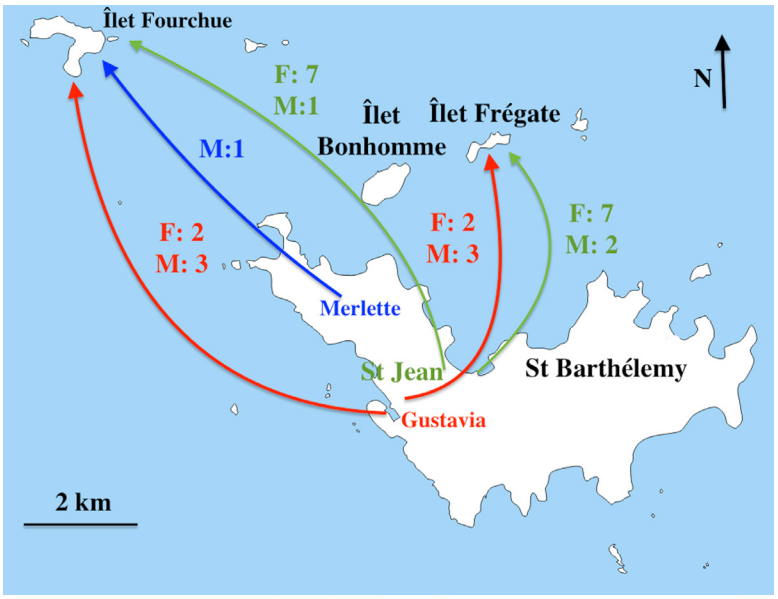

Figure 2. Origins and numbers of the translocated iguanas. The abbreviation M = males, F= females, and the lines with arrows show the translocations from the three localities of the main island to the satellite islets.

hybridized with the endemic population of iguanas (Pounder et al. 2020), leaving few individuals of the endemic species.

The situation on St Maarten is further complicated by invasive alien iguanas from Curaçao, Central America, South America, and the northeastern Andes (Peru, Bolivia; van den Burg et al. 2018b; Vuillaume et al. 2015). The last *I. delicatissima* was recorded there in 1996 (Breuil 2002), however, it is possible some individuals may still be present in the remote parts of the island (Fig. 1). Invasive alien individuals from the complex that can be termed an iguana swarm from St Maarten likely arrived on St Barthélemy in 2005 and hybridized with *I. delicatissima* (Vuillaume et al. 2015); we documented the first hybrid in 2008 (Breuil, unpubl. data).

In St Barthélemy, given the difficulty of regulating invasive alien populations, the Agence Territoriale de l'Environnement, (formerly Réserve Naturelle de St Barthélemy) translocated 28 *I. delicatissima* from the main island to two satellite islets in 2011 (îlet Frégate, îlet Fourchue; Fig. 1) to prevent genetic swamping. While large colonies existed on these islets in the 1960s (Lazell 1973), iguanas were absent from îlet Frégate by the early 20th Century, though a small relict population persisted on îlet Fourchue (Breuil 2002; Anne and Michel Breuil, unpubl. reports). Habitat structure (trees, bushes, cliffs and boulders, diversified food, egg-laying sites) at these sites was deemed suitable for the species (Breuil 2002; Questel and Hochart 2019). Our objectives were to: (1) characterize genetic diversity (nuclear *Cmos* gene, the mitochondrial *ND4* gene, and microsatellites) of *I. delicatissima* on St Barthélemy; (2) compare genetic diversity between founder individuals and their descendants 10–11 y post introductions; (3) detect the arrival of new individuals and hybridization events; and (4) propose methodological refinements for future translocation programs.

Materials and Methods

Although *I. delicatissima* is widely distributed on St Barthélemy, mainly in coastal areas with trees, we selected two primary sites (St Jean and Gustavia) for the founder populations in 2011 because these two areas were undergoing significant anthropogenic degradation due to construction and vehicular traffic. Moreover, one male from an undisturbed area (Merlette) was also included for the constitution of one founder population (Fig. 2). The decision to select these individuals, based on the high risk of mortality they faced, meant that we were unable to take advantage of the full putative genetic diversity of the species throughout St Barthélemy.

Selection of translocated iguanas.—We translocated five males and nine females to îlet Frégate (13.8 ha). On îlet Fourchue (56.7 ha), we translocated five males and nine females to reinforce a relict population of approximately 10 resident adults (Fig. 2). The male-to-female ratio was

selected based on the polygynous mating system of the species (Breuil 2002). Introduced adults varied in size, age class, and reproductive status, and were in good physical condition. These individuals likely differed in their experience with habitat selection and nesting behavior. Consequently, these translocated groups represent artificial populations that may not be in genetic equilibrium.

These two translocations were conducted July 2011 by the Agence Territoriale de l'Environnement with the help of Muséum National d'Histoire naturelle (Paris) without checking the genetic status of the translocated individuals. This was due to a lack of available microsatellites, poor knowledge of the genetic structure of the species, and the absence of funding at that time. Nevertheless, biopsies were taken for all the translocated individuals to prepare their genetic monitoring. To ensure only pure individuals were translocated, we assessed 15 morphological traits (e.g., sub-tympanic plate, tail barring, form of the dewlap, form and numbers of the gular spikes, form and disposition of labial and sub-labial scales, etc.) established in the literature (Breuil 2002, 2013; Pounder et al. 2020; van den Burg et al. 2024). We examined females via palpation and X-ray photography at a veterinary clinic to confirm they were not gravid, thus preventing the introduction of potentially hybridized offspring.

Before translocation, we fitted each iguana with a PIT-tag (passive integrated transponder) injected subcutaneously into the upper left thigh and we photographed it with its PIT-tag number (Questel 2019) to ensure future identification. Between 2012 and 2022, 159 descendants were tagged on îlet Fourchue, and 48 were tagged on îlet Frégate. Precise population estimates were not possible due to the lack of a standardized protocol during the monitoring visits (2012–2014, 2016–2019, 2022). We do not know the precise proportion of the population we were able to catch during each visit.

Genetic analysis.—We performed genetic analyses on 27 of the 28 translocated iguanas (one sample was excluded due to insufficient DNA quality) on biopsies we collected before the translocations. Sampling of descendant populations yielded 23 biopsies from îlet Frégate in 2021 and 28 biopsies from îlet Fourchue in 2022. For each individual, we collected tail tip biopsies (approximately 5 mm) using scalpels. Tail clipping is a standard protocol for squamates for biopsies (e.g., Reynolds et al. 2011; Kindler et al. 2014). Biopsies we used for DNA amplification were taken from inside the tail fragment to prevent cross contamination, following a standardized laboratory protocol (Valette et al. 2013; Vuillaume et al. 2015; Breuil et al. 2019, 2020, 2022; van den Burg et al. 2021). We disinfected scalpels with 90% ethanol and cleaned them with bleach between samples. We also disinfected tail tips after biopsy. We immediately stored all collected tissues in individual tubes containing 70% ethanol, with numbers corresponding to the numbers of the injected PIT-tag. We conducted all the genetic analysis between 2023 and 2025. We extracted total genomic DNA using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, California, USA) following the protocol for animal tissues, which includes a 3-h incubation in proteinase K at 56° C and 30 min of RNase treatment at 37° C.

We used a panel of 10 polymorphic microsatellite loci (L2, L3, L4, L5, L14, L16, L18, L20, L23, and L24) previously validated for *I. delicatissima* (Valette et al. 2013; Vuillaume et al. 2015; van den Burg et al. 2021). These microsatellites are also suitable for detection of possible hybridization. We amplified founder individuals in duplicate at each locus, to ensure consistent allele sizing with published dataset (van den Burg et al. 2021). The presence of null alleles was evaluated with MICRO-CHECKER (Van Oosterhout et al. 2004).

We also partially sequenced the mitochondrial *ND4* gene (NADH dehydrogenase subunit 4), which is widely used in iguana phylogeny studies and has a much larger polymorphism (as shown by this study and unpublished data) than previously identified (Stephen et al. 2013; Martin et al. 2015; van den Burg et al. 2018b; Pounder et al. 2020). According to Stephen et al. (2013), the first described *ND4* haplotype for *I. delicatissima* (AF217783) is 903 bp long but we used a 798 bp length sequence for our analysis. We also sequenced the *Cmos* nuclear gene (oocyte maturation factor), which identifies *I. delicatissima* by its haplotype (HM352538) from other species of the genus *Iguana* (Stephen et al. 2013). This gene, which is 375 bp long, was also used by Pounder et al. (2020), who described a new haplotype on the Anguilla Bank that differs by two mutations (MK605403) from HM352538.

The sequencing protocol for these two genes followed those of Malone et al. (2000) for *ND*4 and Stephen et al. (2013) for *Cmos* genes. We have previously used the protocol of Malone for *ND*4 in Breuil et al. (2019, 2020, 2022) and Vuillaume et al. (2015). To increase accuracy, we sequenced the genes for each iguana in both forward and reverse

directions. The shortest cleaned sequences found in the two founder populations were not used in this analysis and we kept a 362 bp long fragment for all others. To ensure that the new haplotypes found in single individuals were not genotyping errors, we repeated the sequence process twice.

Microsatellite statistical analysis.—We calculated the total, mean and effective number of alleles, as expected (H_E) and observed heterozygosity (H_O), and polymorphism index using the Excel Microsatellite-toolkit 3.1 add-in (created by S.D.E. Park) for the founders (Foun), îlet Frégate descendant (Dfré), and îlet Fourchue descendant (Dfou) populations. This was conducted both before (dHWE) and after a Bonferroni correction (dHWE-Bc; Table 2), using the nine loci that did not deviate significantly from Hardy-Weinberg equilibrium. We calculated the fixation indices by Wright (FIT and FIS) using FSTAT version 2.9.4 (https://www.scienceopen.com/document?vid=79097bb4-ec3c-47c3-94a1-47085d721e6b). We applied Exact Tests for deviation from Hardy-Weinberg equilibrium using the Markov Chain Monte Carlo (MCMC) simulation (5,000 iterations, 100 batches, and a dememorization number of 10,000) implemented in GENEPOP version 4.7.5 (Rousset 2008).

To detect potential hybridization with non-*delicatissima* iguanas, we added 25 representatives of these alien invasive iguanas from the islands of St Martin (French side), Grande-Terre, Basse-Terre, les Saintes, Martinique, and continental pure iguanas from French Guiana and Central America (Vuillaume et al. 2015; van den Burg et al. 2021). As the island of Saba is inhabited by an endemic taxon (Breuil et al. 2020), we added samples from this island. These iguanas have a geographic potential to invade new islands and islets of the Anguilla Bank.

We investigated the genetic structure and admixture patterns of *I. delicatissima* subpopulations and 25 putative invasive alien iguanas (Inva) using the Bayesian clustering algorithm in STRUCTURE version 2.3.4 (Pritchard et al. 2000). We performed 100 independent replications from K = 1 to K = 10 to determine the number (K) of genetically different groups independently of their localities. We performed this analysis on nine of 10 loci that did not deviate from Hardy-Weinberg equilibrium. We used a burn-in period of 25,000 iterations, followed by 100,000 MCMC repeats of each run. This procedure groups individuals into populations and estimates the proportion of membership for each individual in each population. We determined the K value by the log probability of the data (Ln P(D)), based on the rate of change in LnP(D) between successive K values (Earl and vonHoldt 2012). We predicted the optimum K value following the simulation method of Evanno et al. (2005) using the Python script STRUCTURE HARVESTER version v0.7 (July 2022; Earl and vonHoldt 2012). We conducted an Analysis of Molecular Variance (AMOVA) in R 4.2.2 software (R Core Team 2021) using microsatellite data and the POPPR package. This analysis provided details of the variance explained within and between samples and populations (Kamvar et al. 2014) from which we calculated a *P* value using a randomization test with 1,000 permutations. To further visualize the relationships among *Iguana* sp. subpopulations, we performed a Principal Coordinate Analysis (PCoA) using the APE package (Paradis and Schliep 2019). This was based on pairwise F_{ST} calculated between populations to estimate their divergences using the HIERFSTAT package (Goudet et al. 2015). We used the R package GGPLOT2 (Wickham and Sievert 2009) to graph the resulting PCoA, and to graphically display the pairwise F_{ST} values in a heat map matrix.

Sequences analysis.—We aligned each gene separately using the programs SEAVIEW (Gouy et al. 2010) and MEGA11 (Tamura et al. 2021). We compared the haplotypes to published GenBank records and our unpublished *Cmos* and *ND4* sequences. We compared the *Cmos* sequences obtained to published haplotypes, HM352538 (= *Cmos1*) and MK605403 (= *Cmos2*), which are differentiated by two diagnostic positions. For the *ND4* gene, we compared sequences with the two published haplotypes AF217783 (903bp) and KJ561225 (689 bp). New haplotypes were deposited in GenBank, their accession numbers are given below.

Results

Variability of microsatellite loci of the source.—We observed 22 alleles across the *I. delicatissima* subpopulations (Foun, Dfré, and Dfou), with a mean of 2.20 (± 0.79) alleles per locus with a number of alleles from one to four (Table 1). Locus L14 exhibited the highest number of alleles (n = 4). The effective number of alleles (MNE) ranged from 1.00 to 1.86, with a mean of 1.11 (± 0.26), whereas the PIC per marker ranged from 0 (L3) to 0.357 (L20) with a mean of 0.066 (± 0.107; Table 1).

Table 1. Diversity indices of the 10 loci used in this study in terms of Fragment Size (FS), Total Number of Alleles (TNA), Mean Number of Effective alleles over populations (MNE), Polymorphism Index Content (PIC), Overall inbreeding coefficient (F_{IT}), Wright's inbreeding coefficient (F_{IS}), deviations from Hardy-Weinberg equilibrium (*d*HWE), and deviations from Hardy-Weinberg equilibrium after a Bonferroni correction (*d*HWE-Bc). The abbreviations N.S. = not significant and N.A. = not applicable. One asterisk (*) means $P < 0.05$ and three asterisks (***) means $P < 0.001$.

Locus	FS (bp)	TNA	MNE	PIC	F_{IT}	F_{IS}	*d*HWE	*d*HWE-Bc
L2	206–208	2	1.02	0.026	1.000	-0.006	*	N.S.
L3	252	1	1.00	0.000	N.A.	N.A.	N.A.	N.A.
L4	174– 178	2	1.86	0.357	-0.285	0.022	***	***
L5	176– 192	2	1.01	0.013	-0.001	-0.003	N.A.	N.A.
L14	186–196	4	1.06	0.064	0.599	0.027	N.A.	N.A.
L16	180–182	2	1.02	0.026	1.000	-0.006	*	N.S.
L18	192–200	3	1.11	0.107	0.887	0.089	N.A.	N.A.
L20	178–206	2	1.02	0.026	-0.002	0.015	N.S.	N.S.
L23	300–304	2	1.02	0.026	1.000	-0.006	*	N.S.
L24	172–188	2	1.01	0.013	-0.001	-0.003	N.A.	N.A.
	Mean	2.20	1.11	0.066	0.466	0.014		
	Standard Deviation	0.79	0.26	0.107	0.533	0.031		

The inbreeding coefficient within subpopulations (F_{IS}) across loci ranged from -0.006 (L20) to 0.089 (L18), with a mean of 0.014 (± 0.031). After a Bonferroni correction, one locus (L4) deviated significantly from the Hardy-Weinberg equilibrium (Table 1). Finally, the overall inbreeding coefficient (F_{IT}) ranged from -0.285 (L4) to 1 (L2, L16, and L23) across all loci across populations (Table 1).

Microsatellites diversity of iguana populations.— We assessed the genetic variability in each subpopulation of *I. delicatissima* (Foun, Dfré and Dfou) in terms of the mean number of alleles (N_a), Wright's fixation index (F_{IS}), observed (H_O), and expected (H_E) heterozygosity deviations from Hardy-Weinberg equilibrium. Our analysis indicates that the Foun population exhibited the lowest number of alleles, whereas the Dfou population has the highest N_a value (Table 2). Expected heterozygosity (H_E) exceeded mean observed heterozygosity (H_O) in the Foun and the Dfré subpopulations. Across all populations, the mean H_O was 0.066, ranging from 0.029 (± 0.011) in Dfré to 0.118 (± 0.019) in Dfou. F_{IS} values were positive in two of the three subpopulations, ranging from -0.109 (Dfou) to 0.378 (Dfre). Finally, all subpopulations deviated significantly from the Hardy-Weinberg equilibrium, both before and after Bonferroni correction, which indicates a possible underlying population substructure (Table 2).

We used 25 putative invasive alien iguanas (Inva) from different origins, for a total of 101 iguanas, to infer genetic clustering. Based on our analysis and based on the Evanno et al. (2005) method, the most probable number of clusters is K = 2, with the highest ΔK value of 544.9 (Fig. 3). The individuals are clustered (Fig. 4). This analysis shows genetic admixture between at least one, and possibly five Dfou individuals that were captured on îletFourchue where the present-day population is a mix of iguanas present before the translocation, the descendants of introduced *I. delicatissima* and putative invasive

Table 2. Genetic variability of each subpopulation of the Lesser Antilles Iguana (*Iguana delicatissima*) in terms of sample size (n), mean number of alleles (N_a) and its standard deviation (SD), observed (H_O) and expected (H_E) heterozygosity, Wright's inbreeding coefficient (F_{IS}), deviations from Hardy-Weinberg equilibrium (*d*HWE) and deviations from Hardy-Weinberg equilibrium after a Bonferroni correction (*d*HWE-Bc). Three asterisks (***) means $P < 0.001$. The abbreviations Founder = founder iguanas, Dfou = Descendant population of iguanas (îlet Fourchue), and Dfré = Descendant population of iguanas (îlet Frégate).

		N_a		H_O		H_E				
Population	n	Mean	± SD	Mean	± SD	Mean	± SD	F_{IS}	*d*HWE	*d*HWE-Bc
Founder	27	1.20	0.42	0.052	0.013	0.068	0.047	0.243	***	***
Dfou	28	2.10	0.74	0.118	0.019	0.106	0.047	–0.109	***	***
Dfré	21	1.10	0.32	0.029	0.011	0.046	0.046	0.378	***	***

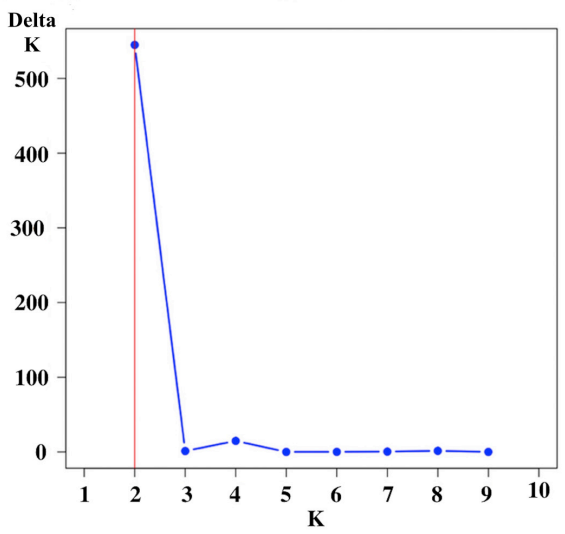

Figure 3. The relationship between K and Delta K (ΔK) used to estimate the optimal K (K = appropriate number of subpopulations). The optimal K estimated by Evanno et al. (2005) is K = 2 (ΔK = 544.9).

alien iguanas, but, in any case, not from the founder individuals.

A comparison of pairwise FST values between populations indicated that most of the genetic variation (74.8%, Σ = 3.114, *P* = 0.001) was attributed to differences among populations. This high among-population variance, however, was primarily driven by the strong genetic differentiation associated with the invasive alien lineage. When considering only the native populations, pairwise FST values remained very low, ranging from 0.0030 (Dfou versus Dfré) to 0.0096 (Foun versus Dfré), indicating weak genetic differentiation among them.

Only 14.8% of the genetic variation occurred among individuals within populations (Σ = 0.616, *P* = 0.001), while 10.4% was distributed within individuals (Σ = 0.435, *P* = 0.001; Table 3). These results are consistent with both the Principal Coordinate Analysis (PCoA) based on pairwise FST values and the heat map of pairwise FST values (Fig. 5). Furthermore, there is low genetic differentiation across the Foun, Dfou and Dfré subpopulations (Fig.3). Most of the genetic variations between populations occurred between the founders and descendant populations (Foun, Dfré and Dfou) and the putative invasive alien iguanas (Inva).

The present-day population of îlet Fourchue has five individuals with alleles that are unknown in the two founder populations: L5-192, L14-186-194-196, L16-180, L18-192, L20-178, L23-304, and L24-172. One individual has these alleles for 5 of the10 loci, two of which are homozygous. The other four iguanas have only one locus with one of these alleles in a homozygous or heterozygous state.

Cmos polymorphism.—Of the 362 bp sequences (20 founder individuals), 16 corresponded to *Cmos2* (MK605403), while four novel *Cmos* haplotypes, named *Cmos3* (GenBank accession number: PV578787), *Cmos4* (PV578788), *Cmos5* (PV578789), and *Cmos6* (PV578790), were identified in a homozygous state (Fig. 6). The seven other individuals could be *Cmos2* rather than *Cmos1* based on the first diagnostic position and the absence of variation along the sequence that differentiates the other haplotypes. Founder individuals for îlet Frégate carried *Cmos2*, and *Cmos5*, whereas these for îlet Fourchue carried *Cmos2*, *Cmos3*, *Cmos4*, and *Cmos6* (Fig. 6). For the *Cmos* nuclear gene, no heterozygous individuals were found at the new diagnostic positions (from one position between *Cmos2* and *Cmos6* to five between *Cmos2* and *Cmos3*, *Cmos3* and *Cmos6*, and *Cmos3* and *Cmos1*).

In the îlet Frégate descendant population, all sampled iguanas (n = 23) were *Cmos2* (Fig. 6). In the îlet Fourchue descendant population, 27 of 28 iguanas were *Cmos2*, and one is homozygous for a novel haplotype (IGI-*Cmos7*: PV578791). This haplotype differs by a single mutation from the non-*delicatissima* reference (HM352539) identified by Stephen et al. (2013; Fig. 6).

ND4 polymorphism.—We identified eight haplotypes in the two founder groups: two

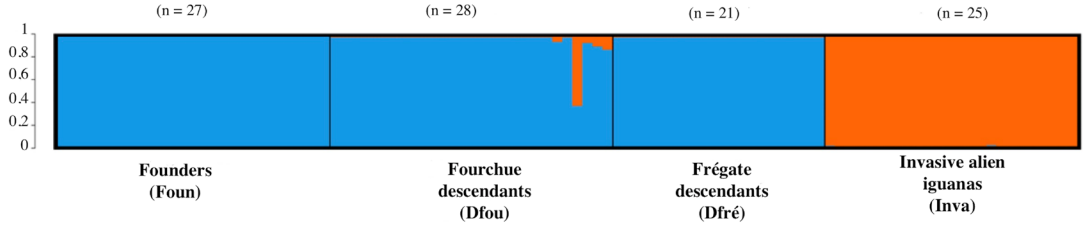

Figure 4. Bayesian model-based estimation of population structure (K = 2) for the 101 *Iguana* isolates in four predetermined populations (x-axis). Each group is separated by a black vertical line. Numbers in the y-axis show the coefficient of assignment and the sample size (n) for each group is shown above the group box.

Table 3. Results of Analysis of Molecular Variance (AMOVA) at nine microsatellite loci within all *Iguana* subpopulations (Founder iguanas, descendant population of iguanas on îlet Fourchue), descendant population of iguanas on îlet Frégate, and invasive alien iguanas). The abbreviations df = degrees of freedom and SS = sums of squares.

Source of variation	df	SS	Variance components (%)	*P*-value
Among populations	3	475.04	3.114 (74.8)	< 0.001
Among individuals within populations	97	161.86	0.616 (14.8)	< 0.001
Within individuals	101	44.00	0.435 (10.4)	< 0.001
Total	201	608.90	4.165 (100)	

previously published (*ND4.1* and *ND4.2*) and six novel haplotypes (*ND4.3–ND4.8,* GB: PV578792–PV5787971; Fig. 7). Of the 25 individuals from the present-day îlet Fourchue population, 15 were KJ561225 (*ND4.2*), seven were AF217783 (*ND4.1*), and three exhibited unique haplotype (*ND4.9–11*, GB: PV578798–PV578800; Fig.7). These three haplotypes were absent in the founder population and may represent relict lineages, unsequenced founder females, or novel immigrants. The three unique haplotypes (*ND4.4–6*) from the female founder population were not found (Fig. 7).

In the îlet Frégate founder population, five iguanas carried KJ561225 (=*ND4.2*), three iguanas had a unique haplotype (*ND4.3*, *ND4.7*, *ND4.8*), and six iguanas could not be assigned. Nineteen individuals representing the present-day îlet Frégate population were all KJ561225 (Fig. 6). Two iguanas had new haplotypes (*ND4.12*, *ND4.13*, GB: PV578801–02) that were found nowhere else. The two haplotypes (*ND4.7* and *ND4.8*) present in the founder females were not found, as was logically the case for *ND4.3* present in a founder male (Fig 7).

Discussion

Polymorphism in the St Barthélemy source population.—Mean (± standard deviation) microsatellite allelic richness (1.20 ± 0.42) in the St Barthélemy *I. delicatissima* population is lower than values reported for other Caribbean islands, such as Basse-Terre (1.47) and Petite Terre (1.80) in the Guadeloupian Archipelago (Vuillaume et al. 2015). In Dominica, Martin-Judson et al. (2018) found an allelic diversity of 2.90 using seven polymorphic microsatellites, while van den Burg et al. (2018b) reported an average of 2.00 alleles on a set of 16 microsatellites for the St Eustatius population. In the St Barthélemy source population, significant deviations from Hardy-Weinberg equilibrium may be attributable to the Wahlund effect (a reduction of heterozygosity and/or an increase of homozygosity in a population), resulting from its artificial composition of individuals from three localities with likely different genetic structures, as well as sampling bias due to its small number.

The most abundant *Cmos* haplotype in the St Barthélemy source population is MK605403 (*Cmos2*). This haplotype was also identified by Pounder et al. (2020) in 22 *I. delicatissima* from Anguilla and 10 from îlet Fourchue. The high *Cmos* variability (*Cmos2-Cmos6*) that we found is unexpected compared to the low polymorphism observed in Anguilla or other parts of the range of the species (Stephen et al. 2013; Pounder et al. 2020). *Cmos3-Cmos6* haplotypes were present only in a homozygous state. A dropout

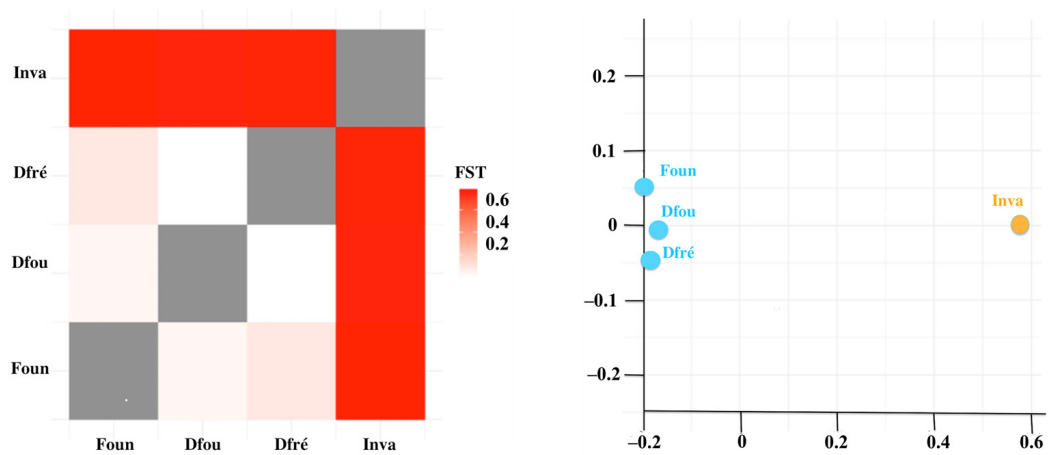

Figure 5. Comparison of pairwise FST between subpopulations of *Iguana*. (Left) The pairwise F_{ST} matrix showing F_{ST} represented by a color gradient. (Right) Principal Coordinate Analysis (PCoA) based on the genetic distance matrix of FST values for microsatellite data.

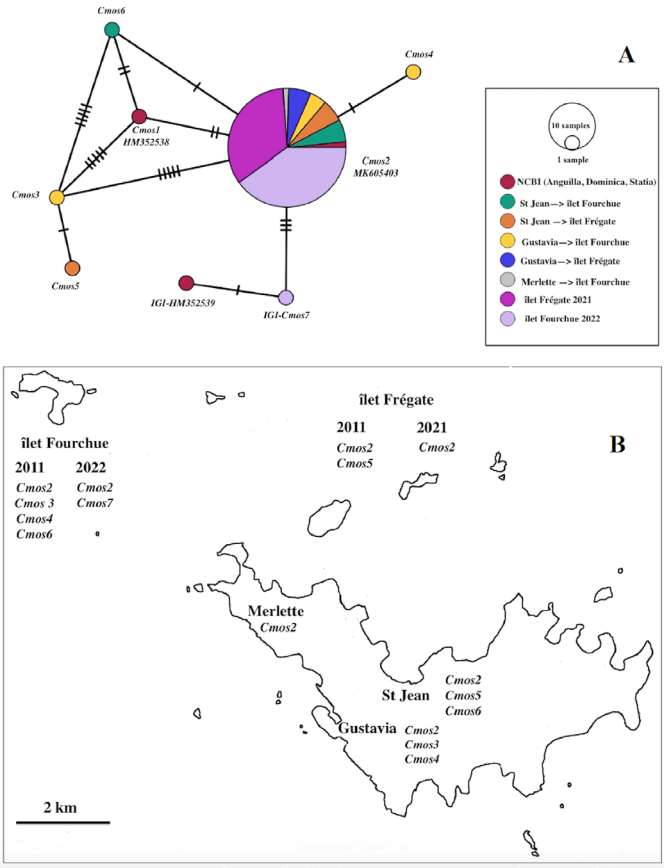

Figure 6. (A) TCS haplotype networks generated using PopArt version 1.7 for the *Cmos* partial gene used in this study and repartition of the haplotypes in the different populations of iguanas. Each circle represents an individual iguana haplotype. The diameter of the circle indicates the relative frequency of sequences belonging to a specific haplotype. Hatch marks along the branches connecting two haplotypes represent the number of mutations. The colors of the pie indicate where the haplotypes are found. (B) Distribution of the *Cmos* haplotypes in St Barthélemy, in the founder populations (2011) and in the descendant populations (2021, 2022).

effect cannot be rejected, but this hypothesis has a low probability, as hybrids between *I. delicatissima* and *I. iguana* from Guadeloupian archipelago were identified on *Cmos* at the three diagnostic positions (Stephen et al. 2013) which differentiate these two species (Michel Breuil et al., unpubl. data).

Low microsatellite diversity contrasts sharply with high mitochondrial (*ND4*) haplotype diversity (n = 8) among the 27 founder individuals. Six were unknown: three were introduced to îlet Fourchue (*ND4.4*, *ND4.5*, *ND4.6*) and three to îlet Frégate (*ND4.3*, *ND4.7*, *ND4.8*). This high mitochondrial diversity is unexpected, especially when compared to the low polymorphism found in the relict population of Anguilla, which had only one *ND4* haplotype (AF217783/KJ56121) among 34 sequenced iguanas (Stephen et al. 2013; Martin et al. 2015; Pounder et al. 2020) or in the other parts of the range of the species (Martin et al. 2015). Compared to the populations of other islands, the *I. delicatissima* population on the St Barthélemy main island did not experience the effect of recent founder effects or bottlenecks like those in Anguilla (Pounder et al. 2020), Petite-Terre and îlet Chancel (Breuil 2002), and St Eustatius (van den Burg et al. 2018a). The identification of these unique haplotypes likely reflects the result of sampling bias or rare ancestral haplotypes that have been lost in most of the population due to genetic drift. With an increase in the number of iguanas sampled per locality and an increase in the number of localities, there is a higher probability of finding rare and unidentified haplotypes.

Evolution of polymorphism on îlet Frégate.—Assuming our samples are representative, the present-day îlet Frégate population exhibits a decline in polymorphism across all markers relative to the founder population, likely driven by genetic

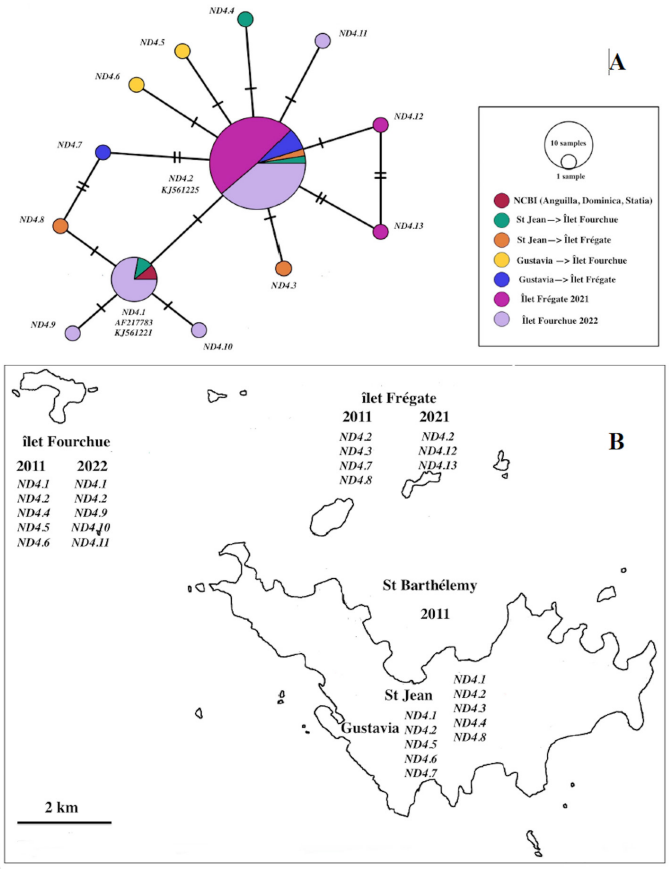

Figure 7. (A) TCS haplotype networks generated using PopArt version 1.7 for the *ND4* partial gene used in this study and repartition of the haplotypes in the different populations of iguanas. Each circle represents an individual iguana haplotype. The diameter of the circle indicates the relative frequency of sequences belonging to a specific haplotype. Hatch marks along the branches connecting two haplotypes represent the number of mutations. The colors of the pie indicate where the haplotypes are found. (B) Distribution of the *ND4* haplotypes in St Barthélemy, in the founder populations (2011) and in the descendant populations (2021, 2022).

drift. The absence of L18-200 allele in descendants suggests that the two homozygous founder females carrying this allele failed to reproduce successfully. The only polymorphic locus in this population (L4) deviated from Hardy-Weinberg equilibrium. This population is 11 y old, and overlapping generations, as well as low density that prevents panmixis, may be responsible for this situation.

All iguanas sampled in the present-day îlet Frégate population were *Cmos2*, whereas the founder population had two haplotypes (*Cmos2*, Cmos5). One founder male homozygous for *Cmos5* and/or its descendants do not appear to have reproduced. For *ND4* gene, two haplotypes (*ND4.7, ND4.8*) from the founder females were not found in the individuals sampled in 2021, with only the most common haplotypes KJ561225 (*ND4.2*) being maintained. These two females or their lineages do not seem to have reproduced.

Considering all the genetic markers, it appears that three females and one male and/or their lineages, have no descendants, assuming our samples are representative of the entire current îlet Frégate population that is impossible to catch entirely. Conversely, we found two novel *ND4* haplotypes called *ND4.12* and *ND4.13* in this present-day population that were not present in the founders. We did not find *I. delicatissima* before the translocation and the persistence of relict iguanas is unlikely. Migration from St Barthélemy and/or another nearby island is a hypothesis worth considering.

Genetic integrity, relict diversity, and loss of polymorphism on îlet Fourchue.—In contrast to îlet Frégate, the population on îlet Fourchue exhibited higher allelic richness and *ND4* haplotype diversity. This is likely due to the contribution of the relict population that existed prior to the 2011 translocation.

The identification of three *ND4* haplotypes (*ND4.9–ND4.11*) not present in the 2011 founders suggests that the original resident population possessed unique genetic lineages that have persisted. This observation highlights the importance of îlet Fourchue as a reservoir of ancestral genetic diversity that may have been lost on the main island of St Barthélemy.

Five iguanas with *I. rhinolophus* genetic markers, captured in 2022, likely represent recent arrivals or the descendant of at least an undetected hybrid. Given that *I. rhinolophus* is established on nearby St Maarten, the possibility of natural dispersal or human mediated introduction (e.g., via pleasure craft) is high. These iguanas may have arrived as the result of deliberate introductions by people, either with good or ill intentions. For instance, we observed one iguana swimming and climbing onto a pleasure boat on St Maarten in summer 2011. Transport by recreational vessels may be responsible for the arrival of invasive alien iguanas on these islets, especially because they have attractive anchor points. They could also have arrived naturally by swimming (Breuil 2002) or on vegetation rafts (Censky et al. 1998; Breuil 1999) such as those potentially generated by Hurricane Irma in 2017. Although photographs of the present-day îlet Fourchue iguanas show no morphological signs of hybridization, it is important to note that these pictures do not show all the diagnostic traits (Breuil 2013) that differentiate *delicatissima* from non-*delicatissima*. This is particularly concerning because it shows that hybrids with multiple backcrosses with *I. delicatissima* are not identifiable by a small number of diagnostic traits.

If we consider the non-problematic *I. delicatissima* individuals, the offspring show an apparent loss of polymorphism in all genetic markers used in this study, assuming our sample is representative of the entire population on îlet Fourchue. Concerning the microsatellites, at the L18 locus, the 200 allele, which was present in the homozygous state in two translocated females, was not found in the descendant population. The L4 microsatellite locus is still polymorphic, however, but not at equilibrium, showing an excess of heterozygous individuals, which was also the case in the founder population. We found only the *Cmos2* haplotype in all present-day individuals. This suggests a loss of *Cmos3*, *Cmos4*, and *Cmos6* haplotypes, which were present in the founder individuals. *Cmos3* and *Cmos4* were present in the homozygous state in two males among the founders, and *Cmos6* was found in one female.

We did not find three haplotypes (*ND4.4–6*), among the five haplotypes introduced. The current population has two haplotypes, *ND4.1* (AF217783) and (*ND4.2*) KJ561225, which are widespread in eight and 14 individuals, respectively. Surprisingly, we identified three new haplotypes (*ND4.9, ND4.10*, and *ND4.11*) in three individuals with no morphological traits from *I. rhinolophus*, even though they were not present in the founder population. These new haplotypes could represent the individuals (males, females, juveniles) that we observed on îlet Fourchue during the spring initial surveys, or migrants from nearby islands.

Population demographic evolution.—From a demographic standpoint, these two translocations can be considered successful even if we observed a loss of polymorphism. We caught 159 iguanas that were born and PIT-tagged on îlet Fourchue from 2012 to 2022, and 48 iguanas that were born and PIT-tagged on îlet Frégate from 2012 to 2021. While available data do not allow for a precise population size estimate, the actual numbers are probably > 50, which is the number of individuals believed necessary to prevent inbreeding (Hvilsom et al. 2022). Biometric data (SVL) also show that the iguanas have reached body lengths and masses comparable to those of iguanas on the main island. This suggests that food conditions are satisfactory and not currently limiting population growth. Furthermore, we found a female iguana dead in 2022 and its PIT-tag identified it as a founder and thus suggested that it had lived on the island since the 2011 translocation. Measuring an increase in population size is often the primary metric for judging the success of reintroduction programs (Ewen et al. 2012; Moseby et al. 2011); however, the long-term survival of a population is also heavily influenced by genetic diversity. In demographic terms, both populations are currently doing well. With the proposed establishment of a natural reserve to protect these two islets, we hope that the surveys of these two populations will be continued to ensure their survival and integrity.

Conclusions.—Our study has revealed that using three categories of genetic markers (mitochondrial, nuclear gene, microsatellites) with different evolution rates and different information contents, in relation to demographic and biological data, complements each other to understand the evolution of an introduced population (Skorupski et al. 2024). These three types of polymorphism are necessary to correctly interpret the presence of certain alleles, as they could be either rare alleles of a species or markers of hybridization

(this study). The use of these three categories of genetic markers allowed us to show complementary phenomena as founder effect, genetic drift, absence of reproduction of some individuals, migration, and hybridization. The development of numerous SNPs and sequencing of the mitogenome could be also interesting options to more deeply describe the species' genetic variability and to understand this divergence.

More than 10 y after these translocations, annual reproduction has occurred on both islets, and new breeding sites have allowed the populations to grow. The vegetation is luxuriant and provides enough food to support two healthy populations of iguanas, although an erosion of the genetic variability seems to have occurred in these two populations across all the genetic markers used. In a translocated population, it is important to consider that some individuals might not reproduce, even if all the introduced iguanas had reached the size of breeding adults, and even if they live for several years after their introduction, or their offspring may fail to reach adulthood or breed. Several factors could theoretically explain this situation, such as low probability of encounter, poor physiological or nutritional conditions, diseases, infections, parasites, infertility, nest destruction, or accidental mortality from falls, fights, or predation. A significant loss of genetic diversity could potentially endanger these populations, making them inbred and reducing their ability to adapt to rapid environmental changes. Nevertheless, it is also important to consider that healthy *I. delicatissima* populations, such as those of îlet Chancel (Martinique; Legouez et al. 2009) and îlets de la Petite Terre (Guadeloupe) have also experienced founder effects and numerous bottlenecks, leading to low genetic diversity but without apparent physical deformities (Breuil 2002, 2021; Vuillaume et al. 2015; unpubl. data).

We recommend that two monitoring efforts per year be conducted on îlets Fourchue and Frégate, with one just prior to the breeding season (February to April), to search only for any invasive alien iguanas and hybrids without disturbing the populations with a Capture-Mark-Recapture program, which may have dramatic consequences by stressing iguanas if conducted during the breeding period. Hybrids and alien invasive iguanas present on these islets must be captured and euthanized, as stipulated by decree N° 2011-152 of the Territorial Collectivity of St Barthélemy. Outside the breeding season, iguanas must be captured and new individuals PIT-tagged, measured (SVL, mass), and checked for parasites and contamination by the bacteria *Devriesea agamorum*.

Thus, based on our experience with translocations (Breuil 2021), including the one conducted in Anguilla (Pounder et al. 2020) with the translocation of 22 *I. delicatissima* to a satellite islet (Prickly Pear East) followed by the reinforcement of this founder population with 10 *I. delicatissima* from Dominica (Farah Mukhida et al., unpubl. data), and various approaches to addressing inbreeding in small populations, we propose a protocol tailored to the following objectives; (1) enhance the genetic diversity of populations with low genetic variation; (2) select iguanas from different cohorts for translocation to a new island or islet; (3) preserve iguanas and their genetic diversity from populations that have already hybridized; and (4) prevent the arrival of invasive alien iguanas.

To do so, we have to consider that *I. delicatissima* inhabit dry islands spanning a few dozen hectares as well as mountainous islands covering several hundred square kilometers, with rainforests. Iguanas on these islands exhibit dramatic differences in size (Breuil 2002; Bochaton et al. 2015), reflecting both their evolutionary histories and the availability and diversity of food resources. Current data (Vuillaume et al. 2015; van den Burg et al. 2021; this study) show that iguanas in the Lesser Antilles are divided into several groups distinguished by diagnostic microsatellite alleles, suggesting different demographic, evolutionary, and adaptive histories. Therefore, to best preserve the uniqueness and genetic diversity of current *I. delicatissima* populations, as well as those resulting from translocation and reinforcement programs, we propose the following guiding principles (Table 4; Appendix).

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Table 4. Guiding principles for translocations and reinforcement programs for the Lesser Antilles Iguana (*Iguana delicatissima*; see Appendix Table for detailed recommendations).

Definitions of population entity	Conservation of genetic integrity	Integrative approach to characterize populations based on:	Pathology, prevention of contamination by	Pathology, prevention of contamination by
Evolutionary Significant Units	Minimization of genetic homogenization	Microsatellites	Mites	Biosecurity protocols at marinas and boat anchorages and harbors close to islets Control and monitoring campaigns on islets twice a year
Management Units		Nuclear and mitochondrial SNPs	*Devriesea agamorum*	
		Morphology		
		Biogeography		
		Ecology		
		Demography		
		Population history		

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Appendix

Appendix Table. Guiding principles to best preserve the uniqueness and genetic diversity of current Lesser Antilles Iguana (*Iguana delicatissima*) populations, as well as those resulting from translocation and reinforcement programs.

The first step involves conducting an assessment of each present-day population based on genetic, morphological, and ecological data, followed by analysis of these data. Each population is thus characterized by its genetic structure and diversity (He, Ho, allelic richness, effective population size, inbreeding coefficient, deviation from Hardy-Weinberg equilibrium, proportion of pure and introgressed individuals, and age of hybridization), as well as by its population size, age structure, and the nature of its habitat. The second step involves establishing kinship relationships among populations using the STRUCTURE software, calculating F_{ST} in conjunction with a phylogenetic analysis based on nuclear SNPs and mitochondrial DNA, which exhibit different rates of evolution. These first two steps make it possible to define evolutionary significant units (ESUs) and management units (MUs) that identify groups of related populations.

For population reinforcement, the goal is to best conserve ESUs/MUs by prioritizing transfers between genetically closely related populations from the same type of habitat to avoid disrupting local adaptation. If possible, individuals with the highest genetic diversity should be selected for translocation. The number of individuals to be introduced depends on the size of the available population and the distribution of genetic variation among individuals. Regular genetic screening is then necessary to ensure the spread of this new genetic diversity within the reinforced population.

When translocating iguanas to establish a new population on an islet with the same ecological characteristics as the source population's habitat, it is necessary to maximize the founder population's genetic diversity, without demographically and genetically spoiling the source population, while ensuring that it is distributed across as many individuals as possible. Furthermore, it is also necessary for this polymorphism to be distributed between the two sexes and across different cohorts to minimize the effects of potential genetic drift. For reinforced and new populations, it is necessary to follow them on a demographic, physiological, and genetic aspect as suggested in our case study (see above).

Moreover, all introduced *I. delicatissima* have to be screened to look for the presence of a pathogenic bacterium (*Devriesea agamorum*) with fatal consequences, particularly in males but also for mites. This bacterium causes spectacular infections (large, hard nodules and abscesses), particularly where the males bite each other during fights (Breuil., unpubl. data; Hellebuyck et al. 2017). This bacterium was identified after the translocations to îlets Frégate and Fourchue. To prevent contamination between the different islands of the Lesser Antilles, it is mandatory to disinfect all materials and contributors' shoes and clothes used during monitoring campaigns across all the Lesser Antilles. It would also be necessary to check for this bacterium in invasive alien iguanas, particularly in St Maarten, to determine if they are healthy carriers that could contaminate *I. delicatissima* throughout the Lesser Antilles.

Faced with the increasingly uncontrolled proliferation of invasive alien iguanas, the numerous hybridizations between different lineages, as seen in Martinique and St Martin (Michel Breuil et al., unpubl. data), increases their genetic diversity and their ability to become increasingly ubiquitous invaders. Our observations, which began in the late 1980s, show that no island or islet is safe from their arrivals. Strict monitoring of these islands and islets is essential, but consideration must also be given to constructing biosecure enclosures within them to prevent the arrival not only of these invasive alien iguanas but also of their predators, particularly rats, dogs, mongooses, raccoons, and cats. Strict biosecurity protocols must be implemented at marinas and popular boat anchorages on satellite islets. Educational outreach targeting pleasure craft operators, regular monitoring of mooring bays, and rapid response eradication networks are mandatory to prevent-human-mediated arrivals of invasive alien iguanas.

As highlighted by Norén and Hassegren (2024), translocation is a tool for genetic rescue, but it is associated with risks. Kyriazis et al. (2021) proposed using animals, for genetic rescue, from moderately sized source populations where recessive deleterious mutations have been purged. We suggest that healthy homozygous adults with no phenotypic defects suggesting that deleterious mutations are present in a homozygous state, and with original haplotypes must be preferred for genetic rescue over heterozygous individuals to prevent introduction of alleles with weakly or deleterious mutations that will become homozygous in small populations and lead to inbreeding depression and extinction. Ronce (2014) considered that the regular arrival of one migrant per generation in small populations with high genetic load is sufficient to replenish genetic variation and counteract the effect of genetic drift, while still preserving local adaptation. The presence of unknown haplotypes in the descendant population of îlet Frégate shows that new bloodlines can arrive from other nearby populations. Thus, the St Barthélemy archipelago functions as a potential metapopulation where occasional natural dispersal between islands and islets may occur. Conservation efforts must therefore balance the need for gene flow among *I. delicatissima* populations with rigorous biosecurity to exclude invasive alien congeners. We suggest that natural displacements between islands with similar natural selection pressure (e.g. dry flat islands vs moist, mountainous islands) may be preferable to human-mediated actions with iguanas from distant localities living in different habitats, with different adaptations as it was realized in Prickly Pear (Anguilla) with ten *I. delicatissima* coming from Dominica (Pounder et al. 2020; Farah Mukhida et al., unpubl. data). Such translocations can lead to a loss of fitness, modification of its genetic signature, and perhaps extinction of the population being saved, even if at first, the population is growing.