Population genetics of Galápagos land iguana (genus *Conolophus*) remnant populations

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Abstract

The Galápagos land iguanas (genus *Conolophus*) have faced significant anthropogenic disturbances since the 17th century, leading to severe reduction of some populations and the extinction of others. Conservation activities, including the repatriation of captive-bred animals to depleted areas, have been ongoing since the late 1970s, but genetic information has not been extensively incorporated. Here we use nine species-specific microsatellite loci of 703 land iguanas from the six islands where the species occur today to characterize the genetic diversity within, and the levels of genetic differentiation among, current populations as well as test previous hypotheses about accidental translocations associated with early conservation efforts. Our analyses indicate that (i) five populations of iguanas represent distinct conservation units (one of them being the recently discovered *rosada* form) and could warrant species status, (ii) some individuals from North Seymour previously assumed to be from the natural *Baltra* population appear related to both Isabela and Santa Cruz populations, and (iii) the five different management units exhibit considerably different levels of intrapopulation genetic diversity, with the Plaza Sur and Santa Fe populations particularly low. Although the initial captive breeding programmes, coupled with intensive efforts to eradicate introduced species, saved several land iguana populations from extinction, our molecular results provide objective data for improving continuing *in situ* species survival plans and population management for this spectacular and emblematic reptile.

Keywords: Galápagos land iguanas, microsatellites, population management, population structure

Introduction

Management of endangered populations and species is an important and pressing challenge. Indeed, 15 589 species are listed as ‘threatened’ by the World Conservation Union (IUCN) (Baillie et al. 2004), and it is estimated that human activities have globally increased species loss rates by a factor of 1000–10,000 (Stuart 1999). The situation is particularly acute for island populations, which are even more susceptible to extinction than their mainland relatives (Frankham 1987; Frankham 1998). Into this category falls the Galápagos land iguanas (genus *Conolophus*) that are endemic to the Galápagos Archipelago and whose numbers have severely declined in the past century.

The Galápagos land iguanas form the sister taxon of the Galápagos marine iguanas (*Amblyrhynchus cristatus*), from which they diverged 10 million to 20 million years ago, as suggested by analyses of osteological, immunological, and mitochondrial DNA data (Wyles & Sarich 1983; de Queiroz 1987; Sites et al. 1996; Rassmann 1997). Given that the present-day islands are < 1 million to 4 million years old,
the split between Galápagos land and marine iguanas either occurred on older, now submerged, islands of the Archipelago following a single colonization event from the mainland (Rassmann 1997), or the two genera may have split prior to colonizing the islands. Despite their ancient divergence, marine and land iguanas are still capable of hybridizing and producing viable offspring.

Land iguanas comprise two recognized species: Conolophus subcristatus, which naturally inhabits five islands (Plaza Sur, Baltra, Santa Cruz, Isabela, and Fernandina), and C. pallidus, which is restricted to the island of Santa Fe (Fig. 1). In addition, there are three translocated populations of C. subcristatus (Islote Caamano, Islote Venecia, and Isla Seymour Norte; Snell et al. 1984, 1996). Contrary to Galápagos marine iguanas, for which the level and pattern of genetic differentiation has been investigated using mitochondrial and nuclear data (Rassmann et al. 1997b; Steinfartz & Glaberman 2007; Parent et al. in press), no extensive population analysis based on molecular genetics data has been published for land iguanas. However, morphological analyses (Snell et al. 1984) challenged current classification and identified three possible distinct groups that may warrant species status: animals from the eastern island of Santa Fe, the central islands (Santa Cruz, Baltra and Plaza Sur) and the western and youngest islands (Isabela and Fernandina), respectively. A preliminary molecular genetic analysis based on a 400-bp fragment of the mitochondrial cytochrome b gene from 94 individuals (Rassmann et al. 2004) indicated a lack of shared haplotypes among these three locations. Note, however, that this criterion is not sufficient for species designation as it ignores relationships among haplotypes and variation within groups (e.g. Milinkovitch et al. 2002).

History of land iguana populations

Most populations of land iguanas, perhaps with the exception of the ones of Fernandina and Plaza Sur (Snell et al. 1984), are experiencing, or have experienced, various anthropogenic disturbances since the arrival of buccaneers in the 17th century, and whalers and settlers in the early 1800s. Besides human predation, introduced animals, such as goats, burros, pigs, cats, dogs and rats, represent a great threat, as they compete with iguanas for food, destroy their habitat and/or prey on them. The impact of predation on iguanas is particularly pronounced because both juveniles and adults are vulnerable, which is not the case, for example, for the Galápagos giant tortoises (Geochelone nigra) facing similar threats in terms of competition and habitat destruction but which escape predation once they reach a sufficient size (Cayot et al. 1994).

Baltra and Seymour Norte. The iguanas of Baltra were extirpated by humans between 1944 and 1954, following the construction of an American military airbase. Fortunately, about 70 Baltra individuals were transferred as an experiment
in 1932 and 1933 to Seymour Norte, a nearby island where land iguanas did not occur naturally (Snell et al. 1984), and were used, in the early 1990s, as a source population for repatriation campaigns to Baltra (Hofkin et al. 2003). In 1997, the Baltra population size was estimated to > 97 (Charles Darwin Foundation 2008). Several aspects of morphological and ecological variation among individuals of *C. subcristatus* inhabiting Seymour Norte, combined with verbal accounts of necessary experimental translocations occurring in late 1976 and early 1977, led to hypotheses that the Seymour Norte population might represent a mixture of iguanas from Baltra and other populations (Hofkin et al. 2003).

Unfortunately, most records of the early conservation activities associated with land iguanas were lost to a fire at the Charles Darwin Research Station in 1984 (Smith 1990), thwarting efforts to absolutely confirm the complete nature of all experimental translocations that may have occurred.

Santa Cruz. The Santa Cruz land iguana population of about 1000 individuals (Conway Bay and Cerro Dragón) was nearly exterminated within less than 1 year in 1976 by feral dogs (Snell et al. 1984; Snell 1994). The 56 remaining iguanas found at Cerro Dragón were transferred to the Charles Darwin Research Station (CDRS) to initiate a captive breeding programme. Furthermore, in 1977, 38 of these individuals were moved to the islets of Venecia, on the northwestern coast of Santa Cruz, where they have been reproducing since then in semi-captive conditions (Snell 1994).

Isabela. As on Santa Cruz, the population of Cartago Bay faced a serious population reduction in the 1970s due to feral dogs. Most other populations of Southern Isabela had previously been extirpated by feral dogs and pigs (Snell et al. 1984). Thirty-seven remaining animals were brought to the CDRS in 1976 to initiate a breeding programme separated from that of the Santa Cruz population. Dogs in the area were eradicated in 1982, and 390 F₁ individuals generated by the Isabela breeding programme were released back to Cartago Bay. Several other Isabela populations, more or less impacted by introduced animals and recent volcanic eruptions, seem isolated from another by lava flows. In particular, a population of phenotypically distinct land iguanas, locally referred to as the *rosada form*, due to its spectacular pink colouration (G. Gentile et al., submitted), occurs in some areas of Volcan Wolf, and has been recorded only since 1986.

Santa Fe. The Santa Fe population has been impacted by human predation and food competition with goats, but the population seems to have stabilized since the eradication of goats in 1972 (Snell et al. 1984; Snell & Christian 1985).

Other islands. Whereas the populations of Plaza Sur and Fernandina probably remained undisturbed by human activities, *C. subcristatus* went extinct on Santiago, as already recorded in 1906 (Van Denburgh & Slevin 1913), most likely due to competition with, and predation by, feral pigs (see Snell et al. 1984). Three or fewer *C. subcristatus* individuals remain on Isote Caamaño, a small islet in Academy Bay, Isla Santa Cruz, apparently translocated there from Plaza Sur in the 1960s (Snell et al. 1984, T. de Roy, personal communication).

Clearly, past captive breeding programmes, coupled with intensive efforts to eradicate exotic species, saved several land iguana populations from extinction. In total, more than 700 captive-bred land iguanas have been repatriated on the sites of Baltra, Isabela and Santa Cruz. Their survival to adult size has exceeded an average of 40% for the three populations and *in situ* reproduction occurs at all three sites (Reynolds 1983; Fritts et al. 2000). However, the captive breeding programmes and the reintroduction plans did not rigorously incorporate pedigree information or molecular genetic data, such that the level of genetic diversity in the captive and repatriated populations was unknown. This situation might be paralleled to that of the Galápagos giant tortoise population found on the island Española which has experienced massive repatriation efforts (Cayot et al. 1994; Fritts et al. 2000). However, molecular genetic analyses indicated that (i) contributions of the Española breeders were highly skewed, leading to a loss of genetic variation that, in turn, might be detrimental to the long-term survival of the tortoise population (Milinkovitch et al. 2004), and (ii) the repatriated population has been contaminated by at least one trans-island hybrid (Milinkovitch et al. 2007) in addition to the erroneously repatriated ‘dome’ tortoise identified morphologically in the late 1970s.

Here, we investigate genetic variation at nine nuclear microsatellite loci among 703 land iguanas from all islands where they occur today. We also genotyped 20 marine iguanas at the same loci for comparison. Molecular data could prove of paramount interest for improving management of *ex situ* captive populations and for guiding the development of proper *in situ* population survival and habitat management plans for this spectacular reptile.

**Materials and methods**

**Sample collection and genotyping**

Between 1997 and 2006, 703 blood samples were collected from all islands where land iguanas occur: Santa Fe (117 samples), Baltra and Seymour Norte (37), Plaza Sur (122), Santa Cruz (212), Isabela (163), and Fernandina (51). Further information on sampling locations is given in Fig. 1. Note that 20 marine iguanas were also sampled in Plaza Sur and genotyped.
Each sample (about 2 mL of blood) was collected from the caudal or brachial vein and preserved in 10 mL lysis buffer (100 mM Tris, 100 mM EDTA, 2% SDS). Total genomic DNA was extracted from blood using the DNeasy Tissue Kit (QIAGEN) according to the manufacturer’s protocol and its quality was checked by agarose gel electrophoresis. Nuclear DNA variation was assessed at nine species-specific microsatellite loci isolated and characterized in Rosa et al. in press) from a genomic library of a Conolophus subrictatus individual from Plaza Sur. All samples were genotyped using conditions given in Rosa et al. (in press) and PCR products were separated by electrophoresis using an ABI 3100 or ABI 3730 sequencer (Applied Biosystems).

Genetic diversity and demographic history

We used the software Convert (Glaubitz 2004) for computing allele frequencies per population per locus and identifying private and diagnostic alleles. The former are alleles present in one population and absent from all others, whereas the latter are private alleles with a frequency > 10%; diagnostic alleles provide a more stringent criterion, reducing chances of confounding allele classification with possible genotyping errors. We used fstat version 2.9.3 (Goudet 1995) to calculate allelic richness per locus and per population according to the smallest number of individuals typed. Using Arlequin version 3.1 (Excoffier et al. 2005), we (i) tested for departure from Hardy–Weinberg (H-W) equilibrium per locus and per ‘sampling unit’ using an exact test (Guo & Thompson 1992) based on 900 000 Markov chain iterations, (ii) tested for independence within each pair of loci using a likelihood-ratio statistic, whose distribution was obtained by a permutation procedure (Slatkin & Excoffier 1996), and (iii) computed observed and expected heterozygosity levels, as well as gene diversity, according to Nei (1987). Critical significance levels for multiple testing were corrected according to the Bonferroni procedure (Rice 1989).

To investigate the impact of recent demographic events on gene diversity, we first used the heterozygosity excess test (Cornuet & Luikart 1996), based on the observation that allelic diversity decreases faster than heterozygosity in recently bottlenecked populations, as implemented in Bottleneck version 1.2.02 (Piry et al. 1999). We used the two-phase model (Di Rienzo et al. 1994) with 20 000 replications, 5% of multistep mutations, and variance among multiple steps of 12, as recommended for microsatellites (Piry et al. 1999). Statistical significance was assessed with a one-tailed Wilcoxon signed rank test, the most powerful and robust statistics when using less than 20 polymorphic loci (Piry et al. 1999). Second, we also used Bottleneck to examine the distribution of the alleles frequencies for a so-called ‘mode-shift’ that discriminates recently bottlenecked from stable populations (Luikart et al. 1998). Finally, we computed M, the mean ratio of the number of alleles at a given locus to the range in allele size, a statistics that can detect population bottlenecks (Garza & Williamson 2001). Indeed, the magnitude of the decrease in M is positively correlated with the severity and duration of the reduction in population size. The ratio was calculated using Arlequin version 3.1.

Population structure

Individuals were assigned to populations based on their multilocus genotypes using a Bayesian model-based clustering method (Pritchard et al. 2000) as implemented in Structure version 2.2. This version of the software also accounts for genotypic ambiguities, such as the possible presence of null alleles (Falush et al. 2007). Analyses were performed under the ‘admixture’ model, an approach tolerating mixed ancestry of individuals. Given that no information is available on the migration rates and evolutionary relationships among populations, we tested both the use of correlated and independent allele frequencies. We performed five independent runs for each value of K (ranging from one to eight when using individuals from all islands or from one to six when using individuals from a single island) with $5 \times 10^6$ iterations and a burn-in of 50 000. The number of populations best fitting our data set was defined both using log probabilities $\log P(X|K)$ and $\Delta K$, as described in Evanno et al. 2005. The latter statistics compare the rate of change in the log probability of data between successive K and the corresponding variance of log probabilities.

F- and R-statistics within and among the groups defined by (i) geographic location, (ii) morphological studies (Snell et al. 1984) and (iii) the Structure version 2.2 analyses using our microsatellite data, were calculated using Arlequin version 3.1 and tested for statistical significance with 20 000 permutations. An allele-size permutation test, as implemented in SPAGeDI (Hardy & Vekemans 2002), was employed to clarify if allele sizes are informative with respect to population differentiation (Hardy et al. 2003). Differentiation among populations was quantified using a hierarchical analysis of variance (AMOVA) using Arlequin version 3.1.

Results

Genetic diversity and demographic history

Deviation from H-W equilibrium was observed for five and seven loci of the Santa Cruz and the Isabela populations, respectively. H-W deviation in Isabela is likely due to the presence of subpopulations (see below), whereas the Santa Cruz population does not fit expectations of random mating in a large population as all individuals originate from the few founders of the captive breeding programme. There is no evidence of linkage disequilibrium among loci. Different populations exhibit different levels of polymorphisms (computed across the nine microsatellite
all diversity indices indicate that Fernandina and Isabela are the most polymorphic populations, Santa Fe and Plaza Sur are the least polymorphic, whereas Santa Cruz and Baltra show intermediate values (Table 1). The much smaller overall observed \( H_O = 0.47 \) than overall expected heterozygosity \( H_E = 0.77 \) suggests the presence of population structure. All island populations, except Baltra and Plaza Sur, exhibit private alleles although most of them are low frequency alleles. Note that more than half of the private alleles in Isabela belong to individuals of the \textit{rosada} form (Table 1 and see below).

Bottleneck analyses did not evidence any significant reduction of population size, neither using the allele-distribution mode-shift criterion nor the one-tailed Wilcoxon signed rank test for heterozygosity excess. On the other hand, Garza-Williamson index values \( (M \text{ in Table 1}) \) are low, ranging from 0.33 to 0.43 across populations, suggesting past population size reductions; case studies presented in Garza \textit{et al.} (2001) indicated \( M \) values > 0.8 for unperturbed populations and values of about 0.6–0.7 for reduced or island populations.

### Population structure

Analyses of morphological data (Snell \textit{et al.} 1984) and of a few mitochondrial haplotypes (Rassmann \textit{et al.} 2004) both suggested the existence of discrete land iguana populations that may even warrant species status. Our Structure version 2.2 analyses, based on the nine microsatellites genotyped in all 703 individuals sampled on all islands where Galápagos land iguanas occur today, indicate an optimal clustering into four distinct groups (both using correlated and independent allele frequencies) (Fig. 2a, b), with an average

<table>
<thead>
<tr>
<th></th>
<th>( N )</th>
<th>( A )</th>
<th>( AR )</th>
<th>( H_O )</th>
<th>( H_E )</th>
<th>( P )</th>
<th>( D )</th>
<th>( M )</th>
<th>Wilcoxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>722</td>
<td>6.4</td>
<td>7.64</td>
<td>0.47</td>
<td>0.77</td>
<td>—</td>
<td>—</td>
<td>0.37</td>
<td>—</td>
</tr>
<tr>
<td>Baltra</td>
<td>37</td>
<td>4.7</td>
<td>4</td>
<td>0.56</td>
<td>0.54</td>
<td>0</td>
<td>0</td>
<td>0.33</td>
<td>0.92</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>212</td>
<td>6.4</td>
<td>5</td>
<td>0.67</td>
<td>0.68</td>
<td>4</td>
<td>3</td>
<td>0.40</td>
<td>0.46</td>
</tr>
<tr>
<td>Isabela</td>
<td>148</td>
<td>10.3</td>
<td>7.05</td>
<td>0.70</td>
<td>0.75</td>
<td>10</td>
<td>0</td>
<td>0.43</td>
<td>0.92</td>
</tr>
<tr>
<td>\textit{rosada}</td>
<td>15</td>
<td>3.8</td>
<td>3.78</td>
<td>0.39</td>
<td>0.41</td>
<td>11</td>
<td>6</td>
<td>0.35</td>
<td>0.90</td>
</tr>
<tr>
<td>Fernandina</td>
<td>51</td>
<td>8.7</td>
<td>7.01</td>
<td>0.75</td>
<td>0.76</td>
<td>1</td>
<td>0</td>
<td>0.42</td>
<td>0.59</td>
</tr>
<tr>
<td>Plaza Sur</td>
<td>122</td>
<td>4.6</td>
<td>2.21</td>
<td>0.27</td>
<td>0.28</td>
<td>0</td>
<td>0</td>
<td>0.29</td>
<td>0.99</td>
</tr>
<tr>
<td>Santa Fe</td>
<td>117</td>
<td>3.6</td>
<td>2.29</td>
<td>0.13</td>
<td>0.13</td>
<td>3</td>
<td>1</td>
<td>0.35</td>
<td>0.99</td>
</tr>
<tr>
<td>Marine</td>
<td>20</td>
<td>6.53</td>
<td>5.63</td>
<td>0.54</td>
<td>0.56</td>
<td>16</td>
<td>3</td>
<td>0.37</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 2  Pairwise $R_{ST}$ (lower diagonal) and $F_{ST}$ (upper diagonal) values between populations identified by the Structure analyses. ‘Isa + Fer’ represents the group of [Isabela (without rosada) + Fernandina]. The Isaabella vs. Fernandina $R_{ST}$ and $F_{ST}$ values are 0.007 and 0.02, respectively. All values are highly significant ($P < 0.001$) except for the pair Fernandina vs. Isabela.

<table>
<thead>
<tr>
<th></th>
<th>Isa + Fer</th>
<th>Baltra</th>
<th>Santa Cruz</th>
<th>rosada</th>
<th>Plaza Sur</th>
<th>Santa Fe</th>
<th>Marine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isa + Fer</td>
<td>0.17</td>
<td>0.15</td>
<td>0.24</td>
<td>0.39</td>
<td>0.45</td>
<td>0.24</td>
<td></td>
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<tr>
<td>Baltra</td>
<td>0.18</td>
<td>0.15</td>
<td>0.42</td>
<td>0.50</td>
<td>0.69</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>0.18</td>
<td>0.15</td>
<td>0.26</td>
<td>0.33</td>
<td>0.45</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>rosada</td>
<td>0.94</td>
<td>0.93</td>
<td>0.92</td>
<td>0.64</td>
<td>0.78</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Plaza Sur</td>
<td>0.54</td>
<td>0.59</td>
<td>0.37</td>
<td>0.96</td>
<td>0.76</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Santa Fe</td>
<td>0.53</td>
<td>0.78</td>
<td>0.53</td>
<td>0.98</td>
<td>0.83</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Marine</td>
<td>0.54</td>
<td>0.33</td>
<td>0.49</td>
<td>0.85</td>
<td>0.60</td>
<td>0.81</td>
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</table>

Table 3  Results of the AMOVA tests for five different clustering hypotheses: Morpho, three groups [Santa Fe vs. (Isabela + Fernandina) vs. (Santa Cruz + Plaza Sur + Baltra)] recognized by analyses of scale morphology; Islands, one group for each of the six islands; Microsats, five groups [Santa Fe vs. Plaza Sur vs. Santa Cruz vs. (Isabela + Fernandina) vs. Baltra] as defined by the Structure analyses of multilocus microsatellite genotypes; Microsats & rosada, same grouping as Microsats but the rosada form is treated as an additional group; and With Marine, same grouping as Microsats & rosada but a group of marine iguanas is included in the analysis. The global $R_{ST}$ is given.

<table>
<thead>
<tr>
<th></th>
<th>Morpho</th>
<th>Islands</th>
<th>Microsats</th>
<th>Microsats &amp; rosada</th>
<th>With Marine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>28.47</td>
<td>32.65</td>
<td>36.74</td>
<td>64.87</td>
<td>63.89</td>
</tr>
<tr>
<td>Among individuals within populations</td>
<td>71.53</td>
<td>67.35</td>
<td>63.26</td>
<td>35.13</td>
<td>36.11</td>
</tr>
<tr>
<td>Global $R_{ST}$</td>
<td>0.28</td>
<td>0.33</td>
<td>0.33</td>
<td>0.65</td>
<td>0.64</td>
</tr>
</tbody>
</table>

assignment probability of 0.95. One of the four groups corresponds to the Santa Fe population, an expected result given that these animals have long been considered as a separate land iguana species (Conolophus pallidus). The remaining three groups are much more unanticipated as they consist of (i) Plaza Sur, (ii) (Isabela + Fernandina + Baltra), and (iii) Santa Cruz individuals. Note that the clustering of Baltra with (Isabela + Fernandina) is less supported in 3 out of 10 Structure runs performed with $K = 4$. In these cases (e.g. Fig. 2b), the 37 Baltra/Seymour Norte individuals are still assigned with the highest probability (65%) to (Isabela + Fernandina) but also with 30% to Santa Cruz.

When each island is analysed separately, the presence of a single population best explains the data, with the exception of Isabela, where an optimal clustering into two groups is detected (Fig. 2a, c). Surprisingly, these two groups consist of (i) 15 animals sampled at Piedra Blanca and (ii) all remaining Isabela (yellow) individuals. Note that a very significant differentiation of the Piedra Blanca group has also been suggested by the analysis of molecular, morphological, and behavioural data (G. Gentile et al. submitted). These 15 individuals have been assigned to the rosada form.

The validity of the clusters identified by Structure was further evaluated by computing $F_{ST}/R_{ST}$ values. $R_{ST}$ was found significantly greater than $pR_{ST}$ (the latter being computed after allele-size permutation), suggesting that allele sizes are informative with respect to genetic differentiation and that the mutation process follows at least partially a stepwise mutation model (SMM); thus, $R_{ST}$ provides a better estimate of gene flow in the case of Galápagos land iguanas. Table 2 shows pairwise $R_{ST}$ values between various land iguana groups. Note that all values are significant ($P < 0.001$). Interestingly, the rosada group is the most differentiated as it even exhibits pairwise $R_{ST}$ values larger than when comparing marine iguanas with any population of land iguanas. Note that the rosada population is distinct from the remaining populations mainly due to the numerous private/diagnostic alleles at one single locus. This probably explains why the Structure software (minimizing H-W and linkage disequilibria) does not assign the 15 rosada samples to a separate population when all samples from all islands are jointly analysed.

The results of the AMOVA tests are presented in Table 3 for four different clustering hypotheses. A closer look at the variation distribution clearly shows that low percentage values are attributed to ‘among populations’ for the clustering hypotheses based exclusively on either scale morphology (Morpho; Snell et al. 1984), or multilocus microsatellite genotypes (Microsats; this study), or island assignment (Islands), indicating that the AMOVA tests do not fully capture population structure unless the rosada form individuals are considered separately (‘among populations’ variation increases from ~30 to 65%). The addition of
marine iguana genotypes makes little difference. Global $R_{ST}$ and their range show a similar pattern: low levels for *Morpho*, *Microsats*, and *Islands* clustering hypotheses, and a considerable increase for the ‘with rosada’ clustering, with marine iguanas included or not.

**Discussion**

In the past two centuries, land iguana populations throughout the Galápagos Archipelago have been confronted with numerous threats, sometimes resulting in severe population declines. Conservation activities based on captive breeding have been established and saved several populations from extinction. However, information crucial for population management, such as population genetic structure within the genus *Conolophus* and the levels of genetic diversity displayed by these perturbed populations, was largely unknown. Here, using nine species-specific microsatellite loci, we analysed samples from 703 individuals from all islands where *Conolophus* persists today. Using a Bayesian model-based clustering method, we identify four clusters corresponding to Santa Fe, Plaza Sur, (Isabela + Fernandina) and Santa Cruz. The surprising differentiation of the Plaza Sur population is strongly supported by the molecular analyses, whereas the Baltra/Seymour Norte population exhibits mixed ancestry with both (Isabela + Fernandina) and Santa Cruz populations (Fig. 2b), a result that probably reflects the true recent history of the mixed Seymour Norte population as a consequence of undocumented translocations of individuals in late 1976 and early 1977, at a time when iguanas from Cerro Dragon on Isla Santa Cruz and Bahia Conway on Isla Isabela were removed from the field where large numbers of iguanas were rapidly being killed by feral dogs. These molecular genetic results confirm hypotheses of accidental translocations of iguanas to Seymour Norte (Hofkin et al. 2003).

Finally, the striking differentiation of 15 animals sampled at the most northern part of Isabel is remarkable. The distinctness of these iguanas, dubbed the *rosada form*, has also been suggested by mitochondrial, morphological, and behavioural data (G. Gentile et al. submitted). Clearly, the status of the various land iguana populations should be carefully reconsidered. For example, the Plaza Sur population and *rosada form* might warrant separate species status (although this result would require confirmation using phylogenetic approaches) as their levels of differentiation approach or exceed those of the Santa Fe recognized separate species of land iguana, or even that of the marine iguana.

**Correlation of genetic data and population history**

As Fernandina and Plaza Sur populations have not been exposed to a significant level of predation or competition by introduced species, both islands were expected to exhibit high levels of genetic diversity, a condition indeed observed for Fernandina but not for Plaza Sur. A possible explanation would be that El Niño events have a higher impact on Plaza Sur because of the island’s small size (12 ha) and dense population (53 iguanas/ha) (Snell & Christian 1985), causing larger fluctuations in population size than on larger islands. Indeed, the heavy rainfall of an El Niño event first favours the growth of the prickly pear cactus *Opuntia*, the main source of food of land iguanas, causing the expansion of the population of iguanas. After the El Niño event, the increased population size of iguanas and the shortage in rainfall may cause the *Opuntia* population to decline, potentially resulting in a later reduction of the land iguana population. If Plaza Sur, given its size and topography, is particularly impacted by El Niño events, recurrent occurrences of this climatic phenomenon might indeed explain the low level of genetic diversity, and the high degree of differentiation, of the current Plaza Sur population.

The population of Isabela displays a high diversity level despite the fact that several central populations have been threatened for over 100 years since the introduction of feral dogs (Kruuk & Snell 1981; Snell et al. 1984). Although our Structure analyses only discriminate the *rosada form*, high diversity levels in Isabela could be due to hidden population structure, as demonstrated in the giant Galápagos tortoises (Ciofi et al. 2006). This point warrants further investigation.

Baltra and Santa Cruz populations display intermediate levels of diversity (Table 1) despite their recovery from 70 and 56 individuals, respectively, after quasi-extinction events (see Introduction). The lowest diversity level is in fact exhibited by the Santa Fe population, an observation that can be explained by two parameters: this population (i) has been largely affected before 1970 by human predation and competition with goats (Snell et al. 1984; Snell & Christian 1985), and (ii) has not been restored (contrary to those of Baltra, Santa Cruz and Isabel) by individuals generated by captive-breeding programmes.

Our analyses suggest that the situation on Plaza Sur and Santa Fe should be investigated further, so as to identify the causes of low levels of genetic diversity. Even if these populations are not under immediate threat, reduced genetic variation and low effective population size can make the population more vulnerable to environmental changes (including infectious diseases), demographic stochasticity, or human-related disturbances (Leberg & Firmin 2008). Although Plaza Sur, Santa Fe, Baltra and Santa Cruz have undergone severe bottlenecks in the past, our inability to detect bottlenecks using allele-distribution mode-shift might be due either to the lack of power of this approach or to the possibility that these demographic events are too recent to have exerted their effect on genetic diversity. On the other hand, the Garza–Williamson index ($M$) exhibits low values for all islands, pinpointing to the probably higher sensitivity of the particular index.
Genetic differentiation among and within islands

The degree of genetic differentiation found among the islands where land iguanas occur today is surprisingly high in comparison with that observed between land and marine iguanas. Comparison among land iguana populations records $R_{ST}$ values ranging from 0.007 (Fernandina vs. Isabela) to 0.98 (rosada vs. Santa Fe). Differentiation among land iguana populations is also higher than that observed among marine iguanas from different islands ($F_{ST} = 0.04–0.18$, Rassmann et al. 1997a). This difference in genetic differentiation can obviously be explained in terms of differences in habitat: while marine iguanas are potentially able to easily migrate among different islands, land iguanas cannot swim (H. Snell, personal observation). It is, therefore, likely that land iguanas can only cross water, even a few hundreds metres, through rafting. Note that the Komodo dragon, which presents even a few hundreds metres, through rafting. Note that the Komodo dragon, which presents

patterns and inter-island migration aptitudes similar to those of land iguanas, displays a similar level of differentiation among islands ($R_{ST} = 0.002–0.8$; Ciofi et al. 2006).

The single not significant pairwise $R_{ST}$ value corresponds to the comparison of Isabela and Fernandina (Table 2). This result is in agreement with preliminary mitochondrial DNA analyses (Rassmann et al. 2004; G. Gentile et al. submitted) and the islands’ geographic history: these are the two youngest islands (< 1 million years) and Fernandina was probably colonized by Isabela individuals. More mysterious is the evolutionary history of the rossada form, as it is found on a young island but is greatly differentiated from all other populations. A possible explanation is that these individuals originate from a now-extinct population from another island. Besides the obvious candidates — Santiago, that has been inhabited by iguanas, and Pinzon, that lies between Santa Cruz and Isabela — other islands, now-submerged due to fluctuating sea levels, might have hosted land iguanas in the past. Figure S1 (Supporting Information) shows the additional islands and the expansion of current land masses caused by a 100-m decrease in sea level, similar to the one that took place during the latest glaciation (Haq et al. 1987).

The overall level of differentiation found for the microsatellite markers ($R_{ST} = 0.65$) is lower than the one for mitochondrial DNA sequence ($F_{ST} = 0.92$) (Rassmann et al. 2004), as also observed for marine iguanas (Rassmann et al. 2004). Such discrepancies may be explained not only by the different modes and tempo of evolution of these markers, but also by the reproductive biology and biogeographic history of the species: as suggested by Caccone et al. (2004) for the giant Galápagos tortoises, if females are able to store sperm from different males, a female colonizing a new location would carry only one mitochondrial lineage and $2 + 2N$ nuclear copies, where $N$ is the number of males who fertilized the female.

In conclusion, our analyses of C. ololophus using microsatellite data suggest that (i) five populations of iguanas [Santa Fe, Plaza Sur, (Isabela + Fernandina), rossada and Santa Cruz] represent distinct conservation units and should be managed separately (the levels of genetic differentiation observed clearly suggest that the species status of these populations should be further investigated), (ii) that the affinities of Baltra/Seymour Norte individuals with both Isabela and Santa Cruz warrant further investigation, and (iii) the populations from distinct islands exhibit considerably different levels of genetic diversity, with two populations (Plaza Sur and Santa Fe) more particularly at risk. Note that, although the fate of Santa Cruz, Baltra and Isabela populations has been largely improved, thanks to past and existing conservation programmes, the polygynous reproductive strategy of land iguanas translates into lowered effective population sizes, stressing the need of continuous and effective management.

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References


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This study is part of ACT & SFPR conservation genetics work in MCM's laboratory. MCM heads the Laboratory of Artificial & Natural Evolution (LANE) at the University of Geneva (Switzerland), and works on various aspects of conservation genetics, phylogenetcs, phyloinformatics, Evo-Devo, and experimental evolution. AF is a researcher at the University of Tor Vergata and her interests include vertebrate molecular ecology, conservation and evolutionary biology. GG teaches conservation genetics at the University of Tor Vergata, his interests are in conservation genetics (in particular of land iguanas from the Galapagos), phylogeography, island biogeography, and cave ecology. HLS’s research interests concentrate on evolutionary and functional ecology, conservation biology and herpetology whereas HMS works on ecology, animal behaviour, and the history of field research in the Galapagos Islands. CM was at the Charles Darwin Foundation and is now volunteer with the Galápagos National Park Service (GNPS). WT is at the Departamento de Conservación y Desarrollo Sustentable of the GNPS.

Supporting Information
Additional Supporting Information may be found in the online version of this article:

Fig. S1 Bathymetric map of the Galápagos archipelago. The current emerged islands are indicated in white, whereas the land masses that would emerge after a 100-m decrease of the sea level (similar to the one that took place during the latest glaciation) are indicated in grey. Bathymetric data compiled by William Chadwick, Oregon State University (http://www.pmel.noaa.gov/vents/staff/chadwick/galapagos.html).

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