GENETIC POPULATION STRUCTURE AND CONNECTIVITY OF AZOREAN LIMPETS

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ABSTRACT
There is growing consensus that anthropogenic activities significantly disrupt the structure and functioning of marine ecosystems. Oceanic islands such as the Azores (NE-Atlantic) are unique habitats with fragile communities, which are highly susceptible to degradation and ecosystem disruption. Patellar limpets have traditionally been collected as a food resource and in 1988 the limpet fishery in São Miguel Island collapsed, after which a one-year ban was implemented allowing the stocks to recover and avoiding catastrophic over-exploitation effects. In 1993, legislation was passed to protect this resource, i.e. limpet no-take areas were created, seasonal harvesting restrictions were applied and minimum legal catch sizes were established. However, a recent survey has shown that limpet populations still show clear signs of over-exploitation and some populations are virtually extinct in some islands. Here we have developed new multiplexed and described microsatellite markers for the species Patella aspera and P. candei and have examined their genetic diversity, gene flow and population connectivity in the Azores Archipelago. Overall, such information is a fundamental asset to inform conservation strategies and to promote the sustainable exploitation of Macaronesian limpets.

METHODS

- Novel microsatellite markers were described for P. aspera and P. candei using 454 next-generation sequencing.
- A total of 798 of P. aspera were collected in Azores (all islands). Additional samples were taken from Canary islands (n=30) and mainland Europe (n=40) (the latter identified as the most recent ancestral species P. asperopatella). Similarly, 962 samples of P. candei were obtained from all islands in Azores (these samples are still being genotyped, so results will only be referred to P. aspera).
- Genotypes of all samples of P. aspera for 17 microsatellite loci were scored using GENEMAPPER V. 4.1 (Applied Biosystem).
- Allele frequencies and observed (Hₒ) and expected heterozygosity (Hₑ) were estimated using GenAlEx (Peakall and Smouse 2006). Allelic richness (Ar) was estimated using the standard method implemented in ALLELE (Stephens et al. 2000). Linkage disequilibrium, inbreeding coefficients (Fᵢ) and deviations from the Hardy-Weinberg equilibrium (HWE) were tested in GENEPOP (Raymond and Rousset 1995).
- The presence and frequency of null alleles were tested for each locus using MICROCHECKER (van Oosterhout et al. 2004). The IM approach (individual-informing model) was used in STRUCTURE (Pritchard et al. 2000) to partition the influence of null alleles on Fₛ values.
- Intraspecific Fₛ values were obtained using FREENA (Chapuis and Estoup 2007). Population structure was also analyzed using the Bayesian model-based clustering approach implemented in STRUCTURE (Pritchard et al. 2000), using prior population information. Selection of the most likely number of genetic clusters (K) was based on the Evanno et al. (2005) method.

- Analysis of molecular variance (AMOVA) was used to partition total molecular variance using ARLEQUIN (Excoffier et al. 1992), on groups identified by STRUCTURE analysis.

RESULTS

- Genetic diversity was affected by the presence of null alleles in many of the loci and populations tested (Table 1). Yet, unbiased Fₛ values show that homogenous excess may be a consequence of actual inbreeding between individuals and populations. In fact, it is thought that only a relatively small reproductive population size exists in the Azores, as a consequence of the high levels of human-exploitation.

- No evidence of genetic differentiation among islands in the archipelago of Azores was found for P. aspera (Table 2). Yet, unbiased Fₛ values show that homogenous excess may be a consequence of actual inbreeding between individuals and populations. In fact, it is thought that only a relatively small reproductive population size exists in the Azores, as a consequence of the high levels of human-exploitation.

CONCLUSIONS

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- Although unbiased pairwise Fₛ values support a small differentiation between Azores populations and Canarias, the STRUCTURE analysis did not provide the same result. When excluding P. asperopatella from the analysis, STRUCTURE reveals differentiation between Azores and Canarias populations, as supported by the unbiased Fₛ values, which would be expected due to the geographical distance between archipelagos (~2300km).

ONGOING RESEARCH

- This study is part of an ongoing research project focusing on the conservation of limpet species in the Azores (www.patelgene.com), but also including other exploited populations from the Macaronesian archipelagos (Canarias, Madeira and Cape Verde).

- A similar approach is also being used for the P. candei complex (ongoing genotyping), a species showing a high level of morphological diversity among and within the Macaronesian archipelagos.

- Spatial and temporal patterns of limpet recruitment are also being examined in parallel.