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MOLECULAR GENETICS AND CONSERVATION IN THE GALAPAGOS

By: Kornelia Rassmann

INTRODUCTION

For more than a century Galápagos has served evolutionary biologists as a natural laboratory for their studies. A major goal has been to describe the unique morphological and behavioral adaptations evolved by the diverse life forms of Galápagos organisms in response to their forbidding environment and to understand how these specializations have come about. Because the basic material of evolutionary changes are genes, understanding the genetic mechanisms of these processes has been an important step. Models derived from theories of population genetics can illustrate the processes influencing the genetic composition of populations or species, and therefore constitute an important part of current evolutionary research. These models attempt to describe the current genetic status of evolutionary systems, e.g. the amount of genetic variation within or among populations, and estimate the future prospects of populations with regard to their genetic composition. At the same time, the genes and the gene composition of a species or population serve as a record of the evolutionary events that occurred in its past and thus provide information on its historical - or phylogenetic - background.

In a rather sad way, population genetic and phylogenetic theory may become increasingly important for the practical aspects of research in the Galápagos. Over the past few centuries the populations of several endemic or native species declined, bringing some of them close to extinction and extirpating a few. The major reasons for these declines include predation by feral animals, habitat destruction and competition for resources by non-indigenous plants and animals introduced to the islands by humans, and human exploitation of indigenous species and their natural habitat (Trillmich 1992). Of course, there are also non-human-induced causes threatening Galápagos' fauna and flora. Sudden catastrophic events such as volcanic eruptions or epidemic diseases may endanger specific populations. Of more global nature are environmental changes such as the recurrent El Niño events -

climatological anomalies which sometimes lead to dramatic rises in the sea surface temperatures in the Galápagos. El Niño events influence the taxa in the Galápagos in different ways (Trillmich 1991). While some species thrive under an abundance of food, others are deprived of their natural resources and experience notable population declines. Thus, low population densities may occasionally occur naturally in some Galápagos species. However, the fossil record suggests that most extinctions in the Galápagos took place after the arrival of humans (Steadman *et al.* 1991). Together with the natural threats to Galápagos' biota, the increasing human impact on this unique ecosystem makes conservation biology an important and pressing field of research in the Galápagos today.

The diversity of the archipelago may now profit from what it taught us in the past. A new discipline, conservation genetics, applies the principles of population genetics and phylogenetics to conservation issues. It documents the amount and the pattern of genetic variation in endangered species and attempts to derive suggestions aiding their preservation. Among its applications are attempts to i) monitor and manage genetic variation in natural and captive populations, ii) predict demography in wild populations (e.g. the population size or the degree of gene flow), iii) recognize evolutionary distinct populations or subpopulations, which may need separate management or conservation, iv) determine the conservation value of populations or taxa based on their degree of genetic deprivation or their phylogenetic distinctiveness, and v) identify individuals or specimens of unknown origin or species affinity (Avise 1994, Hedrick & Miller 1992, Moritz 1994, Dobson *et al.* 1992).

However it is fair to ask, "Can genetic research indeed save endangered species?" Clearly, conservation genetics does not reduce the above mentioned risks of natural or anthropogenic changes of the environment and catastrophic events. Additionally there are two further categories of potential threats to the survival of populations, described as genetic and demographic causes (Shaffer 1981). In particular small populations can face a number of problems related to these categories. A predicted result from models of population genetics is that small populations lose genetic variation faster than large populations. This process is called genetic drift. Low genetic variation is generally assumed to have negative effects on the viability of a population. For instance, a lack of genetic variation may reduce a population's ability to respond quickly to future environmental changes. Also, pedigree inbreeding (e.g. the mating of genetically related animals) is likely in small populations, increasing the probability that deleterious recessive alleles are revealed, which may reduce the fitness of individuals in the population. Because a confusing terminology exists I refer you to a discussion of the different biological meanings of the term 'inbreeding', which need to be kept apart in order to avoid serious errors in management recommendations (Templeton and Read 1994). It is not clear whether the reduction of viability and fecundity in observed in inbred populations - commonly called 'inbreeding depression' - is always a consequence of pedigree inbreeding (Caro & Laurenson 1994, Laurenson *et al.* 1995). However, many authors still consider a management of genetic heterozygosity an important element in the preservation of endangered species (Avise 1994) that deserves greater attention in overall conservation planning (Hedrick & Miller 1992).

Managing the genetic variation in a free-living population is a long-term process, possibly too slow to deal with the population's short-term conservation needs. Also, in populations with low densities reproduction can be reduced for several non-genetic reasons, e.g. simply the lower frequency with which the opposite sexes meet. For such reasons it has been argued that demographic considerations (population growth and age structure) are of greater importance in the direct management of an endangered species than long-term genetic concerns (Lande 1988). Is 'conservation genetics' then of little practical value? Indeed, in the case of the Galápagos the most urgently needed steps towards its rescue might be immediate ecological actions, as well as political decisions and their enforcement. However, I feel that there is also potential in the data accumulating from the increasing number of molecular evolutionary studies in the archipelago.

GALÁPAGOS MARINE IGUANAS

The following serves as an example how a molecular genetic project may help to support the conservation of species. The objective of a study on the marine iguanas (Rassmann, unpubl. data) was to analyze the genetic differentiation within and among populations throughout the Galápagos. Samples of blood from iguanas were collected from 22 populations, including nearly all populations from major islands (Table 1). During the sampling trips in spring 1991 and 1993, populations from islands with introduced predators were observed to be small in numbers and characterized by an absence of juveniles, for example on Islas Isabela and San Cristóbal (Cayot *et al.* 1994). Previously it had been suggested that predation by feral animals was a likely cause for the conspicuous lack of recruitment in some marine iguana populations, and that in extreme cases, such as on Isla Isabela, this would potentially lead to their extirpation (Laurie 1983).

The consequences of artificially increased levels of predation on natural populations are not easily assessed when detailed information on the population's demography and the distribution is missing. It is possible, for example, that migration among different subpopulations from the same or neighboring islands is sufficiently high to make up for the increased losses due to predation. When conservation resources are limited, they need to be directed to the most critical cases. These should encompass not only the populations which are most threatened, but also

Table 1. Genetic variation within and among 22 genetically analyzed marine iguana populations.

Population	N^1_{mic}	N^2_{mt}	H[3]	A[4]	F ST[5]	Phi ST[6]
Española, Cevallos	12	6	0.69	3.25	0.159	0.729
Fernandina, Cabo Hammond	10	10	0.76	4.62	0.080	0.581
Floreana, Punta Montura	10	6	0.77	4.33	0.117	0.403
Fernandina, Punta Espinosa	10	6	0.77	5.44	0.067	0.553
Fernandina, Punta Mangle	13	6	0.77	5.44	0.074	0.468
Genovesa, Campamente	11	12	0.69	3.51	0.149	0.430
Isabela, Caleta Negra	10	6	0.81	5.42	0.060	0.466
Isabela, Caleta Webb	10	6	0.78	5.04	0.071	0.592
Isabela, Punta Albemarle	10	6	0.76	4.67	0.080	0.603
Marchena, Bahia Negra	10	6	0.64	3.97	0.087	0.470
Pinta, Cabo Ibetson	10	6	0.64	2.95	0.121	0.696
Pinzon, Dumb Landing	10	6	0.73	3.69	0.093	0.687
Plaza Sur	10	6	0.74	4.59	0.089	0.483
Santa Cruz, Caamaño	12	6	0.77	4.55	0.072	0.667
Santa Cruz, Estacion	10	6	0.78	4.84	0.064	0.667
Santa Cruz, Punta Estrada	10	6	0.78	4.67	0.063	0.536
Santa Fé, North	10	10	0.71	3.59	0.126	0.659
Santa Fé, South	10	10	0.76	4.33	0.093	0.553
Santiago, James Bay	10	6	0.74	4.03	0.069	0.464
San Cristobal, Loberia	10	6	0.63	2.86	0.168	0.761
San Cristobal, Punta Pitt[7]	10	6	0.50	2.19	0.231	0.887
Seymour Norte	10	6	0.73	4.26	0.088	0.384

[1]Sample size per population for microsatellite data.

[2]Sample size per population for mitochondrial DNA sequence analysis.

[3]Average heterozygosity H, i.e. the percentage of animals heterozygous for a particular microsatellite locus, averaged over three analysed loci.

[4]Number of effective alleles averaged over the three loci (Nei 1987).

[5]Average nuclear genetic distance between a specific population and all other populations, calculated from microsatellite fingerprint data as the average of the FST values of all pairwise comparisons (based on the computer program Fstat, Goudet 1994).

[6]Average mitochondrial genetic distance, calculated from the mitochondrial sequence data as the average of the Phi ST values of all pairwise comparisons (based on the computer program AMOVA, Excoffier 1995).

[7]The population from Punta Pitt on San Cristóbal not only has the lowest average heterozygosity and the lowest number of effective alleles, but also shows the highest degree of evolutionary distinctiveness with respect to its nuclear DNA (average FST = 0.231), and its mitochondrial DNA (average Phi ST =0.887).

those which are most diverged or unique, in order to preserve as much genetic diversity within the species as possible (Avise 1989). Genetic tools can, to some extent, help to find answers to such questions.

In the molecular study of marine iguanas, genetic data were obtained using nuclear DNA markers (three microsatellite fingerprint loci) and mitochondrial DNA markers (sequences of a 450 nucleotide fragment of the cytochrome b gene). Genetic distances calculated from the nuclear data revealed amazingly little differentiation among some of the populations. This suggested that gene flow among neighboring marine iguana populations was sufficiently high to maintain genetic variation even in some of the threatened subpopulations, for example, Isla Isabela. However, it has to be kept in mind that genetic models measuring gene flow or migration do this on an evolutionary rather than on an ecological time scale and thus might not be too meaningful for the current population demography (Moritz 1994). In other words, migration between neighboring populations may be high enough to maintain similarities in their genetic structure, yet it could be too low to prevent a decrease in the actual size of a particular population and thus its possible extirpation. The molecular data on the marine iguana populations do show that gene flow occurs - and where. Field studies can now be targeted to reveal the actual amount of contemporary migration among threatened and non-threatened populations (e.g. among the west coast of Isla Isabela and Fernandina), and thus determine more accurately their conservation status.

Especially disconcerting were the findings for the marine iguanas sampled at Punta Pitt on the easternmost tip of San Cristóbal. Only few animals were encountered and, as was the case on Isla Isabela, no hatchlings were seen and signs of predators were obvious. Results revealed that, whereas the Isla Isabela populations were still among the most variable of all population samples within the archipelago, the Punta Pitt animals showed the least degree of nuclear genetic variation (Table 1). Furthermore, the Punta Pitt animals carried a unique mitochondrial sequence type which did not occur elsewhere in the archipelago, although many other island populations shared specific mitochondrial types. The Punta Pitt population clearly deserves our attention. The reduced degree of nuclear DNA variation indicates that it may indeed consist of only few animals and that it receives little immigration from other populations. The unique type of mitochondrial sequence adds to the population's conservation priority, if our concern is to preserve as much genetic diversity as possible. The results of the molecular data therefore call for immediate action. A detailed census of the actual size of the Punta Pitt population is required, including all neighboring colonies (such as the iguanas on the near islet, Islote Pitt). Further genetic studies might then establish the degree of relatedness among the animals from such neighboring colonies and those from Punta Pitt, indicating which populations may serve as natural (or captive) stock populations. Most importantly, however, the management planning in the Punta Pitt area should include the immediate habitat protection and eradication of all feral animals from this region.

To return to the original question about the role of molecular genetics in conservation - can genetic data save endangered species - does it help rescue the marine iguanas? Ultimately, in the absence of ecological and behavioral studies, genetic data can lead to important, yet only preliminary information on a population's size and demography. Furthermore, recommendations based on molecular studies concerning the management of genetic variation and thus the evolutionary potential of a population are, of course, most relevant to the long-term conservation of a threatened species. Such information may therefore come too late for the survival of some populations.

On the other hand, genetic information provides a solid basis for planning ecological management, and can give support to urgent political decisions. As outlined in the marine iguana example, molecular data may lead to a ranking of populations according to their conservation priority, providing a framework which promotes the best use of limited conservation resources. Clearly, for many of Galápagos' endangered species more genetic information and its implementation is urgently needed. For example, detailed data on the evolutionary distinctiveness of many populations is still lacking, but would be crucial for focusing our present efforts in short-term ecological management and for outlining potential long-term genetic management plans. In the long run, such information may help to preserve as much of Galápagos' biological diversity as possible.

ACKNOWLEDGMENTS

I am grateful to the government of Ecuador, the Galápagos National Park and the Charles Darwin Research Station for sampling permission and continuous support. I am especially indebted to Capitán Fermin Gutierrez and the crew of the *San Juan* for safe sampling trips and assistance in my work. TAME Airlines provided travel to an from the Galápagos Islands at a reduced rate.

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VISITORS AND EVENTS AT THE CHARLES DARWIN RESEARCH STATION, 1995

Compiled by: Heidi Snell and Gayle Davis

January

- Fishermen closed the entrance of CDRS & SPNG for 4 days.
- Gerald Wellington, Univ. Houston, Benjamin Victor & Mark Meekan, assistants arrived to study reef fish.
- The annual flamingo census took place.
- Sabine Tebbich began a study of Woodpecker finches.
- Sandra Guerrero, Catholic Univ. Ibarra volunteered in Environmental Education.
- Sharon Virtue, librarian, Univ. Toronto volunteered for two weeks in the CDRS library.
- Mark Jordan, Univ. New Mexico & Sarah Bouchard Kalamazoo College, MI, arrived to work with Howard & Heidi Snell on the lava lizard study. Monica Calvopiña & Cassie Holman completed the group.
- Peter & Rosemary Grant, Princeton Univ., arrived to continue their studies of Darwin's finches.
- Jorge Gómez-Jurado joined Marine Investigations as a Technical Assistant.
- Milton Arsiniegas, Technical Univ. Esmeraldas arrived as a thesis student in Botany.
- David Hicks, Manchester College, IN spent his sabbatical in Botany working on the status of *Opuntia*.
- Gary McMurtry, Fraser Goff, Univ. Hawaii, & James Sitmac, Los Alamos National Lab., NM; Alfredo Roldan, National Electronics Institution Guatemala, & Rosemary Andrade, Univ. Guayaquil, made a geologic study on Sierra Negra.
- January 24th Fernandina erupted near Cabo Hammond.
- Peruvian troops trespassed into Ecuadorian territory provoking a war & disrupting both countries. Effect in Galápagos was increased patrolling activity.
- CDRS personel presented a Quarantine Workshop held in San Cristóbal.
- Tomato & papaya plants found growing in the fishermen camps on Fernandina were eliminated.

February

- Anna Fitter, Galápagos Conservation Trust of England visited.
- Verónica Toral, Cuenca Univ., volunteered in Marine Biology.
- Sabina Estupiñán, Univ. Luis Vargas Torres, Esmeraldas, volunteered in Botany.
- Olav Oftedal, National Zoo, Wash. DC, & Frank Allen, continued work with the captive reptile program.
- Washington Tapia began thesis work on Isabela tortoises.
- The war between Peru & Ecuador ended.
- Jack Kepper, Canadian Fund & Alfredo Carrasco, CDF Quito, visited the Station.
- Rosemary Andrade volunteered for the Snell/Jordan lava lizard studies before returning to Univ. Guayaquil.
- Bruce Kernan USAID & Alfredo Carrasco checked projects and worked on a Marine Investigation proposal.
- Fernandina continued erupting.

March

- Alegría Mejía & Olga Quevedo, Univ. Guayaquil, arrived to volunteer in Protection & Monitoring.
- Daniel Evans, former Director of CDRS, visited with a group from the Point Reyes Bird Observatory, Calif.
- Roger Tinoco, Inspector National Institution Meteorology & Hydrology gave a course to the personnel of CDRS.
- Verónica Toral represented Ecuador/CDRS at the World Forum for Youth & Development in Israel.
- The National Television of Japan (NHK) made a live telecast from Galápagos from several locations.
- Hugo Valdebenito, Günther Reck & students, Univ. San Francisco Quito, arrived for an ecology course.
- Gustavo Yturralde, Univ. Guayaquil began assisting Mark Jordan with studies of lava lizards.