

USE OF MOLECULAR MARKERS FOR THE STUDY OF MARINE BIODIVERSITY

*Acta of the
1st International Workshop in Marine Molecular Phylogenetics*

Horta 1-6 September 2004

Sérgio Stefanni, Joël Bried & Raul Bettencourt (Eds)



Arquipélago - Life and Marine Sciences

ORGANISATION



SPONSORED BY



Cover drawing by Les Gallagher – ImagDOP in collaboration with Fishpics.

PROGRAM

This workshop aimed to lead PhD students and young scientists through traditional and more up-to-date methodology of analysing molecular data for the study of marine biodiversity. This subject has become an important issue in the scientific community and the use of molecular tools is of invaluable help. Therefore, with this workshop, we gathered together several world-leading experts of marine molecular ecology to give a unique opportunity to students to analyse their own datasets. Seminars followed by tutorials helped the students to become more familiar with the use of designed software. The variety of subjects presented by the invited speakers has covered a large spectrum of organisms inhabiting or associated to the sea.

Description of the Workshop

The structure of the workshop was conducted as follows:

- Introduction to mathematical models and building trees/networks techniques applied on molecular data (sequences and microsatellites)
- Practical exercises on specially prepared datasets for the students.
- Daily seminars with practicals on the different group of marine or sea associated organisms.
- Small groups of students, divided according to type of molecular markers used in their projects, with the aim of analysing their own datasets under the supervision of the related field experts.

Presentation of students' work based on elaboration of their own datasets was expected to conclude the workshop.

REACHED OBJECTIVES

Gathering together world-leading experts working on the area of marine genomics was of great help for all of the participants. Real time feedbacks to cover doubts, to explore newer approaches and evaluate strategies to investigate marine biodiversity were one of the major objectives of this workshop. The interaction between invited scientists and students lead to very constructive exchange of opinions as well as suggestions and the opportunity offered to students to present and discuss their on data set in this environment was of invaluable help. With no other ways we could have reached those targets and the appreciation expressed from everyone present at the event was the best reward for all the effort put into the organization of the 1st workshop in Marine Molecular Phylogenetics.

WORKSHOP SCHEDULE

September 1

9:15 - *Opening session and welcome*

Ricardo S. Santos (Director of Dept. of Oceanography and Fisheries, UAç and vice-president of IMAR)

9:30-12:00 - *lectures (G. Bernardi & S. Stefanni)*

- Biotic and abiotic factors that structure phylogeographic patterns in marine fish species
- Phylogeography of the shanny, *Lipophrys pholis* and the prospect of a new endemism for the Azores

14:30-17:30 - *lecture and tutorial (G. Bernardi)*

- Structural approaches to sequence evolution: Molecules, networks, populations

September 2

9:00-12:00 - *lectures (T. Patarnello & G. Bernardi)*

- Messinian crisis: macro and micro-evolutionary processes that affected Mediterranean marine fauna
- Molecular Phylogenies of *Thalassoma* & *Sparisoma*: Ecology, Evolution, Biogeography

14:30-17:00 - *lectures (A.R. Hoesel, S. Querouil & A. Pinela)*

- Molecular Ecology of Marine Mammals
- Social organization and population genetics of bottlenose dolphins and sperm whales around the Azores

September 3

9:00-12:00 - *lectures (S.A. Karl & H.A. Lessios)*

- Complex population structure in sea turtles
- The use of mitochondrial DNA to detect global-scale barriers to Gene Flow of Shallow Water sea urchins

14:30-17:00 - *Tutorials on students' data sets and students' questions*

September 4

10:00-12:00 - *lectures (R. Bettencourt & D.R. Dixon)*

- Genes that fight infection: what Bivalves say about innate immunity
- Molecular characterisation of pelagic larvae & molecular biology of hydrothermal vent organisms

14:30-17:00 - *Tutorials on students' data sets and students' questions*

September 5

9:00-17:00 - *Excursion*

September 6

9:00-12:00 - *lectures (T.M. Burg & J. Bried)*

- Review of Seabird Genetics
- Combining genetic and demographic data to determine genetic variability and population structure in the wandering albatross (*Diomedea exulans*)

14:30-17:00 - *presentations by students and closing session*



Group photo of the participants on the
1st International Workshop in Marine Molecular Phylogenetics
“Use of Molecular Markers for the Study of Marine Biodiversity”

LIST OF PARTICIPANTS

A. Rus Hoelzel

Biological and Biomedical Sciences, Durham University, Durham, UK

Alexandra Hiller

Department of Animal Ecology, Justus-Liebig-University, Heinrich-Buff-Ring 26-32 (IFZ), D-35392 Giessen, Germany

Ana Pinela

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Ana Verissimo

Department of Animal Biology, Sciences Faculty of the University of Lisbon, Campo Grande, 1148-016, Lisboa, Portugal

David R. Dixon

George Deacon Division for Ocean Processes, Southampton Oceanography Centre, University of Southampton, United Kingdom.

Giacomo Bernardi

Dept. of Ecology and Evolutionary Biology at the University of California, Santa Cruz, CA 95064, USA.

Gilberto M. P. Carreira

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Harilaos A. Lessios

Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama.

Inês Seabra

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Joël Bried

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Marina Ramon

UC Santa Cruz - Ecology and Evolutionary Biology, Long Marine Lab/COH 247, 100 Shaffer Rd, Santa Cruz, CA. 95060

Mirko De Girolamo

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Raul Bettencourt

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Regina L. Cunha

Museo Nacional de Ciencias Naturales Madrid, Spain.

Ricardo Beldade

Instituto Superior de Psicologia Aplicada – ISPA, Rua do Jardim do Tabaco, 34, 1149-041 Lisbon, Portugal

Rita Castilho

Center for Marine Sciences, Biodiversity and Conservation Group, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Sérgio Stefanni

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Sophie Quérrouil

IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

Stephen A. Karl

Department of Biology, University of South Florida, 4202 East Fowler Ave., SCA 110, Tampa, Florida 33620-515, USA

Tawfiq Froukh

Department of Biotechnology and Molecular Genetics Centre for Applied Sensorik (CAG), FB2-UFT, University of Bremen, Leobener Strasse, 28359 Bremen, Germany

Tomaso Patarnello

Department of Biology, University of Padova. Via U. Bassi, 58/B – 35121 Padova, Italy.

Theresa M. Burg

Dept. of Biology, Queen's University, Kingston ON, Canada, K7L 3N6

Vera Domingues

Instituto Superior de Psicologia Aplicada – ISPA, Rua do Jardim do Tabaco, 34, 1149-041 Lisbon, Portugal

EDITORS' MESSAGE

A great deal of effort was dedicated to the organization of the first International Workshop in Marine Molecular Phylogenetics, and the feedback we received was well worthy the energy we had invested. We set out to create a very friendly and familiar contact between speakers and participants as well as flexibility in dedicating time during the afternoon sessions to subjects, questions, doubts and curiosities that rose along with the daily presentations. When applicants contacted us, we asked to send a short description of their projects and expectations from the workshop. We then accommodated their suggestions and project proposals as they were used as basis and criteria for the choice of talks as well as for organizing the practical sessions.

The financial support offered by the sponsors allowed us to offer this workshop at no charges for the participants, and this, in itself represented a great achievement.

Lectures as well as practical sessions were held at the conference room of the "Centro do Mar", a very attractive restored section of the building and embedded in what in the past was the whale factory. There were times when whaling industry was a lucrative source for the economy of the islands.

The choice of organizing this workshop in the Azores had to do with the uniqueness of the place along with the great potentials and scientific interests associated to life diversity encountered on these islands.

The Azores are a large repository of genetic resources. They are rich in biodiversity and the analysis of animal genetic resources provides information on the extent of genetic diversity. During this first International Workshop in Marine Molecular Phylogenetics a series of seminars were aimed at understanding the

applicability of genetic markers in developing a set of criteria and indicators to assess sustainability of marine management, characterization and conservation of genetic diversity. Genetics markers can be used for the identification of species whether at the population or even individual level. Also, genetic markers have also been applied to address questions related to population biology, behavioral ecology, evolution and phylogeny. Therefore, molecular approaches and various laboratory techniques were presented in numerous communications throughout the workshop to exemplify the concept of lineages, relationships, genetic relatedness and pedigree determination. This information, along with new computational techniques for its analysis, has enhanced the study of phylogenetics (species lineages), linkage analysis (co-transmission within families of traits and markers) and yielded information on demographics and population structure that may be of use in evolutionary or ecological studies.

The molecular technologies described were generally hybridization or PCR based. Some of the preferred molecular techniques included RFLP (Restriction fragment length polymorphism), Microsatellites, RAPD (Randomly amplified polymorphic DNA), STMS (Sequence Tagged Microsatellite Sites) and AFLP (Amplified fragment length polymorphism).

Finally, participants were able to explore and learn more of the most updated software used in population genetics and the role of molecular markers to study population biology and make inferences about natural populations. A great help for spreading the attention onto our workshop to International scientific community was offered by EvolDir (Evolutionary Directory coordinated by Brian Golding). Participants came from several countries: USA, Germany, United Kingdom, Belgium, Spain and Portugal.

PHYLOGEOGRAPHY OF THE SHANNY, *Lipophrys pholis*, BASED ON MTDNA CONTROL REGION

S. STEFANNI

STEFANNI, S. 2005. Phylogeography of the shanny, *Lipophrys pholis*, based on mtDNA control region. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 5-8.

The shanny is one of the eight species of blennies inhabiting the Azores. Its distribution is recorded in the NE Atlantic from Norway to Morocco, including the Atlantic islands and the Western Mediterranean. Although this species has been widely inspected from the morphological point of view (LOWE 1843; BATH 1976; ARRUDA 1979), not much has been done on the genetics (GUILLEMAUD et al. 2000). Some authors drew attention on morphological differences between *Lipophrys pholis* of the European continental coasts and the Atlantic islands (LOWE 1843; Bath 1976; ARRUDA 1979). ZANDER (1980) refers to ARRUDA (1979) mentioning "subspeciation of some Azorean fish". Zander justifies the disjunctive distribution for the shanny by differences in competition pressures that lead to the giant race in Madeira (*B. bufo*) and a northern European shanny (*B. pholis*).

In this preliminary study, partial sequences of the mtDNA control region are compared with the aim of resolving the phylogeny of the Azorean shanny. Samples of *L. pholis* were collected from three localities along NE Atlantic coast and two Atlantic islands. 20 fish were successfully sequenced and 17 haplotypes were defined. All sequences correspond to the first hypervariable and part of the central domains of the control region with a length ranging from 450bp (Oban, UK) to 454bp (Is. Azores).

The neighbour-joining (NJ) tree contains thirteen internal branches with bootstrap (BS) values greater than 50% (Fig. 1). The branch separating the Azorean shanny is extremely well supported (BS = 100) while the one including samples from Madeira, mainland Portugal and Scotland has a much lower value (BS = 72). Inside the latter clade, branches with BS values greater than 50% define small (n = 2 or 3) monophyletic groupings of haplotypes from different sampling localities.

Of the 455 sites considered only 79 are parsimony-informative. The maximum parsimony (MP) search found 163 MP trees (L=163, CI=0.859, RI=0.832). The strict component consensus tree of the MP trees which displays all and only those groups found in all the MP trees, resembles the NJ tree. In this tree, the first split separates the Azorean shanny from the monophyletic clade composed by two other groups. One that contains the same three haplotypes from Avencas, Portugal (Av10, Av13 and Av17) as presented in the NJ tree, and the second contains haplotypes from all the localities, except for the Azores.

The statistical parsimony network (SPN) separates the haplotypes in groups when the number of nucleotide difference between even the most similar haplotypes exceeds the parsimony connection limit, which in this case is 9 steps. Six are the groups created by this network, one of which includes the sample from the Azores. None of the other are characterised by grouping of haplotypes from a single sampling site, except for the isolated Av12. The Scottish samples are grouped together with three of the haplotypes encountered in the two localities from mainland Portugal, and the only Madeiran fish is linked to two haplotypes from shannies of Avencas (central Portugal) (Fig. 2).

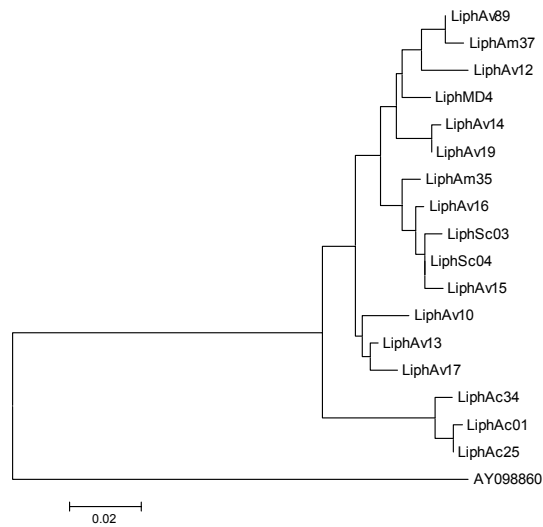


Fig. 1. Neighbour-joining tree constructed from HKY+I+G distances with $t_i/t_v = 2.9294$, $i = 0$ and $\alpha = 0.3877$. Numbers above internal branches indicate bootstrap values out of 1000 replicates (only if greater than 50%). Ac = Azores; Am = Amoreira, PT; Av = Avencas, PT; MD = Madeira Is. and Sc = Oban, UK. Shared haplotypes: Ac25=Ac02+Ac05; Ac34=Ac03+Ac04; Av89=Av08+Av09.

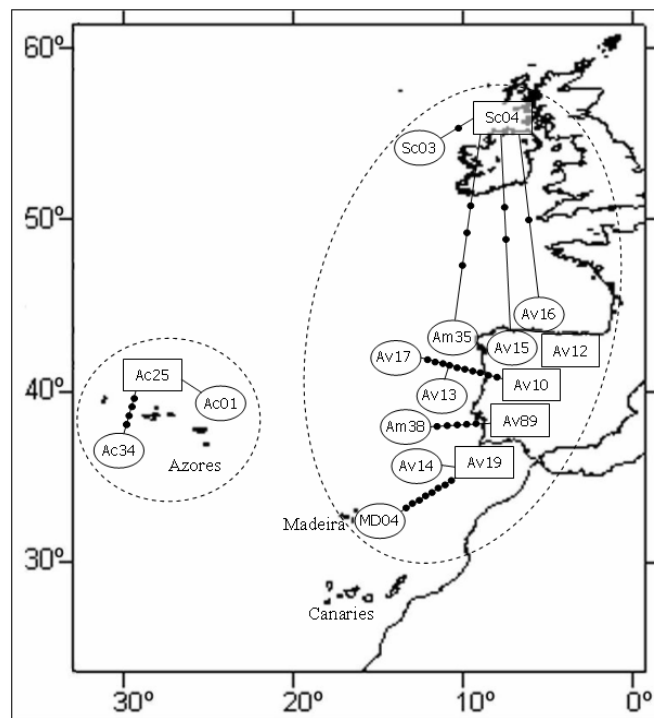


Fig. 2. Statistical parsimony networks from partial D-loop sequences over imposed to a geographical map of the NE Atlantic. Dots indicate the number of nucleotide differences between haplotypes. Ac = Azores; Am = Amoreira, PT; Av = Avencas, PT; MD = Madeira Is. and Sc = Oban, UK. Shared haplotypes: Ac25=Ac02+Ac05; Ac34=Ac03+Ac04; Av89=Av08+Av09.

In the AMOVA analysis the grouping that maximises the value of Φ_{CT} is that in which the Azorean haplotypes are separated from the Madeira, mainland Portugal and Scotland, which are grouped together. This grouping has a Φ_{CT} value of 0.635. Not taking into account the Azorean samples, pairwise Φ_{ST} values between sites varied between 0.093 (Amoreira – Oban) and 0.935 (Oban – Madeira). The latter is of the same order of magnitude when comparing the Azorean haplotypes with the ones from the other localities. However, p -values showed very high significance only when any of the sites is compared with the Azores. The corrected sequence divergence between the Azores and Madeira, mainland Portugal and Scotland sequences is 4.42% while the sequence divergence within these two groups is 0.34% and 2.53%, respectively.

The estimates of the number of migrants (Nm) calculated from the Φ_{ST} values (SLATKIN 1993) are much reduced between the two most remote sites ($Nm = 0.0345$) as well as between the islands of Madeira and Azores ($Nm = 0.0338$). The reduction in gene flow between localities is not supported by the IBD model and the correlation between geographical distances and genetic differentiation is not significant ($p = 0.076$). Phylogenetic analyses and population genetics are strongly supporting the presence of two groupings of shanny, one for the Azores and one for the mainland Europe including the island of Madeira. The clade constituted by the Azorean sample is very highly supported by NJ and MP trees using D-loop and the population statistics further support these findings. The control region describes unique haplotypes for the Azorean shanny.

When all sampling localities are compared one to another, pairwise values of Φ_{ST} result statistically significant only when Azores is included, and as further support, lack of correlation between genetic differentiation and geographical distance. Sequence divergence between Azorean and “continental” shannies averages around 4.4%. At this point, genetics support the existence of two species of shanny, one “continental” whose distribution stretches from northern Europe to the island of Madeira, and a second limited to the Azores. While for the “continental” shanny the name *L. pholis* would stay unaltered, new combinations would become necessary for the Azorean shanny. Since that the Dept. of Oceanography and Fisheries of the University of the Azores has been putting forwards projects for the development of several marine protected areas in the nine islands of the archipelago (SANTOS et al. 1995), it seems appropriate to draw the attention on local endemisms. In the last decades, the number of individual catches and localities where the Azorean shanny was recorded are decreasing drastically. Therefore, the author is strongly supporting to consider the existence of a separate species in the Azores, condition that may also be associated to risk of threat.

ACKNOWLEDGEMENTS:

This work is part of my contribution to a larger project on phylogeography of blennies, in collaboration with Vera Domingues and Dr. Vítor C. Almada from ISPA (Lisbon), Dr. Niels Bouton and Dr. Ricardo S. Santos from IMAR (Azores). I am a postdoctoral fellow funded by FCT (Foundation for Science and Technology, Portugal) and contracted by IMAR, ref SFRH/BPD/14981/2004.

REFERENCE:

- ARRUDA, L.M. 1979. On the study of a sample of fish captured in the tidal range at the Azores. *Boletim da Sociedade Portuguesa de Ciências Naturais* 19: 5-36.
BATH, H. 1976. Revision der Blenniini. *Senckenbergiana Biologica*, 57(4/6): 167-234.

- LOWE, R.T. 1843. Notices of fishes newly observed or discovered in Madeira during the years 1840, 1841, and 1842. *Proceedings of the Zoological Society of London*, 11: 81-95.
- GUILLEMAUD, T., F. ALMADA, R.S. SANTOS, M.L. CANCELA 2000. Interspecific utility of microsatellites in fish: A case study of (CT)(n) and (GT)(n) markers in the shanny *Lipophrys pholis* (Pisces: Blenniidae) and their use in other blennioidei. *Marine Biotechnology* 2(3): 248-253.
- SANTOS, R.S., S.J. HAWKINS, L.R. MONTEIRO, M. ALVES, E. ISIDRO 1995. Marine research, resources and conservation in the Azores. *Aquatic conservation: marine and freshwater ecosystems* 5: 311-354.
- SLATKIN, M. 1993. Isolation by distance in equilibrium and nonequilibrium populations. *Evolution* 47: 264-279.
- ZANDER, C.D. 1980. Zoogeography and speciation of Mediterranean blennioids (Perciformes, Pisces). *Journées d'Etudes de Systématique et Biogéographie Méditerranéenne – Cagliari, C.I.E.S.M.*: 33-38.

Sérgio Stefanni (e-mail: sstefanni@notes.horta.uac.pt), IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

STRUCTURAL APPROACHES TO SEQUENCE EVOLUTION: MOLECULES, NETWORKS, POPULATIONS

G. BERNARDI

BERNARDI, G. 2005. Structural approaches to sequence evolution: Molecules, networks, populations. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 9.

The study of molecular evolution has two main objectives: (i) the reconstruction of the biochemical history of life through the analysis of the macromolecules of existing organisms; (ii) the understanding of the determinants of the evolution at the molecular level. These goals can only be accomplished by a highly interdisciplinary combination of experimental techniques of molecular biology, bioinformatics, and mathematical modeling. The increasing amount of data made available by genome sequencing projects are demanding an increasing integration of these disciplines. In particular, progresses in understanding the structural properties of biological entities at different levels, such as molecules, networks, and populations, can greatly contribute both to elucidate the mechanisms of evolution and to reconstruct its course.

This lecture associated to tutorials will help to become familiar with alignment software programs as ClustalX and also will give an understanding of how various parameters can be changed and how this will potentially affect the resulting alignment. With the program PAUP (probably the most important software program for reconstructing phylogenetic relationships between macromolecular sequences) several parameters will be explored as well.

The issues that are relevant for reconstructing the relationships between protein sequences will be analysed with the help of the program PUZZLE and the MOLPHY package. The aim is to bring together these different main areas: theoretical and empirical studies of population dynamics, computational and experimental studies of the stability and properties of biological macromolecules, and statistical analysis of sequences databases.

G. Bernardi (e-mail: bernardi@biology.ucsc.edu) Dept. of Ecology and Evolutionary Biology at the University of California, Santa Cruz, CA 95064, USA.

ATLANTIC-MEDITERRANEAN DIVIDE: CONTRADICTORY EVIDENCES

T. PATARNELLO

PATARNELLO, T. 2005. Atlantic-Mediterranean divide: contradictory evidences. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 10-11.

The geological history of the Mediterranean basin has been well documented as it is also the present-day currents pattern within Mediterranean Sea and its connection with the Atlantic Ocean (HSU et al. 1977; MILLOT 1999; TINTORE et al. 1988). Despite such wealthy of historical and oceanographic data the Atlantic/Mediterranean transition at the biological level is still controversial as there are discordant results regarding the biogeographic separation between Atlantic and Mediterranean fauna. The opening of Gibraltar strait at the end of the Messinian crisis (some 5.5MYA), removed the land barrier that impeded the marine fauna to freely disperse between the Atlantic and the Mediterranean. However, present day genetic patterns evidence that for some marine vertebrate and invertebrate species there is still a break that prevents population admixture between both sides of the strait (BARGELLONI et al. 2003; ZARDOYA et al. 2004; ZANE et al. 2000). In the last couple of years several papers have challenged the hypothesis of the Gibraltar strait representing a barrier to the gene flow, however, the results appear controversial (Fig. 1). Interestingly, no relation was obvious between dispersal ability or different life histories of a species and partial or complete genetic isolation between Atlantic and Mediterranean gene pools. This issue was investigated in several taxa independently and it appeared that within species of the same family or genus (thus reducing as much as possible biological differences) the results were opposites even comparing sister species. The interpretation of these results is possible if we assume that also closely related species can have different evolutionary histories, for instance events of population bottleneck and expansion, that might explain, at least partially the reported discordant observations. In the species for which the investigation has been carried out at the micro-geographic scale focusing on the possible transition area, changes of allele frequencies appeared associated to the Oran-Almeria front rather than to the Gibraltar strait itself (NACIRI et al. 1999; PEREZ-LOSADA 2002). The Oran-Almeria is an oceanic front exhibiting a strong water current that flows from the Spanish coast toward the African one and also showing a steep temperature and salinity gradient (TINTORE et al. 1988).

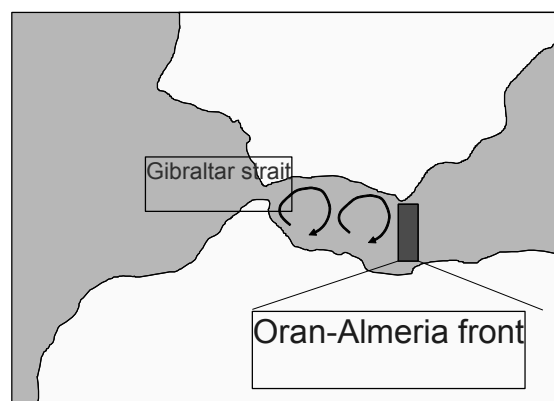


Fig. 1. Map indicating the position of the Oran-Almeria front.

Whether this barriers is effective to reduce the gene flow between Atlantic and Mediterranean populations is under debate since, beside historical factors, species exhibiting an Atlantic/Mediterranean separation should experience in present days a gene flow limitation that prevent genetic homogenisation. This issue has theoretical (ability to discriminate between historical and ongoing processes) and practical (stock identification aimed at proper fishery management) implications. The appearance of a growing number of studies focused on the genetic characterization of Atlantic and Mediterranean populations in vertebrate and invertebrate species offers an excellent opportunity to summarize the state of the art and provide a general view of this matter.

REFERENCES

- BARGELLONI, L., J.A. ALARCON, M.C. ALVAREZ, E. PENZO, A. MAGOULAS, C. REIS & T. PATARNELLO 2003. Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. *Journal of Evolutionary Biology* 6(6): 1149-1158
- HSU, J.K., L. MONTADERT, D. BERNOUILLI, M.B. CITA, A. ERICKSON, R.E. GARRISON, R.B. KIDD, F. MÉLIEREÉS, C. MÜLLER & R. WRIGHT 1977. History of the Mediterranean salinity crisis. *Nature* 267:399-403.
- MILLOT, C. 1999. Circulation in the Western Mediterranean sea. *Journal of Marine Systems* 20: 423-442
- NACIRI, M., C. LEMAIRE, P. BORSA & F. BONHOMME 1999. Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). *Journal of Heredity* 90 (6): 591-596
- TINTORE, J., P.E. LA VIOLETTE, I. BLADE & G. CRUZADO 1988. A study of the intense density front in the eastern Alboran sea: the Oran-Almeria front. *Journal of Physical Oceanography* 18: 1384-1397
- ZANE, L., L. OSTELLARI, L. MACCATROZZO, L. BARGELLONI, J. CUZIN-ROUDY, F. BUCHHOLZ & T. PATARNELLO 2000. Genetic differentiation in a pelagic crustacean (*Meganyctiphanes norvegica*, Euphausiacea) from the North East Atlantic and the Mediterranean Sea. *Marine Biology* 49:191-199
- ZARDOYA, R., R. CASTILHO, C. GRANDE, L. FAVRE-KREY, S. CAETANO, S. MARCATO, G. KREY & T. PATARNELLO 2004. Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. *Molecular Ecology* 13(7):1785-98

Tomaso Patarnello (e-mail: tomaso.patarnello@unipd.it) Department of Biology, University of Padova, Italy.

EVOLUTION OF CORAL REEF FISH *Thalassoma* spp. (LABRIDAE). MOLECULAR PHYLOGENY AND BIOGEOGRAPHY

G. BERNARDI

BERNARDI, G. 2005. Evolution of coral reef fish *Thalassoma* spp. (Labridae). Molecular phylogeny and biogeography. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 12.

Wrasses in the genus *Thalassoma* comprise 27 recognized species that occur predominantly on coral reefs and subtropical rocky reefs worldwide. The phylogenetic relationships for 26 species were examined based on two mitochondrial genes (cytochrome b and 16S rRNA) and one nuclear intron (the first intron of the ribosomal protein S7). Two closely related species, the bird-wrasses (*Gomphosus varius* Lacepede, 1801 and *G. caeruleus* Lacepede, 1801), are also included in the analysis. These species grouped within the genus *Thalassoma*. *Thalassoma newtoni* (Osorio, 1891) from Sao Tome, which is generally synonymized with the Atlantic/Mediterranean *Thalassoma pavo* (Linnaeus, 1758) appears to be a valid species. Using a molecular clock, the genus was estimated to have originally diverged 8-13 million years ago, with *Thalassoma ballieui* (Vaillant and Sauvage, 1875) from Hawaii and *Thalassoma septemfasciata* Scott, 1959 from Western Australia as the ancestral species. Approximately 5-10 million years ago, a sudden burst of speciation resulted in seven clades, which were resolved with the sequence data. The terminal Tethyan event and the closing of the Isthmus of Panama were probably the major historical factors shaping the evolution of species in the genus *Thalassoma*. These data on the spatio-temporal pattern of speciation in the Indo-Pacific indicate that peripheral species have been generated at various times throughout the history of the genus, and that none of the widespread species are relatively young. Thus, there is no clear support for centrifugal (youngest at the periphery) versus centripetal (oldest at the periphery) modes of generation of species, two theories which have been used to account for geographic gradients in species diversity.

G. Bernardi (e-mail: bernardi@biology.ucsc.edu) Dept. of Ecology and Evolutionary Biology at the University of California, Santa Cruz, CA 95064, USA.

POPULATION GENETIC STRUCTURE OF MARINE MAMMAL SPECIES

A.R. HOELZEL

HOELZEL, A.R. 2005. Population genetic structure of marine mammal species. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 13-14.

Marine mammals have adapted to marine habitats in ways that to varying degrees reflect their earlier evolutionary history in terrestrial environments. This is especially relevant to the evolution of population genetic structure when reproductive constraints affect patterns of dispersal, movement and demography. In this lecture I will consider some of the implications of adaptation to the marine habitat for the spatial and temporal patterns of population genetic structure in marine mammals.

Some marine mammal taxa give birth on land or ice (all pinnipeds species and the polar bear), others give birth at sea (all cetaceans and sirenians), and one reproduces near shore, but is not dependent on land for breeding (the sea otter). Those species that mate and give birth on land or ice are tied to breeding sites, and suitable habitat is limiting. Therefore, geographic range, philopatry and mating system are likely to be especially important in shaping genetic population structure for these species. Species that give birth at sea are typically less restricted, but still require suitable habitat with respect to resource and possibly thermal requirements. Even so, population boundaries are often less predictable for the fully aquatic marine mammals.

Site fidelity is generally high in otariids where the same animals may breed within the same small part of a colony from one year to the next. Some pack-ice breeding seals, which tend to be monogamous, breed over a broad area and are less site faithful. Among the fully aquatic species, one group of sirenians (the manatees) and some species of cetaceans (in the genus *Platanistidae*) inhabit rivers and estuaries, while most species have oceanic distributions. The extent and pattern of dispersal varies greatly, with some species having a nearly global distribution (such as sperm whales), while others are more restricted (such as the Florida Manatee and Commerson's dolphin). A further complexity involves the regular migrations undertaken by some species, in some cases over vast distances.

For many marine mammals, another important factor is their history of extensive and indiscriminate harvesting for various products including fur and oil. In some species this resulted in genetic population bottlenecks, reductions in range and local extinctions. For several species, including the Stellar sea cow (*Hydrodamalis gigas*), the Caribbean monk seal (*Monachos tropicalis*) and the Japanese sea lion (*Zalophus japonicus*), it resulted in extinction. Some species were depleted to the extent that their populations have remained small long after the hunting ceased. For example, the northern right whale, once abundant in the North Atlantic, now numbers only about 500 whales, and the population appears to be in decline.

The development during the past decade of powerful techniques that can assay the variability of molecular genetic markers has greatly facilitated studies about the evolution of behaviour and population structure in these difficult study subjects. The methods include the enzymatic amplification of DNA by the polymerase chain reaction (PCR), automated DNA sequencing (in particular of highly variable mitochondrial DNA) and assays of size variation in variable number of tandem repeat (VNTR) markers (in particular

microsatellites). The molecular approach is an essential component of our understanding and conservation of natural populations, as it allows the determination of otherwise intractable information, from paternity and kin associations to the pattern and level of genetic diversity within and among populations. We need to conserve diversity at this level, as this is the raw material that permits a species to adapt to a changing environment over time.

A.R. Hoelzel (e-mail: a.r.hoelzel@dur.ac.uk) Biological and Biomedical Sciences, Durham University, Durham, UK

POPULATION GENETICS AND SOCIAL ORGANISATION OF THE BOTTLENOSE DOLPHIN AND THE SPERM WHALE AROUND THE AZORES

S. QUÉROUIL, A. PINELA, M. SILVA, S. MAGALHÃES, R. PRIETO, J. MATOS, D. MENDONÇA & R.S. SANTOS

QUÉROUIL, S., A. PINELA, M. SILVA, S. MAGALHÃES, R. PRIETO, J. MATOS, D. MENDONÇA & R.S. SANTOS 2005. Population genetics and social organisation of the bottlenose dolphin and the sperm whale around the Azores. *Arquipélago. Life and Marine Sciences* 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 15-16.

Whilst assessment of cetacean stocks by direct observation is difficult and time-consuming, genetic analyses can readily provide information on population structure and dynamics, as well as patterns of genetic relatedness between individuals. Within the frame of an FCT funded project (CETAMARH), we analysed the population structure and social organisation of two widespread species of Cetaceans, the bottlenose dolphin (*Tursiops truncatus*) and the sperm whale (*Physeter macrocephalus*), around the Azores. While the bottlenose dolphins are present year-round, the sperm whales only visit the archipelago during their migrations. The archipelago of the Azores consists of three groups of islands separated by hundreds of kilometres, and surveys are carried out in all three groups. Three complementary methods are used: photo-identification, genetics and acoustics. Genetic analyses are performed on skin samples and consist in molecular sexing, sequencing of 627 bp of the mitochondrial hyper-variable region (for bottlenose dolphins only, as the D-loop is hardly variable in the sperm whale), and analysis of c.a. ten microsatellite loci.

For bottlenose dolphins, both DNA sequences and microsatellites indicate a lack of population structure within the Azores ($n = 66$). Consistently, photo-identification techniques show that some individuals move between groups of islands. Analysis of samples from animals stranded along the coast of mainland Portugal ($n = 8$) indicates that the Azorean population is differentiated from that of the mainland. Comparison with DNA sequences available in GenBank reveals that the Azorean population is discrete, but is only slightly differentiated from the offshore population of the North West Atlantic. Contrary to what was found in the North West Atlantic (HOELZEL et al. 1998), behavioural and genetic data do not indicate any differentiation between nearshore and offshore ecotypes in the Azores. Azorean bottlenose dolphins would be of the offshore type, despite the fact that they are mostly spotted within five miles from the coast (SILVA et al. 2003). Pairwise genetic relatedness coefficients indicate that individuals sighted together are no more related than expected by chance, which is a likely consequence of the "fission-fusion" social structure of this species. A regression of relatedness coefficients against distance between sampling locations does not reveal any geographical pattern, suggesting that the Azorean population may represent a single community.

For the sperm whale, genetic analyses were based both on biopsy and sloughed skin samples. Sloughed skin samples preserved in ethanol amplify as well as biopsy samples. They are easier to collect, but usually cannot be allocated to a given individual and are prone to replication. Microsatellite analyses do not reveal any geographical or inter-annual population structure in the Azores ($n = 92$). This result is in agreement with photo-identification surveys, which indicate that some individuals come back to the Azores repeatedly throughout the years, but do not demonstrate fidelity to a given group of islands.

Primary social units are characterized by a high degree of genetic relatedness, which supports the hypothesis that these units consist of related females and offspring (WHITEHEAD & WEILGART 2000). Whatsoever, a single female-offspring pair could be identified in the data set. Secondary associations of primary units (individuals seen in the same area on the same day) are also made up of related individuals.

For both species, future prospects will focus on the relationship between association patterns and genetic relatedness between individuals, which will require the analysis of a higher number of samples.

REFERENCES

- HOELZEL, A.R., C.W. POTTER & P.B. BEST 1998. Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proceedings of the Royal Society of London* 265: 1177-1183.
- SILVA M.A., R. PRIETO, S. MAGALHÃES, R. CABECINHAS, A. CRUZ, J.M. GONÇALVES & R.S. SANTOS 2003. Occurrence and distribution of cetaceans in the waters around the Azores (Portugal), Summer and Autumn 1999-2000. *Aquatic Mammals* 29: 77-83.
- WHITEHEAD, H. & L. WEILGART 2000. The sperm whale: social females and roving males. Pp. 154-172 in: MANN, J., R.C. CONNOR, P.L. TYACK & H. WHITEHEAD (Eds). *Cetacean societies. Field studies of dolphins and whales*. The University of Chicago Press, Chicago. 448 pp.

Sophie Quéroutil (e-mail: squerouil@notes.horta.uac.pt), Ana Pinela, Mónica Silva, Sara Magalhães, Rui Prieto, & Ricardo Santos - IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal; Mónica Silva, Sea Mammal Research Unit, Gatty Marine Laboratory, University of St. Andrews, St. Andrews KY16 8LB, Scotland; José Matos, Diogo Mendonça, Laboratório de Biotecnologia, INETI, Estrada do Paço do Lumiar 22, PT - 1649-038 Lisboa, Portugal.

COMPLEX POPULATION STRUCTURE IN SEA TURTLES

S.A. KARL

KARL, S.A. 2005. Complex population structure in sea turtles. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 17.

Sea turtles have a complex population structure that is exemplified by the loggerhead turtle (*Caretta caretta*) in the North Atlantic. Hatchlings leave the nesting beach and feed in the North Atlantic gyre for up to ten years. During this period, there is no discernable population structure, as juveniles from all regional rookeries are found together in feeding habitats. Subsequently, the subadult turtles move to a neritic (benthic) feeding strategy on shallow continental coastlines.

Based on mtDNA surveys, the subadult (neritic) cohorts have low but significant population structure ($F_{st} = 0.01$, $P < 0.005$), indicating that immature turtles are homing to the vicinity of their natal rookery. Upon reaching sexual maturity, turtles migrate to breeding aggregates and adjacent nesting beaches, and one consequence is strong population structure in adult females ($F_{st} = 0.29$, $P < 0.001$). Thus the degree of population structure increases through progressive life history stages, creating a novel challenge to wildlife management programs. Disturbances to the juvenile populations will impact nesting colonies across the North Atlantic (and possibly the Mediterranean). Disturbance to the subadult populations will have a more direct impact on nearby nesting colonies. Disturbance to the adult nesting population will yield pinpoint damage to the corresponding nesting colony.

While surveys of (maternally inherited) mtDNA show strong population structure among nesting colonies, corresponding surveys of (biparentally inherited) nuclear DNA show no population structure across the same nesting colonies (Pearce 2001, unpublished MS thesis, University of Florida). Apparently loggerhead females home faithfully to their natal nesting colony, but males provide an avenue of gene flow between these nesting colonies, probably via opportunistic mating on feeding grounds and migratory corridors. These findings create a second major challenge for wildlife management. A conventional interpretation of the nDNA data would be that the nesting colonies along the southeastern U.S. comprise a single management unit. However, the natal homing behavior of females (as demonstrated with the mtDNA data) indicates that nesting colonies are demographically independent, and should be managed as isolated populations.

S.A. Karl (e-mail: skarl@hawaii.edu) The Hawai'i Institute of Marine Biology, University of Hawaii at Manoa, Coconut Island, P.O. Box 1346, Kane'ohe, Hawai'i 96744, USA

THE USE OF MITOCHONDRIAL DNA TO DETECT GLOBAL-SCALE BARRIERS TO GENE FLOW OF SHALLOW WATER SEA URCHINS

H.A. LESSIOS

LESSIOS, H.A. 2005. The use of mitochondrial DNA to detect global-scale barriers to Gene Flow of Shallow Water sea urchins. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 18-19.

The world's oceans contain both present-day and historical barriers to dispersal for marine organisms, which have resulted in the isolation of populations and speciation. The ones potentially most important for shallow water tropical species in the last 20 my have been: (1) The Miocene closure of the Tethys; (2) The Pliocene closure of the Isthmus of Panama; (3) The Miocene collision of Australia with Asia; (4) The Miocene initiation of the Benguela upwelling off South Africa; (5) The isolation of the Red Sea during low sea-level stands; (6) The long stretch of deep water between central and eastern Pacific (present for most of the Cenozoic); (7) Sea-level fluctuations from the Messinian to the Holocene. My colleagues and I have evaluated the importance of such barriers for shallow water tropical species by constructing mitochondrial DNA phylogenies of four pantropical genera of sea urchins: *Eucidaris*, *Diadema*, *Echinometra*, and *Tripneustes*, and asking how each barrier has affected each genus. Because all of these genera are strictly tropical, they are unable to maintain contact between the Atlantic and the eastern Pacific around the S. tip of America. The only completely impermeable barrier is the Isthmus of Panama. The Benguela upwelling is also very effective, but there is one instance, in *Diadema* of gene flow around the S. tip of Africa that continued into the Pleistocene. The Eastern Pacific Barrier is a haphazard filter that keeps many, but not all, species from crossing, as seen in *Tripneustes*, in which eastern, central and western Pacific, as well as Indian Ocean populations show very little genetic divergence. The sill of Bab el Mantab has separated shallow water species of *Diadema* when sea levels were low. Many taxa show genetic breaks between the Western Pacific and the Indian Ocean, but as a rule, W. Australian haplotypes group with the W. Pacific. The existence of many species of Pleistocene age indicates that glaciations and associated sea-level fluctuations were the cause of recent speciation in the tropical seas. Formation of all extant species we have examined is too recent to have been caused by the closure of the Tethys. The effectiveness of the barriers as derived from phylogenies and Analysis of Molecular Variance is presented in Table 1.

Table 1
Effectiveness of global barriers for each species of four genera of sea urchins, as determined by genetic discontinuities in mtDNA.

	Panama	East Pacific	Australia	S. Africa	Mid-Atlantic	Ascension	Amazon	Red Sea
<i>Eucidaris</i>								
<i>E. metularia</i>	NA	YES	YES	YES	NA	NA	NA	?
<i>E. thouarsi</i>	YES	YES	NA	NA	NA	NA	NA	NA
<i>E. galapagensis</i>	YES	YES	NA	NA	NA	NA	NA	NA
<i>E. tribuloides</i>	YES	NA	NA	NA	NO	YES	NO	NA
<i>Echinometra</i>								
<i>E. mathaei</i>	NA	YES	?	YES	NA	NA	NA	?
<i>E. oblonga</i>	YES	YES but breached	YES	YES	NA	NA	NA	NA
<i>E. vanbrunti</i>	YES	YES	NA	NA	NA	NA	NA	NA
<i>E. lucunter</i>	YES	NA	NA	NA	NO	NO	YES	NA
<i>E. viridis</i>	YES	NA	NA	NA	YES	YES	YES	NA
<i>Diadema</i>								
<i>D. setosum</i>	NA	YES	YES	YES	NA	NA	NA	YES
<i>D. savignyi</i>	YES	YES but breached	NO	YES	NA	NA	NA	YES
<i>D. paucispinum</i>	NA	YES	YES	YES	NA	NA	NA	YES
<i>D. antillarum</i>	YES	NA	NA	YES	YES	YES	YES	NA
<i>Tripneustes</i>								
<i>T. gratilla</i>	YES	NO	NO	YES	NA	NA	NA	?
<i>T. ventricosus</i>	YES	NA	NA	YES	YES	?	YES	NA

Abbreviation for each barrier: Panama: Isthmus of Panama; East Pacific: East Pacific Barrier; Australia: separation between Indian and Pacific Oceans; S. Africa: tip of S. Africa and Benguela upwelling; Mid-Atlantic: Mid-Atlantic barrier; Ascension: isolation of Ascension and St. Helena islands; Amazon: fresh-water plume of Amazon and Orinoco rivers; Red Sea: separation between the Red Sea and the Indian Ocean. Abbreviations for the action of each barrier: YES: Acts as a barrier either by delimiting the distribution of the species, or by splitting clades within the species. NO: there is no phylogenetic or high F_{ST} effect in mtDNA. NA: not applicable, because species does not occur on either side of the barrier. YES but breached: barrier has caused phylogenetic splitting, but isolated haplotypes of a clade on one side are now found on the other side. ? No data.

H.A. Lessios (e-mail: lessiosh@stri.org) Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama.

GENES THAT FIGHT INFECTION: WHAT BIVALVES SAY ABOUT INNATE IMMUNITY

R. BETTENCOURT

BETTENCOURT, R. 2005. Genes that fight infection: what bivalves say about innate immunity. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 20-24.

The innate immune responses in early Metazoans consist of diverse and effective reactions aimed at the elimination of pathogens. Their survival is thus ensured through efficient recognition of microbes and the activation of host defense mechanisms which involves cell-mediated and humoral reactions. Invertebrates are now emerging as newly suitable genetic and molecular models for the study of innate immune reactions. Many gene products and signaling pathways share striking similarities between Mammals and Invertebrate innate immune reactions suggesting conserved mechanisms throughout evolution, in particular those mechanisms responsible for the induction and transcriptional control of immune genes (HOFFMANN & REICHHART 2002). Perhaps the best example of this is illustrated by the Toll receptor, first discovered and characterized in *Drosophila* and more recently in Mammals. NF- κ B homologues have been extensively studied in Insects and recently characterized in Molluscs, providing further evidence for the conservation of signaling pathways between Mammals and Invertebrates (MONTAGNANI et al. 2004).

Molluscs and Equinoderms were among the first groups in which cytokine-like molecules analogous to mammals were functionally demonstrated in Invertebrates. Interleukin-1 like activity has been identified in sea stars and in mussels using mammalian lymphocytes in mitogenic and antibody cross-reactivities experiments. The mammalian alternative complement pathway has also its homologous counterpart in sea urchins. The C3 protein homologue is inducible in sea urchins coelomocytes (Echinoderm blood cell type) by challenge with lipopolysaccharide (GROSS et al. 1999). Molluscs evolved effective set of immune reactions consisting of complement-like molecules, proteases inhibitors or serpins, metal-binding proteins, lectins, free circulating effector molecules such as lytic enzymes and antimicrobial peptides, immune recognition molecules and cytokines to orchestrate the immune response and ensure communication between different cellular compartments. In addition, invertebrate blood cells or hemocytes, exhibit cytotoxic reactions (oxidative burst) involving NADPH-oxidase, superoxide dismutase, to generate toxic oxygen radicals and nitric oxide, detrimental to microbes (BAYNE 2001). Mollusc hemocytes, as in insects, are also capable of producing antimicrobial peptides and represent one of the hallmarks of the antimicrobial host defense (MITTA et al. 2000).

Molecular data in Bivalves, have been originated from species with commercial value and used for human consumption such as the mussels, *Mytilus edulis* and *Mytilus galloprovincialis* and the oysters *Crassostrea virginica* and *Crassostrea gigas*. *Mytilus* is still one the most studied species with respect to environmental monitoring of pollutants. Recently, molecular studies of bivalve humoral defense clearly indicate that Mollusk as in Insects, are equipped with signaling mechanisms homologous to that of Mammalian nuclear factor kappa-B (NF- κ B) signaling pathway. Thus, it is not surprising that antimicrobial peptides and cytokine-like molecules have been found and are now the subject of gene regulation studies in *Mytilus*.

Bivalve species play an important role with respect to marine environment and human health. Numerous biomedical problems arise from marine environments including infectious diseases transmitted by filter-feeding marine species used for consumption. Bivalves can transmit infectious agents such as viruses and bacteria, and accumulate harmful algae (CANESI et al. 2001). *Salmonella* and *campylobacter* are examples of two bacteria pathogenic to humans and are often the cause of food-borne illness around the world. It is evident that shellfish, such as oysters and clams are increasingly becoming harbingers of these microbes and pose serious risk, especially since they are often eaten raw. Thus, Bivalves have developed intrinsic and dynamic relationships with the infectious agents that they accumulate while feeding. It is important to understand how their biological systems sustain parasitic infestation and how Human health can be affected by the decline of marine coastal environments. Bivalves are also highly efficient bioaccumulators of toxic heavy metals which can lead to poisoning of human communities who consume oysters, clams or mussels from contaminated sites. Consequently, the study of bivalve immunity is fundamental to better address a variety of problems related to the management of those food resources, particularly the problems related to the prevention and spread of invertebrate diseases and human pathogens and to design optimal conditions for cultured marine invertebrates. Hence the urgency to study the invertebrate immune responses at a basic level and develop an appropriate knowledge necessary to better assist decision and policies makers and prevent future outbreak and spread of diseases and preserve the ecology of coastal environments.

OBJECTIVES AND METHODOLOGIES OF MY PROPOSED RESEARCH ACTIVITIES AT OUR DEPARTMENT

Despite an increasing interest for functional genomics and comparative immunobiology studies among invertebrates our knowledge of innate immune mechanisms in Bivalves is still scarce and poorly understood. Given the growing concerns on marine biotope imbalances and their implication on human health, as well as aquaculture and fishing industries constantly challenged by the threat of microbial aggressions, it seems fundamental to develop marine model systems to study and provide new insights into the basic physiological principles that govern the defense mechanisms in Molluscs and in particular the Bivalves.

This project will include the study of two bivalve species: *Ruditapes decussatus* (European clam) and *Bathymodiolus azoricus* (hydrothermal vent mussel). Both species are found in the Azores archipelago. The former is found in a coastal lagoon in São Jorge island. The latter is a dwelling species belonging to the fauna found around deep-sea hydrothermal vents near the mid-Atlantic ridge in the Azores region. The physiological differences that might be found between these two species will probably reflect the distinct environmental conditions in which *R. decussatus* and *B. azoricus* live. On the one hand *R. decussatus* is well acclimatized to the shallow depression that the pool occupies and in that sense the clam population is subjected to the tidal effect unperturbed by the effects of either currents or waves. On the other hand, *B. azoricus* lives under extreme physical and chemical conditions, tolerating unusual levels of heavy metals, pH, temperature, CO₂ and sulphide. How the immune system of deep-sea hydrothermal vents animals is regulated is to the best of our knowledge, unknown. Deep-sea hydrothermal vent environments are considered as highly toxic by human standards, yet the animals dwelling around the vents exhibit high productivity and therefore must efficiently cope with environmental microbes.

In addition to providing evidence for humoral and cellular defense reactions in the two aforementioned bivalves, my study will focus on molecular mechanisms behind host resistance to pathogen infections and ways of entry of the parasite in different clam strains. To target up-regulated genes, whether involved in susceptibility to parasitic infection or

innate immune defenses, the suppression-subtractive hybridization (SSH) technique will be used. SSH clones will be sequenced and compared through BlastX analysis against GenBank databases. Given the fact that a considerable wealth of information is already available from public data bases (e.g. *Drosophila* genome, ESTs clones from the Pacific oyster *Crassostrea gigas*) it will be possible to design cloning strategies for the identification of homologous immune genes in *R. decussatus* and *B. azoricus*. Thus, it will be possible to use degenerated primers, in PCR experiments, based on the latest homology sequences available for genes known to be involved in signal transduction and transcriptional regulation mechanisms participating in host immune defenses such as molecules from the MAP kinases and NF- κ B signaling cascades. With these experiments in mind and as a first step, I expect to shed light into molecular changes associated with the bivalve exposure to pathogens whether in a natural or laboratory settings and dissect the biological process underlying the microbe ability to produce disease in a host organism and the degree of resistance or susceptibility of the host, due mainly to the effectiveness of its defense mechanisms. Finally, it is likely that bivalve immunity studies will benefit from public genomic databases, molecular and genetic tools readily available for the oyster *Crassostrea gigas*. The analysis of EST clones in *C. gigas* constitutes an invaluable comparison tool for gene cloning strategies and cDNA libraries screenings as well as PCR based cloning techniques. A number of genes with putative functions in immunity have been identified in this organism lending support to their involvement in bivalve innate immunity. Work by ESCOUBAS et al (1999); GUEGUEN et al (2003); MONTAGNANI et al (2001, 2004) and TANGUY et al (2004), refer to the use of RT-PCR experiments and the construction of subtractive hybridization cDNA libraries as a means to study differentially expressed genes involved in innate immune responses or in response to exposure to environmental pollutants and stressors. Moreover, DNA micro-array technology, often considered as the next revolution in Molecular Biology, will soon be available to study the expression of genes in bivalves allowing a better characterization of immune genes. Results from gene expression analyses and suppression-subtractive hybridization experiments will be confirmed by Northern-blot experiments. The newly discovered genes or newly characterized signalling and/or inflammatory molecules will be chosen for subsequent studies and analyses considering a potential use in biotechnology. New sequences obtained will be deposited in public databases and results submitted for publication.

SIGNIFICANCE OF THE BIVALVE IMMUNITY PROJECT

The proposed research activity will present our research group with the unique opportunity to investigate new putative genes involved in host-parasite relationships and unravel molecular mechanisms behind host resistance to pathogen infections. This is particularly relevant given growing concerns regarding the impact that microorganisms have on marine organisms and marine fisheries. The quahog parasite unknown (QPX) and the *Perkinsus* parasite are believed to cause significant mortality of aquacultured stocks of clams and quahog fisheries. The knowledge on dynamics of infection and pathogenicity of the parasites is likely to benefit from the proposed research activities and will bring insights as to how ameliorate the management of the parasite in cultured shellfish. The establishment of new molecular diagnostic tools and new methods for health risk assessment in marine invertebrate species of commercial interest is of particular importance given the rapid decline of marine ecosystems as a result of human activities. New therapeutic agents or strategies could be envisaged from our studies to reverse the severe losses of shellfish stocks and other edible mollusks. For the institution's own scientific and technological developments and advancements the opportunity to work in the field of bivalve immunity and in collaboration with other marine science research centers will be beneficial for the subsequent work in the Azores islands. This work will give us for the first time the opportunity to learn and develop knowledge on pathogenic microorganisms, including the mechanisms of resistance to pathogens and diseases, and the effect of environmental factors

on these interactions. Given the north Atlantic localization of the Azores islands, the proposed research activity is well suited for the implementation of a local core facility for continuous monitoring of diseases and to provide advice on management of edible mollusc populations. In addition, *in vivo* and *in vitro* assays to assess healthy and infected populations will reveal differences as to how immune genes are selectively modulated by environmental conditions (Azores versus other regions in the north Atlantic) without the need for establishing elaborated experimental animal models.

CONCLUDING REMARKS

The newly created bivalve immunity area of interest is justified by marine bioscience studies currently being developed at DOP (University of the Azores). My research proposal is in accordance with growing expectations as to implementing and developing scientific programs utilizing increasingly more molecular biology tools to address important subjects regarding preservation of the marine biodiversity in Azores, marine ecosystems and economical assets dependent on fishery practices. A small population genetics core facility is in place since 2000 and has since being developed and expanding by hands of Dr. Sergio Stefanni at first and later with the arrival of Dr. Raul Bettencourt. The work to be conducted in the Azores will be beneficial to the global interests of the Department of Oceanography and Fisheries, and will meet a local growing demand for specialized skills as well as a wish to keep up with the new challenges set by a growing interest on functional genomics studies and the exploitation of new potential applications offered by marine biotechnology sciences.

ACKNOWLEDGEMENTS

This work is supported by a fellowship from the Portuguese Foundation for Science and Technology granted to Raul Bettencourt: SFRH/BPD/14896/2004

REFERENCES

- BAYNE C.J., U.K. HAHN & R.C. BENDER 2001. Mechanisms of molluscan host resistance and of parasite strategies for survival. *Parasitology* 123: 159-167.
- BAYNE C.J. 2002. Origins and Evolutionary Relationships between the Innate and Adaptive Arms of Immune Systems. *Integrative and Comparative Biology* 43(2): 293-299.
- BECK G. 1998. Macrokines: Invertebrate cytokine-like molecules? *Frontiers in Bioscience* 3: 559-569
- BECK G., T.W. ELLIS & N. TRUONG 2000. Characterization of an IL-1 receptor from *Asterias forbesi* coelomocytes. *Cellular Immunology* 203: 66-73
- CANESI L., C. PRUZZO, R. TARSİ & G. GALLO 2001. Surface Interactions between *Escherichia coli* and Hemocytes of the Mediterrean Mussel *Mytilus galloprovincialis* Lam. Leading to Efficient bacterial Clearance. *Applied and Environmental Microbiology* (Jan): 464-468
- ESCOUBAS J.M, L. BRIANT, C. MONTAGNANI, S. HEZ, C. DEVAUX & P. ROCH 1999. Oyster IKK-like protein shares structural and functional properties with its mammalian homologues. *FEBS letter* 453: 293-298
- GROSS P.S., W.Z. AL-SHARIF, L.A. CLOW & L.C. SMITH 1999. Echinoderm immunity and the evolution of the complement system. *Developmental & Comparative Immunology* 23: 429-442
- GUEGUEN Y., J.P. CADORET, D. FLAMENT, C. BARREAU-ROUMIGUIERE, A.L. GIRARDOT, J. GARNIER, A. HOAREAU, E. BACHERE & J.M. ESCOUBAS 2003. Immune gene discovery by expressed sequence tags generated from hemocytes of the bacteria-challenged oyster, *Crassostrea gigas*. *Gene* 303: 139-145.

- HOFFMANN J.A. & J.M. REICHHART 2002. Drosophila innate immunity: an evolutionary perspective. *Nature Immunology* 3(2): 121-126
- LACOSTE A., A. CUEFF & S.A. POULET 2002. P35-sensitive caspases, MAP kinases and Rho modulate B-adrenergic induction of apoptosis in mollusc immune cells. *Journal of Cell Science* 115: 761-768
- LAVINE M.D. & M.R. STRAND 2002. Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology* 32(10):1295-1309.
- LEITE R.M., P.M. RODRIGUES, L.M. ELANDALLOUSSI, R.M. AFONSO, P.A. NUNES & M.L. CANCELA 2003. *Perkinsus atlanticus* - Desenvolvimento de um método de diagnóstico para protecção da infecção em moluscos bivalves. *Sociedade Portuguesa de Biotecnologia. Boletim de Biotecnologia* 75: 8-10.
- LOPEZ C., M.J. CARBALLAL, C. AZEVEDO & A. VILLALBA 1997. Morphological characterization of the hemocytes of the clam, *Ruditapes decussatus* (Mollusca: Bivalvia). *Journal of Invertebrate Pathology* 69: 51-57
- MONTAGNANI C., F. LE ROUX, F. BERTHE & J.M. ESCOUBAS 2001. Cg-TIMP, an inducible tissue inhibitor of metalloproteinase from the Pacific oyster *Crassostrea gigas* with a potential role in wound healing and defense mechanisms. *FEBS letter* 500: 64-70
- MONTAGNANI C., C. KAPPLER, J.M. REICHHART & J.M. ESCOUBAS 2004. Cg-Rel the first Rel/NF- κ B homolog characterized in a mollusk, the Pacific oyster *Crassostrea gigas*. *FEBS Letters* 561(1-3): 75-82.
- Marine Biotechnology: An European Strategy for Marine Biotechnology. ESF Marine Board Feasibility Study Group Report. December 2001. <http://www.esf.org/publication/127/biotech.pdf>
- MITTA G., F. VANDENBULCKE, T. NOEL, B. ROMESTAND, J.C. BEAUVILLAIN, M. SALZET & P. ROCH 2000. Differential distribution and defence involvement of antimicrobial peptides in mussel. *Journal of Cell Science* 113: 2759-2769.
- Proposal for the construction of BAC libraries from the Eastern Oyster *Crassostrea virginica* and the Pacific Oyster *Crassostrea gigas*. <http://www.genome.gov/Pages/Research/Sequencing/BACLibrary/OysterBACconstruction.pdf>
- TANGUY A., X. GUO & S.E. FORD 2004. Discovery of genes expressed in response to *Perkinsus marinus* challenge in Eastern (*Crassostrea virginica*) and Pacific (*C. gigas*) oysters. *Gene* 338(1): 121-31.
- WOOTTON E.C., E.A. DYRYNDA, R.K. PIPE & N.A. RATCLIFFE 2003. Bivalve Immunity: comparisons between the marine mussel (*Mytilus edulis*), the edible cockle (*Cerastoderma edule*) and the razor-shell (*Ensis siliqua*). *Fish and Shellfish Immunology* 15: 195-210

R. Bettencourt (e-mail: raul@notes.horta.uac.pt) - IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal

MOLECULAR CHARACTERISATION OF PELAGIC LARVAE & MOLECULAR BIOLOGY OF HYDROTHERMAL VENT ORGANISMS

D.R. DIXON

DIXON, D.R. 2005. Molecular characterisation of pelagic larvae & molecular biology of hydrothermal vent organisms. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 25-26.

I would wish to cover the work we have been doing on deep-sea vent mussels, *Bathymodiolus azoricus*, and their larvae from three contrasting vent fields on the mid Atlantic Ridge. This includes molecular analyses of individual mussel larvae trapped at hydrothermal sites SW of the Azores Triple Junction, and uses PCR amplification of diagnostic ITS2 regions as species and population markers. I will also include some information relating to our current work on the chromosomes of deep-sea vent organisms, and include recent evidence relating to the analysis of chromosome polymorphisms using the state-of the art techniques of chromosome microdissection and FISH (fluorescent labelling of specific chromosome arms).

Different levels of DNA strand breakage were measured, using the comet assay, in untreated mussels, shortly after collection, and it was animals from the shallowest, and less active, Menez Gwen vent field (840-m depth), which showed the greatest amount of damage. In contrast to animals from two deeper and putatively more toxic sites, Menez Gwen animals went on to repair this damage and were able to survive under laboratory conditions at 1 bar pressure for several months. Animals from the two deeper sites showed both higher levels of initial mortality and a much reduced capacity for survival at 1 bar. The differences in DNA damage levels at the time of collection were interpreted as an expression of differences in cell viability/enzyme activity rather than a reflection of any differences in their natural environmental conditions. Small *B. azoricus* showed a capacity to repair DNA damage, whereas this ability appeared to be lacking in large individuals. By reproducing at a relatively early age, the deep-sea vent fauna may be able to resist the toxic effects of its environment by exploiting this natural, stage specific capacity to repair damaged DNA.

MILEIKOVSKY (1971) proposes four main types of development in benthic invertebrates: pelagic (e.g. vent mytilids, vestimentiferans), demersal (*Alvinella*), direct and viviparous (*Ventrella*). Further complications are: entire or partial pelagic larvae, short or long pelagic development, and feeding or non-feeding larvae (all of which are found in vent invertebrates). Hence, there is no single reproductive strategy which is representative of vent species.

Genetic differentiation at the ridge scale
Gst or Fst values <0.05 signify little or no differentiation

<i>R. pachyptila</i> Nei' Gst	EPR 0.022	Entire range 0.031
<i>R. piscesae</i> Wright's Fst	JdF 0.07	Entire range 0.10
<i>V. sulfuris</i> Gst	EPR 0.046	Entire range 0.441
<i>P. grasslei</i> Gst	EPR 0.029	Entire range 0.069

Gene flow and population differentiation are inversely related: high levels of gene flow result in homogenisation of subpopulations (low F_{st} values), whereas interrupted or restricted gene flow allows independent genetic evolution and differentiation (high F_{st} values). How much gene flow is required to prevent genetic differentiation taking place? Answer: as little as 0.1 migrants per generation.

GENETIC VARIABILITY OF VENT ORGANISMS

Based on the percentage of polymorphic loci (from allozyme studies), the range is from 6% in the clam *Calyptogena magnifica* to 65% in the polychaete *Paralvinella grasslei*. Vagile species such as the amphipod *Ventiella sulfuris* have the same level of polymorphism as some sessile species. Reproductive strategy is clearly a dominant influence on genetic differentiation levels. In such an unstable environment, dispersal is essential to ensure colonisation of new sites and prevent extinction.

DOES GENE FLOW PREVENT ADAPTATION TO LOCAL CONDITIONS?

Where there are low levels of gene flow, well-adapted combinations of genes have the potential to become fixed locally through the combined action of genetic drift and natural selection. If gene flow levels are always high, well-adapted combinations of genes cannot become established in any single population, and if levels of gene flow are always low, these combinations could become established in one population but would not necessarily spread to another. Gene flow in some species appears sporadic, with dispersal over short distances occurring over most of the time but with dispersal over longer distances occurring frequently enough to produce widespread genetic homogeneity. It is possible to detect genetic variation at or below the segment scale even in some planktonic spawning species (e.g. *Ridgeia*), but how significant or important this is in relation to the evolution and biogeography of hydrothermal-vent organisms is not known at the present time. Transient isolation, at the segment and subsegment scale, may be important for maintaining the high levels of genetic variation seen in some species (e.g. *Alvinella*). This may be of some evolutionary significance, although other species (e.g. *Bathymodiolus thermophilus*) appear to do perfectly well without. Identified barriers to gene flow in the Pacific include Hess Deep separating the EPR and Galapagos Rift (e.g. *Ventiella*: topography) and the 300 km transform fault separating the Juan de Fuca and Gorda Ridges on the MAR (e.g. *Ridgeia*: distance and/or hydrography). Comparing the Atlantic and the Pacific, some evidence suggests that the marked depth differences between vent fields in the Atlantic (e.g. 1600 metres between Lucky Strike and Snake Pit), coupled with the barrier effects of transform faults and segment walls, plus the longer time scale of venting (thousands of years), may have led to endemism on a much smaller scale in the Atlantic. We await the results of further research with great interest.

D.R. Dixon (e-mail: drd@soc.soton.ac.uk); George Deacon Division for Ocean Processes, Southampton Oceanography Centre, University of Southampton, United Kingdom.

OVERVIEW OF SEABIRD GENETICS

T.M. BURG

BURG, T.M. 2005. Overview of seabird genetics. *Arquipélago*. Life and Marine Sciences 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 27-29.

Seabirds are a diverse group of organisms exhibiting a large amount of variation in life history traits and population dynamics. These species are found in a variety of habitats ranging from remote oceanic islands to coastal waters and can sometimes be found in inland areas. While, they are capable of dispersing vast distances, many species are highly philopatric. Recently, seabirds have become the focus for genetic studies to examine systematics, population structure and mating systems. Seabirds, especially those breeding on oceanic islands, are ideal study organisms because of their varying characteristics and the fact that they form discrete breeding populations.

Patterns of population structure in seabirds seem to be as varied as the birds themselves (Fig. 1). Physical barriers, such as land appear to be effective barriers to gene flow for most seabirds. Studies on globally distributed species in the Northern Hemisphere and tropics, suggest that gene flow is severely restricted between the Indo-Pacific and Atlantic Oceans. Genetic studies on sooty terns and three species of boobies have shown clear phylogenetic splits between the Indo-Pacific and Atlantic populations (AVISE et al. 2000; STEEVES et al. 2003). Within ocean basins and in the Southern Ocean, where there are no physical barriers, the pattern is more variable. For example, sub-antarctic species, such as shy albatross (*Thalassarche cauta*) in the Tasman Sea, exhibit high levels of population differentiation with each island forming a genetically distinct population (ABBOTT & DOUBLE 2003). In sharp contrast, the closely related grey-headed albatross (*T. chrysostoma*) is completely panmictic and the level of gene flow between geographically distant islands is high (BURG & CROXALL 2001). A similar pattern is found within the wandering albatross complex. The widely distributed *Diomedea exulans* shows low levels of population differentiation between breeding sites (<7000 km), compared to high levels of differentiation between *D. antipodensis* breeding on Antipodes and Adams Island (<750 km) (BURG & CROXALL 2004). In contrast, several northern species of seabirds show no genetic differentiation within the North Atlantic. The common murre (*Uria aalge*), black-legged kittiwake (*Rissa tridactyla*) and northern fulmar (*Fulmarus glacialis*) were all found to be panmictic across their range (Riffault pers. comm., McCoy pers. comm., BURG et al. 2003). Within ocean basins, there is evidence of isolation by distance (black-legged kittiwake and wandering albatross complex), long distance dispersal (great frigatebirds, *Fregata minor*; black-browed albatross, *T. melanophris*) and range expansion (northern fulmars, *Fulmarus glacialis*) (BURG et al. 2003; BURG & CROXALL 2001; DEARBORN et al. 2003; MOORE et al. 2001).

Clearly, genetic approaches add important insights into the biology of seabirds and combined with demographic and behavioural studies, they will continue to improve our understanding of population dynamics and life history traits of this vast group.

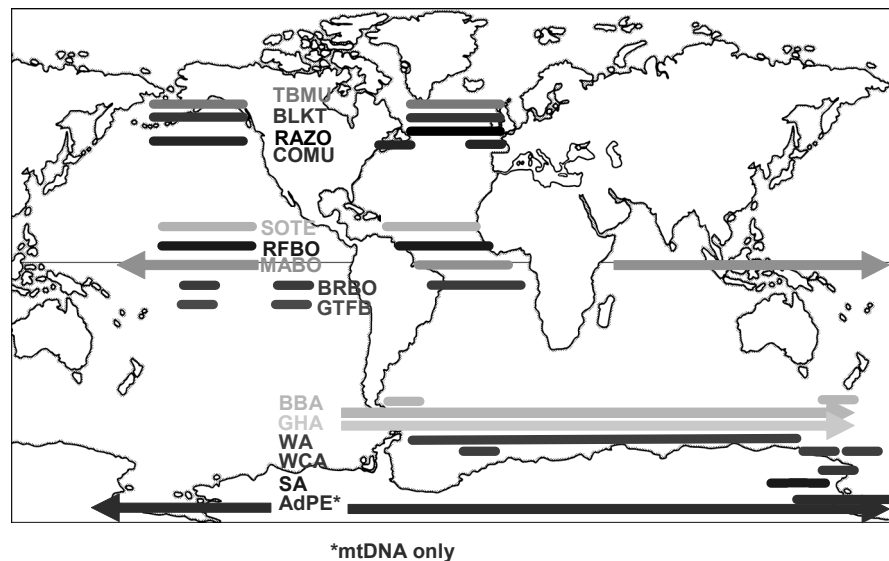


Fig. 1. Summary of global phylogenetic studies on seabirds (ABBOTT & DOUBLE 2003; BURG & CROXALL 2001; BURG & CROXALL 2004; DEARBORN et al. 2003; MOUM & ARNASON 2001; RITCHIE et al. 2004; STEEVES et al. 2003). Each continuous line represents genetically discrete populations. Species include thick-billed murre (*Uria lomvia*, TBMU), black-legged kittiwake (*Rissa tridactyla*, BLKT), razorbills (*Alca torda*, RAZO), common murre (*Uria aalge*, COMU), sooty tern (*Sterna fuscata*, SOTE), red-footed booby (*Sula sula*, RFBO), masked booby (*Sula dactylatra*, MABO), brown booby (*Sula leucogaster*, BRBO), great frigatebird (*Fregata minor*, GTFB), black-browed albatross (*Thalassarche melanophris* and *T. impavida*, BBA), grey-headed albatross (*Thalassarche chrysostoma*, GHA), wandering albatross (*Diomedea exulans*, *D. dabbenena*, *D. antipodensis*, WA), white-capped albatross (*Thalassarche steadi*, WCA), shy albatross (*Thalassarche cauta*, SA) and Adelie penguin (*Pygoscelis adelia*, AdPE).

REFERENCES:

- ABBOTT, C.L. & M.C. DOUBLE 2003. Genetic structure, conservation genetics and evidence of speciation by range expansion in shy and white-capped albatrosses. *Molecular Ecology* 12: 2953-2962.
- AVISE, J., W. NELSON, B. BOWEN & D. WALKER 2000. Phylogeography of colonially nesting seabirds, with special reference to global matrilineal patterns in the sooty tern (*Sterna fuscata*). *Molecular Ecology* 9: 1783-1792.
- BURG, T.M., J. LOMAX, R. ALMOND, M.L. BROOKE & W. AMOS 2003. Unraveling dispersal patterns in an expanding population of a highly mobile seabird, the northern fulmar (*Fulmarus glacialis*). *Proceedings of the Royal Society, Series B*, 270 979-984.
- BURG, T.M. & J.P. CROXALL 2001. Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. *Molecular Ecology* 10: 2647-2660.
- BURG, T.M. & J.P. CROXALL 2004. Global population structure and taxonomy of the wandering albatross species complex. *Molecular Ecology* 13: 2345-2355.
- DEARBORN, D.C., A.D. ANDERS, E.A. SCHREIBER, R.M.M. ADAMS & U.G. MUELLER 2003. Inter-island movements and population differentiation in a pelagic seabird. *Molecular Ecology* 12: 2835-2843.
- MOORE, P.J., T.M. BURG, G.A. TAYLOR & C.D. MILLAR 2001. Provenance and sex ratio of Black-browed Albatross *Diomedea m. melanophris* breeding on Campbell Island. *Emu* 101: 329-334.

- MOUM, T. & E. ARNASON 2001. Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. *Molecular Ecology* 10: 2463-2478.
- RITCHIE, P., C. MILLAR, G. GIBB, C. BARONI & D. LAMBERT 2004. Ancient DNA enables timing of the Pleistocene origin and Holocene expansion of two Adelie penguin lineages in Antarctica. *Molecular Biology and Evolution* 21: 240-248.
- STEEVES, T., D. ANDERSON, H. McNALLY, M. KIM & V. FRIESEN 2003. Phylogeography of *Sula*: the role of physical barriers to gene flow in the diversification of tropical seabirds. *Journal of Avian Biology* 34: 217-223.

T.M. Burg (e-mail: burgt@biology.queensu.ca or theresaburg@yahoo.com) Dept. of Biology, Queen's University, Kingston ON, Canada, K7L 3N6

COMBINING GENETIC AND DEMOGRAPHIC DATA TO DETERMINE GENETIC VARIABILITY AND POPULATION STRUCTURE IN THE WANDERING ALBATROSS (*Diomedea exulans*)

J. BRIED, M. NICOLAUS, P. JARNE & P. JOUVENTIN

BRIED, J., M. NICOLAUS, P. JARNE & P. JOUVENTIN 2005. Combining genetic and demographic data to determine genetic variability and population structure in the wandering albatross (*Diomedea exulans*). *Arquipélago. Life and Marine Sciences* 22A. Acta of the 1st International Workshop in Marine Molecular Phylogenetics "Use of Molecular Markers for the Study of Marine Biodiversity": 30-32.

The Wandering Albatross *Diomedea exulans* exhibits interesting, and somewhat paradoxical, traits from a population biology perspective, such as (i) a limited population size (during a given year, about 20000 pairs attempt to breed, with no more than 6000 breeding pairs at any locality, TICKELL 2000) which has markedly decreased over the last three centuries due to human activities (harvesting for food, albatross fishing and shooting, fisheries; JOUVENTIN et al. 1988; MEDWAY 1998; WEIMERSKIRCH et al. 1997; CROXALL & GALES 1998), (ii) strong philopatry associated with quasi-absolute fidelity to its breeding islands (TICKELL 2000; RYAN et al. 2001; COOPER & WEIMERSKIRCH 2003), very high site and mate fidelity (both > 92%) from one breeding cycle to the next (BRIED et al. 2003), delayed maturity (individuals start breeding when *ca* 10 years old on average, TICKELL 2000), high longevity (more than 50 years, WEIMERSKIRCH & WILSON 2000), low fecundity (pairs breed every other year when they manage to fledge their single chick but annually when they fail during incubation, TICKELL 2000; on average, a pair successfully raises a chick every third year, JOUVENTIN & WEIMERSKIRCH 1988), and (iii) foraging at sea several thousands of kilometres away from its breeding localities all year round (WEIMERSKIRCH et al. 1993; WEIMERSKIRCH & WILSON 2000).

Using both genetic and demographic methods, we evaluated how these traits might affect the structure within (inbreeding, low variability) and among (restricted gene flow) populations. Our study was conducted on Possession Island, Crozet archipelago. The genetic approach was based on two colonies (Pointe Basse and Baie du Marin) sampled in 2001-2002 and six microsatellite loci: De1, De2, De3, De7, De18, isolated from the Wandering Albatross, plus De5 which was isolated from the Grey-headed Albatross *Thalassarche chrysostoma* (BURG 1999; BURG & CROXALL 2001). The demographic approach used data from a 36-year capture-mark-recapture survey of the entire population from Possession island.

The breeding pairs from each colony did not exhibit a more extreme (higher or lower) genetic relatedness than would pairs drawn at random from the same population based on a permutation test: 7.2% of lower relatedness values were obtained in Pointe Basse, and 15% of higher values in Baie du Marin. Our demographic data revealed only one pair out of 355 was inbred in 2001-2002, which did not differ from the expected frequency under random mating. Therefore, inbreeding neither occurred more often than expected by chance nor was avoided. Within-population genetic variability was very limited. However no genetic bottleneck was detected, a likely explanation being small effective population size due to low census size and slow demography.

No significant genetic differentiation occurred between the two colonies (exact test, adults: $P = 0.128$; chicks: $P = 0.525$). The estimates of F_{st} were 0.008 for adults ($n = 102$) and -

0.009 for chicks ($n = 51$), consistent with our demographic estimates of dispersal (about one individual per year). Demographic data confirmed that males were more philopatric than females when performing their first breeding attempt (95%, $n = 499$ recruits, vs 92%, $n = 493$; $G_1 = 5.72$, $P = 0.017$), but the genetic differentiation between colonies was not higher in males and within each colony, no differentiation between sexes was found (exact test, all: $P \geq 0.1$; all F_{st} values were < 0.01 , $n = 51$ for each sex): the demographic difference is probably too small to be detected given the amount of available genetic variability. Although low genetic variability is often associated with low adaptability in response to environmental perturbations (FRANKLIN 1980), the foraging ecology of Wandering Albatrosses is exceptionally buffered against environmental instability caused by fluctuations in prey availability (XAVIER et al. 2003).

ACKNOWLEDGEMENTS

The French Polar Institute (IPEV) and the INSU Department “Environnements et Sociétés” of the CNRS (“Zone-Atelier de Recherches sur l’Environnement Antarctique et Subantarctique”) supported this study, which was also part of JB’s post-doctoral contract at the Instituto do Mar (IMAR/FCT-PDOC-001/2001-BirdEco and FCT grant SFRH/BPD/20291/2004) and of MN’s diploma (DEA). Bird banding and blood-sampling were approved by the Ethical Committee of IPEV. We thank F. Pawlowski and F.S. Dobson for field assistance. C. Debain, F. Di Giusto, P. Sourrouille, A. Charmantier and K. Belkhir helped during the genetic analyses, and T. Lenormand wrote the permutation program. A. Charmantier made helpful comments.

REFERENCES

- BRIED, J., D. PONTIER & P. JOUVENTIN 2003. Mate fidelity in monogamous birds: a re-examination of the Procellariiformes. *Animal Behaviour* 65: 235-246.
- BURG, T. M. 1999. Isolation and characterization of microsatellites in albatrosses. *Molecular Ecology* 8: 338-341.
- BURG, T. M. & J. P. CROXALL 2001. Global relationships amongst Black-browed and Grey-headed Albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. *Molecular Ecology* 10: 2647-2660.
- COOPER, J. & H. WEIMERSKIRCH 2003. Exchange of the Wandering Albatross *Diomedea exulans* between the Prince Edward and Crozet Islands: implications for conservation. *African Journal of Marine Science* 25: 519-523.
- CROXALL, J.P. & R. GALES 1998. An assessment of the conservation status of albatrosses. Pp. 46-65 in: ROBERTSON, G. & R. GALES (Eds). *Albatross biology and conservation*. Surrey Beatty & Sons, Chipping Norton, 299 pp.
- FRANKLIN, I.R. 1980. Evolutionary change in small populations. Pp. 135-149 in: SOULÉ, M.E. & B.A. WILCOX (Eds). *Conservation Biology: an evolutionary approach*. Sinauer, Sunderland, MA.
- JOUVENTIN, P., J.-C. STAHL & H. WEIMERSKIRCH 1988. La conservation des oiseaux des Terres Australes et Antarctiques Françaises. *Livre rouge des oiseaux menacés des régions françaises d’Outre-mer. ICBP Technical Publications* 5: 225-251.
- JOUVENTIN, P. & H. WEIMERSKIRCH 1988. Demographic strategies of southern albatrosses. *Proceedings of the 19th International Congress of Ornithology (Ottawa 1986)*: 857-865.
- MEDWAY, D.G. 1998 Human-induced mortality of Southern Ocean albatrosses in the 19th century: a brief historical review. Pp. 189-198 in: ROBERTSON, G. & R. GALES (Eds). *Albatross biology and conservation*. Surrey Beatty & Sons, Chipping Norton, 299 pp.

- RYAN, P.G., J. COOPER & J.P. GLASS 2001. Population status, breeding biology and conservation of the Tristan Albatross *Diomedea (exulans) dabbenena*. *Bird Conservation International* 11: 35-48.
- TICKELL, W.L.N. 2000. *Albatrosses*. Pica Press, The Banks, Mountfield, East Sussex, 448 pp.
- WEIMERSKIRCH, H., N. BROTHERS & P. JOUVENTIN 1997. Population dynamics of Wandering Albatross *Diomedea exulans* and Amsterdam Albatross *D. amsterdamensis* in the Indian Ocean and their relationships with long-line fisheries: conservation implications. *Biological Conservation* 79: 257-270.
- WEIMERSKIRCH, H., M. SALAMOLARD, F. SARRAZIN & P. JOUVENTIN 1993. Foraging strategy of Wandering Albatrosses throughout the breeding season: a study using satellite telemetry. *Auk* 110: 325-342.
- WEIMERSKIRCH, H. & R.P. WILSON 2000. Oceanic respite for Wandering Albatrosses. Birds taking time off from breeding head for favourite long-haul destinations. *Nature* 406: 955-956.
- XAVIER, J.C., J.P. CROXALL, P.N. TRATHAN & P.G. RODHOUSE 2003. Inter-annual variation in the cephalopod component of the diet of the Wandering Albatross, *Diomedea exulans*, breeding at Bird Island, South Georgia. *Marine Biology* 142: 611-622.

Joël Bried (e-mail: bried@notes.horta.uac.pt), IMAR Centre of the University of the Azores, Department of Oceanography and Fisheries, PT - 9901-862 Horta, Azores, Portugal; Marion Nicolaus, Philippe Jarne and Pierre Jouventin, Centre National de la Recherche Scientifique, Centre d'Ecologie Fonctionnelle et Evolutive, 1919 route de Mende, 34293 Montpellier Cedex 5, France