THE ANNUAL BALZAN LECTURE

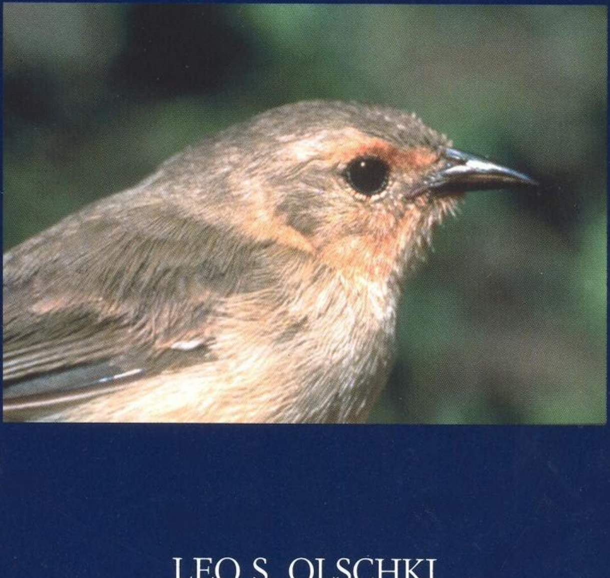
1

THE EVOLUTION OF DARWIN'S FINCHES, MOCKINGBIRDS AND FLIES

by

PETER and ROSEMARY GRANT

2005 Balzan Prizewinners



LEO S. OLSCHKI
2010

Peter and Rosemary Grant

The Evolution of Darwin's Finches, Mockingbirds and Flies

13 May 2010, Rome, Accademia Nazionale dei Lincei

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Swiss Academies of Arts and Sciences

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ALBERTO QUADRIO CURZIO
Chairman of the Joint Commissions established by
the International Balzan Foundation "Prize", the Accademia Nazionale dei Lincei
and the Swiss Academies of Arts and Sciences

FOREWORD

It is a great honour and a remarkable satisfaction for me to write the foreword to this first volume in "The Annual Balzan Lecture" series.

This lecture series is the fruit of the Agreements on Collaboration[1] between the International Balzan Foundation "Prize"[2] the Accademia Nazionale dei Lincei[3] and the Swiss Academies of Arts and Sciences.[4]

As Chairman of the two Joint Commissions, established to give definitive shape to such collaboration, it is both my pleasure and my duty to illustrate the origin and the aim of this initiative.

Since becoming a member of the Balzan "Prize" Board, I have appreciated the fact that its Chairman, Ambassador Bruno Bottai, has always stressed the inherent international nature of the Balzan, while at the same time recognizing its strong historical roots in Italy and Switzerland. This sentiment – shared by the whole Balzan "Prize" Board, which, I would like to stress, includes Achille Casanova, who is also Chairman of the Balzan "Fund" Board in Zurich – expresses the wishes of the Foundress of the "Balzan" and is actively supported by the Governments of the two Countries.

In order to give practical expression to this, I proposed that the Balzan "Prize" Board should evaluate a possible collaboration with the Accademia Nazionale dei Lincei and the Swiss Academies of Arts and Sciences. Both of which are highly regarded internationally.

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[1] See p. 11.

[2] See p. 12.

[3] See p. 13.

[4] See p. 13.

This collaboration has now taken concrete shape through the constructive role of the Balzan "Prize" Board.[5] Its final form is the result of discussions I had with Professor Dr. René Dändliker, former President of the Swiss Academies of Arts and Sciences and Dr. Markus Zürcher, Head of Administration of the Swiss Academies of Arts and Sciences, where this proposal was fully elaborated with the collaboration of Dr. Suzanne Werder, Secretary General of the Balzan "Prize" Foundation. I am grateful for the emphatic support given by the former President of the Accademia Nazionale dei Lincei, Professor Giovanni Conso, to these endeavours.

I am confident that the opportunities for collaboration provided by the Agreements will be beneficial and will strengthen the international role of the Balzan Foundation in promoting the research endeavours of the Balzan Prizewinners and the public diffusion of the results of these activities.

The institutional activities of the Balzan Foundation will obviously remain untouched by these Agreements where the authority of the General Prize Committee – composed of twenty eminent European scholars and scientists – remains absolute. It must be stressed here that the selection of the Balzan Prizewinners is strictly reserved to the autonomous Balzan General Prize Committee which retains its own full statutory competence within the Balzan Foundation.

The tri-polar collaboration will certainly contribute in its own way to the spreading of the renown of the Balzan Foundation internationally and will embellish its image as one of the most prestigious forms of recognition in the sciences and the humanities.

* * *

Practical collaboration was initiated with a splendid event, held at the Swiss National Science Foundation in Bern last year which took the form of an interdisciplinary Forum involving the 2009 Balzan Prizewinners.

Following on from that, this first Annual Balzan Lecture delivered by Professors Peter and Rosemary Grant on *The Evolution of Darwin's Finches, Mockingbirds and Flies*, is indeed a very appropriate point of departure for this series of lectures. It exemplifies the central purpose

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[5] For composition of members see p. 71.

of the Agreements, to promote the diffusion of cutting edge research and provide a setting for fruitful academic exchanges. Indeed, such exchanges are greatly enhanced by the research projects funded out of the second half of the Grants' Balzan Prize which involve four young researchers.

Peter and Rosemary Grant[6] were awarded the 2005 Balzan Prize for Population Biology *for their remarkable long-term studies demonstrating evolution in action in Galápagos finches*. This work has assured the Professors Grant a prestigious position in the scientific pantheon. Thus, we are very pleased that they have accepted our invitation to initiate this series of lectures.

I state this both as President of the Joint Commissions overseeing the Agreements and as President of the Class of Moral, Historical and Philological Sciences of the Accademia Nazionale dei Lincei. As an aside I would like to thank my two distinguished colleagues of the Lincei, Professors Giuseppina Barsacchi and Ernesto Capanna for having accepted with enthusiasm to be discussants and to welcome the presence at this annual lecture, of Professor Dr. Peter Suter, President of the Swiss Academies of Arts and Sciences. Finally I would like to thank also Professor Lamberto Maffei, President of the Accademia Nazionale dei Lincei, for his hospitality in hosting the first "Annual Balzan Lecture".

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[6] For biographical and bibliographical data see p. 49.

AGREEMENTS ON COLLABORATION BETWEEN THE INTERNATIONAL BALZAN FOUNDATION "PRIZE" THE ACCADEMIA NAZIONALE DEI LINCEI AND THE SWISS ACADEMIES OF ARTS AND SCIENCES

(hereafter referred to as the 'Balzan', the 'Lincei' and the 'Swiss Academies', respectively)

The main points of the agreements between the Balzan, the Lincei and the Swiss Academies are the following:

1) The promotion of the Balzan Prize and the presentation of the Prizewinners through the academies' channels of communication, in Italy and Switzerland as well as further abroad. By virtue of the relations of the Swiss Academies and the Lincei with academies of other countries and with international academic organizations, they will contribute to raising the profile of the Balzan.

2) On the occasion of the Balzan Awards ceremony, held on alternating years in Bern and Rome, each academy will contribute to the scientific organization of an interdisciplinary Forum, in the course of which the Prizewinners of that year will present their scientific work and discuss it with other scientists proposed by the academies. Furthermore, in the years when the ceremony is held in Rome, one of the Prizewinners will give a Balzan Distinguished Lecture in Switzerland, and when the ceremony is held in Bern, a Balzan Distinguished Lecture will be organized at the headquarters of the Lincei in Rome;

3) The academies will contribute to a series of publications in English (ideally with summaries in Italian, German and French), created by the Balzan, with the collaboration of the Balzan Prizewinners.

To promote and supervise all these initiatives, two Commissions have been set up, one between the Balzan and the Swiss Academies (composed of Professors René Dändliker and Peter Suter as well as Dr. Markus Zürcher) and another between the Balzan and the Lincei (composed of Professors Sergio Carrà, Lellia Cracco Ruggini and formerly Claudio Leonardi †, now Carlo Ossola). Both commissions are chaired by Professor Alberto Quadrio Curzio as a representative of the Balzan, which is also represented by Professors Enrico Decleva and Paolo Matthiae, while the Balzan Secretary General, Dr. Suzanne Werder, has been appointed Secretary of both Commissions.

International Balzan Foundation

The International Balzan Foundation was established in Lugano in 1956 thanks to the generosity of Lina Balzan, who had come into a considerable inheritance on the death of her father, Eugenio. She decided to use this wealth to honour his memory.

Eugenio Francesco Balzan was born in Badia Polesine, near Rovigo (Northern Italy), on 20 April 1874 into a family of landowners. He spent almost his entire working life at Milan's leading daily newspaper, *Corriere della Sera*. After joining the newspaper in 1897, he quickly worked his way up from editorial assistant, to news editor and special correspondent. In 1903 editor Luigi Albertini made him managing director of the paper's publishing house; he then became a partner and shareholder in the company. He was not only a skilful manager but also a leading personality in Milanese society. In 1933 he left Italy due to opposition from certain quarters hostile to an independent *Corriere*. He then moved to Switzerland, living in Zurich and Lugano, where for years he had invested his fortune with success. He also continued his charitable activities in favour of institutions and individuals. He officially returned to Italy in 1950. Eugenio Balzan died in Lugano, Switzerland, on 15 July 1953.

The *International E. Balzan Prize Foundation – "Prize"* aims to promote, throughout the world, culture, science, and the most meritorious initiatives in the cause of humanity, peace and brotherhood among peoples, regardless of nationality, race or creed. This aim is attained through the annual award of prizes in two general fields: literature, the moral sciences and the arts; medicine and the physical, mathematical and natural sciences. Nominations for the prizes in the scientific and humanistic fields are received at the Foundation's request from the world's leading learned societies. Candidates are selected by the *General Prize Committee*, composed of eminent European scholars and scientists. Prizewinners must allocate half of the Prize to research work, preferably involving young researchers. At intervals of not less than three years, the Balzan Foundation also awards a prize for Humanity, Peace and Brotherhood Among Peoples.

The *International E. Balzan Prize Foundation – "Prize"* attains its financial means from the *International E. Balzan Prize Foundation – "Fund"* which administers Eugenio Balzan's estate.

Accademia Nazionale dei Lincei

The Accademia Nazionale dei Lincei, founded in 1603 by the Roman aristocrat and scientist Federico Cesi and the Dutch physician Johann Eck, is the oldest scientific academy in the world. It promotes academic excellence through its Fellows who have included, among many other renowned names, Galileo Galilei.

Since 1992, the Academy has provided specialized advice to the President of the Italian Republic and has recently received the 'High Permanent Patronage' of the President of the Italian Republic.

The Academy's mission is "to promote, coordinate, integrate and spread scientific knowledge in its highest expressions in the context of cultural unity and universality".

To this end, the Accademia Nazionale dei Lincei organises national and international conferences, meetings and seminars and its members participate in similar Italian and foreign initiatives. The Academy promotes and carries out research activities and missions, confers awards and grants, publishes the proceedings of its own conferences, meetings and seminars. The Academy further provides – either upon request or on its own initiative – advice to public institutions and when appropriate drafts relevant proposals.

Swiss Academies of Arts and Sciences

The Association of the "Swiss Academies of Arts and Sciences" includes the Swiss Academy of Sciences (SCNAT), the Swiss Academy of Humanities and Social Sciences (SAHS), the Swiss Academy of Medical Sciences (SAMS), and the Swiss Academy of Engineering Sciences

(SATW) as well as the two Centres for Excellence TA-SWISS and Science et Cité. Their collaboration is focused on methods of anticipating future trends, ethics and the dialogue between science and society. It is the aim of the Swiss Academies of Arts and Sciences to develop an equal dialogue between science and society and to advise Government and society on scientifically based, socially relevant questions. The academies stand for an open and pluralistic understanding of science. Over the long-term, they mutually commit to resolving interdisciplinary questions in the following fields:

- They offer knowledge and expertise in relation to socially relevant subjects in the fields of Education, Research and Technology.
- They adhere to the concept of ethically-based responsibility in gaining and applying scientific knowledge.
- They build bridges between Science, Government and Society.

OPENING REMARKS BY BRUNO BOTTAI

Chairman of the International Balzan Foundation "Prize"

Professor Alberto Quadrio Curzio read the following message from Ambassador Bruno Bottai:

"President Maffei, thank you for the renewed hospitality of the Accademia Nazionale dei Lincei. Thank you in particular for having accommodated this first Annual Balzan Lecture in the ancient and beautiful Hall of Physical Sciences, in Rome. Thanks also to Professors Peter and Rosemary Grant, Balzan Prizewinners in 2005 for Population Biology, for accepting the invitation to give this lecture.

The authoritativeness of the International Balzan Foundation 'Prize' derives from the calibre of its prizewinners. It is their commitment which invigorates research and deepens knowledge.

Professors Grant of Princeton University, both born in England, have distinguished themselves through their important studies by which they have demonstrated the evolution in action of finches in the Galápagos. This was the motivation for the awarding of the Balzan Prize to them in 2005. They have since received many other awards and honours. Young people are inspired by their example. This example was clearly evident last year in Milan, when in honour of the bicentenary of the birth of Charles Darwin, they lectured at universities in Milan and at the Civic Museum of Natural History.

Thanks to the second part of the Balzan Prize, for research projects involving young people, the Grants have another opportunity to shape the next generation of evolutionary scholars".

WELCOME MESSAGE BY LAMBERTO MAFFEI

President of the Accademia Nazionale dei Lincei

It is a great honour and pleasure to welcome two such prestigious and eminent scientists as Peter and Rosemary Grant, recipients of the 2005 Balzan Prize for Population Biology for their very important observational research. Their life seems to us both scientific and "romantic", living in a tent while working in the Galápagos.

If I were to summarize their seminal work, I would say that they have brilliantly continued the evolutionary work of Charles Darwin on size and shape variations in finches' beaks. Charles Darwin visited the Galápagos for five weeks, while the Grants have been visiting the Galápagos back and forth for thirty years. Their findings have brought Darwin's work into the 21st century, confirming and expanding his research using a combination of scientific approaches which range from behavioral to molecular biology. This includes experimental observation of the molecular mechanisms underlying them. They have shown that natural selection concerning the beak and body of finches can be very rapid when the food supply of the birds changes drastically, as for example when climatic conditions have changed due to El Niño events. This is indeed remarkable.

Quoting from their recent research report regarding this mechanism: "*bone morphogenetic protein 4 (BMP4) and its differential expression during development resulted in variation of beak size and shape among finches. BMP4 acts in the developing embryo to lay down skeletal features, including the beak. The different beak shapes of Darwin's finches are also influenced by slightly different timing and spatial expression of a gene called calmodulin (CaM)*": This, incidentally, occurs in many other developments, including the brain.

The Grants' work on Galápagos finches is probably the most important study on 'evolution in action', and this 'evolution in action' is a fascinating subject which defines the cutting edge of biological research today.

Lecture by PETER and ROSEMARY GRANT

THE EVOLUTION OF DARWIN'S FINCHES, MOCKINGBIRDS AND FLIES

Thank you for the very kind introduction, and for the invitation to come to the Academy and speak to you today about our research and the research of four young researchers who we have supported with funds from the International Balzan Foundation.

The central task of evolutionary biologists is to explain the enormous biological diversity of the world. There are literally millions of species, and they vary in size from viruses to whales, they vary in shape, in colour, and in pattern, and they vary in numerous ways in which they exploit the environment for food and avoid being eaten by their enemies. How do we explain this variety? Almost 40 years ago Rosemary and I (Peter) chose to address this fundamental question in a model system of diversity, Darwin's finches on the Galápagos Islands. There are 14 species derived from an ancestral species in the last 2-3 million years. Their populations are convenient for the study of evolutionary processes because they are relatively small, isolated, and live in environments that have been scarcely altered by human activity. Five years ago we were fortunate to receive the Balzan Prize in Population Biology for our research. We used half of the prize money to support the research of four young researchers. In the first part of our lecture I will summarize their main findings. In the second part Rosemary will discuss what Darwin's finches have taught us about evolution and the origin of new species.

MOCKINGBIRDS ON GALÁPAGOS

The research topics of the four researchers are not linked together in a linear chain, but they are unified in being related to different aspects, relating to our interest in evolution in small populations. Paquita Hoeck (Fig. 1) of the University of Zurich chose to study two small populations of mockingbirds in the Galápagos archipelago.

Four species occur in the archipelago with no more than one per island (Figs. 2a-2b).

She studied many populations, but with a focus on the Floreana mockingbird (Fig. 3) because it now exists on only two satellite islands of Floreana, the main population having become extinct about 130 years ago through, I regret to say, human agency. Given their small size, the question is whether they are vulnerable to extinction through

Fig. 1. Paquita Hoeck with a mockingbird. Photo P. Hoeck.

Fig 2a. Four species of Galápagos mockingbirds.

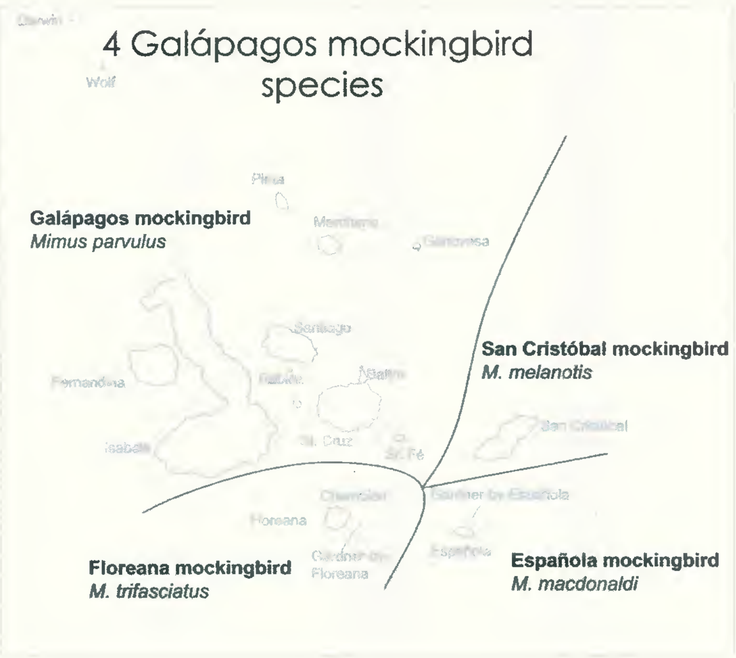


Fig. 2b. The islands on which they occur. Photos P. Hoeck.

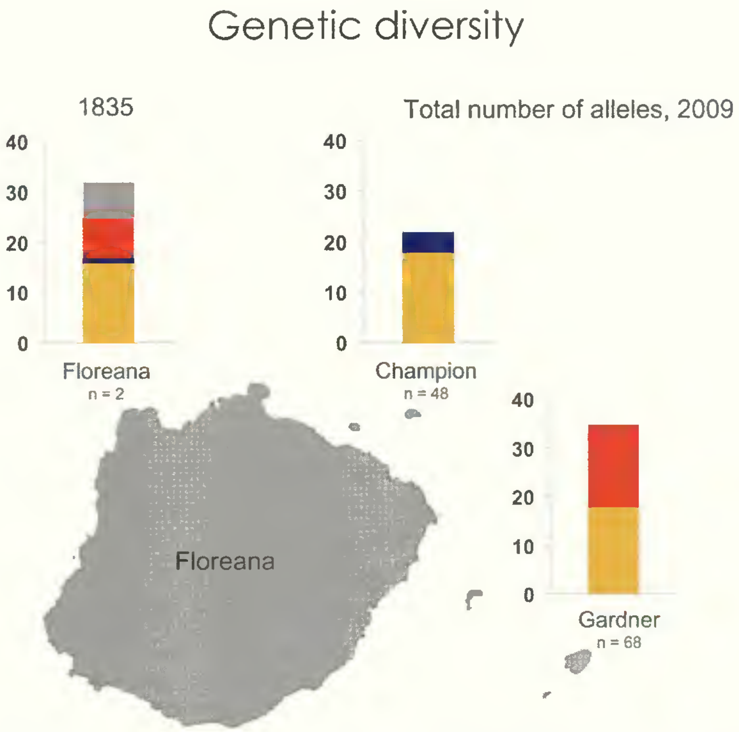


Fig. 3. Genetic diversity of Champion and Gardner mockingbirds. Key: shared alleles in yellow, alleles unique to each island in red or blue. Adapted from Hoeck *et al.* (2010).

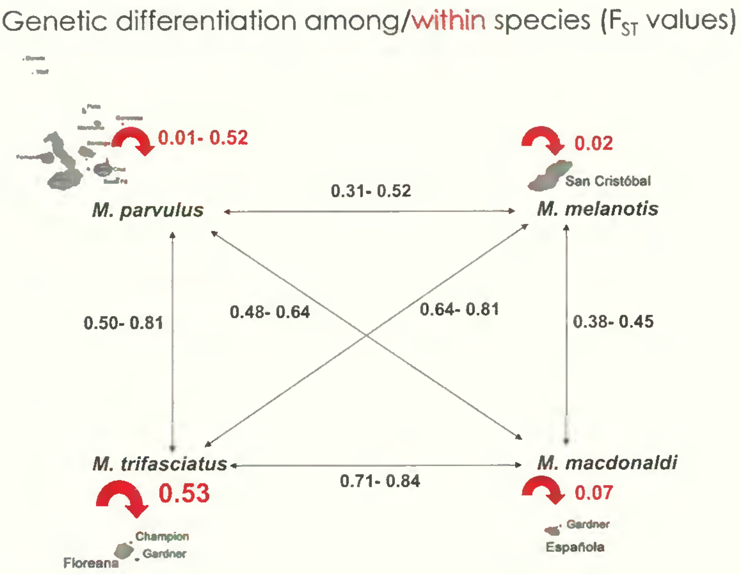


Fig. 4. Genetic differentiation of mockingbird populations as measured by the F_{ST} index (among/within population variance). Populations of species (black) generally differ more than populations of the same species (red), but the Floreana mockingbirds (*M. trifasciatus*) are exceptional. Illustration by P. Hoeck.

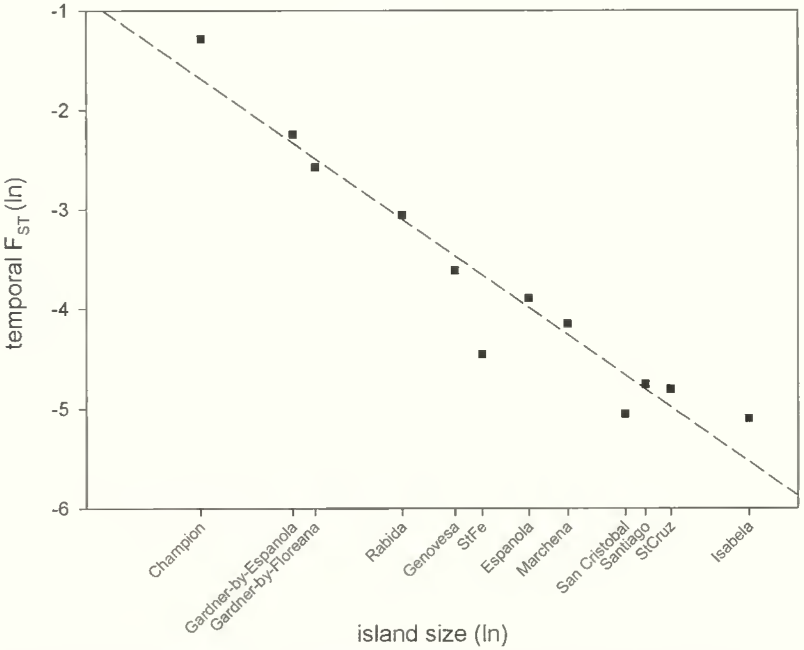


Fig. 5. Genetic differences (F_{ST}) between populations in time are larger on small islands such as Champion and Gardner than on large islands. From HOECK *et al.* (2010).

inbreeding and problems attending random loss of genetic variation. If they are ultimately doomed can the species be rescued by transferring some individuals to the nearby island of Floreana? If so, which should be the source population? This research, inspired by a general evolutionary problem, has practical managerial applications.

Paquita studied microsatellite DNA as genetic markers. She found (Fig. 3) that the two populations shared many alleles (in yellow) but also differed in others (red and blue). The difference between the populations (Fig. 4) is very large (*M. trifasciatus*) when compared with pairs of populations of the other species in close proximity (e.g. *M. macdonaldi*), and comparable in magnitude to differences between species (numbers in black). Is the large difference ancient or a consequence of the extinction of the parent population on Floreana Island? To answer that question she extracted DNA from museum specimens, including two specimens from Darwin's visit on the *Beagle* in 1835 (Fig. 3). From her results it seems likely that the three island populations were once connected as a result of individuals from one island dispersing occasionally to another, but since the time when the Floreana population became extinct, alleles once present on Floreana have been lost on the satellite islands. This conclusion is supported (Fig. 5) by a comparison of genetic variation today and 100 years ago. Champion, the smallest island, has undergone the greatest change; it has lost a substantial amount of variation. This study is one of the best to show how precarious populations are when they become small and isolated. As a protective counter-measure, genetic results justify using individuals from both satellite populations in a program of reintroduction to the large island of Floreana.

GENETICS OF *DROSOPHILA* COLOR VARIATION

Margarita Womack-Ramos (Fig. 6) of Princeton University addressed a problem of understanding phenotypic variation in genetic terms. She chose *Drosophila* to work on because of the convenience of a large amount of genomic data – 12 species have been completely sequenced – and the convenience of keeping large populations in the lab for breeding purposes and genetic analysis. The task of identifying individual genetic factors responsible for phenotypic differences is easiest with

Fig. 6. Margarita Womack Ramos. Photo M. Womack-Ramos.

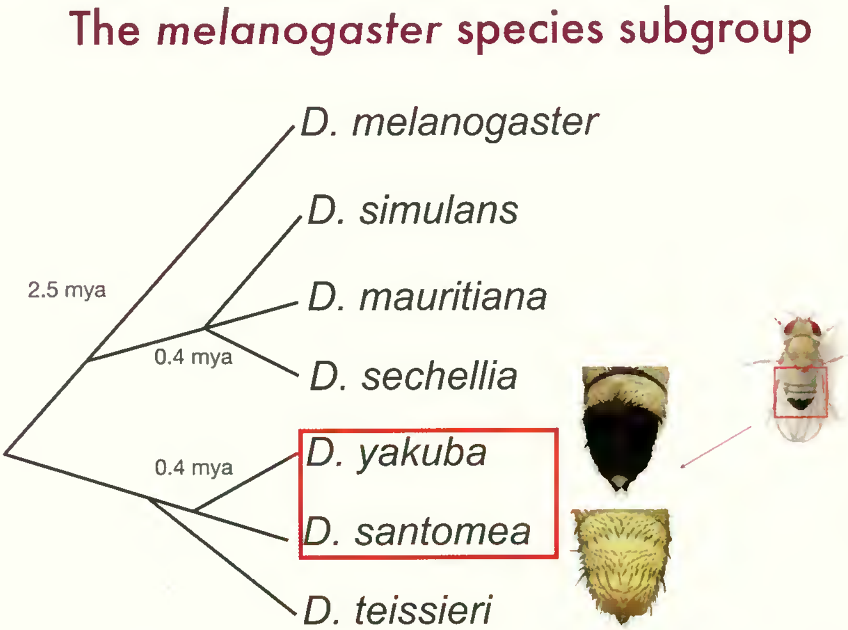


Fig. 7. The *Drosophila melanogaster* species subgroup. Illustration by M. Womack-Ramos.

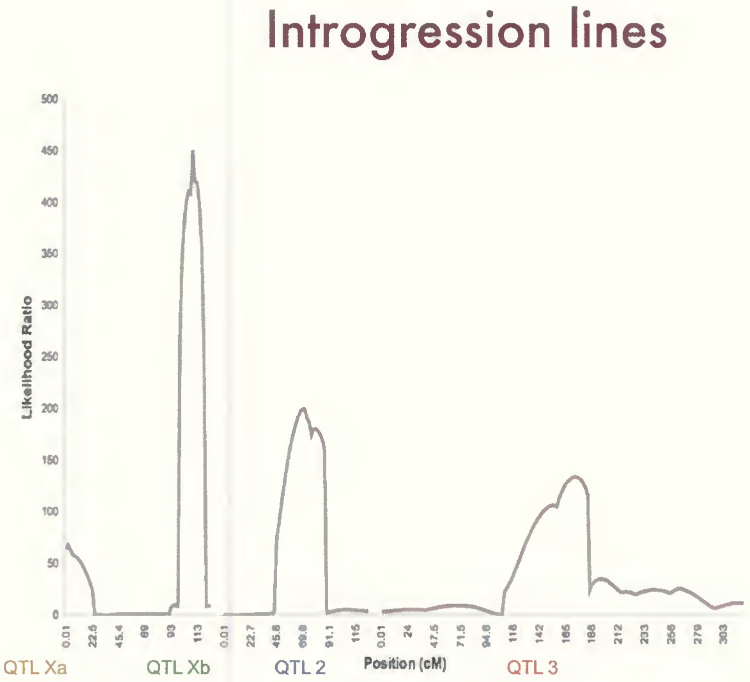


Fig. 8. Four chromosomal regions (QTL) of *D. yakuba* and *D. santomea* are associated with differences in their abdomen colors. From CARBONNE *et al.* (2005).

closely related species for two reasons: first they should differ by only a small number of genes, and second hybrid offspring are likely to be fertile, so inheritance can be determined by crossing experiments in the laboratory. A suitable pair of species occurs on the island of Sao Tomé in the Gulf of Guinea (Fig. 7). *D. yakuba*, a species with a dark abdomen, lives at high elevations and *D. santomea*, lacking dark pigment, occupies habitat at lower elevation. Already it was known from a statistical correlation between regions of the genome and phenotypes that the difference between the species was attributable not to just a single Mendelian genetic factor, but to at least four so-called quantitative trait loci (QTL) or restricted regions of the genome (Fig. 8).

This is depicted on a map that lines up all the genes on three chromosomes in a linear sequence. At least three of the pigmentation QTLs have small but recognizably different phenotypes (Fig. 9). But the regions are large. Margarita wanted to do better, and narrow the search to individual genes. Her research question was therefore how many genes are involved, what are they and how do they function? Finding and identifying individual genes is important for evolutionary studies, just as it is important in medicine and agriculture.

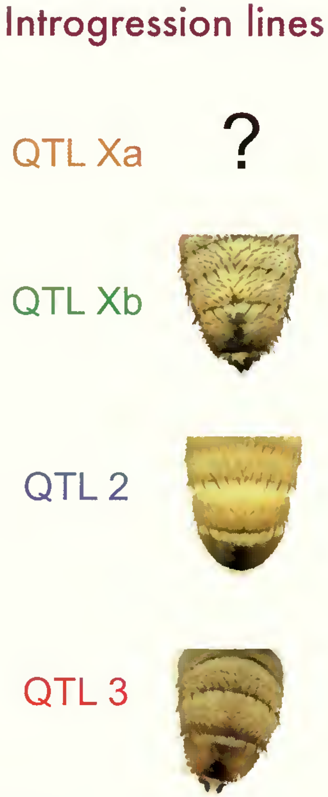


Fig. 9. Different chromosomal regions (QTL) have different effects on abdomen color of *D. yakuba* and *D. santomea*. Illustration by M. Womack-Ramos.

To answer these questions she used a breeding program illustrated in Figure 10. This involved producing hybrids, with half their genes from each of their parents, breeding the hybrids with a *santomea* male and thereby producing offspring with fewer *yakuba* genes, and doing this repeatedly until the fraction of the *yakuba* genome was extremely small yet contained genes for pigmentation. This is possible to do because in sexual reproduction, when a chromosome from the mother pairs with a chromosome from the father, a small piece of paternal and maternal chromosomes are exchanged through crossing-over. By selecting flies for breeding with pigmentation but only small pieces of the *yakuba* genome Margarita was able to reduce the contribution of the *yakuba* genome to a very small amount. The next step was to use different hybrid lines with slightly different pieces of the *yakuba* genome to narrow the significant region by elimination of the non-essential parts. The result was a 2-orders of magnitude higher resolution of the map. One example is shown in Figure 11. With regard to QTL3 she was able to reduce the starting complexity to very few genes in a narrow region, and in doing so she discovered another locus nearby. The first one is known as ebony; her research pinpointed its location. The new one is called truffle. By drawing upon the better resources of *Drosophila melanogaster* she has been able to demonstrate that truffle affects pigmentation, moreover it does so by suppressing pigment deposition. And finally she

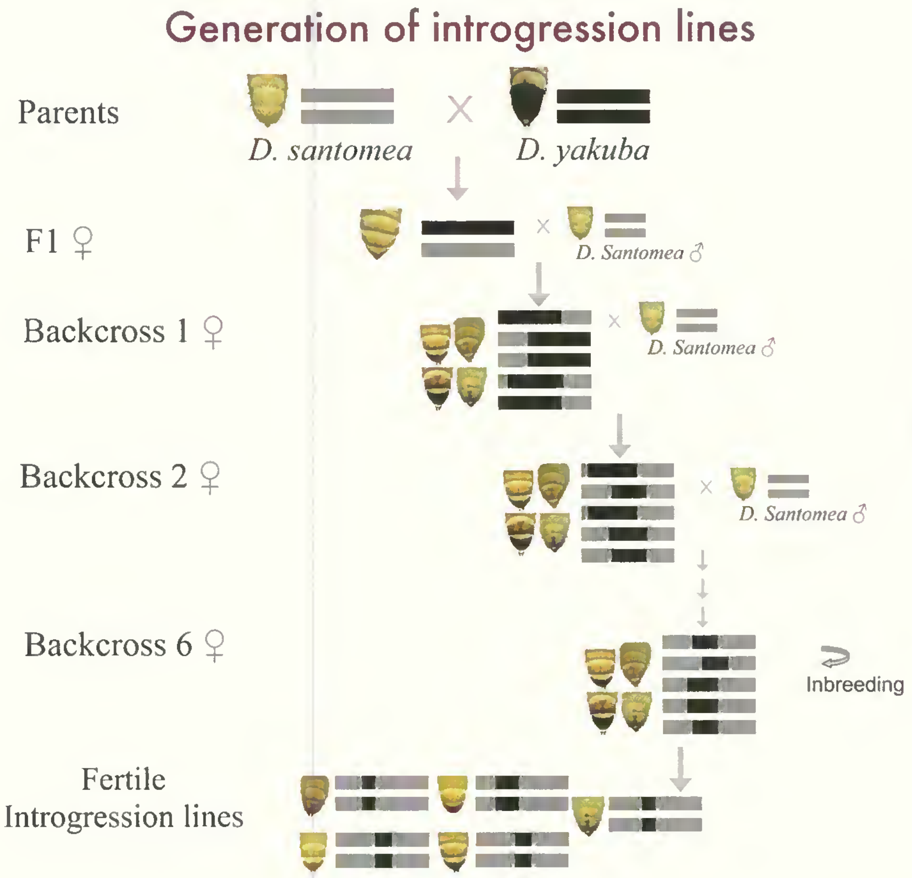


Fig. 10. Breeding design and analysis of introgression of *D. santomea* alleles into the *D. yakuba* genome. Illustration by M. Womack-Ramos.

discovered the mode of epistatic interaction of two genes on the X chromosome: the expression of one of them is affected by whether the other is functioning or not. The research is continuing.

The significance of this work is two-fold. First she has developed a novel and powerful technique for dissecting the genetic underpinning of complex, continuously varying traits. Second she has shown that such traits may be governed by only a handful of interacting genes.

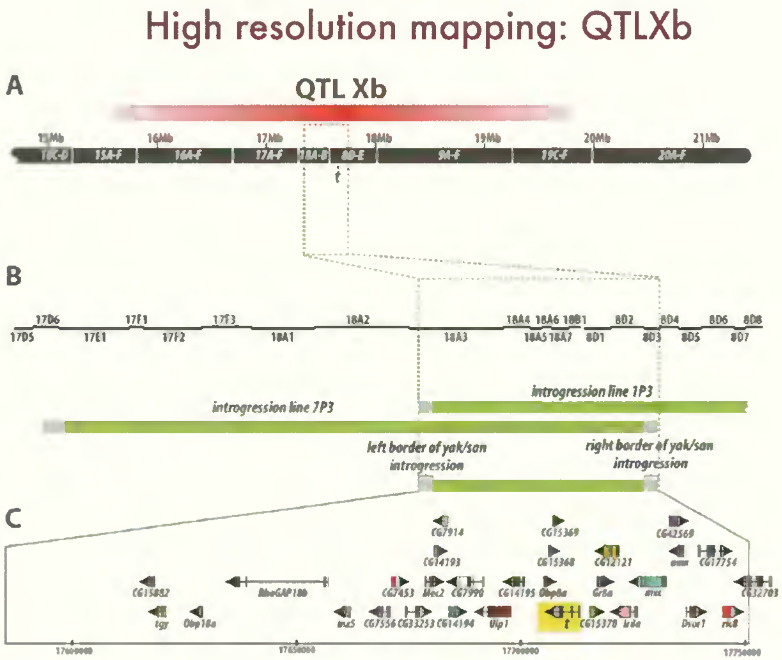


Fig. 11. High resolution map of a quantitative trait locus, QTLXb. From Rebeiz *et al.* (2010).

BEAK DIVERSITY: GENE REGULATION

The other two projects directly stem from our research on Darwin's finches. The species differ in their beak sizes and shapes. Our fieldwork has demonstrated that beak size and shape vary in relation to diet, and beaks undergo evolutionary change when the environment changes: Rosemary will talk more about this later. Two important genes involved in beak development were identified by our collaborators, Cliff Tabin and Arhat Abzhanov at Harvard University. These genes produce two signaling molecules, bone morphogenetic protein 4 (*Bmp4*) and Calmodulin (*CaM*). *Bmp4* is associated with deep and broad beaks, whereas Calmodulin is associated with long beaks. *Bmp4* is strongly expressed in *Geospiza magnirostris* on day 5 of embryonic development, but less strongly and later in other species. At the same time Calmodulin is strongly expressed in the two species of cactus finches with elongated beaks. One question posed by these discoveries is how are the genes regulated in such a way as to produce beaks of different size in different species?

Céline Clabaut (Fig. 12) of Harvard University has spearheaded the search for an answer. Species may differ in three ways: in the structure of a gene such as *Bmp4*, in the promotor in the immediate vicinity that contains the binding sites for the transcription of the gene, or in one or more enhancers at a greater distance that regulate the expression of the gene. Céline first sequenced the *Bmp4* gene of all 14 species of Darwin's finches, and found they were almost identical. Differences between species in beak depth and width therefore cannot be explained by differences in the structure of this gene. Instead, species differ in their beaks because expression of the same gene is regulated in different ways. Céline next examined the promotor region, looking for differences between the species, and found some promising candidates. The differences between species are promising areas for research into how the gene is transcribed. For example the factor Sp1 is apparently present only in *G. scandens* and therefore might contribute to its unique long and pointed beak.

For the third possibility of variation in the enhancers of gene activity Céline drew upon detailed knowledge in the chicken, mouse and humans. Just like finches, we also have the *Bmp4* gene and it is active in our own cranio-facial development – in fact much of the research is carried out in Dental School Research Departments. As expected from the relative evolutionary histories, humans and mice have much more genetic material in common near the *Bmp4* gene than do humans and chickens (Fig. 13), but there are at least nine regions of similarity between chickens and humans. These evolutionarily conserved regions are probably crucial for establishing features that are common to all vertebrates, including cranio-facial bone. In the eighth conserved region Céline found two types of differences between Darwin's finch species; first a simple base pair identity change, and second a change in the number of a particular nucleotide (Fig. 14).

The first determines whether a binding site will be present or not, while the second affects the number of binding sites. The final step in the investigation that is currently underway is to test experimentally for enhancer activity and *Bmp4* expression at the time that beaks are

Fig. 12. Céline Clabaut. Photo by C. Clabaut.

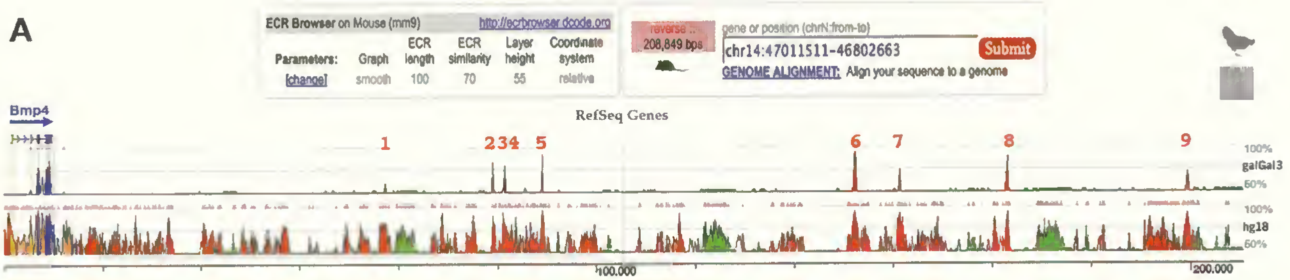


Fig. 13

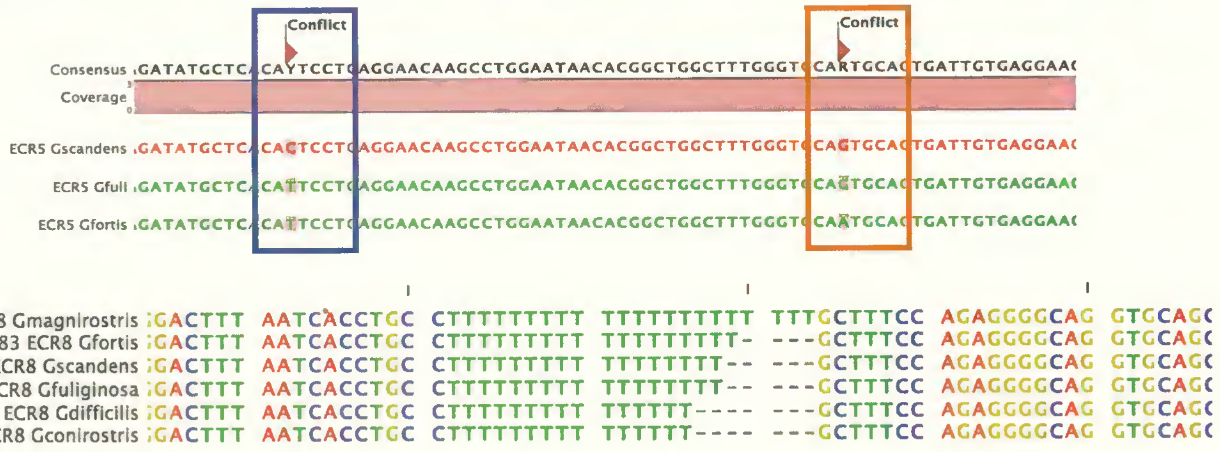


Fig. 14

Fig. 13. Evolutionarily conserved regions (ECR) are far fewer in a comparison of birds and humans (upper line, ECR shown in red) than in a comparison of mice and humans (lower line, shown in red and green). Illustration by C. Clabaut.
Fig. 14. Transcription factor analysis in Darwin's finches: the identity and number of nucleotide base pairs in an enhancer vary among species. Illustration by C. Clabaut.

formed during embryo development. Transgenic chickens and mice are being used for this purpose.

BEAK DIVERSITY: ELONGATION OF BEAKS

Jennifer Gee (Fig. 15) undertook a parallel study in the same laboratory of Arhat Abzhanov at Harvard University. The oldest species in Darwin's finch evolution is the warbler finch (Fig. 16).

Its beak is quite unlike all the other species in being thin and pointed and not robust. Correspondingly its diet is different. It feeds on spiders, small insects and nectar and not on seeds. The question she addressed is how is such an unusual beak produced. Either calmodulin, a beak-lengthening gene, is unusually active, or *Bmp4*, which normally affects beak depth, is inactive; or both processes are involved. Her first surprise was the discovery that calmodulin is hardly expressed at all, and then only late in development. This means that *Bmp4* is also not likely to be expressed, and that is exactly what she found. This made sense when she discovered that *noggin*, the gene that antagonizes expression of *Bmp4*, is expressed throughout beak development. In other words the long and thin beak of the warbler finch is not

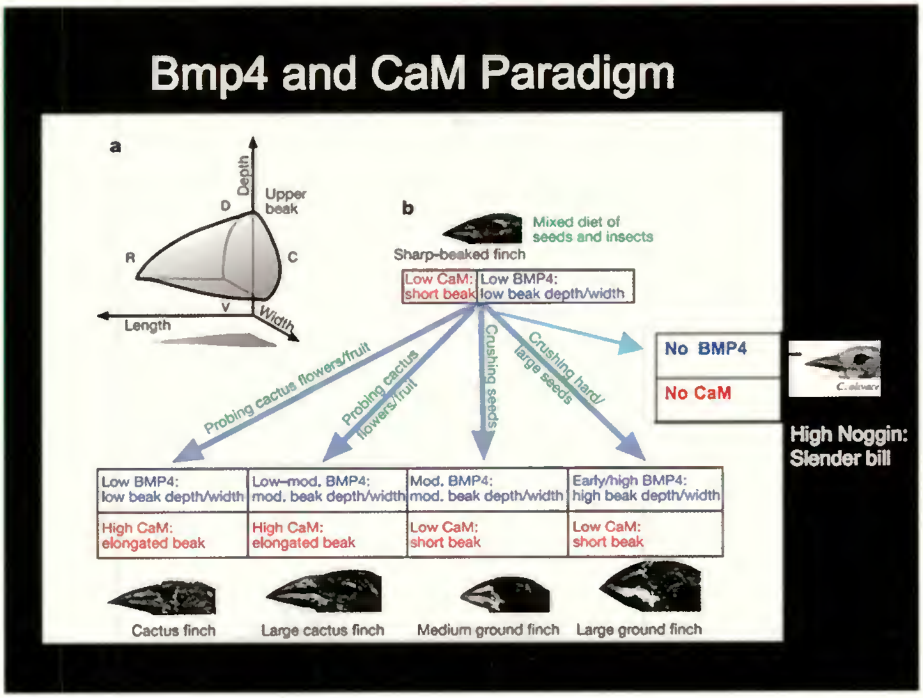
Fig. 15. Jennifer Gee (left) with students. Photo J. Gee.

Fig. 16. Warbler finch, *Certhidea olivacea*. From GRANT and GRANT (2008).

produced by a gene responsible for making the beak long but by a gene that suppresses the effects of a gene responsible for making the beak deep. This adds a new dimension to our understanding of evolutionary divergence (Fig. 17).

Jennifer then took the first step to examine species outside the group of finches on the Galápagos (Fig. 18).

The closest relatives are on the South American continent and in the Caribbean. Most of them have robust beaks like most of Darwin's finches but one of them, the bananaquit, has a long thin beak like the warbler finch only down-curved. Is this the result of the same genes active at the same time? Yes and No. Expression of *Bmp4* and *noggin* are similar in the two species; that explains why the two species have narrow, slender beaks. But unlike the warbler finch the bananaquit has strong expression of calmodulin. In this respect it resembles the cactus finches of the Galápagos, although the gene is expressed later in the bananaquit and in a different part of the beak. Like the other three studies, Jennifer's has followed a problem arising from a field study of small populations into the detailed realm of molecular genetics.



Bmp4 and CaM Paradigm

Fig. 17. Expression during development of genes that affect the depth and length of beaks in the warbler finch in the context of variation established among ground finches (*Geospiza*). Adapted from Abzhanov *et al.* (2006).

To summarize, all four young researchers addressed problems of understanding how evolution works in small island populations of animals. All learned modern molecular techniques of genetic analysis. All produced surprises in their research. And all are on the way to professional careers.

As a postscript to this brief account of their research I should add a few comments on how their research is being disseminated. At the conclusion of the supported research, with prize money received from the International Balzan Foundation, we supported a conference, entitled *Evolution in Small Populations*, to give the young researchers the opportunity to present their results to an invited audience of approximately 100 scientists with similar research interests. The conference was held at Princeton University on September 5th and 6th 2008. It consisted of thirty-two presentations. Twenty-four were lectures

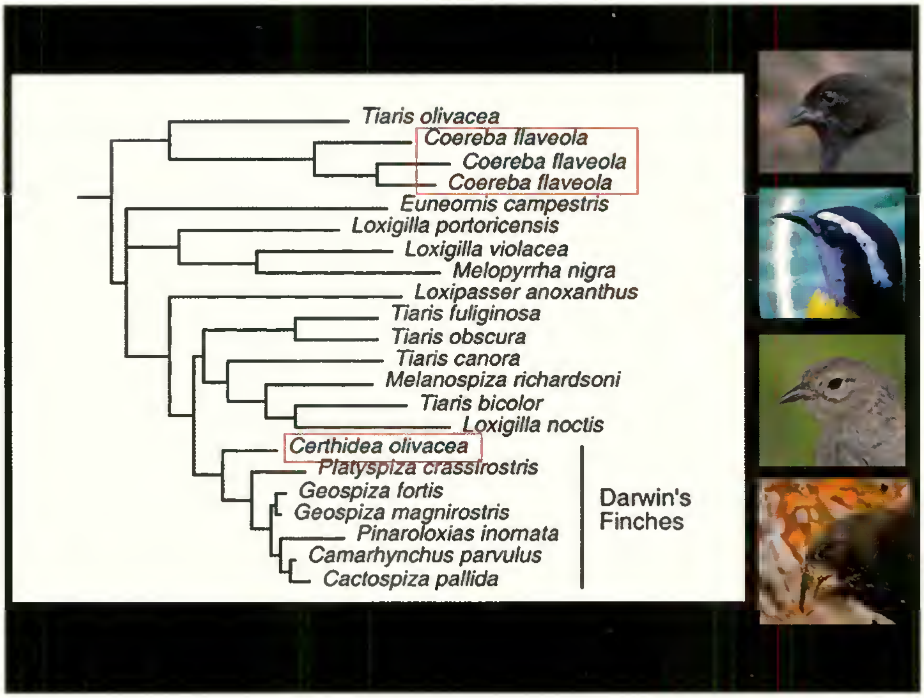


Fig. 18. Darwin's finches compared with Caribbean relatives in a reconstructed molecular phylogeny. Species with pointed beaks in each group are highlighted. Adapted from BURNS *et al.* (2002).

given by established scientists in ecology, behavior and evolution, from Costa Rica, Britain, Switzerland and Germany, as well as from the U.S. and Canada. Four more were lectures given by the young researchers, and another four were short summaries of research given by recent graduates from Ecuadorian Universities, supplementing their poster presentations. We invited the Ecuadorian students to attend as a means of transmitting some of the benefits we have reaped from our research back to the country that has helped us. We believe the conference was an outstanding success, both in the lecture hall and outside during coffee breaks and meals when students and senior research workers were able to interact in small groups and establish valuable professional contacts as well as friendships.

The Evolution of Darwin's Finches

The reason we started our long-term study of evolution was to find out more about speciation – how and why species multiply (Grant and Grant 2008). The question of when exactly one species becomes two is not the question we are addressing in our research. Instead we are trying to understand the whole process of two species being formed from one. Darwin was very clear about the first part of this process, and much less clear about the second. He wrote about the first part as follows: "Those cases in which a species splits into two or three or more species, I should think near perfect separation would greatly aid in their specification, to coin a new word". Well fortunately the word specification never caught on, we call it speciation, but the fundamental importance of that separation, meaning spatial or geographical separation in the earliest stages of speciation, has passed down to modern times as a core feature of the process of speciation. With regard to the second part, how the process of speciation ends, we had to wait for the discovery of genetics before an explanation could be given.

The simplest way of answering that question was provided by Hermann Muller, a Nobel-prize winning population geneticist. He wrote as follows: "A long period of non-mixing of two groups is inevitably attended by the origination of actual immiscibility". By this he meant that genetic isolation arises just as a result of random change through mutation in each of the separate lineages. A point is reached when the differences that have accumulated in geographical isolation are sufficient to constitute a barrier to the exchange of genes if those populations ever came together and tried to breed. So that is the short answer to the question of how two species are formed from one. The long answer is much more interesting, and we have been seeking it in studies of Darwin's finches over a period of thirty-eight years.

The three-step framework we use was actually first suggested by Charles Darwin. In an archipelago such as the Galápagos archipelago, (Fig. 19) the three steps would be:

(1) colonization: members of a population move to a new environment, initially from the mainland, and encounter new ecologies, new predators, new diseases, etc.

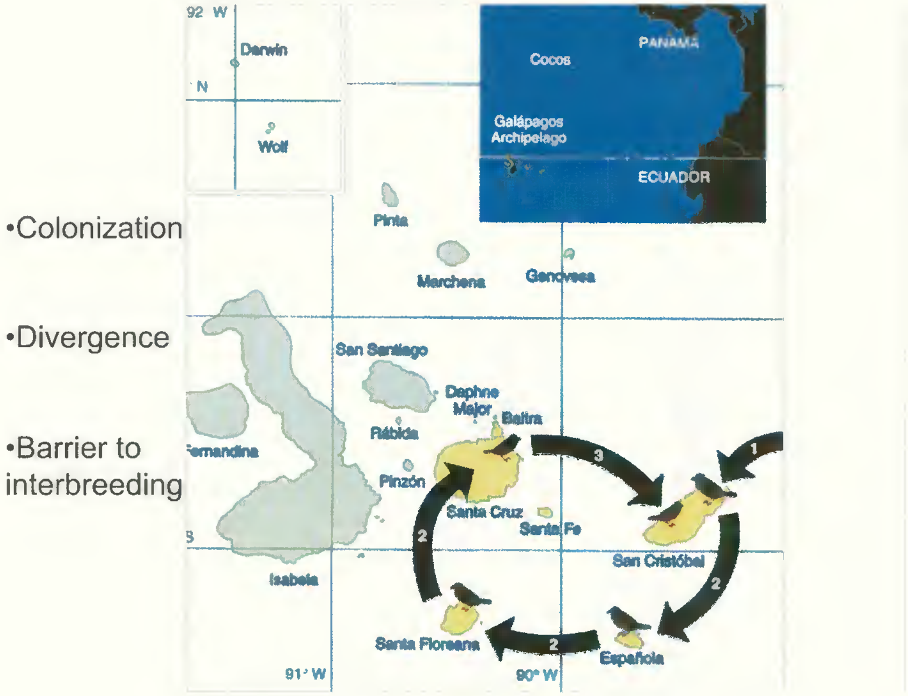


Fig. 19. Allopatric and sympatric phases of speciation. Note San Santiago should be Santiago and Santa Floreana should be Floreana. From GRANT and GRANT (2002).

(2) divergence: the population will gradually change through a combination of natural selection and random genetic drift.

(3) a barrier to interbreeding: differences in traits will have arisen in geographical isolation, and these constitute a barrier to the exchange of genes through interbreeding between populations. For Darwin's finches the components of the barrier are differences in their song and morphology.

Step one and step two will be repeated over and over again, as birds from different populations move to different island environments until eventually two populations come together. If divergence has been pronounced members of the two lineages will not recognize each other as the same species; speciation will have already been completed. But the process is unlikely to be that simple, because often there is divergence in some characteristics and not in others, and then we need to find out the factors that build the barrier to interbreeding between two populations.

To investigate this and related problems we chose the island of Daphne because of its small size. When we first began there were two, clearly distinct, finch species on Daphne: *Geospiza fortis* the medium ground finch, and *G. scandens* the cactus ground finch. We banded and measured hundreds of birds, sampled blood for DNA analyses and measured changes in food supply. Medium ground finches are extremely variable in body size and beak shape.

The Galápagos archipelago sits astride the equator and as such is subject to the Southern Oscillation phenomenon, which causes some years to be extremely wet and other years to be completely dry. Some droughts last for as long as two and a half years, and it is during these droughts that large numbers of birds die. Populations can drop to less than a hundred individuals. In 1977, four years after our research began, we had the first major drought, when almost no rain fell on the island and 80 percent of the medium ground finch population died. We noticed that the majority of seeds on the ground were large and hard. There were a few smaller and softer seeds, but they were quickly depleted. As these were depleted, more smaller-beaked birds died than large-beaked birds, because the smaller-beaked birds were unable to crack the large and hard *Tribulus* seeds, which were the predominant seeds in the environment. This is natural selection. Although it is sometimes called the survival of the fittest, I think it ought to be called the mortality of the unfit, because it comes about, basically, through the mortality of the birds not fit for their environment.

Natural selection is not the same as evolution. Natural selection occurs within a generation whereas evolution is a change in the genetic composition of a population from one generation to the next. For evolution to occur, the trait in question must be heritable. Knowing this we had actually looked at the heritability of all the traits we were interested in, from body size to beak depth, beak width, and beak length, by associating measurements of the offspring with their parents' measurements, and found that all traits were heritable: small parents produced on average small offspring and large parents produced large offspring. We measured heritabilities and found they were high; for example the heritability of beak depth was about 0.74 on a scale from 0 to 1.

So, by knowing the heritability and the strength of natural selection we could predict the evolutionary response to selection by using what is called the breeder's equation – that is, the response in the next generation to selection (R) is equal to the heritability (h^2) times the selection differential (s). The response we predicted was very close to what we observed in the next generation produced in 1978.

Well, Darwin would not have been surprised at this. He was asked by one of his correspondents to explain exactly what he meant by natural selection. Darwin wrote in reply the following: "I would now say that of all birds annually born, some will have a beak a shade longer, and some a shade shorter, and that under conditions and habitats of life favoring longer beak all individuals with beaks a little longer will be more apt to survive than those with beaks shorter than average." Remarkable: just substitute depth for length and he said exactly what we were able to show!

We learned more about evolution by natural selection following an extremely intense El Niño event in 1983 when rain fell over an eight-month period. Normally the wet season lasts for only a month or two. *Tribulus* plants, which produce large and hard seeds, were quickly covered by grasses, vines, and other small seed-producing plants. So the island was converted from a producer of mainly large and hard seeds to a producer of small and soft seeds. There followed another drought in 1985 when seeds in the seed bank in the ground were mainly small and soft. This time small members of the medium ground finch population with small beaks were at an advantage because they could rapidly pick up and exploit the small seeds faster than large birds could, moreover being smaller their energy needs were lower. As there were very few large and hard seeds the large-beaked birds died disproportionately.

El Niño events are often followed by droughts. We have experienced fluctuations between the two environmental extremes of El Niño rainfall and droughts several times over a period of more than 30 years.

From these we know that the conditions for finches during a drought depend on the types and quantities of seeds in the soil at the beginning of the drought, which in turn depend on preceding conditions. Natural selection occurred repeatedly during these droughts, and selection oscillated in direction: over the 30 years there was a net decrease, or decline in the average body size of the birds. *G. fortis* decreased in average body size, whereas beak size increased and beak shape changed; beaks are now more pointed than they were 30 years ago. Body size of *G. scandens*, the cactus finch, also decreased, bill size decreased and the beaks became much blunter. Thus in both populations birds are not quite the same as they were at the beginning of our research. Both have undergone evolutionary change.

Step two in the speciation cycle: divergence in sympatry.

At the time previously separated populations come into contact they may be subject to divergent selection that minimizes ecological interactions and competition between them, as well as interbreeding. Competition that results in divergence of a trait involved in feeding is known as ecological character displacement. An example of this arose after two decades of research.

Between 2003 and 2005 another very strong drought occurred on the island. It was the most severe drought we had ever experienced: more than 90 percent of the medium ground finches died during this time. Now, the conditions were nearly the same as in 1977: the seed bank was composed of predominantly large and hard seeds, so all else being equal we would expect the average bill size of medium ground finches to increase once again as happened in 1977. But this time, the small birds survived best. Why was this? The answer is that all else was not equal. A population of large ground finches, *Geospiza magnirostris*, had become established in the amazing El Niño of 1983, built up numbers in the following 20 years, so that by 2003 there were more than 200 individuals present. *G. magnirostris*, a specialist on *Tribulus* seeds, equipped with a much larger beak and almost twice the body size of *G. fortis*, outcompeted the large members of the *G. fortis* population. Therefore large *G. fortis* died to a disproportionate extent. Natural selection had occurred again, resulting in an enhancement of the difference between two competitor species as envisaged in the model of speciation (Fig. 19); in other words character displacement had occurred.

Small birds also died, but more of them survived than the larger ones. Interestingly their survival was aided by a behavioral mechanism. Some of them foraged close to large ground finches, and as *G. magnirostris* crushed and shattered the large and hard *Tribulus* seeds, fragments would fly out and be seized by the smaller *G. fortis*.

Step three in the speciation cycle: the barrier to interbreeding.

It is important to understand the barrier to interbreeding between closely related species, because this is fundamental to the formation of species. All six species of Darwin's ground finches are very similar in plumage – males are black, females are brown. All Darwin's finches build similar nests, and they all have similar courtship displays, or as far as we can see they do. But they differ in song and morphology, and these are the elements of the barrier to interbreeding. These two attributes have been investigated experimentally by Laurene Ratcliffe and ourselves.

We first asked whether individuals can discriminate on the basis of body shape and beak size in the absence of any vocal cues. We placed two museum specimens, a female of one species and a female of another species, on a rod mounted on a tripod in a bird's territory and asked the territory owner if he could discriminate between the two females. The answer was a resounding yes. During these experiments, birds were very active and they courted vigorously the female of their own species, even though it was a stuffed museum specimen, and the female of the other species much less often. The experiment was repeated many times and on several islands with different species and not just on Daphne. Then we asked if individuals can discriminate purely on the basis of song in the absence of any morphological cues? To test this, we tape-recorded songs and then played back the songs in various territories. When we played back a cactus finch (*G. scandens*) song, a cactus finch would fly in towards the loudspeaker. The medium ground finch would completely ignore the song. When we did this with the song of the medium ground finch (*G. fortis*) a medium ground finch would come in towards the loudspeaker, whereas the cactus finch would completely ignore the song.

So clearly they can also discriminate on the basis of song in the absence of any morphological cues. And it turns out that the song is very important in their lives. The song differs very much between the three species on Daphne. *Geospiza fortis*, the ground finch, we call the 'Swiss bird' because some of them sing 'mostly müesli, mostly müesli'. The *G. scandens* has a loud 'ch ch ch ch ch ch' song, and the immigrant *G. magnirostris* sings a low-pitched 'chee-urr chee-urr'. Thus the species differ in their songs, as well as in beak sizes and shapes, and in body size.

Half the birds alive in the world today learn their songs. These are the passerine songbirds, hummingbirds and parrots. But amongst these birds that learn their song, some – the so-called closed learners – learn during a short sensitive period of life and afterwards they learn no more. Darwin's finches are among this group. They have a short sensitive period, which lies between day 10 after hatching and day 40. This coincides with the last few days in the nest up to the endpoint of the period when, as fledglings, they are being fed by their parents. Their father is singing at this time, therefore it is not too surprising they learn their song mainly from him. And once their song is learnt it is retained for life, and their life can be as long as 17 years if they manage to survive the droughts.

Adult finches choose a mate and pair according to the morphology and the song they have learnt early in life. These two features constitute a pre-mating barrier between the species. We can ask how robust this barrier is, and whether it ever leaks. After all the barrier is based on learning, and learning is very vulnerable to disruption if a young bird hears and learns the song of another species during its short sensitive period. Disruption does in fact occasionally happen; the barrier does leak, rarely, and birds hybridize. For example *G. scandens*, being slightly larger than *G. fortis*, sometimes takes over a *G. fortis* nest, and it usually evicts all the eggs. However, an egg may be left behind, in which case the finch that emerges grows up as a *G. fortis* in a *G. scandens* nest, and learns its foster father's song. As another example, learning the wrong song can happen following the death of the father. Females do not sing, therefore young birds in the nest without a father learn a neighbor's song instead. If the neighbor is a bird of another species, the young bird will learn the song of that species.

When size differences between species are large, even learning another species' song does not lead to hybridization. We have had an example of this. In the last 25 years nine *G. fortis* have learned the song of *G. magnirostris*. Instead of singing 'mostly müesli' they sang 'chee-urr chee-urr', but none has bred with the *G. magnirostris.* The reason is that every time a *G. fortis* sang this song, a male *G. magnirostris* would fly in and chase it away. One of these *G. fortis* individuals was very persistent – it sang a *G. magnirostris* song and got chased time and time again; it crouched down behind a bush and sang but still got harassed. Eventually it stopped singing altogether and got a mate, and the mate was not a *G. magnirostris* but a member of its own species.

To summarize, if the size difference between the species is very small and if the male learns another species' song, then hybridization may occur. This happens in about one percent of breeding birds. But if the size difference is large they do not hybridize despite learning the 'wrong' song.

What are the consequences of hybridization? How fit are the hybrids: how viable and fertile are they? During the first ten years of our study hybrids were rarely produced, and the few that were produced failed to survive long enough to breed. We thought at first their poor survival might reflect a weakness caused by some degree of genetic incompatibility. But alternatively, since hybrids have intermediate beak size between their parent species, they might not have survived because seeds of the appropriate size were scarce. The second possibility seems to be the correct one because after 1983, when small and soft seeds became plentiful, hybrids survived long enough to breed, and since then they have done so as well as the parental species. There is no statistical difference in survival between hybrids and their parental species. Hybrids obtain mates as well as the parental species, and also produce as many eggs, nestlings, fledglings and recruits. Importantly, there is no evidence of genetic incompatibility between species. Hence species of Darwin's finches, after becoming diagnosably different from each other, have not reached the point at which they cannot interbreed for reasons of genetic incompatibility.

Hybrids breed with one or the other of the parental species, according to the song type of the fathers, and this means that genes trickle from one species to another episodically. This episodic input of genes, we have been able to show, increases the genetic variation – we have done this by recording the increase in heterozygosity at 16 micro-satellite loci – and also increases the morphological variation on which selection can act. Thus hybridization can be a very rapid route to change. It means that in changing or new environments, it allows a population to move along a new evolutionary trajectory. This could be important, broadly and generally, during the early stages of speciation. For example, there is indirect, genetic, evidence that rare hybridization of finches occurs on all islands in the Galápagos archipelago. Hybridization and introgression of genes through back-crossing, may be more widely important than is generally believed. In the literature there are numerous instances of introgressive hybridization in many groups of organisms:

in bacteria through horizontal gene transfer, in plants, insects, fish, amphibians, reptiles, birds, mammals and even in primates, such as the lemurs of Madagascar. It has even been recorded in our own human lineage, with molecular genetic evidence of introgressive hybridization between Neanderthals and our ancestors. So clearly it is a force to be reckoned with. It is even important in our own work in the formation of a new lineage of finches.

A NEW LINEAGE OF DARWIN'S FINCHES

A very unusual finch arrived on Daphne in 1981. It was a male, in immature plumage, and looked more like a medium ground finch (*G. fortis*) than a cactus finch (*G. scandens*), but it was much larger, weighing about 28 grams; on Daphne the average weight of *G. fortis* is about 18 grams. This bird arrived at a time when most of the birds on the island had been banded, so we were almost sure it was an immigrant. A genetic analysis with microsatellite alleles revealed two facts about its origin. First, it was indeed an immigrant, and with a high probability it came from Santa Cruz, a neighboring and much larger island. Second, also with a high probability, it was the product of an initial hybridization between *G. fortis* and *G. scandens* followed by back-crossing to *G. fortis*. Not only was it exceptionally large, it had a recognizably distinctive song, and it was uniquely homozygous (183/183) at one of the microsatellite loci. This hybrid bred initially with a *G. fortis* individual without much success, then later with another hybrid of the same back-cross constitution (*fortis-fortis-scandens*) that hatched on Daphne and whose parents were known. With its hybrid mate it produced eight offspring. Four of them survived, all males, and like their father they all carried the 183 genetic marker and sang his song (and so did their sons).

Many years later, when the island experienced a severe drought (2003-05), only two descendants of this lineage survived, and they happened to be a brother and a sister. Remarkably they are still alive on the island, are still breeding, and have so far produced 15 offspring on Daphne. Not only that, their offspring have bred with each other and not with any of the medium ground finches. In other words they are an endogamous population, reproductively isolated from other species, and distinguished from them by the large size of their beaks and by their unique song. They and all their offspring carry the diagnostic genetic marker (183/183), and all the males sing the original immigrant male's song. Thus, there has been a cultural transmission of the song, from father to son, all the way down six generations, as well as genetic transmission.

Territorial behavior provides another indication that the hybrid lineage is functioning as a separate species. Pairs hold large, contiguous, territories, which they defend against each other, and while some *G. fortis* and *G. scandens* territories overlap theirs, the new immigrants ignore their owners when they intrude. Members of the hybrid lineage have all the attributes of a separate species. Now, it is too early to say if the current situation is transitory or if it will endure. It could disappear through inbreeding depression and poor survival – we have seen no sign of that so far. It could disappear through interbreeding with another species – so far we have seen no sign of that either. Regardless of the future, the current state provides insight into how reproductive isolation can develop. The essential factors for the formation of a barrier to interbreeding were a size difference and a song difference. Contributing factors were hybridization with back-crossing, which provided the lineage with a lot of genetic variation that has probably sustained it through a period of intense of inbreeding. Another important factor was the drought of 2003-05, which resulted in large-scale mortality, constrained mating of lineage members to a brother and sister, but made available a vacant ecological niche to birds of their size through natural selection against *G. fortis* of large size.

A CONSERVATION MESSAGE

Drawing upon our own research as well as the work of the young researchers we supported with funds from the International Balzan Foundation, we would like to leave you with one message, and it is a conservation message. What our work has shown is that neither species nor environments are static entities, but dynamic and constantly changing. To conserve species and the environments they occupy we must keep them both capable of further change. And a special thank you to the Balzan Foundation for supporting four excellent young scientists and their studies that link genetics, behavior and ecology.

Their studies and ours have contributed to a more mechanistic and evolutionary understanding of the diversity of life. Thank you.

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Peter and Rosemary Grant

BIOGRAPHICAL AND BIBLIOGRAPHICAL DATA

Peter R. Grant was born in Norwood, London, on 26 October 1936.

He is Class of 1877 Professor of Zoology Emeritus, Princeton University (since 2008).

After receiving his BA (Honors) in 1960 from Cambridge University and his Ph.D. in 1964 from the University of British Columbia, he earned a post-doctoral Fellowship at Yale University (1964-1965). At McGill University he was Assistant Professor (1965-1968), Associate Professor (1968-1973) and full Professor (1973-1977). He was Professor at the University of Michigan (1977-1985) prior to his appointment to Princeton University (1985), where he became Class of 1877 Professor of Zoology (1989). He was also Visiting Professor at the Universities of Uppsala (1981, 1985) and Lund (1981).

He is a member or a fellow of numerous US and European societies and academies, such as the Royal Society of London, the Royal Society of Canada, the American Philosophical Society, the American Academy of Arts and Sciences, the American Association for the Advancement of Science, the American Society of Naturalists (President in 1999, Honorary member since 2008), and the American Academy of Sciences. He is also a member of the Society for the Study of Evolution, the Ecological Society of America, the American Ornithologists' Union, the Linnean Society of London, the Society for Behavioural Ecology and Charles Darwin Foundation, where he is a member of the General Assembly. He has received honorary degrees from the Universitiy of Uppsala (1986), Universidad San Francisco, Quito (2005), and the University of Zurich (2008). He was and currently is an associate editor of scientific journals, including *Ecology* (1968-1970), *Evolutionary Theory* (since 1973), *Biological Journal of the Linnean Society* (since 1984), and *Philosophical Transactions of the Royal Society of London* (1990-1993). He was made Honorary Citizen of Puerto Bacquerizo, I. San Cristóbal, Galápagos, in 2005.

B. ROSEMARY GRANT was born in Arnside, England, on 8 October 1936.

She is Research Scholar and Professor of Zoology Emeritus, Princeton University (since 2008).

After receiving her BSc (Honors) in 1960 from Edinburgh University, Scotland, she became a Research Associate at the University of British Columbia, Canada (1960-1964), at Yale University (1964-1965), at McGill University (1973-1977) and at the University of Michigan (1977-1985). She earned her Ph.D. in 1985 from Uppsala University. At Princeton University she became Research Scholar and Lecturer (1985), and then Senior Research Scholar and Professor in the Department of Ecology and Evolutionary Biology (1997). She was Visiting Professor at the University of Zurich, Switzerland (spring-summer 2002 and summer 2003).

She is a member of the American Academy of Arts and Sciences (1997) and of the Charles Darwin Foundation (2002), Foreign Fellow of the Royal Society of Canada (2004) and Fellow of the Royal Society of London (2007), Foreign Member of the American Academy of Sciences (2008) and honorary member of the American Society of Naturalists (2008). She has received honorary degrees from McGill University (2002), Universidad San Francisco, Quito (2005), and the University of Zurich (2008). She was made Honorary Citizen of Puerto Bacquerizo, I. San Cristóbal, Galápagos, in 2005.

PETER R. GRANT and B. ROSEMARY GRANT were awarded the Linnean Society of London's Darwin-Wallace Medal of 2009 on the occasion of Charles Darwin's 200th birthday, 12 February, 2009.

They have received several joint honours, including the 2009 Kyoto Prize in the category of Basic Sciences, the Municipality of Puerto Ayora Science Award, I. Santa Cruz, Galápagos (2006), the American Institute of Biological Sciences Outstanding Scientist Award for 2005, the Distinguished Scientists Award for 2005 from the American Institute of Biological Sciences, the Grinnell Award from the University of California at Berkeley (2003), the Loye and Alden Miller Award from the Cooper Ornithological Society (2003), the Darwin Medal from the Royal Society of London (2002), the E.O. Wilson Prize from the American Society of Naturalists (1998) and the Leidy Medal from the Academy of Natural Sciences of Philadelphia (1994).

For their book *Evolutionary Dynamics of a Natural Population: The Large Cactus Finch of the Galápagos* (Chicago, University of Chicago Press, 1989) they received the Wildlife Society's 1991 Wildlife Publication Award. The book by Jonathan Weiner, *The Beak of the Finch: A Story of Evolution in Our Time* (New York, Alfred Knopf, 1994), in which Rosemary and Peter Grant discuss their 20 years of fascinating research, was awarded the Pulitzer Non-Fiction Prize in 1995. Their most recent book *How and Why Species Multiply. The Radiation of Darwin's Finches* (Princeton University Press, Princeton, New Jersey) was published in

2008. Their new book *In Search of the Causes of Evolution, From Field Observations to Mechanisms* (Princeton University Press, Princeton, NJ), is forthcoming.

They have advised the California Science Center on the construction of an Evolution Exhibit featuring Darwin's Finches and own their research on them, as well as an exhibit entitled "Explore Evolution" sponsored by the National Science Foundation, and also the American Museum of Natural History on their "Darwin" exhibit.

Peter and Rosemary Grant have jointly published numerous journal papers. Among the most recent we should mention:

- *Saving Darwin's Muse: evolutionary genetics for the recovery of the Floreana mockingbird*, «Biology Letters», 6, pp. 212-215, 2010 (with others).
- *Conspecific versus heterospecific gene exchange between populations of Darwin's finches*, «Philosophical Transactions of the Royal Society B», 365, pp. 1065-1076, 2010.
- *Multilocus genotypes from Charles Darwin's finches: biodiversity lost since the voyage of the Beagle*, «Philosophical Transactions of the Royal Society B», 365, pp. 1009-1018, 2010 (with others).
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- *The secondary contact phase of allopatric speciation in Darwin's finches*, «Proceedings of the National Academy of Sciences USA 106», 20141-20148, 2009.
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- *The origin and diversification of Galápagos mockingbirds*, «Evolution», 60, pp. 370-382, 2006 (with others).
- *Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation*, «Mol. Ecol.», 14, pp. 2943-2957, 2005 (with others).
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- *Simulating secondary contact in allopatric speciation: an empirical test of premating isolation*, «Biological Journal of the Linnean Society», 76, pp. 542-556, 2002.
- *Unpredictable evolution in a 30-year study of Darwin's finches*, «Science», 296, pp. 707-711, 2002.
- *Adaptive radiation of Darwin's finches*, «American Scientist», 90, pp. 130-139, 2002.
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- *Hybridization and speciation in Darwin's finches: the role of sexual imprinting on a culturally transmitted trait*, pp. 404-422, in D.J. Howard and S. Berlocher (eds.), *Endless forms: species and speciation*, Oxford University Press, 1998.
- *Genetics and the origin of bird species*, «Proceedings of the National Academy of Sciences, USA», 94, pp. 7768-7775, 1997.
- *Hybridization, sexual imprinting and mate choice*, «American Naturalist», 149, p. 128, 1997.
- *Cultural inheritance of song and its role in the evolution of Darwin's Finches*, «Evolution», 50, pp. 2471-2487, 1996.
- *Predicting microevolutionary responses to directional selection on heritable variation*, «Evolution», 49, pp. 241-251, 1995.
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- *Hybridization of bird species*, «Science», 256, pp. 193-197, 1992.

GIUSEPPINA BARSACCHI
Member of the Accademia Nazionale dei Lincei

MORPHOLOGICAL TRANSITIONS AND THE CONCEPT OF "DEEP HOMOLOGY"

I wish to thank the President and the Vice-President of the Accademia for giving me the opportunity to take part in this Conference, and I also wish to thank the Chairman of the Balzan "Prize" Foundation, and especially you, Professor Rosemary Grant, and Professor Peter Grant – you have beautifully demonstrated how we can actually witness evolution at work, provided we comply with at least the following three conditions:

- choose small populations in nature, such as Darwin's finches in the Galápagos;
- be able to ask the appropriate questions, and
- perform a field study in the light of ecology, which in the terrific job you have performed meant minutely observing and amassing data for more than 30 years – with no apparent intervals, as one can infer from your reports.

Your work has produced great insights into the processes that create biodiversity by applying basic and apparently simple techniques, such as banding all individuals with rings of different colours, locating their nests, and measuring their beaks and other body parts, and the like.

More recently, as you have shown us today, questions raised by your field studies have greatly influenced molecular genetics.

Some of your results are now presented in an Italian University textbook on Developmental Biology (Fig. 1). I think however that the molecular information you provided would not have the same relevance

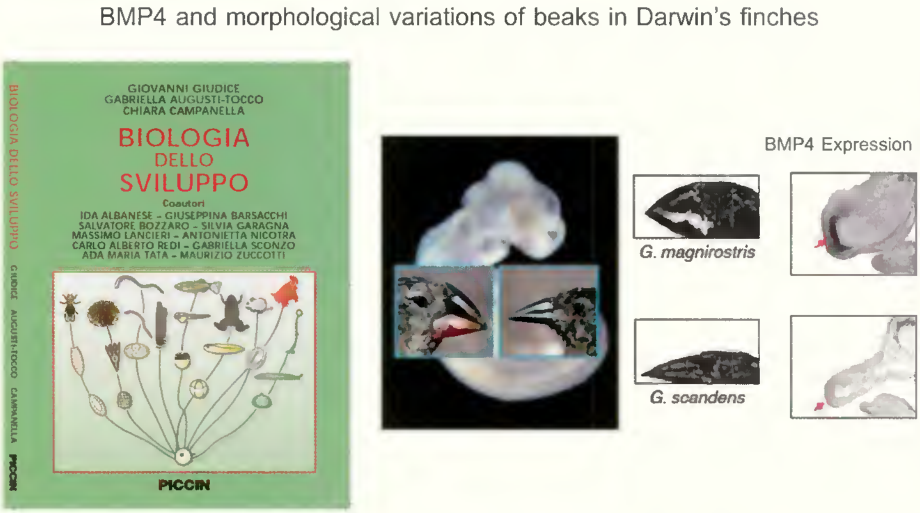


Fig. 1. From A. Abzhanov *et al.*, «Science», 305, pp. 1462-1466, 2004.

if it were not in the context of evolution on the Galápagos, with all the variables at play that you have taken into consideration, such as climate change, vegetation, bird song, competition, mate choice, hybridization etc. One wishes that all developmental molecular studies would demonstrate similar depth stemming from such a wide cultural background.

In particular, you used analysis of candidate genes to figure out the developmental genetic mechanisms driving the construction of the different beak shapes during embryo development, and were able to show that the differential expression of the BMP4 protein is responsible for the different shapes of the finches beak: a higher amount of BMP4 produces a larger and deeper beak, a lower expression of BMP4 is related to a longer and thinner beak (Fig. 1). In addition, another signaling protein, calmodulin (CaM), cooperates to give rise to the long and thin beaks of some finch species. You even managed to transform a chick beak into a finch beak by manipulating BMP4 or CaM expression during embryo development.

At present, it is recognized that major morphological transitions can be accommodated by a few key developmental genetic changes, as the ones you have shown, and now I'm going to briefly present a few additional examples for the audience and especially for the students who are here today.

As a first example, formation of the webbed feet in some birds, such as the duck, appears to have been brought about by a change in gene expression occurring in the developing foot during embryo development (Fig. 2).

In the early embryo, the interdigital membrane – the structure that gives rise to the webbed foot – is present in the hind limb bud of both chick and duck, but subsequently it is destroyed by apoptosis – that is programmed cell death, and dying cells are marked in red – in the chick, but not in the duck.

In the duck, in fact, apoptosis is prevented by the differential expression of a secreted protein called gremlin, while gremlin is not expressed in the interdigital membrane of the chick, where therefore apoptosis can take place, thus separating the digits one from another.

In fact, the forced expression of gremlin in the interdigital space of the developing chick hind limb generates a webbed foot (Fig. 3), thus mimicking the foot of a duck!

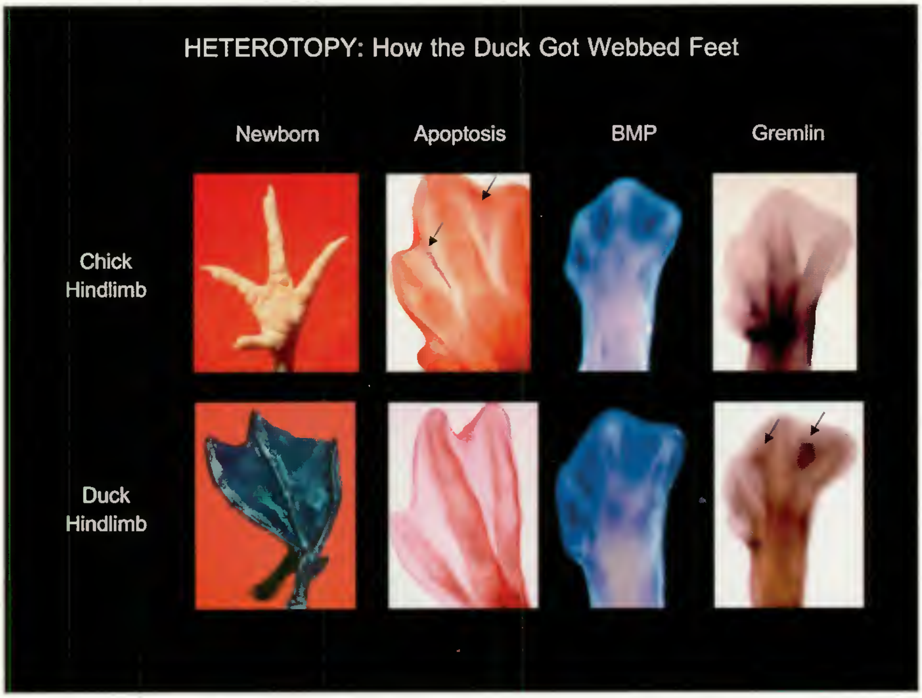


Fig. 2. From R. Merino *et al.*, «Development», 126, pp. 5515-5522, 1999.

How the Duck Got its Webbed Feet

Experimental Manipulation of Chick Feet

Untreated
Chick Hindlimb
(Saline bead)

Chick Hindlimb Treated
with Gremlin-Containing Bead in
Interdigital Space

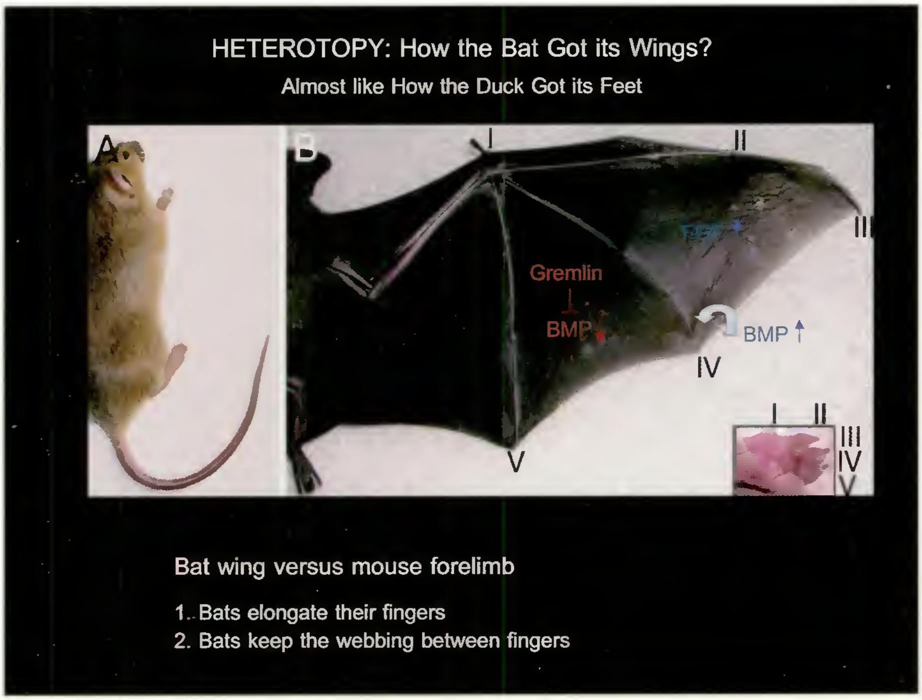
Fig. 3. From R. MERINO *et al.*, «Development», 126, pp. 5515-5522, 1999.

The inference in this "case study" is that a change in gene expression – namely, the novel expression of gremlin – may have contributed to the evolution of webbed feet in birds.

A similar mechanism operates to produce chiropatagium in the bat forelimb, since again gremlin, by inhibiting the secreted protein BMP, contributes to protecting the interdigital webbing from apoptosis in the bat forelimb, but not in mouse limbs nor in the bat hind limbs (Fig. 4).

Conversely, BMP signaling plays a role in supporting the disproportionate elongation of the fingers that sustain the chiropatagium. This data provides a potential explanation as to how bats were able to achieve powered flight soon after they diverged from other mammals, nearly 65 MY ago.

Gene expression changes such as those producing the different beaks of finches, or the duck's webbed feet or the bat's chiropatagium, must occur each in an otherwise conserved genetic program that



HETEROTOPY: How the Bat Got its Wings?

Almost like How the Duck Got its Feet

Bat wing versus mouse forelimb

1. Bats elongate their fingers
2. Bats keep the webbing between fingers

Fig. 4. From S.D. Weatherbee *et al.*, «Proc. Natl. Acad. Sci. U.S.A.», 103, pp. 15103-15107, 2006.

generates the very basic structure of a beak or a foot or a forelimb, respectively.

The concept of "deep homology" has been proposed precisely to indicate the conserved genetic regulatory apparatuses that are used to build specific animal features during development – and the features produced by "deeply homologous" genes, can be "morphologically and phylogenetically disparate" (N. Shubin *et al.*, «Nature», 457, pp. 818-823, 2009).

Perhaps the most famous case that fits with the "deep homology" description is that of the eye, since very similar genetic networks of interacting genes specify formation of eyes as varied as the compound eye of insects or the camera eye of vertebrates (Fig. 5).

Conservation of individual genes in the network is so extreme, that the *Pax6* gene of a jellyfish, or a squid, or an ascidian or a mouse, are each able to form ectopic eyes in transgenic flies – on the antenna, or

A genetic network for eye specification is conserved between Invertebrates and Vertebrates

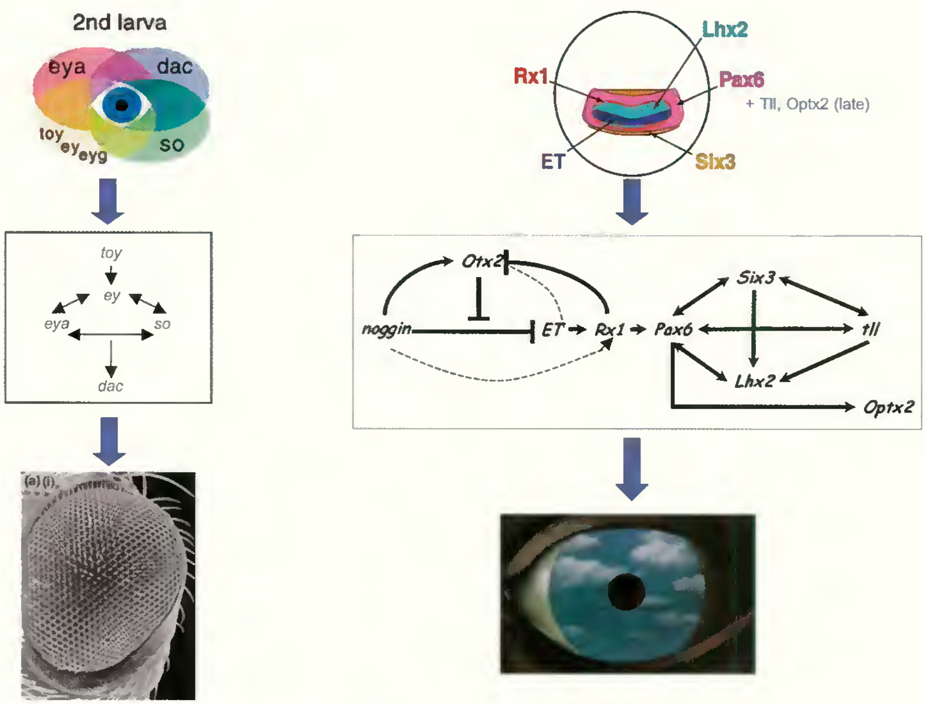
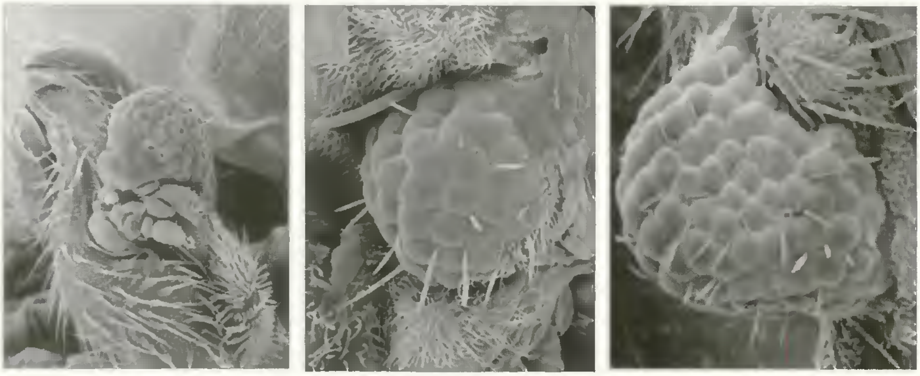


Fig. 5. From W. Gerhing and K. Ikeo, «Trends Genet.», 15, pp. 371-377, 1999; M.E. Zuber *et al.*, «Development», 130, pp. 5155-5167, 2003.

The *Pax6* Genes of Mice, Squids and Jellyfishes elicit ectopic *Drosophila* eye formation



Jellyfish *PaxB* into fly — Squid *Pax6* into fly — Mouse *Pax6* into fly

The *Pax6* function for eye specification is evolutionarly conserved

Fig. 6. From S.I. Tomarev *et al.*, «Proc. Natl. Acad. Sci. U.S.A.», 94, pp. 2421-2426, 1997.

on a leg, or on a wing of a fly – and all these ectopic eyes have the structure of the compound eye of *Drosophila*, regardless of the provenance of the *Pax6* gene (Fig. 6).

On such grounds, it seems to me that remaining, major difficult questions might include:

– Firstly: How does natural selection shape gene expression? A large field may have to be explored by searching for the links between proximate causes of development and ultimate causes of natural selection. I would like to have your comments on this point.

– Secondly: How can one reconcile the "deep homology" of genes working through development with classic homology, so as to conform to Darwin's understanding of the importance of Embryology for Evolution?

CHARLES DARWIN

"Embryology is to me by far
the strongest class of facts
in favour of change of forms."

Letter to A. Gray, 1860

"Community of embryonic structure
reveals community of descent."

On the Origin of Species, 1859

Charles Darwin. By George Richmond, late 1830s.

Acknowledgements. I wish to thank Professor Scott Gilbert for the generous gift of some slides.

ERNESTO CAPANNA
Member of the Accademia Nazionale dei Lincei

MOLECULAR DARWINISM: A MILESTONE OR A MILLSTONE?

It is a hard task to speak after Rosemary and Peter Grant's presentation of their splendid work. In fact, there is very little to add.

I only want to suggest a few reflections on the great gift that molecular biology has given to scholars of biological evolution. The molecular genetics of development expressed so well in the Grants' presentation on the formation of the beak of Certhidea and again confirmed in the discussion by Giuseppina Barsacchi highlights this very fact: the introduction of biomolecular techniques has profoundly influenced knowledge of the processes of evolution. It has truly been a revolution. I can say this on the basis of my training as an anatomist who has taught comparative anatomy for half a century, and who, already as a student, raised many questions to which no one knew the answer in those days, or better, to which palaeontologists had not offered completely satisfying answers.

Let me just cite two examples, which are from comparative anatomy itself. Homology, as defined by Sir Richard Owen: "The same organ in different animals under every variety of form and function",[1] became an important paradigm for Thomas Huxley to protect Darwinian evolutionism. Homology as evidence of origin from one same common ancestor.

Homology between fish fins and tetrapods' limbs has been, however, a complex problem. It has been tackled on the basis of evidence from vertebrate palaeontology, but not definitively concluded. The

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[1] R. OWEN, *Lectures on Invertebrate Animals*, London 1843, p. 379.

comparison between the fleshy fin of the Crossopterigi Ripidisti (fleshy-finned fish) and the limb of the first labyrinthodonts (Eryops, Paleogyrinus, etc.), and the first truly terrestrial tetrapods has lead to the formulation of theories, at the time antithetical to each other, like Westoll's and Holmgreen's, but nevertheless unable to define the processes of development underlying a "shared" ontogenesis. Between 1995 and 1996,[2] Sordino demonstrated, with an elementary experiment on the molecular genetics of development, the deep homology between fish fins and tetrapods' limbs. The same Hox genes (Hoxa-11 and Hoxd-13) that codify the regulation of the development of tetrapods' limbs, express their genic product in the embryonic fin of the zebrafish (Danio rerio). A significant difference, however, was brought out: while the disposition of the genic expression of these genes is arranged in a linear fashion in fish, in the stylopod-zeugopod direction, in the tetrapod limb, it is also arranged so as to cover the entire apical portion of the embryonic limb. Hence, besides stylopod-zeugopod, it is also expressed for the formation of the basipod and the metapod, of the carpus and metacarpus. There is still no certainty about digits; digits are the true novelty of tetrapods. There are those who think there is homology between the lepidotrichs of fish and digits, but on this point I do not completely agree. Digits develop through an autostotic process, that is to say, they are derived from a previous cartilaginous embryonic endoskeleton, while the lepidotrichs of fish fins are absolutely allostotic; they are derived from dermal formations. Homology cannot yet be established, but our faith in the molecular approach to the problems of comparative morphology gives us some hope.

I will cite another example that is much more complex and mysterious, and that is why it is so fascinating: the arrangement of the front limbs of tetrapods. They fall along the outside of the rib cage. I am not saying anything new – anyone can see that the scapula rests on the ribs and that the girdle closes in front of the rib cage. In turtles, however, and in the entire evolutionary line of Chelonians, the opposite

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[2] P. Sordino – F. Van der Hoeven – D. Duboule, *Hox genes expression in teleost fins and the origins of digits*, «Nature», 375, pp. 678-681 (1995).

P. Sordino – D. Duboule, *A molecular approach to the evolution of vertebrate paired appendages*, «Trends in Ecology and Evolution», 11, pp. 114-119 (1996).

is true: the girdle is inside the rib cage; the scapula, in fact, lies in relation to the inner face of the ribs, and other bones of the girdle are inside the carapace, which includes the ribs dorsally and the sternum ventrally. How did this come about? George Cuvier, the undisputed father of comparative anatomy, was also concerned with this problem. When I, still a student, asked my professors of comparative anatomy how in the world this could happen, they answered, "It's obvious: since the turtle must withdraw his limb back into the carapace, it is clear that the girdle must stay inside the carapace". This was the same answer that Cuvier gave too, a result of his functionalist mentality. But a functional answer is a non-answer for those with an evolutionary outlook. In this case, too, the problem was confronted on a paleontological basis, with a great methodological handicap: that even the most ancient fossils of turtles, that is, Odontochelis semitestacea[3] of the late Triassic, had already acquired this peculiar situation. Hence it was not possible, as in the previous example related to the phyletic line of tetrapods, to draw the terms of passage through intermediate forms of contemporary or previous Anamni. In this case, too, studies in biology solved the problem. A series of research projects carried out by Shigeru Kuratani and his group clearly identified the process of the formation of the carapace and of the incorporation of the limbs and girdle under it.[4] To make a long story short, an ecto-mesodermic folding was created, and it took the name "carapacial ridge", which shifted dorsally and dragged with it the embryonic ribs and involved them in the formation of the carapace. The embryonic limb developed ventrally to this carapacial ridge, and so when the carapace was joined together with the breastplate, the girdle and the limb remained inside the rib cage.

This and other successes of the molecular approach to problems of evolutionary morphology generated a great deal of enthusiasm: "We have finally understood how things work".

In his *Dictionnaire Philosophique*, Voltaire wrote: "L'enthousiasme est précisément comme le vin: il peut exciter tant de tumulte

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[3] C. Li – X.-C. Wu – O. Rieppel – L.-T. Wang – L.-J. Zhao, *An ancestral turtle from the late Triassic of southwestern China*, «Nature», 456, pp. 497-501 (2008).

[4] H. Nagashima – S. Kuraku – K. Uchida – K. Ohyay – Y. Narita – S. Kuratani, *On the Carapacial ridge in turtle embryos; its developmental origin, function and the chelonian body plan*, «Development», 134, pp. 2219-2226 (2007).

dans les vaisseaux sanguigns, et de si violentes vibrations dans les nerfs, que la raison en est tout à fait détruite" (i.e. enthusiasm is precisely like wine, it has the power to excite such a ferment in the blood-vessels, and such strong vibrations in the nerves, that reason is completely destroyed by it). Thus, has this enthusiasm for a genetic approach to development destroyed us? It has not let us notice that it threatens the foundations of at least two parameters of Darwinian orthodoxy: phyletic gradualism and natural selection. In fact, if an alteration in the processes of development linked to the change in the functional role of genes can create structural novelty and thus suddenly open up to phyletic lineage, what is the role of natural selection? Phyletic gradualism, indeed, does not have fundamental importance in the Darwinian model; Huxley had already criticized his friend Darwin. Later, too, George G. Simpson, the palaeontologist among the founders of Modern Synthesis, did not believe in phyletic gradualism, just as recently, other palaeontologist evolutionists like Stephen J. Gould and N. Eldridge do not.

Phyletic gradualism, I repeat, is not one of the main pilasters of Darwinism; it is a fringe element in the protective wall of theory. But natural selection, on the contrary, is the kingpin around which the Darwinian conception of evolution revolves. If at a certain moment an entire phyletic line like the turtle's can come to an end because there is developmental novelty that we do not find in all other terrestrial vertebrates, and a simple apical extension of the expression of a few Hox genes is enough to equip the line of vertebrates for tetrapod locomotion, then where is the fundamental role of Darwinian natural selection? The heresy of the neutralism of Motoo Kimura,[5] and of Masatoshi Neil[6] is born precisely of a position of over-evaluation of the role of molecular processes in ontogenetic development. With his hypothesis of "molecular drive",[7] Gabriel Dover maintains that evolution moves where molecules drag it.

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[5] M. KIMURA, *The Neutral theory of molecular evolution and the world view of a neutralist*, «Genome», 31, pp. 24-31 (1989).

[6] M. NEIL, *Selectionism and neutralism in molecular evolution*, «Molecular Biology and Evolution», 22, pp. 2318-2342 (2005).

[7] G. DOVER, *Concerted evolution, molecular drive and natural selection*, «Current Biology», 4, pp. 1165-1166 (1994).

The sensational entrance of molecular biology in the study of biological evolution forced Richard C. Lewontin[8] to reflect on the matter and wonder whether the molecular approach to evolution represented a milestone or a millstone to Darwinism – an element of suicide, as I have entitled my own reflections, stealing his words.

This is not the way things are. Natural selection is fundamental, as much on the micro-evolutionary level as on the macro-evolutionary one. Importance on the micro-evolutionary level has clearly been demonstrated in Rosemary and Peter Grant's presentation; if Darwin's finches had not found favourable ecological and ethological conditions, they would never have been able to fix such easily accomplished molecular novelty. The isolation of a small deme, which also plays an important role, is not enough, as the Grants emphasize in their work. The molecular phase in micro-evolution merely represents the trigger phase of a process of speciation, analogous to other processes in the molecular re-ordering of genetic structures, like those that produce chromosomal rearrangements.

One can say that the same thing happens on a macro-evolutionary level: if there are no special adaptive conditions that make the formation of a basipod and a metapod, or the formation of a carapace "favoured in the struggle for life", to use Darwin's words, the entrance of new anagenetic lines like those of terrestrial Vertebrates or Chelonians would never have happened. The bizarre nature of a polydactyl limb, or of a heavy bony shell, would inexorably be discarded in the process of natural selection.

The verses of an ancient author seem to synthesize this idea, and I would like to conclude my reflections with them. I would like to deliver them in Latin, since I love the musicality of the classic couplet. The passage is taken from Titus Lucretius Carus' De Rerum Natura. Thus we read

> Cetera de genere hoc monstra ac portenta creabat /
> nequiquam quoniam natura absterruit auctum.

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[8] R.C. Lewontin, *Twenty five years ago in Genetics. Electrophoresis in development of evolutionary genetics: milestone or millstone?*, «Liber» V, pp. 845-846.

Here Lucretius is talking about Mother Earth, who is the mother of all living beings, as Democritus of Abdera maintained. In her frenzy to create living beings, Mother Earth generates monsters and marvels, but in vain, because nature hinders their growth.

This is natural selection.

QUESTIONS AND ANSWERS – FINAL REMARKS

Peter Grant: Well, thank you very much for your remarks which nicely broaden the context of our research. You are raising questions and topics that we have certainly discussed with students when we've been teaching. In contrast, when we're out on the little island of Daphne, we're not thinking deeply about Darwinian questions such as whether he got it right or wrong about embryology, for example, nor are we thinking very deeply about the deep questions that you have raised, such as deep homology. You asked us to comment on deep homology, on the ideas of Neil Shubin and Cliff Tabin and Sean Carroll. I think the papers they've written are very nice, in fact I adopt their framework in looking at problems of evolution and development. Basically, in the past the approach was to take a trait, like a limb, for example, and try to look for homologous relationships, among species of vertebrate animals and trace a homological development from primitive to ancestral structures. But now in the modern era of molecular genetics we have a quite different view of homology, through detailed studies of development, and of how adult forms are produced, and this is revealing all sorts of fascinating surprises – facts that confirm intuition in some cases and facts that are completely different in others. One of the surprising things is – and you mentioned this – the pax 6 gene is so beautifully conserved across a number of organisms. I find just as interesting the change in gene function across organisms, which presents us with a real challenge when we come to try to fit in molecular genetic findings with homology. This is something Rosemary and I have talked about, but we're not very knowledgeable about it. I don't know, for example, how BMP4 would ever start out with one function and finish up with another one. I don't know how genes get coopted for new functions. Expression patterns change, yes, but how? Perhaps you'd like to comment on that yourself, but I'll just make the one general point that this is one area in which molecular biology (molecular genetics) is revealing to us a much more complex picture of developmental processes than we had originally thought existed. And I find that just absolutely fascinating.

Question from the audience: As you certainly know, already in 1865 or 70, Darwin himself noted that the selection of animals between male and female cannot be explained exclusively by means of sexual selection; he was not speaking about genes, but simply that it cannot be explained by natural selection. And he suggested 150 years ago that the nervous system was necessary in order to explain some of the differences in evolution. Now my question is very simple: do you think that all these differences between birds are only due to genetic properties, or let's say expression of genetic properties, or is there also some contribution of the nervous system, which exists in the birds as it exists in all living entities?

Peter Grant: Well, the nervous system is vitally important to the evolution of behaviour, just as genes are essential for evolution to occur. Nevertheless, to broaden the question a little, whether genetics alone will explain different morphological patterns, or whether something else needs to be invoked to account for great differentiation including the nervous system, is an example of a general problem in evolutionary biology: can you explain everything in genetic terms, which means genes affected by natural selection, sexual selection, which we haven't mentioned so far, and random genetic drift, or do you have to invoke other genomic factors like chromatin architecture, genomic imprinting, and environmental influences on both of those that might possibly be inherited in a non-Mendelian fashion? That is a very general issue, beyond just the one you have raised in connection with Darwin's point about the nervous system.

I think that Rosemary wants to add something.

Rosemary Grant: I would like to add something about the neurological part of a bird's brain. The FOXP2 gene, which is in our own brain, is also expressed in the song centre of a bird's brain. The song centre is very much under hormonal control, to the extent that a female bird that does not normally sing, can be brought into singing condition with an injection of testosterone. This has not been done with Darwin's finches, but in other species of song birds, demonstrating that females like males can learn song from a tutor and have the potential to sing. During springtime when the birds start to sing, increasing daylight stimulates hormonal action in males, which in turn stimulates the song centres in the brain. Half the birds in the world today learn their song; these are, songbirds, parrots and hummingbirds. There are some rudimentary similarities in the language centres of our own brain and that of the song centres of these songbirds in that we both have sensory and motor connections and expression of some common genes. There is variation between different species of birds regarding their ability to learn songs, and this is an interesting evolutionary question that deserves further research. Some species having the ability to learn and extemporize throughout life, others learn elements of new songs every spring and yet others such as Darwin's finches learn in a short sensitive period early in life, and we don't understand why. This variation in vocal learning has suggested to some researchers that investigation into how birds learn and produce song should provide insight into understanding the evolution and mechanisms of language in humans.

Peter Grant: Well there remains little left to say. The discussants have raised some very interesting issues, central to our work, which has resulted in a very illuminating discussion. This occasion clearly illustrates the value of the Balzan Prize. It is not simply a recognition of academic achievement but a unique promoter and incubator of academic research. In a world where research funds are increasingly being directed only to research which may prove remunerative in the short term, it is refreshing to see the Balzan Prize support such a wide range of endeavours both of a pure and applied nature. This annual lecture series is an ideal way for the results of such research to be made available to a wider public. Thank you ladies and gentlemen.