Charles Darwin’s life and legacy are well known. You get the impression that not a single day in his life passed without being scrutinized by some scholar somewhere. And for the good reason that his towering scholarship laid the foundation for almost all aspects of modern evolutionary biology.

In this lecture we will tell you about one way we have built on the foundation, by following in his footsteps to the Galápagos. He visited the islands for 5 weeks in 1835 on board the Beagle, with mainly geological questions in mind. But he also collected specimens of birds and plants, and made observations that he later drew upon when sorting his views on how species changed and new ones were formed.

For example, with regard to a group of finches, he wrote (1842) “Seeing this gradation and structure [of beak sizes] in one intimately related group of birds, one might fancy that, from an original paucity of birds in this archipelago, one species has been taken and modified for different ends.”

Here was the seed or germ of two ideas, the concepts of natural selection (“taken and modified”) and adaptation (“for different ends”). 17 years later the two concepts became the pillars of his theory of speciation, which he developed at length in his book On the Origin of Species.
We began our studies in 1973, motivated in part by his insightful suggestion to pick closely related species to explore his ideas on the origin of species. One suitable group for study is a cluster of Darwin’s finches, named in his honor by Percy Lowe 100 years after Darwin returned to England with the specimens he collected and, by coincidence, the same year that Rosemary and I were born! 14 or more species evolved from a common ancestor, which we know happened in the last 2 million years from mitochondrial DNA dating.

Darwin’s ideas on how they evolved can be illustrated with a diagram and a hypothetical scheme. A species disperses from one island to another, diverges through natural selection, and eventually the previously separated populations encounter each other through additional dispersal, and diverge even more by natural selection that minimizes two things: interbreeding and competition for food. This happened in the past. What can be learned about such processes by studying contemporary populations? To answer this question we chose the small island of Daphne Major.

Daphne is in the center of the Galápagos archipelago. It is only about three quarters of a kilometer long and 120 meters high, and it has never had a human settlement. Before describing our results we will share some of our experiences, illustrated with short sequences from videos we made and licensed to Pearson Education and the Films Media Group for educational purposes.

Volcanic in origin, Daphne was formed underwater, perhaps as recently as 20 thousand years ago. In the center is a crater where seabirds breed. The most important clue for understanding the lives of finches is the effect of rainfall on the vegetation and their food supply. In a typical year Daphne receives rain anytime between January and April or May, and nothing for the rest of the year. In some years there is no rain. In years of the El Niño phenomenon there is an abundance of rain and birds breed for many months in succession.

Before we come our supplies are quarantined for a couple of days, then loaded onto the boat at the Charles Darwin Research Station on nearby Santa Cruz Island. Once we are ashore, our task is to get everything up a 4-meter cliff. This entails hauling up all our food, water, clothes, scientific equipment, and camping and medical supplies, for a field season that lasts anywhere from a few weeks to several months. When we have established the camp, the white metal boxes will store our food and scientific equipment. Like everything else we bring, they have been in quarantine before arriving on the island. Even so, we check everything to minimize the chances of accidentally bringing alien insects or plants to the island.

The kitchen is in a cave. We bring fresh water from the Research Station in 30 or more large plastic containers. With a built in safety margin, this amount will be enough to last us for a month. Since fresh water is at a premium, all water for washing dishes, as
well as clothes, has to come from the sea....... So part of the daily routine is a return to the landing. There is only one place on the island to put up a tent.

Since the main goal of the research is to understand how finches live we need to capture, mark, release and observe them. Capturing birds in mist nets is the key activity. We then put them in light cloth bags and take them to a shaded place. There we measure them. Banding begins with a numbered metal band. Three colored bands made of PVC plastic are placed on their legs. The colors are coded to correspond to the last three numbers on the metal band. We then take a small drop of blood by pricking the wing vein with a fine needle, transferring the blood to filter paper, and storing it in a jar of drierite to keep it dry for later analysis of DNA. The procedure is very similar to the taking of blood from newborn babies for characterizing their blood group. Another major task is to find each nest and tie flagging to a nearby bush so we can find it again. 95% of all nests are built in cactus bushes throughout the island. The nests are accessible: bushes are no more than two and a half meters high, so we don’t need to use nest boxes. There are no nest predators on the island. We band all the nestlings, and knowing the parents we build up a pedigree of relatedness.

There are three species on the island: *fortis*, the medium ground finch, is the commonest; *magnirostris*, the large ground finch; and *scandens*, the cactus finch. Most *fortis* feed on small seeds. They have small beaks. *magnirostris* feed on the hard seeds of *Tribulus*. They have large beaks. *scandens* specialize on cactus pollen, nectar and seeds. They have long beaks.

A key difference between the species is their diet in the dry season, and that difference is due to a difference in beak shape. An important question is how does a new beak size or shape evolve? A drought in 1977 gave us an answer, when four out of every five *fortis* died of starvation. We had previously captured and measured a large number of finches, and banded them with a unique combination of colored and metal leg bands. From this we knew that the few *fortis* that survived were unusually large birds with large and deep beaks. Average beak size gradually increased during the drought. Finches with large beaks survived because they could crack open the woody fruits of a plant called *Tribulus*. *fortis* with smaller beaks could not do this: they could only feed on small seeds. Many of them died of starvation. And because beak shape is heritable the offspring of the survivors, produced in the following year, likewise had unusually large and deep beaks. Beak size and shape had evolved as a result of natural selection.

Six years later the archipelago experienced an extraordinarily intense and prolonged El Niño event. More than a meter of rain fell over 8 months on the semi-arid island of Daphne, resulting in an enormous growth of plants. *Tribulus*, the plant whose woody fruits had made the difference between survival and starvation in 1977, was smothered by an abundance of vines and other species producing small seeds. Thus the island was transformed from a large seed producer to a
predominantly small seed producer. Then a La Niña drought occurred two years later. As the seed supply declined finches with small beaks survived better than finches with large beaks, so the average beak size became smaller in this and the next generation. Evolution had occurred again.

We discovered that beaks also evolve when species compete for food. This happened in the year 2005 as a result of an important event 23 years earlier. In that extraordinary El Niño event of 1982-83 *magnirostris*, the large ground finch, colonized the island. Two females and three males bred successfully and produced a total of 17 offspring. *magnirostris* numbers gradually built up over the years. By 2003, when a long drought began, there were at least 250 *magnirostris* on the island. They are much faster at cracking open *Tribulus* mericarps than *fortis*. The greater efficiency of *magnirostris* turned out to be a crucial disadvantage to those *fortis* with large beaks. They were outcompeted by *magnirostris* for the dwindling supply of *Tribulus*. *fortis* with large beaks suffered heavier mortality than *fortis* with small beaks. As a result *fortis* beaks became smaller on average, and the difference between *fortis* and *magnirostris* became magnified.

This confirms and demonstrates an important ingredient of Darwin’s theory of speciation, the Principle of Character Divergence. The next generation of *fortis* also had small beaks like their parents. Beak size had evolved once again as a result of natural selection. And by collaborating with Swedish scientists we discovered an important gene, HMGA2, that affects beak size and was involved in this evolution.

These results illustrate the two main environmental factors involved in the evolutionary diversification of the finches: a fluctuating food supply and competition. They vindicate Darwin, and provide a more detailed understanding of the process of evolution in a way that he anticipated but could only speculate about. This is what he wrote when challenged to explain exactly what he meant by natural selection.

“I would now say that of all birds annually born, some will have a beak a shade longer, & some a shade shorter, & that under conditions or habitats of life favouring longer beak, all the individuals, with beaks a little longer would be more apt to survive than those with beaks shorter than average.”

Remarkably prescient! But food and competition are not the only factors on Daphne.

Emeritus Professor Rosemary Grant

Another factor influencing divergence and speciation is rare gene flow between closely related species. Introgression or gene flow between species comes about through hybridization followed by backcrossing to one or other of the parental species. The small amount of gene flow introduces new genetic combinations and increases the genetic
and phenotypic variation of the population. We have observed such events, albeit rarely, on Daphne Major and on other islands in the Galápagos. The outline of my talk is first an investigation of the causes and evolutionary consequences of rare introgression, and then I will describe one of the most exciting parts of our 40 year study on the finches, which is the origin of a new lineage that we have observed and followed from its inception for six generations.

The barrier to interbreeding between species in all Darwin’s finches is song and appearance (phenotype) particularly body size and beak shape. In all other respects species of Darwin’s ground finches are similar to each other. They have similar plumage, males are black, females are brown; they build similar domed nests and have similar courtship behavior. We can tell the difference between the species using song and appearance, but can the birds? To test if birds themselves could discriminate between their own and another species on the basis of appearance alone in the absence of song, we used stuffed museum specimens of two females, one of their own species and one of another species mounted at either end of a branch. We then took this contraption into a bird’s territory. The answer showed strong discrimination. Males vigorously courted the female of its own species, even though she was just a stuffed museum specimen, and ignored the other species. We then used playback of tape-recorded song to test whether birds could discriminate between their own and another species on the basis of song alone, and in the absence of any morphological cues. Again the answer was positive. Males flew towards the recorder when their own species song was played and ignored the other species song. These experiments were repeated many times with controls and clearly showed that birds could discriminate on the basis of song and appearance. The question then is how do the birds acquire this knowledge? Robert Bowman in the 1960’s provided the answer with experiments on captive reared birds.

Bowman showed that in all Darwin’s finch species song is learned from a singing male early in life during a short receptive period from approximately day 10 to day 40 after hatching. In the wild this period corresponds to the last few days in the nest and when, as a fledglings, they are out of the nest being fed by their parents. All this time the father is singing, females do not sing. Therefore it is not surprising that sons learn and sing almost perfect rendering of their fathers’ song. Once learned, the song is retained for life and life can be as long as 17 years. They pair according to their learned species song in association with appearance. This mating pattern results in a to interbreeding between species. However because this barrier is based on learning it can be breached if a young bird hears and learns another species song during the short receptive period early in life. This happen occasionally when an egg is left behind after a nest is taken over by another species. Then the young learn their foster father’s song. It also can occur when the father dies and the mother is left to rear the offspring. Females do not sing, as a result the young learn their natal neighbor’s song, and if that neighbor is another species they learn that species song. In each breeding season approximately 1% of birds learn another species song.
The colonization of Daphne by the large ground finch *magnirostris* gave us the opportunity to test the robustness of this barrier to interbreeding. *Magnirostris*, at approximately 30-32 grams, is much larger than the 17-18 gram *fortis*, its closest relative, and the 19 - 22 gram *scandens*. It is aggressive with a loud song. Over the years 8 *fortis* and 2 *scandens* learned a perfect rendering of a *magnirostris* song, but this never led to interbreeding because as soon as these little birds sang a *magnirostris* song a *magnirostris* would fly in and beat them up. It seems that the size difference is important for maintaining a robust barrier, because learning another species song can lead to hybridization between the smaller species, *fortis* and *scandens*. Approximately 1% of *fortis*-*scandens* hybrids are produced each breeding season. However for hybrids with their intermediate beak size to survive their first dry season requires seeds of the appropriate size. For the first 10 years of our study, when the seed supply consisted predominately of large hard *Tribulus* seeds that they were unable to crack, none survived. Hybrids did survive with increased availability of small seeds after the El Niño event of 1983, when Daphne Island was converted from a large hard seed producer to a small seed producer. We found no evidence of genetic incompatibility when we comparing hybrid survival with survival of the parental species hatched in the same year during times of abundant small seeds. Hybrids not only survived as well as the parental species under these new conditions but they also produced as many eggs, fledglings and recruits into the breeding population as the pure species. Hybrids did not breed with each other, but backcrossed with one or other of the parental species according to their learned song. Thus, a trickle of genes passed from one species to the other and continued to do so for the next 30 years, resulting in the two species *fortis* and *scandens* converging genetically and morphologically, particularly in beak shape.

Using DNA samples obtained from small drops of blood from each individual we were able to track the genes flowing from one species into the other. One gene, *ALX1*, found by our Swedish collaborators was particularly important. This gene comes in two forms, one associated with blunt beaks the other with pointed beaks. We found that the *ALX1* pointed variety was introduced from *scandens* into the *fortis* population and contributed to *fortis* beaks becoming more pointed and *scandens*-like, whereas genes introduced into *scandens* from *fortis* contributed to *scandens* beaks becoming blunter and more *fortis*-like.

We proposed the possibility that if such backcross or introgressed birds flew to another island with different ecological conditions their novel genetic composition and phenotypic characteristics could be the starting point of a new evolutionary trajectory leading to a new lineage. Amazingly and unexpectedly we witnessed exactly this in the origin of the new lineage we now call the Big Bird lineage.

The lineage began with a bird arriving on Daphne at a time when we had nearly all the birds banded. It looked like a *fortis* but at 28 grams was much larger than the 17-18
gram Daphne fortis. We caught and banded it 5110 and took a small drop of blood for DNA analysis. By matching its genetic profile with birds on the island and on the surrounding islands we found it was most probably a fortis/scandens/fortis backcross hatched on Santa Cruz. It had a distinctive genetic marker, being homozygous for a rare allele and a unique song. He took a long time to breed, but when he did he bred with a fortis/scandens/fortis backcross born on Daphne and then later with 2 fortis females. This was the only out-breeding that occurred. For the next 3 generations there was considerable inbreeding. Then a two and a half year drought from 2003 to 2005 caused almost 90% of all birds on the island to die of starvation. This mortality included all the big birds, except for two, a brother and sister.

When the rains returned the brother and sister paired with each other, and produced 26 offspring, all but 9 of which survived to breed. We documented intense inbreeding for the next three generations. All offspring were homozygous (had two copies of) the rare allele as a result of previous inbreeding. All males sang 5110’s unique song and all were large. Thus from the original colonist 5110, there was both genetic transmission and cultural transmission.

Is this new lineage behaving as a separate species? It is larger than its nearest relative fortis in size, lying between magnirostris and fortis. It differs in song from the other three species on the island. It defends large contiguous territories against other members of its lineage. These territories overlap those held by fortis, scandens and magnirostris, the other 3 species on the island, who it ignores and is ignored by them. Thus in all respects it is behaving like a separate species. Will it survive? We do not know. It could die out through inbreeding depression or through introgression with another species but there is no sign of either so far. Whether it survives or not this new lineage gives insights into how a new species could arise and either persist or become extinct.

In summary, we began our study in 1973 on Daphne major with 2 species, fortis and scandens. This situation lasted for 10 years until a massive El Niño event, estimated from coral core dating to be the most severe in 400 years, changed the island from a large hard seed producer to a small soft seed producer. Under these new conditions hybrids between fortis and scandens survived and backcrossed to one or other species according to the song they learned early in life. As a result the two species began converging genetically and morphologically. Magnirostris colonized the island in 1983, increased in numbers and outcompeted the large fortis for Tribulus seeds during a severe drought in 2003-2005. This character displacement event expanded the gap between fortis and magnirostris, which is now occupied by the new big bird lineage.

Our work has given insights into three methods of speciation. It supports Darwin’s allopatric model in all details, including a robust barrier to interbreeding between
*magnirostris* and *fortis*, and a character displacement event through competition. Our study also reveals two different routes to speciation unknown to Darwin; these are two arising from introgression. First, introgression can lead to the fusing of two species into one unique lineage of mixed genetic composition, while the two species remain separate on other islands, as seen in the fusion of *fortis* and *scandens* on Daphne. Second, when an introgressed population with its novel genetic composition and morphological characteristics enters a new environment natural selection takes it off on a new trajectory, as demonstrated by the expansion of the Big Bird lineage.

How common is introgression in other organisms? A review of the literature in the last 15 years shows increasing number of examples of introgression in many organisms from horizontal DNA transfer in bacteria to interbreeding in our own early human ancestors.

Darwin’s famous diagram from his notebook B in 1837 shows him pondering over the relationship between species and drawing a branching tree. If he was able to return to this world would he now join some of these branches to indicate introgression?

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