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Evolution of Darwin's finches

(Ernst Mayr Lecture am 4. November 2004)

For three reasons it is a quite exceptional honour for us to be presenting the Ernst Mayr Lecture in the year 2004. First, Ernst Mayr has been the most influential synthesizer of evolutionary thought in the twentieth century, and a major influence on our own thinking. Second, he is 100, which is a different type of magnificent achievement. And third, of all locations this is the environment that nurtured him in his formative years. The time and the place are just right, and we are truly honored to be invited here to give a lecture in the name of Ernst Mayr at the Berlin-Brandenburg Academy.

For the first thirty years of his professional career Ernst Mayr was preoccupied with the Darwinian questions of what species are and how they are formed. His year-long collecting experience in New Guinea, the Solomons and the Bismarck islands gave him raw material for interpreting the products of evolutionary diversification: that is, different but related species of birds occurring in different combinations in different habitats on different islands. What would have bewildered many he reduced to order. In 1940 he helped to establish Dobzhansky's biological species concept, based on the criterion of reproductive isolation, and he spelled out a scheme, which we now call the allopatric model of speciation, by which a single species splits into two non-interbreeding populations; in other words two species from one. By establishing this framework he left to others the task of studying evolution directly to reveal which processes really matter, the genetical details that underlie reproductive isolating mechanisms, the environmental circumstances of speciation, and the specific causes. We are grateful to him for leaving something for us to do!

Our studies began where part of his career left off: with fieldwork. Our chosen 'field', the Galápagos islands, differed from his. Some of their occupants, Darwin's finches, are an almost ideal or model system for carrying the study of speciation to a deeper level of understanding. Even though there are no fossils to guide us, the environment of several of the Galápagos islands has not been disturbed by humans, and no Darwin's finch species has become extinct through human activity. The spe-

cies carry traces of their history in their molecules, and environmental history can be reconstructed from a knowledge of Galápagos geology, plant species diversity and global temperature fluctuations.

Our presentation will be in two parts. I (Peter) will discuss how 14 species came to be derived from a single ancestral species, and Rosemary will describe how those species manage to coexist and persist.

History of an adaptive radiation

The first finches arrived two-three million years ago by over-water flight from the South American mainland, perhaps impelled by fires associated with volcanic activity in the foothills of the Andes. Our estimate of the time is based on the difference in mitochondrial DNA among the modern finches on the Galápagos and between them and a mainland group of seed-eating tanagers that constitute the genetically closest relatives. At their time of arrival there were far fewer islands than today, perhaps only five. The number of islands subsequently increased as a result of volcanic activity centered on and near a hotspot beneath the western island of Fernandina. Global temperatures were higher then than now, and permanent El Niño conditions are thought to have occurred until about the time glaciation began 2.75 MYA (million years ago). This leads us to believe that the ancestral finches encountered a climate and vegetation more like those of modern-day Cocos island: warmer, wetter and more humid conditions, fostering rain forest from coast to island peaks. Cocos Island has temperatures similar to coastal habitats in the Galápagos, and rainfall equivalent to what the Galápagos now receives in an El Niño year at high elevations. Reasoning from the oldest of the modern finches, *Certhidea* warbler finches, we believe the initial evolutionary pathway taken by the finches was towards the exploitation of small arthropods, and nectar and pollen from small flowers, in a rain-forest-like environment. Morphologically this involved a change from a general seed-eating beak in the ancestral species to a smaller and more pointed beak. As the climate cooled the vegetation changed and two evolutionary developments unfolded, producing five new finch species adapted to exploiting arthropods from trees, mainly at high elevations, and six additional species (ground finches) adapted to feeding on seeds in lowland habitats.

How and why did the radiation unfold in such a way as to result in 14 species adapted to different ways of life? A primary driving force was environmental change, providing opportunity and creating necessity. Not all of the contemporary ecological niches were available when the ancestors arrived. Rather, the numbers and types of opportunities for finch evolution increased as the number of islands increased and the food of finches – plants and arthropods that feed on them, and on each other – increased in diversity and changed in distribution. The scarcity of other, potentially competitive, species must have been an important facilitating factor.

The multiplication of species

Darwin's finch species multiplied by repeated speciation, that is the formation of two species from one repeated several times. According to the allopatric model (Fig. 1), speciation begins with the establishment of a new population, continues with the divergence of that population and newly derived ones from the parent population, and is completed when members of two diverged populations coexist without interbreeding in sympatry. To coexist they must share the environment without intensely competing for resources. They do so as a result of acquiring different feeding habits in allopatry. The six existing populations of *Geospiza difficilis*, the sharp-beaked ground finch, illustrate this stage of the speciation cycle very well.



Figure 1

Allopatric model of speciation, in three stages: initial colonization (1), establishment of a second population (2) and secondary contact between the two populations (3). The choice of islands is arbitrary. Repetition of stages 2 and 3 in other parts of the archipelago would have given rise to more species. From Grant & Grant (2002a).

Three of the populations have persisted at mid- and upper-elevations in *Zanthoxylum* forest, which we believe to be old. Three entered arid lowland habitat, perhaps when the *Zanthoxylum* forest gradually disappeared from low islands as the climate changed. Different populations of this species feed in different ways on different foods with beaks of different size and shape. On the high islands of Santiago, Ferdinandina and Pinta they have relatively blunt beaks, and feed on arthropods and mollusks, as well as fruits and seeds in the dry season. On the low island of Genovesa, where they are much smaller in beak and body size, they are more dependent on small seeds, as well as nectar and pollen from plants including *Opuntia* cactus. Apparently uniquely on the low island of Wolf they exploit seabirds (boobies) in two dramatic ways. They gain moisture and protein from membranes around the egg as it is being laid. From this simple habit has developed a deeper interest in the egg itself. They kick the egg until it falls or hits a rock and cracks, enabling the finches to open it and consume the contents. Even more bizarre than this, they inflict wounds at the base of wing feathers of the sitting booby and consume the blood. This habit has almost certainly been derived from feeding on hippoboscids flies that suck blood from boobies, much as mosquitoes do from us. By feeding directly on the boobies' blood the finches have bypassed the flies and shortened the food chain. On this island their beaks are long. Thus members of the same species of Darwin's finches are versatile in their feeding habits and vary adaptively in beak morphology from island to island. Versatility is fostered by ecological opportunity and driven by food scarcity in the harsh conditions of dry seasons and dry years. Differences among populations of the same species in beak size and shape such as those of *Geospiza difficilis* became magnified to form differences between species, and this happened repeatedly during the adaptive radiation.

Molecular genetics of beak development

Recent molecular genetic analyses of ground finches (*Geospiza* spp.) throw light on what was involved in the transformation of small differences between populations into large differences between species. They were carried out by two of our colleagues Arhat Abzhanov and Cliff Tabin at Harvard University.

At the start of avian beak development two signaling molecules, fibroblast growth factor 8 (FGF8) and sonic hedgehog (SHH), have adjacent, non-overlapping, domains in the epithelium covering neural crest-derived mesenchyme that gives rise to the skeletal projections of upper and lower mandibles. At about day 3–4 in the life of the embryo the *Fgf8* domain is the dorsal fronto-nasal primordium (FNP) and the ventral mandibular nasal primordium (MNP). The intervening region is the domain for *Shh*. By misexpressing these two genes with retroviral vectors injected into the neighborhood of the developing beak Abzhanov and Tabin were able to show that together the two molecules induce cartilage outgrowth where the domains

meet. This is the origin of the beak. They also synergistically induce expression of other factors such as the signaling molecule *Bmp4* (bone morphogenetic protein 4) in the underlying neural crest mesenchyme. Differences among the species in the sizes of beak primordia begin to appear at day 5, and it takes only two-three more days for the species to attain their distinctive morphologies. Hatching takes place after 12 days.

Now, an important factor in the origin of these differences is *Bmp4*. At day 5 its expression is detectable at low levels in the sub-ectodermal mesenchyme of *G. difficilis* and other species, but at a dramatically higher level in the largest species *G. magnirostris*. At day 6 *Bmp4* expression is elevated in three ground finch species (*G. magnirostris*, *G. fortis* and *G. fuliginosa*) but not in the two cactus finches (*G. conirostris* and *G. scandens*) that have relatively long and shallow beaks. *Bmp4* expression occurs earlier in *G. magnirostris* than its relatives, over a greater spatial domain and at a higher quantitative level. The effect of *Bmp4* expression on beak depth development was neatly demonstrated by our colleagues who used a retroviral vector to misexpress *Bmp4* in chickens solely in the distal mesenchyme of the upper beak at day 6. This experiment mimics the natural occurrence of elevated levels of *Bmp4* at the same stage in *G. magnirostris*, and it produced very *magnirostris*-like beaks, both in width and in depth of the upper mandible. Injecting *Noggin* retrovirally, which is a *Bmp4* antagonist, led to a dramatic decrease in the size of the upper beak and to a much smaller skeletal element in the beak.

Thus variation in *Bmp4* regulation appears to be one of the principal molecular factors that provided the morphological variation acted on by natural selection in the evolution of the beaks of the Darwin's Finch species. This is an exciting beginning to understanding the molecular genetic basis of beak size variation. There are many more interacting genes to be discovered. A clear need for the future is to answer the question of how *Bmp4* is regulated differently in the different species.

Adaptive evolution when the environment changes

Inferences about evolution in the past are much more plausible if supported by a demonstration of evolution in the present. It is possible to do this owing to strongly fluctuating environmental conditions: climatically the Galápagos environment oscillates greatly and more or less regularly (Fig. 2).

We have documented evolutionary change on the small island of Daphne where the rainfall data in Figure 1 were obtained. This pristine island is about three-quarters of a kilometer long, 120 meters high, and is situated in the center of the archipelago. Humans have neither settled on the island nor introduced alien organisms. We began a study of finches there in 1973. In 1977 we were fortunate to witness a severe drought. Not so fortunate for the finches, 85 % of the medium ground finch population died! Those that survived had particularly large beaks. As a result of this dif-

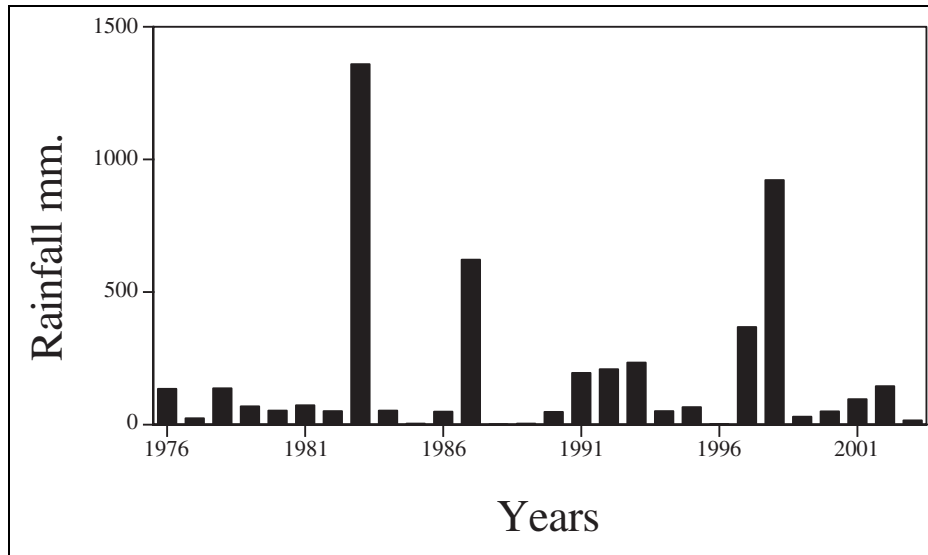


Figure 2
Annual rainfall on Daphne Major Island.

ferential mortality average beak size as well as body size increased, and continued doing so until the rains resumed at the beginning of 1978. Natural selection had occurred.

Let's recall Darwin's three requirements for evolution: traits must vary, be inherited, and be subject to natural selection. Figure 3 helps to visualize these three components.

The upper panel demonstrates natural selection. It is the difference in average beak depth between the survivors in black bars and the total population before the drought. Inheritance of beak characteristics is reflected in the similarity between parents and their offspring. Evolution takes place from one generation to the next, and this is shown by a comparison of the offspring born in 1978 in the bottom panel with the previous generation at the top before the advent of selection. Birds of the next generation, like their parents who had survived the drought, had large beaks.

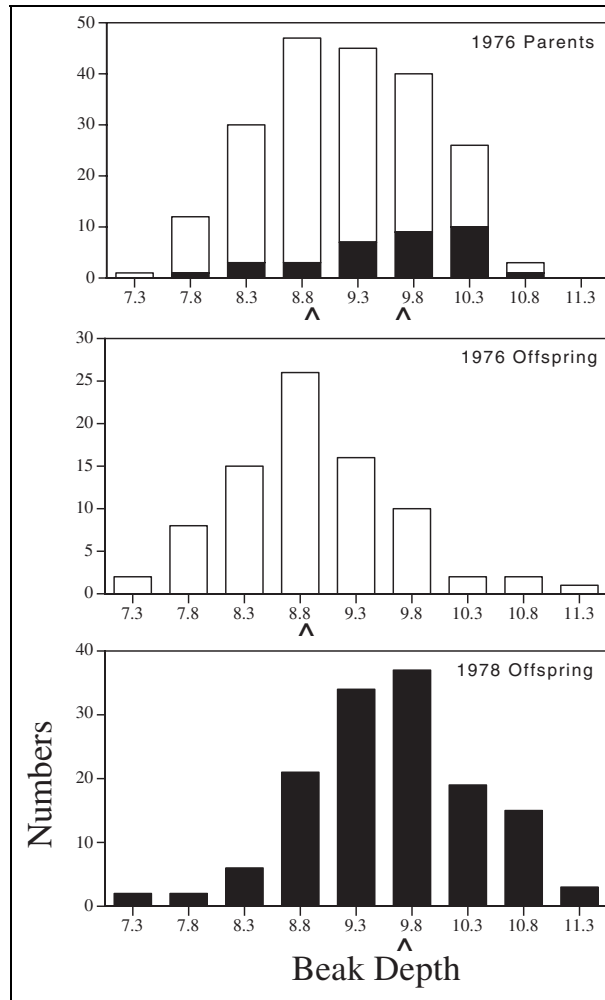


Figure 3

Result of natural selection on beak variation on the population of *Geospiza fortis* on Daphne Major Island. In the upper panel, survivors of a drought in 1977 are shown as solid bars, those which did not survive are shown by open bars, and their mean beak depths are indicated by carats (^) below the axis. The 1976 offspring did not survive (middle panel). The 1978 offspring, below, resembled their parents, above, in beak depth. From Grant & Grant (2003).

Thus evolution had occurred as a result of natural selection on the inherited trait, beak size. This was not a unique event. Evolutionary change occurred several times later as a result of changes in rainfall and vegetation (Fig. 4).



Figure 4
Vegetation on the 'plateau' of Daphne Major island in the dry season (upper), the wet season of a normal year (middle) and an El Niño year of abundant rain (lower). Photos by the authors.
From Grant et al. (2000).

The most dramatic change took place in 1983, the year of an exceptional El Niño event that has been described as the most severe event in 400 years on the basis of coral core records. It affected *Tribulus*, the plant that was so crucial to the survival of large-beaked finches in the drought of 1977 because it produces large and hard seeds protected by woody tissue that only birds with large and deep beaks can crack or tear open. These plants were smothered in the extensive growth in the El Niño year of 1983. As the rains continued for 8 months rampant growth of vines covered everything, including cactus bushes, and even in the following years the effects of El Niño could easily be seen. The important effect was this. As a result of the smothering of *Tribulus* plants and cactus bushes, and prolific growth of small seed-bearing plants, the island was converted from a predominantly large-seed environment to a small-seed environment. Under these altered conditions now small beaked birds had a selective advantage over the large beaked birds. The direction of evolution had been reversed.

These two events were not the only ones. We have been fortunate to witness several El Niño events on Daphne, and several droughts (Fig. 2). Natural selection has occurred repeatedly (Fig. 5), on both the medium ground finch and the cactus finch.

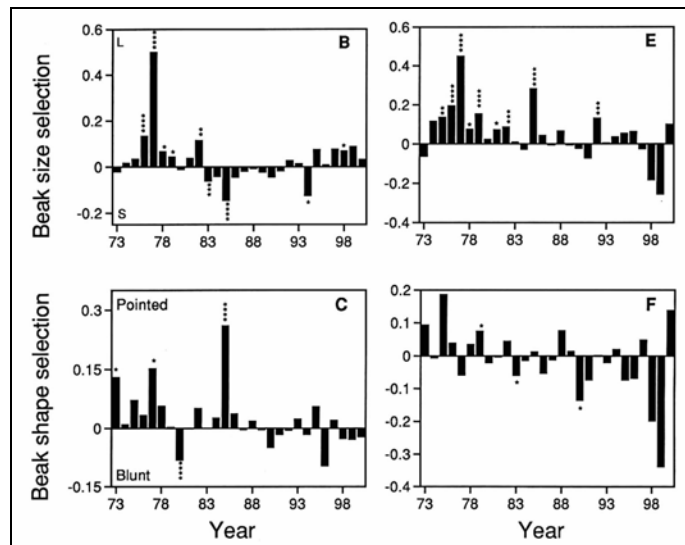


Figure 5

Repeated natural selection on Daphne Major Island: *Geospiza fortis* (left two panels) and *G. scandens* (right two panels). Selection coefficients that are significantly different from zero are indicated by one ($P < 0.05$), two ($P < 0.01$) or three ($P < 0.001$) asterisks. L and S stand for large and small respectively. Panels A and D have been omitted. Modified from Grant & Grant (2002b).

It has affected body size, beak size and beak shape. The magnitude and direction of the selection coefficients are shown by the size of the bars, positive or negative, above or below a zero line that runs from 1973 to the present. They oscillate in direction. Frequent selection implies frequent evolutionary change, and that in fact is what has happened. Remarkably the finches are not the same now as they were when we began the study. With one exception their morphological trajectories have taken them out of the 95 % confidence limits to the estimates of mean morphology in 1973, the first year of adequate sampling. For example, *G. fortis* now have smaller and more pointed beaks than they did in 1973.

Summarizing to this point, the 30 years of research on the island of Daphne Major has demonstrated four important points: that evolution by natural selection is an observable, measurable and interpretable process in a natural environment. It oscillates in direction. It occurs when the environment changes and it has evolutionary consequences.

Coexistence in sympatry

Speciation is completed when members of two diverged populations coexist without interbreeding in sympatry. To coexist they must share the environment without intensely competing for resources. Sympatric species feed in different ways, or they feed on different types of foods, according to their particular beak sizes and shapes. They probably do compete for foods when the food supply is limiting in dry years because their diets overlap, but evidently not so severely that extinction is the result, at least not according to our observations.

How do they coexist without interbreeding? This is a more challenging question to answer because groups of related species such as the ground finches are very similar in general appearance (plumage) and courtship behaviour. Our studies on Daphne have focused on *G. fortis* and *G. scandens*. Although closely related, having shared a common ancestor in the last million years, they are not sister species. They differ in two important respects, in song and in adult beak morphology. *G. scandens*, a ~ 22 gram bird, has a longer and more pointed beak than *G. fortis*, an ~ 18 gram bird (Fig. 6).

Furthermore they hold overlapping territories, which is an indication that they perceive each other as distinct species. Experiments using mounted museum specimens and song playback have confirmed that finches can discriminate between conspecific and heterospecific individuals on the basis of morphology alone, and they can also do so on the basis of song in the absence of morphological cues. Thus the two factors, morphology and song, can act alone or together as barriers to interbreeding. However, as demonstrated below, song takes precedence over morphological cues in the discrimination between *G. fortis* and *G. scandens*.



Figure 6
Geospiza fortis (above) and *G. scandens* (below). Photos by the authors.

Song as a cultural barrier between the species

Many years ago Robert Bowman showed with song playback to captive young finches that song is learned in an imprinting-like manner during a short sensitive period from day 10 after hatching to approximately day 30. This time corresponds to the last two to three days in the nest and the period of dependency on their parents as fledglings. Both parents feed the young, especially the father, who repeatedly sings. Young finches learn their species-specific song through interacting with their fathers: mothers do not sing. In about 80 % of the cases in both species sons sing a perfect rendering of their father's song (Fig. 7).

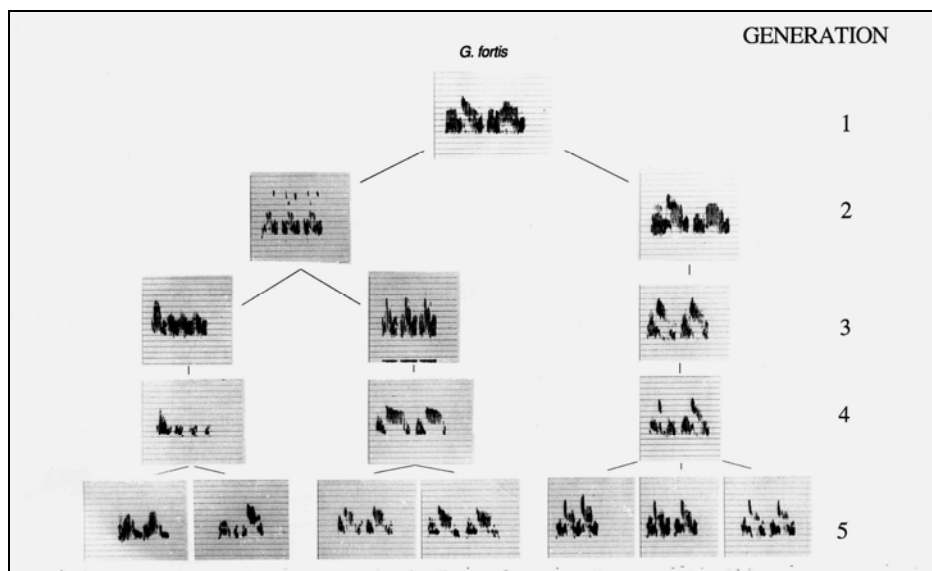


Figure 7

Inheritance of songs of *G. fortis* on Daphne Major Island. From Grant & Grant (1996).

The remaining 20 % learn the species-specific specific song but copy slightly different variants sung by neighbours or others. Furthermore repeated recordings of the same individuals over 10 or more years demonstrate that once the song is learned it remains unaltered throughout life. Daughters, like sons, learn their father's song in an imprinting-like manner and when adult they use it in selecting a mate.

The barrier leaks

An imprinting-like mechanism being learned is vulnerable to perturbation if the young bird hears a heterotypic song rather than a conspecific song during the short sensitive period of imprinting. It then misimprints on heterospecific song. This occurs rarely, in less than one percent of young, and under a variety of circumstances: for example, following the death of the father; or as a consequence of an egg being left in the nest after the nest was dispossessed by the male of another species; or when a loud male of one species repeatedly drives away the male of another species from its unusually close nest, and persistently sings.

Misimprinted birds sometimes, but not always, mate with a member of the species on which they have imprinted: they hybridize. We recorded the songs of both the father and the mate of 482 female finches on Daphne Major, and of these 16 hybridized,

all mating according to song type and not according to morphology. In 12 cases females mated with misimprinted males, and in the remaining four cases females were the offspring of a misimprinted father. Thus misimprinting on song can lead to hybridization.

Hybrid fitness

This raises the question of the fitness of hybrids compared with the parental species. From 1976 to 1982 none of the hybrids survived long enough to breed. There were two possible explanations for this: either hybrids were genetically incompatible, or the sizes of seeds available were not suited to birds of intermediate beak size (the hybrids) to survive the dry season. There is evidence for the second explanation. The large and hard *Tribulus* seed on which *G. fortis* were surviving were too large for hybrids to crack, even though they were observed trying to do so. Moreover although hybrids could exploit *Opuntia* seeds, the main dry season food of *G. scandens*, they did so significantly less efficiently than *G. scandens*.

After the 1983 El Nino event ecological conditions changed profoundly, and the seed bank became dominated by small and soft seeds from 22 species of plants. Under these conditions survival of hybrids with their intermediate bill sizes and shapes was high. A comparison of pure species, hybrids and backcrosses in the 1983, 1987 and 1991 cohorts, chosen for the years of maximum fledgling production, revealed that hybrids and backcrosses survived as well as, if not slightly better than, the pure species hatched at the same time and experiencing the same environmental conditions (Fig. 8).

Furthermore there was no significant difference between pure species, their hybrids and backcrosses in egg production, number of offspring hatched and those fledged. Thus in neither survival or reproductive output were hybrids and backcrosses at a fitness disadvantage. This demonstrates that *G. fortis* and *G. scandens* are genetically compatible.

Occurrence and significance of introgression

High survival of hybrids and backcrosses from 1983 to 2004 and frequent breeding resulted in introgression. F₁ hybrids were too rare to breed with each other, instead they backcrossed to one species or the other according to the type of song they learned from their fathers. Introgressive gene flow occurred in both directions, although from 1990 onwards it was three times greater from *G. fortis* to *G. scandens* than vice versa. By 2003 approximately 30 % of *G. scandens* individuals contained some *G. fortis* genes, and the two species had become more similar to each other both genetically and morphologically.

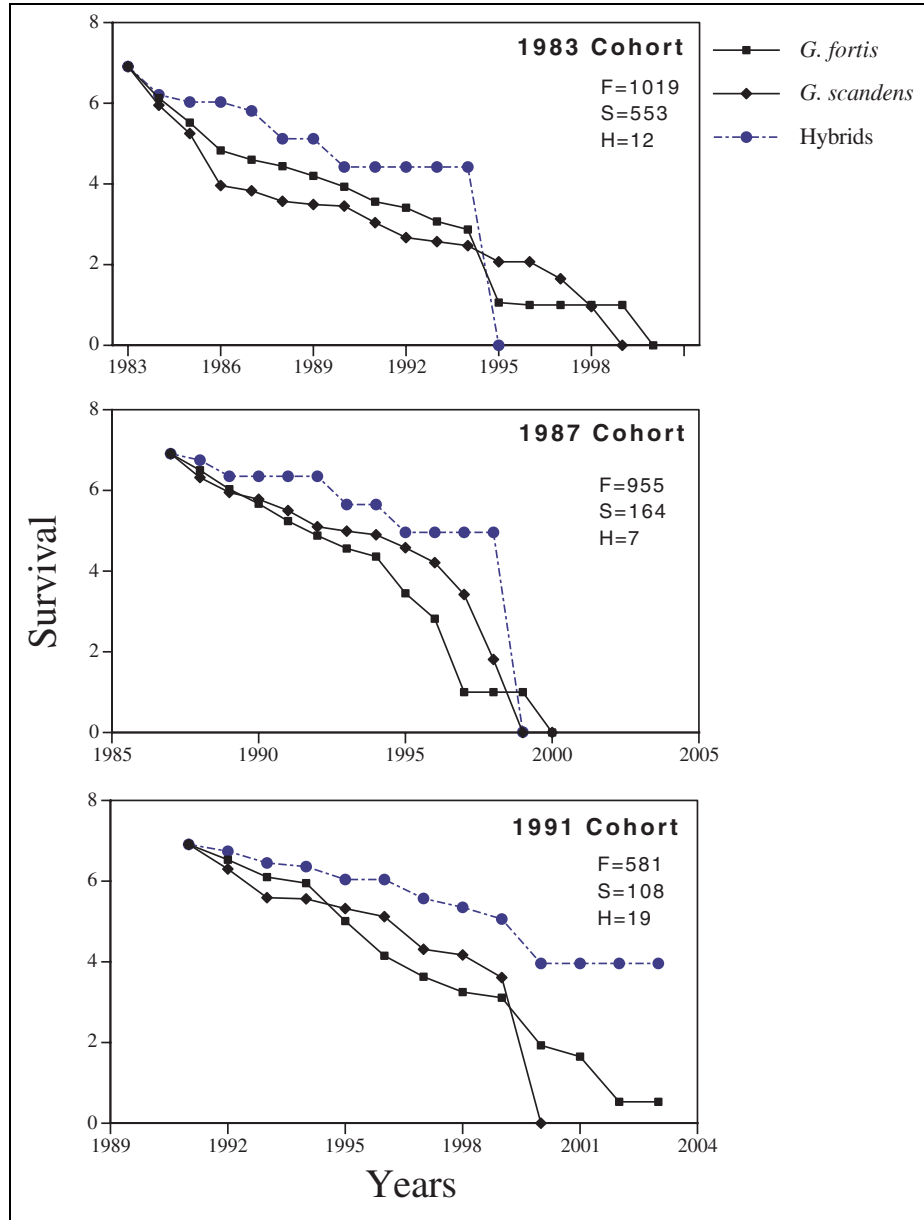


Figure 8
Survival of hybrids (including backcrosses) in three cohorts on Daphne Major Island. Survival is on a natural log scale, with initial numbers scaled to 1000.

These unexpected findings have two interesting implications in the context of speciation and the maintenance of genetic variation. First, to the extent that they can be generalized they imply a dynamic tension between ecologically differentiated species that are derived from a common ancestor and differ reproductively only in how they choose mates. The process of speciation is often viewed as a steady increase in differences between populations leading eventually to the complete cessation of interbreeding, both actual and potential. Our results show the process can be put into reverse under particular environmental conditions. Complete fusion of the previously reproductively separate populations might be one outcome, but if the environment changes again divergence might once again resume, though perhaps along slightly different pathways. An oscillation between divergence and convergence might characterize the process of speciation better than uniform divergence or acceleration in divergence just after the time that sympatry is established.

Second, introgressive hybridization throws light on the question of how genetic variation is maintained. Evolution by natural selection requires genetic variation, and if as we have seen selection oscillates in direction we might expect genetic variation to be gradually eroded over time. The enigma of how it is maintained in a fluctuating environment is at least partly answered by introgression, because this has the power to replenish the supply of alleles in a population. It might play a creative role too if a genetically augmented population can more easily respond to selection and evolve along a novel trajectory than would be the case in the absence of introgression. We suggest that in the past episodic introgression could have played a role in the adaptive radiation of the finches by maintaining variation in small populations subjected to climatic perturbations. The greatest evolutionary effect of introgression may have occurred after some genetic difference had arisen between species, but before the point at which interbreeding incurred a fitness cost.

The main lesson we draw from these facts and observations is that species are not evolutionarily independent of each other in adaptive radiations.

Summary

In summary, Darwin's finches provide a model of speciation and adaptive radiation in an isolated archipelago. Mitochondrial DNA data suggest the radiation took place during the last 2–3 million years. Three million years ago the archipelago consisted of perhaps only five islands. As more islands were added through volcanic activity, species of finches diversified, resulting in a total of fourteen species derived from one, each adapted to different ecological niches. Paleoclimatic data suggest that environmental change was an important factor in this radiation. This is reinforced by a detailed study of uniquely ringed finches on the uninhabited island of Isla Daphne Major over the last 32 years which has demonstrated that finch populations track environmental change through evolution by natural selection, especially in beak size

and shape. Implicated in the evolutionary diversification of the finch species is an important signaling molecule, *Bmp4*, which is now known to be involved in beak development. Species rarely interbreed because they differ in their songs, which are learned early in life by an imprinting-like mechanism. Very rarely a finch misimprints on the song of another species, and this leads to hybridization and introgression. The importance of introgressive hybridization lies in the enhancement of a population's genetic variation on which selection acts. Introgression has possibly contributed significantly to the radiation of the finches. A theme of our talk is that neither environments nor species are static entities: they are dynamic and constantly changing through the interplay of changing ecological conditions, genetics, and learned, culturally transmitted, traits. To conserve both species and their environments we must keep them capable of further change.

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