

Chapter 1

Introduction: The Bloodless Fish of Bouvet Island

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When we no longer look at an organic being as a savage looks at a ship, as at something wholly beyond his comprehension; when we regard every production of nature as one which has had a history; when we contemplate every complex structure and instinct as the summing up of many contrivances, each useful to the possessor, nearly in the same way as when we look at any great mechanical invention as the summing up of the labor, the experience, the reason, and even the blunders of numerous workmen; when we thus view each organic being, how far more interesting, I speak from experience, will the study of natural history become!

—Charles Darwin,
On the Origin of Species (1859)

IT MAY BE THE MOST REMOTE PLACE ON EARTH.

Tiny Bouvet Island is a lone speck in the vast South Atlantic, some 1600 miles southwest of the Cape of Good Hope (Africa) and almost 3000 miles east of Cape Horn



Bouvet Island. Photographed by Ditlef Rustad, Norwegian expedition, 1928.

Bouvet Island, as seen and photographed by Ditlef Rustad on the 1928 Norwegian expedition. *Photograph from Scientific Results of the Norwegian Antarctic Expeditions, 1927-28, published by I. Konnusion. Hos Jacob Dybwad of Oslo, 1935.*

Ruud returned to Norway the following year and mentioned the tale to Rustad. Much to his surprise Rustad told Ruud, "I have seen such a fish," and showed him the photographs he had taken on his expedition.

Ruud heard nothing more about the bloodless fish for twenty years. Then, another Norwegian biologist returned from an Antarctic expedition with white-blooded fish from a different location. His curiosity reawakened, Ruud began to ask other colleagues voyaging to the Antarctic to be on the lookout for what the whalers called "devilfish" or, because of their near transparency, "icefish." Finally, Ruud returned to the Antarctic himself in 1953, almost twenty-five years after his first journey, with the hope of catching and studying these fish and resolving the mystery of their blood.

He set up a makeshift laboratory on South Georgia Island (the island to which explorer Ernest Shackleton rowed in 1916 in order to save the stranded crew of the *Endurance*). He promptly received a few precious specimens and carefully analyzed their odd blood. His findings, reported in 1954, are still a shock for any biologist reading them for the first time. The fish completely lacked red blood cells, the pigmented oxygen-carrying cells that, until the discovery of these Antarctic icefish, had been found in every living vertebrate. Indeed, no other case of bloodless vertebrate has ever been discovered outside of the fifteen or so species of icefish now known.

Red blood cells contain large amounts of the hemoglobin molecule, which binds oxygen as blood cells circulate through the lungs or gills, and then releases it as red cells circulate through the rest of the body. The hemoglobin molecule is made up of a protein called globin and a small molecule called heme. The red color of blood is due to the heme that is buried in the hemoglobin molecule and actually binds the oxygen. We would, and do, die without red cells (anemias are conditions of low red cell numbers). Even close relatives of the icefish, such as Antarctic rock cod and New Zealand black cod, are red-blooded.

The existence of these remarkable fish provokes many questions. Where, when, and how did they evolve? What happened to their hemoglobin? How can the fish survive without it or red blood cells?

The typical place one would begin to explore the origin of a species would be the fossil record. However, that is completely lacking for these fish and their relatives. And, even if we had fossils, we would not be able to tell, from the remnants of their bones, what color their blood was and when it changed. But, there is a record of the history of icefish that we can access—in their DNA.

The clear, stunning answer to the question of what happened to their hemoglobin came from the study of icefish DNA more than forty years after Ruud first sampled their blood. In these amazing fish, the two genes that normally contain the DNA code for the globin part of the hemoglobin molecule have gone extinct. One gene is a molecular fossil, a mere remnant of a globin gene—it still resides in the DNA of the icefish, but it is utterly useless and eroding away, just as a fossil withers upon exposure to the elements. The second globin gene, which usually lies adjacent to the first in the DNA of red-blooded fish, has eroded away completely. This is absolute proof that the icefish have abandoned, forever, the genes for the making of a molecule that nurtured the lives of their ancestors for over 500 million years.

What would provoke such a dramatic rejection of a way of life that serves every other vertebrate on the planet?

Necessity and *opportunity*, both of which sprang from dramatic, long-term changes in ocean temperature and currents.

Over the past 55 million years, the temperature of the Southern Ocean has dropped, from about 68 degrees F to less than 30 degrees in some locales. About 33 to 34 million years ago, in the continual movement of the Earth's tectonic plates, Antarctica was severed from the southern tip of South America, and became completely surrounded by ocean. Ensuing changes in ocean currents isolated the waters around the Antarctic. This limited the migration of fish populations such that they either adapted to the change, or went extinct (the fate of most). While others vanished, one group of fish exploited the changing ecosystem. The icefish are a small family of species, within the larger suborder Notothenioidae, that altogether contains about 200 species and now dominates the Antarctic fishery.

types of amino acids. Since warm-water fish have nothing of this sort, the antifreeze genes were somehow invented by Antarctic fish. Where in the world did antifreeze come from?

Chi-Hing Cheng, Arthur DeVries, and colleagues at the University of Illinois discovered that the antifreeze genes arose from part of another, entirely unrelated gene. The original gene encoded a digestive enzyme. A little piece of its code broke off and relocated to a new place in the fish genome. From this simple nine-letter piece of DNA code, a new stretch of code evolved for making the antifreeze protein. The origin of the antifreeze proteins stands out as a prime example of how evolution works more often by tinkering with materials that are available—in this case a little piece of another gene's code—rather than by designing new things completely from scratch.

As a resident of a cold climate, I have to admire the icefishes' grit and ingenuity. We take various measures to keep our cars running on subzero Wisconsin days, but the icefish has managed to change its whole engine *while the car was running*. It invented a new antifreeze, changed its oil (blood) to a new grade with a remarkably low viscosity, enlarged its fuel pump (heart), and threw out a few parts along the way—parts that had been used in every model of fish for the past 500 million years.

The DNA record of icefish, and of all other species, is a whole new level of evidence of the evolutionary process. It allows us to see beyond the visible bones and blood, directly into the fundamental text of evolution. The making of the extraordinary icefish illustrates the ordinary, if somewhat messy, course of the making of the fittest at the DNA level. Icefish evolved from warm-water, red-blooded ancestors ill suited to life in the cold. Their adaptation to the changing environment of the Southern Ocean was not a matter of instant design, nor just a one-way "progressive" process. It was an improvised series of many steps, including the invention of some new code, the destruction of some very old code, and the modification of much more.

By comparing the states of genes in different icefish, their closest red-blooded relatives, and other Antarctic fish, we can see that certain

changes occurred at different stages in icefish evolution. All 200 or so Antarctic notothenioid species have antifreeze genes, so that was an early invention. So, too, were the modifications of microtubule genes. But only the fifteen or so icefish species have fossil hemoglobin genes. This means that the hemoglobin genes must have been abandoned by the time the first icefish evolved. Furthermore, while some icefish can't and don't make myoglobin, others do. This reveals that the changes in the myoglobin genes are more recent than the origin of icefish, and that the use (or disuse) of myoglobin is still evolving. By examining other DNA sequences from each species, it is possible to map these events onto the timeline of the geology of the South Atlantic—with the origin of the Antarctic Nototheniidae occurring about 25 million years ago, and the origin of the icefish only about 8 million years ago (figure 1.3). The DNA record tells us that the icefish crossed the divide between a warm-water, hemoglobin-dependent lifestyle, and a very-cold-water, hemoglobin- (and for some myoglobin-) independent lifestyle in many steps, not in a leap.

The DNA record of the many modifications accumulated by the icefish in the long course of its descent from red-blooded, warm-water ancestors vividly demonstrates the two key principles of evolution—natural selection, and descent with modification—first articulated by another zoology student, Charles Darwin, who journeyed around the South Atlantic a century before Rustad and Ruud. In order to fully appreciate the power of this new DNA record I am going to describe throughout this book, and its place in the larger picture of the evolutionary process, it is important to refamiliarize ourselves with these two principles and their initial statement in *On the Origin of Species*.

Darwin Redux

Darwin boarded the HMS *Beagle* in December 1831, at age twenty-two, for what would eventually become a five-year voyage that circumnavigated the globe. The bulk of the voyage was spent in and around

Malay Archipelago). Darwin brought *evidence*. Mounds and mounds of observations, fact upon fact, ingenious experiments, clever analogies, and twenty years of finely crafted argument.

The esteem we biologists have for Darwin is manifold. Sure, *On the Origin of Species* is the most important single work in biology. Darwin's "long argument" is brilliantly constructed, supported by a dazzling breadth of facts, and the product of a heroic individual effort. It is also very readable today, with its passion still resonant. But the full body of his contributions filled many books, from insights into the building of coral reefs, to the importance of sexual selection, and the biology of orchids, barnacles, and much more. It just dwarfs what the merely talented or industrious might achieve.

So why have his great ideas endured such a struggle?

Seeing the Steps

Darwin understood all too well, and therefore correctly anticipated, most of the objections that could or would be raised against his ideas. Many of the attacks, of course, were from those who found Darwin's view of life's history repulsive and demeaning on nonscientific grounds. Most scientists fairly readily accepted the reality of evolution, that is, that species did change. But even Darwin's supporters had difficulties with the *how*—with the mechanism he proposed.

Questioning the *how* was quite understandable. I believe that most people, scientists or laypeople, initially struggle to get their heads around Darwin's picture of natural selection, or what also became known as "the survival of the fittest." (An interesting note: Darwin did not coin that famous phrase, the philosopher Herbert Spencer did. It did not appear in *On the Origin of Species* until the fifth edition of 1869, at Wallace's suggestion.) Darwin's process of evolution involved three key components—variation, selection, and time. Each of these presented some conceptual or evidential problems, and all were potential sources of incredulity. Darwin was asking his readers, in essence,

tions? If such do occur, can we doubt (remembering that many more individuals are born than can possibly survive) that individuals having any advantage, however slight, over others, would have the best chance of surviving and of procreating their kind? On the other hand, we may feel sure that any variation in the least degree injurious would be rigidly destroyed. *This preservation of favorable variations and the rejection of injurious variations I call Natural Selection* [emphasis added].

—Ch. IV, *On the Origin of Species*

Darwin then leaped to the bold conclusion that this process would connect all life's forms via their descent from common ancestors:

Several classes of facts . . . seem to me to proclaim so plainly, that the innumerable species, genera, and families of organic beings, with which this world is peopled, have all descended, each within its own class or group, from common parents, and have all been modified in the course of descent.

—Ch. XIII, *On the Origin of Species*

And then, even bolder:

Therefore I should infer from analogy that probably all the organic beings which have ever lived on this earth have descended from one primordial form, into which life was first breathed.

—Ch. XIII, *On the Origin of Species*

This is the essence of Darwinian evolution—that natural selection for incremental variation forged the great diversity of life from its beginning as a simple ancestor. Simple logic, scientific immortality. No wonder Huxley was chiding himself.

But there was much more in *On the Origin of Species* than these few conclusions (some of which Alfred Russel Wallace had independently reached as a result of his studies in South America and the

meal, I want to take some care in explaining the main ingredients of evolution—variation, selection, and time—so that we fully appreciate how they interact in the making of the fittest.

The late Nobel laureate Sir Peter Medawar once remarked that “the reasons that have led professionals without exception to accept the hypothesis of evolution are in the main too subtle to be grasped by laymen.”

I don't believe that this is true. However, if it is at all true, this is a failure on the part of scientists to clearly explain the power of natural selection, compounded by time, to make all things great and small—from whales to bloodless icfish.

To redress this shortcoming, I will explain the everyday math of evolution (chapter 2). This is the best way to get a good feel for the power of natural selection and to vanquish some of the misleading arguments against the probability of events in evolution. This simple math is generally not explained in popular accounts of evolution. It is important, however, for grasping not just the plausibility of natural selection, but also the real-world interplay of chance, time, and selection. I know, you are saying, “Math?! Forget it.” Don't worry. At the very minimum, that chapter might help you become a better gambler or investor.

The main body of the book will be a six-course meal, served in six chapters. The focus of each chapter will be on how the new DNA record reveals a particular aspect of evolution, with new kinds of evidence that neither Darwin nor his mathematically gifted disciples could have dreamed of.

I will begin by illustrating how the DNA record documents the processes of natural selection and descent with modification on a vast geological timescale. I will show unimpeachable evidence of how natural selection acts to remove, in Darwin's words, injurious change (chapter 3). This evidence is manifest in the form of genes that have been preserved across kingdoms of life for two *billion* years or longer. The text of these “immortal” genes is stuck “running in place” under the conservative surveillance of natural selection. Immortal genes are

that pioneer paves the way for much faster analysis of its relatives. By comparing genes and genomes between relatives of different ranks, we can pinpoint important changes and spot the mark of natural selection. The view can be as humbling as it is exciting. We can peer back a few million years to track the changes that took place in the evolution of the line that led to us from our common ancestor with the chimpanzee, our closest relative on the planet. We can look back 100 million years or so to see what gave rise to the differences between marsupial and placental mammals. We can even glimpse before the dawn of animals and find hundreds of genes in simple, single-celled organisms that evolved more than two billion years ago and still carry out the same jobs in our bodies today.

The ability to see into the machinery of evolution transforms how we look at the process. For more than a century, we were largely restricted to looking only at the outside of evolution. We observed external change in the fossil record and assessed differences in anatomy. But before this new molecular age there was no way to make genetic comparisons between species. We could study the reproduction and survival of organisms and infer the forces at work. However, we had no concrete knowledge of the mechanism of variation or the identity of the meaningful differences between species. Yes, we understood that the outcome was the survival of the fittest, but we did not know *how the fittest are made*. Just as for any work of human creation, we so much better understand how complex things have come to be—cars, computers, spacecraft—when we understand how they are made, and how each new model is different from its predecessors. We are no longer savages staring at passing ships.

The focus of this book will be to peer into the DNA record to see how evolution works. Along the way we will explore how some of the most interesting and important capabilities of some fascinating creatures arose. The book is organized into three main parts. I would like to think of them as being like the three parts of a good and memorable meal—a little bit of prepatation, plenty of food, and some meaningful conversation. First, in order to prepare for the