'Beyond fascinating. An amazing book'

HELEN MACDONALD, AUTHOR OF *H IS FOR HAWK*

CONTAIN MULTITUDES

THE MICROBES WITHIN US AND A GRANDER VIEW OF LIFE

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PROLOGUE: A TRIP TO THE ZOO

Baba does not flinch. He is unfazed by the throng of excited kids who have gathered around him. He is unperturbed by the Californian summer heat. He does not mind the cotton swabs that brush his face, body and paws. His nonchalance makes sense, for his life is safe and cushy. He lives in San Diego Zoo, wears an impregnable suit of armour, and is currently curled around the waist of a zookeeper. Baba is a white-bellied pangolin – an utterly endearing animal that looks like a cross between an anteater and a pine cone. He's about the size of a small cat. His black eyes have a doleful air, and the hair that frames his cheeks looks like unruly mutton chops. His pink face ends in a tapering toothless snout that's well adapted for slurping up ants and termites. His stocky front legs are tipped with long, curved claws for clinging to tree trunks and tearing into insect nests, and he has a long tail for hanging off tree branches (or friendly zookeepers).

But his most distinctive features, by far, are his scales. His head, body, limbs and tail are covered in them – pale orange, overlapping plates that create an extremely tough defensive coat. They are made of the same material as your nails – keratin. Indeed, they look and feel a lot like fingernails, albeit large, varnished, and badly chewed ones. Each one is flexibly but firmly attached to his body, so they sink down and spring back as I run my hand down his back. If I stroked him in the opposite direction, I'd probably cut myself – many of the scales are sharp-edged. Only Baba's face, belly and paws are unprotected, and if

he chose to, he could easily defend them by rolling up into a ball. It's this ability that gives his kind their name: pangolin comes from the Malay word *pengguling*, meaning 'something that rolls up'.

Baba is one of the zoo's ambassador animals – exceptionally docile and well-trained individuals who take part in public activities. Keepers frequently take him to nursing homes and children's hospitals to brighten up the days of sick people, and to teach them about unusual animals. But today, he gets the day off. He just sits around the keeper's midriff, like the world's strangest cummerbund, while Rob Knight gently dabs a cotton swab against the side of his face. 'This is one of the species that I've been captivated by since I was a kid – just that something like that exists,' he says.

Knight, a tall, lanky New Zealander with buzzcut hair, is a scholar of microscopic life, a connoisseur of the invisible. He studies bacteria and other microscopic organisms – microbes – and he is specifically enthralled by those that live in or on the bodies of animals. To study them, he must first collect them. Butterfly collectors use nets and jars; Knight's tool of choice is the cotton swab. He reaches over with a small bud and rolls it over Baba's nose for a couple of seconds, long enough to infuse the end with pangolin bacteria. Thousands, if not millions, of microscopic cells are now entangled in the white fuzz. Knight moves delicately so as not to perturb the pangolin. Baba couldn't look less perturbed if he tried. I get the feeling that if a bomb went off next to him his only reaction would be to fidget slightly.

Baba is not just a pangolin. He is also a teeming mass of microbes. Some of them live inside him, mostly in his gut. Others live on the surface, on his face, belly, paws, claws, and scales. Knight swabs each of these places in turn. He has swabbed his own body parts on more than one occasion, for he too hosts his own community of microbes. So do I. So does every beast in the zoo. So does every creature on the planet, except for a few lab animals that scientists have deliberately bred to be sterile.

All of us have an abundant microscopic menagerie, collectively known as the *microbiota* or *microbiome*.[1] They live on our surface,

inside our bodies, and sometimes inside our very cells. The vast majority of them are bacteria, but there are also other tiny organisms including fungi (such as yeasts) and archaea, a mysterious group that we will meet again later. There are viruses too, in unfathomable numbers – a *virome* that infects all the other microbes and occasionally the host's cells. We can't see any of these minuscule specks. But if our own cells were to mysteriously disappear, they would perhaps be detectable as a ghostly microbial shimmer, outlining a now-vanished animal core.[2]

In some cases, the missing cells would barely be noticeable. Sponges are among the simplest of animals, with static bodies never more than a few cells thick, and they are also home to a thriving microbiome.[3] Sometimes, if you look at a sponge under a microscope, you will barely be able to see the animal for the microbes that cover it. The even simpler placozoans are little more than oozing mats of cells; they look like amoebae but they are animals like us, and they also have microbial partners. Ants live in colonies that can number in their millions, but every single ant is a colony unto itself. A polar bear, trundling solo through the Arctic, with nothing but ice in all directions, is completely surrounded. Bar-headed geese carry microbes over the Himalayas, while elephant seals take them into the deepest oceans. When Neil Armstrong and Buzz Aldrin set foot on the Moon, they were also taking giant steps for microbe-kind.

When Orson Welles said 'We're born alone, we live alone, we die alone', he was mistaken. Even when we are alone, we are never alone. We exist in symbiosis – a wonderful term that refers to different organisms living together. Some animals are colonised by microbes while they are still unfertilised eggs; others pick up their first partners at the moment of birth. We then proceed through our lives in their presence. When we eat, so do they. When we travel, they come along. When we die, they consume us. Every one of us is a zoo in our own right – a colony enclosed within a single body. A multi-species collective. An entire world.

These concepts can be hard to grasp, not least because we humans are a global species. Our reach is boundless. We have expanded into

every corner of our blue marble, and some of us have even left it. It can be weird to consider existences that play out in an intestine or in a single cell, or to think about our body parts as rolling landscapes. And yet, they assuredly are. The Earth contains a variety of different ecosystems: rainforests, grasslands, coral reefs, deserts, salt marshes, each with its own particular community of species. But a single animal is full of ecosystems too. Skin, mouth, guts, genitals, any organ that connects with the outside world: each has its own characteristic community of microbes.[4] All of the concepts that ecologists use to describe the continental-scale ecosystems that we see through satellites also apply to ecosystems in our bodies that we peer at with microscopes. We can talk about the diversity of microbial species. We can draw food webs, where different organisms eat and feed each other. We can single out keystone microbes that exert a disproportionate influence on their environment – the equivalents of sea otters or wolves. We can treat disease-causing microbes – pathogens – as invasive creatures, like cane toads or fire ants. We can compare the gut of a person with inflammatory bowel disease to a dying coral reef or a fallow field: a battered ecosystem where the balance of organisms has gone awry.

These similarities mean that when we look at a termite or a sponge or a mouse, we are also looking at ourselves. Their microbes might be different to ours, but the same principles govern our alliances. A squid with luminous bacteria that glow only at night can tell us about the daily ebbs and flows of bacteria in our guts. A coral reef whose microbes are running amok because of pollution or overfishing hints at the turmoil that occurs in our guts when we swallow unhealthy food or antibiotics. A mouse whose behaviour changes under the sway of its gut microbes can show us something about the tendrils of influence that our own companions insinuate into our minds. Through microbes, we find unity with our fellow creatures, despite our incredibly different lives. None of those lives is lived in isolation; they always exist in a microbial context, and involve constant negotiations between species big and small. Microbes move between animals, too, and between

our bodies and the soils, water, air, buildings, and other environments around us. They connect us to each other, and to the world.

All zoology is really ecology. We cannot fully understand the lives of animals without understanding our microbes and our symbioses with them. And we cannot fully appreciate our own microbiome without appreciating how those of our fellow species enrich and influence their lives. We need to zoom out to the entire animal kingdom, while zooming in to see the hidden ecosystems that exist in every creature. When we look at beetles and elephants, sea urchins and earthworms, parents and friends, we see individuals, working their way through life as a bunch of cells in a single body, driven by a single brain, and operating with a single genome. This is a pleasant fiction. In fact, we are legion, each and every one of us. Always a 'we' and never a 'me'. Forget Orson Welles, and heed Walt Whitman: 'I am large, I contain multitudes.'[5]

1. LIVING ISLANDS

The Earth is 4.54 billion years old. A span of time that big is too mind-boggling to comprehend, so let's collapse the planet's entire history into a single calendar year.[1] Right now, as you're reading this page, it is 31 December, just before the stroke of midnight. (Thankfully, fireworks were invented nine seconds ago.) Humans have only existed for 30 minutes or fewer. The dinosaurs ruled the world until the evening of 26 December, when an asteroid hit the planet and wiped them out (except for the birds). Flowers and mammals evolved earlier in December. In November, plants invaded the land and most of the major animal groups appeared in the seas. Plants and animals are all made up of many cells, and similar multicellular organisms had certainly evolved by the start of October. They may have appeared before that – the fossils are ambiguous and open to interpretation – but they would have been rare. Before October, almost every living thing on the planet consisted of single cells. They would have been invisible to the naked eye, had eyes existed. They had been that way ever since life first emerged, some time in March.

Let me stress: all the visible organisms that we're familiar with, everything that springs to mind when we think of 'nature', are latecomers to life's story. They are part of the coda. For most of the tale, microbes were the only living things on Earth. From March to October in our imaginary calendar, they had the sole run of the planet.

During that time, they changed it irrevocably. Bacteria enrich soils and break down pollutants. They drive planetary cycles of carbon, nitrogen, sulphur and phosphorus, by converting these elements into

compounds that can be used by animals and plants and then returning them to the world by decomposing organic bodies. They were the first organisms to make their own food, by harnessing the sun's energy in a process called photosynthesis. They released oxygen as a waste product, pumping out so much of the gas that they permanently changed the atmosphere of our planet. It is thanks to them that we live in an oxygenated world. Even now, the photosynthetic bacteria in the oceans produce the oxygen in half the breaths you take, and they lock away an equal amount of carbon dioxide.[2] It is said that we are now in the Anthropocene: a new geological period characterised by the enormous impact that humans have had on the planet. You could equally argue that we are still living in the Microbiocene: a period that started at the dawn of life itself and will continue to its very end.

Indeed, microbes are everywhere. They live in the water of the deepest oceanic trenches and in the rocks below. They persist in belching hydrothermal vents, boiling springs, and Antarctic ice. They can even be found in clouds, where they act as seeds for rain and snow. They exist in astronomical numbers. Actually, they far exceed astronomical numbers: there are more bacteria in your gut than there are stars in our galaxy.[3]

This is the world in which animals originated, one smothered in and transformed by microbes. As palaeontologist Andrew Knoll once said, 'Animals might be evolution's icing, but bacteria are really the cake.'[4] They have always been part of our ecology. We evolved among them. Also, we evolved *from* them. Animals belong to a group of organisms called *eukaryotes*, which also includes every plant, fungus and alga. Despite our obvious variety, all eukaryotes are built from cells that share the same basic architecture, which distinguishes them from other forms of life. They pack almost all their DNA into a central nucleus, a structure that gives the group its name – 'eukaryote' comes from the Greek for 'true nut'. They have an internal 'skeleton' that provides structural support and shuttles molecules from place to place. And they have mitochondria – bean-shaped power stations that supply cells with energy.

All eukaryotes share these traits because we all evolved from a single ancestor, around two billion years ago. Before that point, life on Earth could be divided into two camps or *domains*: the bacteria, which we already know about, and the archaea, which are less familiar and have a fondness for colonising inhospitable and extreme environments. These two groups both consisted of single cells that lack the sophistication of eukaryotes. They had no internal skeleton. They lacked a nucleus. They had no energy-providing mitochondria, for reasons that will soon become abundantly clear. They also looked superficially similar, which is why scientists originally believed that archaea *were* bacteria. But appearances are deceptive; archaea are as different from bacteria in biochemistry as PCs are from Macs in operating systems.

For roughly the first 2.5 billion years of life on Earth, bacteria and archaea charted largely separate evolutionary courses. Then, on one fateful occasion, a bacterium somehow merged with an archaeon, losing its free-living existence and becoming entrapped forever within its new host. That is how many scientists believe eukaryotes came to be. It's our creation story: two great domains of life merging to create a third, in the greatest symbiosis of all time. The archaeon provided the chassis of the eukaryotic cell while the bacterium eventually transformed into the mitochondria.[5]

All eukaryotes descend from that fateful union. It's why our genomes contain many genes that still have an archaeal character and others that more resemble those of bacteria. It's also why all of us contain mitochondria in our cells. These domesticated bacteria changed everything. By providing an extra source of energy, they allowed eukaryotic cells to get bigger, to accumulate more genes, and to become more complex. This explains what biochemist Nick Lane calls the 'black hole at the heart of biology'. There's a huge void between the simpler cells of bacteria and archaea and the more complex ones of eukaryotes, and life has managed to cross that void exactly once in four billion years. Since then, the countless bacteria and archaea in the world, all evolving at breakneck speed, have never

again managed to produce a eukaryote. How could that possibly be? Other complex structures, from eyes to armour to many-celled bodies, have evolved on many independent occasions but the eukaryotic cell is a one-off innovation. That's because, as Lane and others argue, the merger that created it – the one between an archaeon and a bacterium – was so breathtakingly improbable that it has never been duplicated, or at least never with success. By forging a union, those two microbes defied the odds and enabled the existence of all plants, animals, and anything visible to the naked eye – or anything with eyes, for that matter. They're the reason I exist to write this book and you exist to read it. In our imaginary calendar, their merger happened some time in the middle of July. This book is about what happened afterwards.

After eukaryotic cells evolved, some of them started cooperating and clustering together, giving rise to multicellular creatures, like animals and plants. For the first time, living things became big – so big that they could host huge communities of bacteria and other microbes in their bodies.[6] Counting such microbes is difficult. It's commonly said that the average person contains ten microbial cells for every human one, making us rounding errors in our own bodies. But this 10-to-1 ratio, which shows up in books, magazines, TED talks, and virtually every scientific review on this topic, is a wild guess, based on a back-of-the-envelope calculation that became unfortunately enshrined as fact.[7] The latest estimates suggest that we have around 30 trillion human cells and 39 trillion microbial ones – a roughly even split. Even these numbers are inexact, but that does not really matter: by any reckoning, we contain multitudes.

If we zoomed in on our skin, we would see them: spherical beads, sausage-like rods, and comma-shaped beans, each just a few millionths of a metre across. They are so small that, despite their numbers, they collectively weigh just a few pounds in total. A dozen or more would line up cosily in the width of a human hair. A million could dance on the head of a pin.

Without access to a microscope, most of us will never directly glimpse these miniature organisms. We only notice their consequences, and especially the negative ones. We can feel the painful cramp of an inflamed gut, and hear the sound of an uncontrollable sneeze. We can't see the bacterium *Mycobacterium tuberculosis* with our naked eyes, but we can see the bloody spittle of a tuberculosis patient. *Yersinia pestis*, another bacterium, is similarly invisible to us, but the plague epidemics that it causes are all too obvious. These disease-causing microbes – pathogens – have traumatised humans throughout history, and have left a lingering cultural scar. Most of us still see microbes as germs: unwanted bringers of pestilence that we must avoid at all costs. Newspapers regularly churn out scare stories in which everyday items, from keyboards to mobile phones to doorknobs, turn out to be – gasp! – covered in bacteria. Even more bacteria than on a toilet seat! The implication is that these microbes are contaminants, and their presence a sign of filth, squalor, and imminent disease. This stereotype is grossly unfair. Most microbes are not pathogens. They do not make us sick. There are fewer than 100 species of bacteria that cause infectious diseases in humans;[8] by contrast, the thousands of species in our guts are mostly harmless. At worst, they are passengers or hitchhikers. At best, they are invaluable parts of our bodies: not takers of life but its guardians. They behave like a hidden organ, as important as a stomach or an eye but made of trillions of swarming individual cells rather than a single unified mass.

The microbiome is infinitely more versatile than any of our familiar body parts. Your cells carry between 20,000 and 25,000 genes, but it is estimated that the microbes inside you wield around 500 times more.[9] This genetic wealth, combined with their rapid evolution, makes them virtuosos of biochemistry, able to adapt to any possible challenge. They help to digest our food, releasing otherwise inaccessible nutrients. They produce vitamins and minerals that are missing from our diet. They break down toxins and hazardous chemicals. They protect us from disease by crowding out more dangerous microbes or killing them directly with antimicrobial chemicals. They produce

substances that affect the way we smell. They are such an inevitable presence that we have outsourced surprising aspects of our lives to them. They guide the construction of our bodies, releasing molecules and signals that steer the growth of our organs. They educate our immune system, teaching it to tell friend from foe. They affect the development of the nervous system, and perhaps even influence our behaviour. They contribute to our lives in profound and wide-ranging ways; no corner of our biology is untouched. If we ignore them, we are looking at our lives through a keyhole.

This book will open the door fully. We are going to explore the incredible universe that exists within our bodies. We'll learn about the origins of our alliances with microbes, the counter-intuitive ways in which they sculpt our bodies and shape our everyday lives, and the tricks we use for keeping them in line and ensuring a cordial partnership. We'll look at how we inadvertently disrupt these partnerships and, in doing so, jeopardise our health. We'll see how we might reverse these problems by manipulating the microbiome for our benefit. And we'll hear the stories of the gleeful, imaginative, driven scientists who have dedicated their lives to understanding the microbial world, often in the face of scorn, dismissal, and failure.

We won't focus only on humans, either.[10] We'll see how microbes have bestowed on animals extraordinary powers, evolutionary opportunities, and even their own genes. The hoopoe, a bird with a pickaxe profile and a tiger's colours, paints its eggs with a bacteria-rich fluid that it secretes from a gland beneath its tail; the bacteria release antibiotics that stop more dangerous microbes from infiltrating the eggs and harming the chicks. Leafcutter ants also carry antibiotic-producing microbes on their bodies, and use these to disinfect the fungi that they cultivate in underground gardens. The spiky, expandable pufferfish uses bacteria to make tetrodotoxin – an exceptionally lethal substance which poisons any predator that tries to eat it. The Colorado potato beetle, a major pest, uses bacteria in its saliva to suppress the defences of the plants that it eats. The zebra-striped cardinalfish houses luminous bacteria, which it uses to attract its prey. The ant lion, a predatory

insect with fearsome jaws, paralyses its victims with toxins produced by the bacteria in its saliva. Some nematode worms kill insects by vomiting toxic glowing bacteria into their bodies;[11] others burrow into plant cells, and cause vast agricultural losses, using genes stolen from microbes.

Our alliances with microbes have repeatedly changed the course of animal evolution and transformed the world around us. It is easiest to appreciate how important these partnerships are by considering what would happen if they broke. Imagine if all microbes on the planet suddenly disappeared. On the upside, infectious diseases would be a thing of the past, and many pest insects would be unable to eke out a living. But that's where the good news ends. Grazing mammals, like cows, sheep, antelope, and deer would starve since they are utterly dependent on their gut microbes to break down the tough fibres in the plants they eat. The great herds of Africa's grasslands would vanish. Termites are similarly dependent on the digestive services of microbes, so they would also disappear, as would the larger animals that depend on them for food, or on their mounds for shelter. Aphids, cicadas, and other sap-sucking bugs would perish without bacteria to supplement the nutrients that are missing from their diets. In the deep oceans, many worms, shellfish, and other animals rely on bacteria for all of their energy. Without microbes, they too would die, and the entire food webs of these dark, abyssal worlds would collapse. Shallower oceans would fare little better. Corals, which depend on microscopic algae and a surprisingly diverse collection of bacteria, would become weak and vulnerable. Their mighty reefs would bleach and erode, and all the life they support would suffer.

Humans, oddly, would be fine. Unlike other animals, for whom sterility would mean a quick death, we would get by for weeks, months, even years. Our health might eventually suffer, but we'd have more pressing concerns. Waste would rapidly build up, for microbes are lords of decay. Along with other grazing mammals, our livestock would perish. So would our crop plants; without microbes to provide plants with nitrogen, the Earth would experience a catastrophic

de-greening. (Since this book focuses entirely on animals, I offer my sincerest apologies to enthusiasts of botany.) 'We predict complete societal collapse only within a year or so, linked to catastrophic failure of the food supply chain,' wrote microbiologists Jack Gilbert and Josh Neufeld, after running through this thought experiment.[12] 'Most species on Earth would become extinct, and population sizes would be reduced greatly for the species that endured.'

Microbes matter. We have ignored them. We have feared and hated them. Now, it is time to appreciate them, for our grasp of our own biology is greatly impoverished if we don't. In this book, I want to show you what the animal kingdom really looks like, and how much more wondrous it becomes when you see it as the world of partnerships that it actually is. This is a version of natural history that deepens the more familiar one, the one laid down by the greatest naturalists of the past.

In March 1854, a 31-year-old British man named Alfred Russel Wallace began an epic eight-year trek through the islands of Malaysia and Indonesia.[13] He saw fiery-furred orang-utans, kangaroos that hopped in trees, resplendent birds of paradise, giant birdwing butterflies, the babirusa pig whose tusks grow up through its snout, and a frog that glides from tree to tree on parachute-like feet. Wallace netted, grabbed, and shot the wonders he saw, eventually amassing an astonishing collection of over 125,000 specimens: shells; plants; thousands of insects, pinned in trays; birds and mammals, skinned, stuffed, or preserved in spirits. But unlike many of his contemporaries, Wallace also labelled everything meticulously, noting *where* each specimen was collected.

That was crucial. From these details, Wallace extracted patterns. He noticed a lot of variation in the animals that live in a certain place, even among those of the same species. He saw that some islands were home to unique species. He realised that as he sailed east from Bali to Lombok – a distance of just 22 miles – the animals of Asia suddenly gave way to the very different fauna of Australasia, as if these two islands were separated by an invisible barrier (which would later be called the Wallace Line). For good reason, Wallace is today heralded

as the father of biogeography – the science of where species are, and where they are not. But as David Quammen writes in *The Song of the Dodo*: 'As practiced by thoughtful scientists, biogeography does more than ask *Which species?* and *Where?* It also asks *Why?* And, what is sometimes even more crucial, *Why not?*'[14]

The study of microbiomes begins in exactly this way: cataloguing the ones that are found on different animals, or on different body parts of the same animal. Which species live where? Why? And why not? We need to know their biogeography before we can gain deeper insights into their contributions. Wallace's observations and specimens led him towards *the* defining insight of biology: that species change. '*Every species has come into existence coincident both in space and time with a pre-existing closely allied species,*' he wrote, repeatedly and sometimes in italics.[15] As animals compete, the fittest individuals survive and reproduce, passing their advantageous traits to their offspring. That is, they evolve, by means of natural selection. This was as important an epiphany as science has ever produced, and it all began with a restless curiosity about the world, a desire to explore it, and an aptitude for noticing what lives where.

Wallace was just one of many naturalist explorers who traipsed around the world and catalogued its riches. Charles Darwin endured a five-year, round-the-world voyage aboard the HMS *Beagle*, in which he would discover the fossilised bones of giant ground sloths and armadillos in Argentina, and encounter the giant tortoises, marine iguanas, and diverse mockingbirds of the Galapagos Islands. His experiences and collections planted the intellectual seeds of the same idea that had independently germinated in Wallace's mind – the theory of evolution, which would become inextricably linked with his name. Thomas Henry Huxley, who became known as 'Darwin's bulldog' for his ferocious advocacy of natural selection, sailed to Australia and New Guinea and studied their marine invertebrates. The botanist Joseph Hooker meandered his way to Antarctica, collecting plants along the way. More recently, E. O. Wilson, after studying the ants of Melanesia, wrote the textbook on biogeography.

It is often assumed that these legendary scientists focused entirely on the visible worlds of animals and plants, ignoring the hidden worlds of microbes. That is not entirely true. Darwin certainly collected microbes – he called them 'infusoria' – that blew onto the deck of the *Beagle*, and he corresponded with the leading microbiologists of the day.[16] But there was only so much he could do with the tools available to him.

By contrast, today's scientists can collect samples of microbes, break them apart, extract their DNA, and identify them by sequencing their genes. In this way, they can do exactly what Darwin and Wallace did. They can collect specimens from different locations, identify them, and ask the fundamental question: what lives where? They can do biogeography – just on a different scale. The gentle caress of a cotton bud replaces the swing of a butterfly net. A read-out of genes is like a flick through a field guide. And an afternoon at the zoo, walking from cage to cage, can be like the voyage of the *Beagle*, sailing from island to island.

Darwin, Wallace and their peers were particularly fascinated by islands, and for good reason. Islands are where you go if you want to find life at its most outlandish, gaudy, and superlative. Their isolation, restricted boundaries, and constrained size allow evolution to go to town. The patterns of biology resolve into sharper focus more readily than they would do on the extensive, contiguous mainland. But an island doesn't have to be a land mass surrounded by water. To microbes, every host is effectively an island – a world surrounded by void. My hand, reaching out and stroking Baba at San Diego Zoo, is like a raft, conveying species from a human-shaped island to a pangolin-shaped one. An adult being ravaged by cholera is like Guam being invaded by foreign snakes. No man is an island? Not so: we're all islands from a bacterium's point of view.[17]

Each of us has our own distinctive microbiome, sculpted by the genes we inherited, the places we've lived in, the drugs we've taken, the food we've eaten, the years we've lived, the hands we've shaken. Microbially, we are similar but different. When microbiologists first

started cataloguing the human microbiome in its entirety they hoped to discover a 'core' microbiome: a group of species that everyone shares. It's now debatable if that core exists.[18] Some species are common, but none is everywhere. If there is a core, it exists at the level of *functions*, not organisms. There are certain jobs, like digesting a certain nutrient or carrying out a specific metabolic trick, that are always filled by *some* microbe – just not always the same one. You see the same trend on a bigger scale. In New Zealand, kiwis root through leaf litter in search of worms, doing what a badger might do in England. Tigers and clouded leopards stalk the forests of Sumatra but in cat-free Madagascar that same niche is filled by a giant killer mongoose called the fossa; meanwhile, in Komodo, a huge lizard claims the top predator role. Different islands, different species, same jobs. The islands in question could be huge land masses, or individual people.

In fact, every individual is more like an archipelago – a *chain* of islands. Each of our body parts has its own microbial fauna, just as the various Galapagos islands have their own special tortoises and finches. The human skin microbiome is the domain of *Propionibacterium*, *Corynebacterium*, and *Staphylococcus*, while *Bacteroides* lords over the gut, *Lactobacillus* dominates the vagina, and *Streptococcus* rules the mouth. Every organ is also variable in itself. The microbes that live at the start of the small intestine are very different from those in the rectum. Those in dental plaque vary above and below the gumline. On the skin, microbes in the oily lakes of the face and chest differ from those in the hot and humid jungles of the groin and armpit, or those colonising the dry deserts of the forearms and palms. Speaking of palms, your right hand shares just a sixth of its microbial species with your left hand.[19] The variations that exist between body parts dwarf those that exist between people. Put simply, the bacteria on your forearm are more similar to those on my forearm than to those in your mouth.

The microbiome varies in time as well as space. When each baby is born, it leaves the sterile world of its mother's womb and is immediately colonised by her vaginal microbes; almost three-quarters of

a newborn's strains can be traced directly back to its mother. Then follows an age of expansion. As the baby picks up new species from its parents and environment, its gut microbiome becomes gradually more diverse.[20] The dominant species rise and fall: as the baby's diet changes, milk-digesting specialists like *Bifidobacterium* give way to carbohydrate-eaters like *Bacteroides*. And as the microbes change, so do their antics. They start making different vitamins and they unlock the ability to digest a more adult diet.

This period is turbulent but follows predictable stages. Imagine watching a forest recently scoured by fire, or a fresh island newly risen from the sea. Both would quickly be colonised by simple plants like lichens and mosses. Grasses and small shrubs would follow. Taller trees would arrive later. Ecologists call this *succession*, and it applies to microbes too. It takes anywhere from one to three years for a baby's microbiome to reach an adult state. Then, a lasting stability. The microbiome may vary from day to day, from sunrise to sunset, or even from meal to meal, but such variations are small compared to the early changes. This dynamism of the adult microbiome conceals a background of constancy.[21]

The exact pattern of succession will vary between different animals, because we turn out to be picky hosts. We are not just colonised by whatever microbes happen to land on us. We also have ways of selecting their microbial partners. We'll learn about these tricks, but for now let us simply note that the human microbiome is distinct from the chimpanzee microbiome, which looks different from the gorilla microbiome, just as the forests of Borneo (orang-utans, pygmy elephants, gibbons) are distinct from those in Madagascar (lemurs, fossas, chameleons) or New Guinea (birds of paradise, tree kangaroos, cassowaries). We know this because scientists have swabbed and sequenced their way around the entire animal kingdom. They have described the microbiomes of pandas, wallabies, Komodo dragons, dolphins, lorises, earthworms, leeches, bumblebees, cicadas, tube worms, aphids, polar bears, dugongs, pythons, alligators, tsetse flies, penguins, kakapos, oysters, capybaras, vampire bats, marine iguanas,

cuckoos, turkeys, turkey vultures, baboons, stick insects, and so many more. They have sequenced the microbiomes of human infants, premature babies, children, adults, the elderly, pregnant women, twins, city dwellers from the USA or China, rural villagers from Burkina Faso or Malawi, hunter-gatherers from Cameroon or Tanzania, Amazonian people who had never been contacted before, lean and fat people, and those in perfect health versus those with disease.

These kinds of studies have blossomed. Even though the science of the microbiome is actually centuries old, it has picked up tremendous pace in the last few decades, thanks to technological improvements and the dawning realisation that microbes matter enormously to us – especially in a medical setting. They affect our bodies so extensively that they can determine how well we respond to vaccines, how much nourishment children can extract from their food, and how well cancer patients respond to their drugs. Many conditions, including obesity, asthma, colon cancer, diabetes, and autism, are accompanied by changes in the microbiome, suggesting that these microbes are at the very least a sign of illness, and at most a cause of it. If it's the latter, we might be able to substantially improve our health by tweaking our microbial communities: by adding and subtracting species, transplanting entire communities from one person to another, and engineering synthetic organisms. We can even manipulate the microbiomes of other animals, breaking partnerships that allow parasitic worms to afflict us with horrendous tropical diseases, while forging new symbioses that allow mosquitoes to fight off the virus behind dengue fever.

This is a rapidly changing field of science, and one still shrouded in uncertainty, inscrutability, and controversy. We cannot even identify many of the microbes in our bodies, let alone work out how they affect our lives or our health. But that is exciting! It is surely better to be on the crest of a wave, looking at the ride ahead, than to have already washed up on shore. Hundreds of scientists are now surfing that wave. Funds are flowing in. The number of relevant scientific papers has risen exponentially. Microbes have always ruled the planet but for the first time in history, they are *fashionable*. 'This was completely

We walk past a gang of meerkats, some upright and alert, others playing together. The lone female – the group's matriarch – is the only one Knight could potentially swab but she is old and has a heart condition. That's not uncommon. Meerkats will sometimes attack each other's pups or abandon their own, and when this happens, the zoo steps in to hand-raise the youngsters. They survive, but the keeper tells us that, for unknown reasons, they often develop heart problems when they get older. 'That's very interesting,' says Knight. 'Do you know anything about meerkat milk?' He asks because mammalian milk contains special sugars that infants cannot digest, but that certain microbes can. When a human mother breastfeeds her child, she isn't just feeding it; she is also feeding the child its first microbes, and ensuring that the right pioneers settle inside its gut. Knight wonders if the same applies to meerkats. Do the abandoned pups start their lives with the wrong microbes because they don't get mother's milk? Do those early changes affect their health in later life?

Knight is already working on other projects to improve the health of the zoo's animals. As we walk past a cage full of silvered langurs – beautiful, pewter-furred monkeys with electric facial fuzz – he tells me that he is trying to work out why some monkey species frequently suffer from inflammation of the colon (colitis) in captivity, while others do not. There's good reason to think that their microbes are involved. In people, cases of inflammatory bowel disease are usually accompanied by an overabundance of bacteria that provoke the immune system and a lack of those that restrain it. Several other conditions show similar patterns, including obesity, diabetes, asthma, allergies, and colon cancer. These are health problems re-envisioned as ecological ones, where no single microbe is at fault, yet an entire community has shifted into an unhealthy state. They are cases of symbiosis gone wrong. And if these distorted microbiomes actually cause the various conditions, it should be possible to restore good health by manipulating the microbes. Even if the microbial communities are changing as a *result* of a disease, they could still be useful in diagnosing a condition

before symptoms become apparent. That's what Knight hopes to see in the monkeys; he is comparing animals with and without colitis, across different species, to see if there are signatures of disease that keepers could use to identify a symptomless animal at risk. Such studies might also help us to understand how the microbiome changes in people with inflammatory bowel disease.

Finally, we walk into a back room where several animals are being temporarily housed out of the public eye. One of the cages houses a giant shadow: a three-foot-long, black-furred creature that has the shape of a weasel but the countenance of a bear. It's a binturong: a large, shaggy civet which Gerald Durrell described as a 'badly made hearthrug'. The keeper reckons that we could easily swab its face and feet, but the real action lies further down. Binturongs have scent glands on either side of their anus, which produce a smell that's reminiscent of popcorn. Again, it seems likely that bacteria create the odours. Scientists have already characterised the microbial scents that drift from the scent glands of badgers, elephants, meerkats, and hyenas. The binturong awaits!

'Could we swab the anus?' I ask.

The keeper looks at the intimidating animal in the cage and then slowly back at us. He says, 'I . . . don't think so.'

When we look at the animal kingdom through a microbial lens, even the most familiar parts of our lives take on a wondrous new air. When a hyena rubs its scent glands on a blade of grass, its microbes write its autobiography for other hyenas to read. When a meerkat mother breastfeeds its pups, it builds worlds within their guts. When an armadillo slurps down a mouthful of ants, it feeds a community of trillions that, in turn, provide it with energy. When a langur or human gets sick, its problems are akin to a lake that's smothered by algae or a meadow that's overrun with weeds – ecosystems gone awry. Our lives are heavily influenced by external forces that are actually inside us, by trillions of things that are separate from us and yet very much a part of us. Scent, health, digestion, development, and dozens of other traits that

are supposedly the province of *individuals* are really the result of a complex negotiation between host and microbes.

Knowing what we know, how would we even define an individual?[25] If you define an individual anatomically, as the owner of a particular body, then you must acknowledge that microbes share the same space. You could try for a developmental definition, in which an individual is everything that grows from a single fertilised egg. But that doesn't work either because several animals, from squids to mice to zebrafish, build their bodies using instructions encoded by both their genes *and* their microbes. In a sterile bubble, they wouldn't grow up normally. You could moot a physiological definition, in which the individual is composed of parts – tissues and organs – that cooperate for the good of the whole. Sure, but what about insects in which bacterial and host enzymes work together to manufacture essential nutrients? Those microbes are absolutely part of the whole, and an indispensable part at that. A genetic definition, in which an individual consists of cells that share the same genome, runs into the same problem.

Any single animal contains its own genome, but also many microbial ones that influence its life and development. In some cases, microbial genes can permanently infiltrate the genomes of their hosts. Does it really make sense to view them as separate entities? With your options running out, you could pass the buck to the immune system, since it supposedly exists to distinguish our own cells from those of intruders, to tell self from non-self. That's not quite true, either; as we will see, our resident microbes help to build our immune system, which in turn learns to tolerate them. No matter how we squint at the problem, it is clear that microbes subvert our notions of individuality. They shape it, too. Your genome is largely the same as mine, but our microbiomes can be very different (and our viromes even more so). Perhaps it is less that I *contain* multitudes and more that I *am* multitudes.

These concepts can be deeply disconcerting. Independence, free will, and identity are central to our lives. Microbiome pioneer David Relman once noted that 'loss of a sense of self-identity, delusions of

self-identity and experiences of "alien control"' are all potential signs of mental illness.[26] 'Small wonder that recent studies of symbiosis have engendered substantial interest and attention'. But he also added that '[Such studies] highlight the beauty in biology. We are social creatures and seek to understand our connections to other living entities. Symbioses are the ultimate examples of success through collaboration and the powerful benefits of intimate relationships.'

I agree. Symbiosis hints at the threads that connect all life on Earth. Why can organisms as disparate as humans and bacteria live together and cooperate? Because we share a common ancestor. We store information in DNA using the same coding scheme. We use a molecule called ATP as a currency of energy. The same is true across all life. Picture a BLT sandwich: every component, from the lettuce and tomatoes to the pig that produced the bacon, to the yeast that baked the bread, to the microbes that surely sit on its surface, speaks the same molecular language. As Dutch biologist Albert Jan Kluyver once said, 'From the elephant to the butyric acid bacterium – it is all the same!'

Once we understand how similar we are, and how deeply the ties between animals and microbes extend, our view of the world will become immeasurably enriched. Mine certainly has. All my life, I have loved the natural world. My shelves are lined with wildlife documentaries and books bursting with meerkats, spiders, chameleons, jellyfish, and dinosaurs. But none of these talk about how microbes affect, enhance, and direct the lives of their hosts, and so they are incomplete – paintings without frames, cakes without icing, Lennon without McCartney. I now see how the lives of all these creatures depend upon unseen organisms that they live with but are unaware of, that contribute to and sometimes entirely account for their abilities, and that have existed on the planet for far longer than they have. It is a dizzying change in perspective, but a glorious one.

I have been visiting zoos ever since I was too small to remember (or to know that you shouldn't climb into the giant tortoise enclosure). But my visit to San Diego Zoo with Knight (and Baba) feels different.

2. THE PEOPLE WHO THOUGHT TO LOOK

Bacteria are everywhere, but as far as our eyes are concerned, they might as well be nowhere. There are a few extraordinary exceptions: *Epulopiscium fishelsoni*, a bacterium that lives only in the guts of the brown surgeonfish, is about the size of this full stop. But the rest cannot be seen without help, which means that for the longest time they weren't seen at all. In our imaginary calendar, which condenses Earth's history into a year, bacteria first appeared in mid-March. For virtually their entire reign, nothing was consciously aware of their existence. Their anonymous streak broke just a few seconds before the very end of the year, when a curious Dutchman had the whimsical notion of examining a drop of water through handmade lenses of world-beating quality.

In 1632, Antony van Leeuwenhoek was born in the city of Delft, a bustling hub of foreign trade permeated by canals, trees, and cobbled paths.[1] By day, he worked as a city official and ran a small haberdashery business. By night, he made lenses. It was a good time and place to do so: the Dutch had recently invented both the compound microscope and the telescope. Through small circles of glass, scientists were peering at objects too far or too small to see with the naked eye. The British polymath Robert Hooke was one. He gazed at all manner of minute things: fleas, lice clinging to hairs, the points of needles, peacock feathers, poppy seeds. In 1665 he published his observations in a book called *Micrographia*, complete with gorgeous and extraordinarily

In October 1676, Leeuwenhoek told the Royal Society about what he'd seen.[5] All of his missives were utterly unlike the stuffy scientific discourse of academic journals. They were full of local gossip and reports about Leeuwenhoek's health. ('The man needed a blog,' observed Anderson.) The October letter, for example, tells us about the weather in Delft that summer. But it also contains fascinatingly detailed accounts of the animalcules. They were 'incredibly small; nay, so small, in my sight, that I judged that even if 100 of these very wee animals lay stretched out one against another, they could not reach the length of a grain of coarse sand; and if this be true, then ten hundred thousand of these living creatures could scarce equal the bulk of a coarse grain of sand'. (He later noted that a sand grain is around 1/80th of an inch across, which would make these 'wee animals' 3 micrometres long. That is, more or less, the length of an average bacterium. The man was *astonishingly* accurate.)

If someone suddenly announced to you that they had seen a group of wondrous, invisible creatures that no one else had ever witnessed, would you believe them? Oldenburg certainly had his doubts, as he did about Leeuwenhoek's earlier descriptions of the 'animalcules'. Still, he published Leeuwenhoek's letter in 1677, in what Nick Lane calls 'an extraordinary monument to the open-minded scepticism of science'. Oldenburg did, however, add a cautionary note, saying that the Society wanted details of Leeuwenhoek's methods so that others could confirm his unexpected observations. Leeuwenhoek didn't exactly cooperate. His lens-making technique was a closely guarded secret. Instead of divulging it, he showed the animalcules to a notary, a barrister, a physician, and other gentlemen of repute, who assured the Royal Society that he could indeed see what he claimed to have seen. Meanwhile, other microscopists tried to duplicate his work – and failed. Even the mighty Hooke struggled at first, and succeeded only when he turned to the single-lens microscopes he so hated. His success vindicated Leeuwenhoek, and cemented the Dutchman's reputation. In 1680, this untrained draper was elected a Fellow of the Royal Society. And since he still couldn't read Latin or English, the Society agreed to write the diploma of membership in Dutch.

Having already become the first human to see microbes, Leeuwenhoek then became the first to see his own. In 1683 he noticed white, batter-thick plaque lodged between his teeth and, as was his wont, he looked at it through his lenses. More living things, 'very prettily a-moving'! There were long, torpedo-shaped rods that shot through the water 'like a pike', and smaller ones that spun around like a top. 'All the people living in our United Netherlands are not as many as the living animals that I carry in my own mouth this very day,' he reported. He drew these microbes, creating a simple image that has become the *Mona Lisa* of microbiology. He studied them in the mouths of local Delft citizens: two women, an eight-year-old child, and an old man who had reputedly never cleaned his teeth. He even added wine vinegar to his own scrapings and saw that the animalcules fell dead – the first account of antisepsis.

By the time he died in 1723, at the age of 90, Leeuwenhoek had become one of the Royal Society's most famous members. He bequeathed to them a black lacquered cabinet containing 26 of his amazing microscopes, complete with mounted specimens. Bizarrely, the cabinet disappeared and was never recovered; an especially tragic loss, since Leeuwenhoek never told anyone exactly how he made his instruments. In one letter, he complained that students were more interested in money or reputation than in 'discovering things hidden from our sight'. 'Not one man in a thousand is capable of such study, because it needs much time, and spending much money,' he lamented. 'And over and above all, most men are not curious to know: nay, some even make no bones about saying: What does it matter whether we know this or not?'[6]

His attitude almost killed his legacy. When others looked through their inferior microscopes they saw nothing, or imagined figments. Interest waned. In the 1730s, when Carl Linnaeus began classifying all life, he lumped all microbes into the genus *Chaos* (meaning formless) and the phylum Vermes (meaning worms). A century and a half would pass between the discovery of the microbial world and its earnest exploration.

* * *

Microbes are now so commonly associated with dirt and disease that if you show someone the multitudes that live in their mouth, they will probably recoil in disgust. Leeuwenhoek harboured no such revulsion. Thousands of tiny things? In his drinking water? In his *mouth*? In *everyone's mouth*? How exciting! If he suspected that they might cause disease, it didn't manifest itself in his writing, which was notable for its lack of speculation. Other scholars were not so restrained. In 1762, the Viennese doctor Marcus Plenciz claimed that microscopic organisms could cause sickness by multiplying in the body and spreading through the air. 'Every disease has its organism,' he said, presciently. Sadly, he had no evidence, and so no way of persuading others that these insignificant organisms were significant. 'I shall not waste time in efforts to refute these absurd hypotheses,' wrote one critic.[7]

Things started changing in the mid-nineteenth century, thanks to a cocky, confrontational French chemist named Louis Pasteur.[8] In short succession, he demonstrated that bacteria could sour liquor and putrefy flesh. And if they were responsible for both fermentation and decay, Pasteur contended, they might also cause disease. This 'germ theory' had been championed by Plenciz and others, but was still controversial. People more commonly thought that diseases were caused by bad air, or *miasma*, released from rotting matter. Pasteur showed otherwise in 1865, when he discovered that two conditions afflicting France's silkworms were caused by microbes. By isolating infected eggs, he stopped the illnesses from spreading and saved the silk industry.

Meanwhile, in Germany, physician Robert Koch was working on an epidemic of anthrax that was sweeping local farm animals. Other scientists had seen a bacterium, *Bacillus anthracis*, in the victims' tissues. In 1876, Koch injected this microbe into a mouse – which died. He recovered it from the dead rodent and injected it into another one – which also died. Doggedly he repeated this grim process for over 20 generations and the same thing happened every time. Koch had unequivocally shown that the bacterium caused anthrax. The germ theory of disease was right.

he also believed that some microbes could *prolong* life. In this, he was inspired by Bulgarian peasants, who regularly drank soured milk and lived well past the age of 100. The two traits were connected, said Metchnikoff. The fermenting milk contained bacteria, including one that he called the Bulgarian bacillus. These made lactic acid, which killed the harmful life-shortening microbes in the peasants' intestines. Metchnikoff was so convinced by this idea that he started regularly quaffing sour milk himself. Others were so convinced by Metchnikoff – a respected scientist – that they did the same. (His claims even started a fashion for colostomy, and inspired Aldous Huxley to write *After Many a Summer*, in which a Hollywood tycoon injects himself with carp guts to alter his gut microbes and achieve immortality.) Humans had, of course, been drinking fermented dairy products for thousands of years, but they were now doing so with microbes in mind. This fad outlasted Metchnikoff himself, who died of heart failure at the age of 71.

Despite the efforts of Kendall, Metchnikoff and others, the study of the symbiotic bacteria, in both humans and other animals, was steamrollered by the increasing focus on pathogens. Public health messages started encouraging people to scour germs from their bodies and surroundings with antibacterial products and a regime of hyper-hygiene. Meanwhile, scientists discovered and mass-manufactured the first antibiotics – substances that overwhelmed both germs and the narrative around them. Finally, we had a chance of vanquishing these tiny foes. And with that chance, the study of symbiotic bacteria lapsed into a long drought, which continued well into the latter half of the twentieth century. A detailed history of bacteriology, published in 1938, failed to mention our resident microbes at all.[21] The leading textbook in the field gave them a lonely chapter, but mainly talked about how to distinguish them from pathogens. They were notable only because they had to be separated from their more interesting peers. If scientists studied bacteria, they mostly did so to understand other organisms better. It turned out that many aspects of biochemistry, like how genes are switched on or how energy is stored, were

and infections. 'Several kinds of microbes play an essential role in the development and physiological activities of normal animals and man,' he wrote.[30]

But Dubos knew that he was just scratching the surface. 'It is certain that [the bacteria identified so far] present but a very small part of the total indigenous microbiota, and not the most important,' he wrote. The rest – perhaps as many as 99 per cent of them – simply refused to grow in a lab. This 'uncultured majority' was a daunting obstacle. Despite everything that had happened since Leeuwenhoek's day, microbiologists still knew nothing about most of the organisms they were meant to be studying. Powerful microscopes couldn't solve the problem. Techniques for culturing microbes couldn't solve the problem. A different approach was needed.

In the late 1960s a young American named Carl Woese began a weirdly niche project: he collected different species of bacteria and analysed a molecule called 16S rRNA, which was found in all of them. No other scientists saw the value of this work and Woese had no competitors: 'It was a one-horse race,' he would later say.[31] The race was expensive, slow, and dangerous, involving worrying amounts of radioactive liquids. But it was also revolutionary.

At the time, biologists relied solely on physical traits to deduce the relationships between species, comparing minutiae of size, shape, and anatomy to work out who was related to whom. Woese felt he could do a better job with the molecules of life: DNA, RNA, and proteins, which are universal to all living things. These molecules accumulate changes over time, so closely related species have more similar versions than distantly related ones. If Woese compared the right molecule across a diverse enough range of species, he believed, the branches and trunks of the tree of life would reveal themselves.[32]

He settled on 16S rRNA, which is produced by a gene of the same name. It forms part of the essential protein-making machinery that is found in all organisms, and so provided the unit of universal comparison that Woese craved. By 1976, he had profiled 16S rRNA from around

30 different microbes. And in June of that year he started work on the species that would change his life – and biology as we know it.

It came from Ralph Wolfe, who had become an authority on an obscure group of microbes called methanogens. These bugs could survive on little more than carbon dioxide and hydrogen, which they converted into methane. They lived in marshes, oceans, and human guts; the one Wolfe sent over – *Methanobacterium thermoautotrophicum* – was found in hot sewage sludge. Woese assumed, as did everyone else, that it would be just another bacterium, albeit one with strange proclivities. But when he looked at its 16S rRNA, he realised that it was decidedly un-bacterial. Accounts differ as to how fully he grasped what he saw, how exuberant or cautious he was, and whether he asked for the experiments to be repeated. But what is clear is that by December his team had sequenced several more methanogens and found the same pattern in all of them. Wolfe remembers Woese telling him, 'These things aren't even bacteria.'

Woese published his results in 1977, in a paper that rebranded the methanogens as the *archaebacteria*, later renamed simply as *archaea*.[33] They weren't weird bacteria, Woese insisted, but an entirely different form of life. It was an astonishing claim. Woese had lifted these obscure microbes out of muck and given them equal billing to the ubiquitous bacteria and the mighty eukaryotes. It was as if everyone was staring at a world map, only for Woese to quietly unfold a full third that had been hidden underneath.

As expected, his claims drew vociferous criticism, even from fellow iconoclasts. The journal *Science* would later dub him 'microbiology's scarred evolutionary', and he bore those scars right up to his death in 2012.[34] Today, his legacy is undeniable. His assertion that archaea are distinct from bacteria was correct. Perhaps more importantly, the approach he championed – comparing genes to work out how species are related to each other – is one of the most important in modern biology.[35] His methods also paved the way for other scientists, like his long-time friend Norman Pace, to *really* start exploring the microbial world.

In the 1980s, Pace started studying the rRNA of archaea that lived in extremely hot environments. He was especially excited by Octopus Spring, a deep blue cauldron in Yellowstone National Park whose water reached a scalding 91 degrees Celsius. The spring was full of unidentified heat-loving microbes, which grew in such huge swarms that they manifested as visible pink filaments. Pace remembers reading about the spring and rushing into his lab, shouting, 'Hey, guys, look at this! Kilogram quantities! Let's get a bucket and go up there.' One of his team said, 'But you don't even know what the organism is.'

And Pace replied: 'That's okay. We can sequence for it.'

He might as well have shouted, 'Eureka!' Pace had realised that, with Woese's methods, he no longer needed to *grow* microbes to study them. He didn't even need to *see* them. He could just pull DNA or RNA right out of the environment and sequence the lot. That would reveal what was living there *and* how they fitted into the microbial tree of life – biogeography and evolutionary biology, in one fell swoop. 'We took our bucket up to Yellowstone and did it,' he says. From the waters of that 'still, beautiful, and lethal place', Pace's team identified two bacteria and an archaeon. None of them had been cultured. All were new to science. The results, published in 1984,[36] marked the first time that anyone had discovered an organism from its genes alone. It would not be the last.

In 1991, Pace and his student Ed DeLong analysed samples of plankton, fished out of the Pacific Ocean. They found an even more complex community of microbes than in Yellowstone: 15 new species of bacteria, two of which were distinct from any known group. Slowly, the sparse bacterial tree of life sprouted new leaves, twigs, and sometimes entire trunks. In the 1980s, all known bacteria had fitted nicely into a dozen major groups, or phyla. By 1998, that number had blossomed to around 40. When I spoke to Pace, he told me that we now are up to 100, and around 80 of those have never been cultured at all. A month later, Jill Banfield announced the discovery of 35 new phyla from a single aquifer in Colorado.[37]

Freed from the yoke of cultures and microscopy, microbiologists could now carry out a more comprehensive census of the planet's microbes. 'That was always the goal,' says Pace. 'Microbial ecology had become a moribund science. People went out, overturned a rock, found a bacterium and thought it exemplary of what's out there. It was stupid. From the very first days of this, we just blew open the doors of the natural microbial world. I want that on my epitaph. It was a wonderful feeling and still is.'

They weren't restricted to 16S rRNA. Pace, DeLong and others soon developed ways of sequencing *every* microbial gene in a dollop of soil or a scoop of water.[38] They would extract the DNA from all the local microbes, cut it into small fragments, and sequence them together. 'We could get any damn gene we wanted,' says Pace. They could see who was there using 16S rRNA, but they could also work out what the local species were capable of by searching for vitamin synthesis genes or fibre-digesting genes or antibiotic resistance genes.

This technique promised to revolutionise microbiology; all it needed was a catchy name. Jo Handelsman provided one in 1998 – *metagenomics*, the genomics of *communities*.[39] 'Metagenomics may be the most important event in microbiology since the invention of the microscope,' she once said. Here, finally, was a way of understanding the full extent of life on Earth. Handelsman and others started studying the microbes that lived in Alaskan soils, Wisconsin grasslands, the acidic run-off from a Californian mine, the water from the Sargasso Sea, the bodies of deep-sea worms, and the guts of insects. And, of course, in the style of Leeuwenhoek, some microbiologists turned to themselves.

Like Dubos and many others who eventually fell in love with microbes, David Relman originally planned to kill them, having begun his career as a clinician working on infectious diseases. In the late 1980s, he used Pace's new technique to identify unknown microbes behind mysterious human diseases. At first he was deeply frustrated because every tissue sample that might harbour a new pathogen was always swamped by our normal microbiota. These residents were an annoying distraction – until Relman realised that they were interesting