Taken from *Slayers, Saviors, Servants, and Sex: An Exposé of Kingdom Fungi* by David Moore; published by *Springer-Verlag, New York: 2001,* ISBN 0387950982.

Chapter 9 Birds do it. Bees do it. Even educated fleas do it. But why?

Look around. There are living organisms everywhere. We have a special word for it - ‘biodiversity’. We even have international conventions about it, and Article 2 of the 1988 Convention helpfully tells us that ‘Biological diversity means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic systems and the ecological complexes of which they are a part; this includes diversity within species, between species and of ecosystems.’ All that boils down to: the life support system of planet Earth on which we depend for the air we breathe and the food we eat. We worry about it, of course, because we have recognized over the past few years that human activities are destroying ecosystems. We make deserts out of fertile plains, turn tropical forests into pastures and pollute whole oceans. Then we worry about it.

One important, and useful, outcome of the worrying has been the recognition that a problem might exist. Another is the realization that we need to know what the situation is now before we can measure how much change occurs over the next year, or decade, or century. In other words, we’ve got to start with the question: ‘just how diverse is biology?’ We might approach an answer to that question by counting how many names we have for different organisms. We have been naming things ever since we’ve been able to communicate, but really scientific naming has been going on only since Carl Linnaeus established the first widely-agreed set of rules in the middle of the eighteenth century, just about two hundred and fifty years ago. We give a species a particular name in order to catalogue the accumulated knowledge about it and provide a means to recognize it when it is found again. Amazingly, there is no single catalogue yet in existence of the species we already know about. Lists and catalogues cost money, and nobody has yet put their hand in their pocket for anything like an index of species known in the world. It would be a pretty large undertaking. About twenty-thousand new species are named each year, and the total we currently know about is about one and a half million. Sounds a lot? Maybe, but the best guess at the moment is that we have given a name to little more than ten percent of the species that actually exist. The United Nations Environment Program has estimated that there are fifteen million species of organisms on planet Earth right now. If that’s true, then, at our current rate of progress, it will take another seven hundred years to find and name each one that’s unnamed at the moment.

Knowing how many species there are in nature is important to appraisals about the impact of human activities on the natural environment. Even more important is the judgement that has to be made about whether we should change our activity to alter that impact; and how the change is to be accomplished (and at what cost - and to whom) if we decide to change. These considerations are crucial to our future on the planet, but they are outside the scope of this book! I have ventured down this line of thought for a different reason. A useful approximate definition of a species is that it is a collection of individuals who are able to interbreed. This implies that the ‘rule’ is that two different species cannot interbreed, whereas members of the same species can interbreed. In other words the whole definition of ‘species’ is bound up with the process of sexual reproduction. There are exceptions. Some genuine species hybrids are possible. But these are exceptions and the ability to reproduce sexually is a very reliable guide to species identity. In a very real sense, therefore, sexual reproduction is the process which drives biodiversity. There *are* fifteen million organisms on Earth *because* of the numerous rounds of sexual reproduction which have taken place in the 3.8 billion years that life has been evolving on the Earth. Obviously, sex is important to living things, and an important question to ask is: why? Why do organisms reproduce sexually? Like every other attribute of living creatures, sexual reproduction must have evolved, so there must have been a time before it evolved when the simple creatures of the day didn’t have sex. So when and why did sex begin?

The answer to the 'when' is reasonably straightforward, it must have been a long, long time ago; but the answer to the 'why' is much more difficult because it leads us into deep philosophical waters. The difficulty is that sex involves the genetic material of one organism combining with the genetic material of another. Inevitably, sex decreases the representation of an individual's genes in the next generation, so what is the selective advantage that enabled it to evolve and become such a dominant force? Let's leave the 'why' to one side for a moment and try to find out 'when'.

Plants and animals make pretty good fossils and a lot of people expect fossils, particularly fossils of animals, to answer most questions about evolution. And, yes, you can find evidence of sexual activity in fossilized things that sound as though they are very old. For example, several museums have pieces of amber that contain pairs of insects which were mating when they were trapped in the resin which became amber. These may be forty, fifty or sixty million years old. Old, but not *that* old. The earliest fossil dragonflies, which were flying around about three hundred million years ago had complex male and female genitals. And we can see the sex business even further back than that. Four hundred to five hundred million years ago there were small, shelled creatures called ostracods, related to the *Daphnia* 'water fleas' we use to feed goldfish, which had clearly different males and females. The females with large brood pouches and the males with a double penis up to a third of the animal's body length. Very extravagant sexual development half a billion years ago. But it seems to have paid off. These creatures colonized every aquatic environment on the planet and have survived every cataclysmic extinction to occur in that half billion years and they're still going strong today. That sort of persistence is evidently one of the advantages of sex - it helps a species to survive. At the other extreme, another advantage of sex is that it promotes variety of life. And we can see this because during the same five hundred million years that ostracods have been bumbling around, other lines of evolution have produced fish, amphibians, reptiles, birds, mammals - and us.

Obviously we have to go much further back in time than a mere five hundred million years, so let's try another tack. In chapter 8 we saw that there is evidence for fossil microbes in rocks that are three-and-a-half billion years old. They don't look like much more than circular or cylindrical blobs of stone between one and five thousandths of a millimetre long, but they are thought to be the oldest bacteria yet found. Some of these blobs have constrictions along their length that have been interpreted as indicating that the cells were in the process of dividing - just splitting into two to reproduce themselves - at the time they were killed. Bacterial cells are too simple to have enough organized structure for us to recognize differences that might be 'sexual' so to make a guess at when and why cells like this started to share their genetic material with a mate we will have to look at what happens in present day bacteria.

Sharing genetic material requires the physical mixing of genes from two different sources. In present day animals and plants it's eggs and sperms and pollen that bring together genes from 'mother' and 'father'. Bacteria don't have such highly evolved machinery, but they do seem to have a more basic, down to earth relationship with their surroundings. So basic that they can absorb genes directly from the fluid they are living in. Viruses can also carry bacterial genes from one bacterium to another. Both of these events can result in a bacterium having a mixture of genes from two sources - the essence of sexual reproduction. But the process in bacteria which is closest to a sexual one is when two bacterial cells join together and genes from one flow into the other. This is called conjugation. The remarkable thing about this is that it's not controlled by bacterial genes, but by an alien genetic element called a plasmid. Plasmids are stripped-down parasites. They don't make cells of their own, instead they live inside other cells, mostly bacteria. They are all quite simple, and the very simplest ones only have genes for processes needed for their own reproduction. But some also organize cell to cell contact, that is conjugation, between their host cells. What's in it for the plasmid is that conjugation allows the plasmid to pass like an infection from one bacterium to another of the same generation. Plasmids that do not organize conjugation are trapped within their host and can only get into a new cell when their host cell reproduces. So conjugation can open up the whole population to infection, giving the plasmid an enormous biological advantage. What's in it for the bacterium is that occasionally the plasmid genes get stuck onto the bacterial genes and the plasmid transfers these to the other bacterial cell. That second bacterial cell then ends up containing genes from two sources - it's got its own and it has other bacterial genes from its partner. The genetic information is mixed and the necessary prerequisites of sexual reproduction are established. These sorts of event occur in all groups of bacteria we know of today, even those that are thought to represent the most ancient and primitive forms.

So it could be that sexual reproduction originated when bacteria took advantage of an infection mechanism evolved by plasmids originally for their own purposes. Remember that they had a long time to achieve this. Bacterial evolution occupied a period of about one and a half billion years before the higher organisms arose from their bacterial ancestors about two billion years ago. And the first higher organisms then had another billion years to perfect and adapt the process even before plants, animals and fungi started to separate from each other about one billion years ago.

Today, plants, animals and fungi undergo sexual reproduction using essentially the same biochemical tools. So if they all have a common process, it's reasonable to assume that it *is* common because the process evolved before evolution separated these three higher Kingdoms. This must mean that sex was 'invented' by those early organisms that were neither animal, plant nor fungus but some primitive compromise between all three. Sex reared its ugly head *that* long ago. We've had one billion years of fooling around! Maybe it's about time we asked why. To deal with this I really need to talk about one group of organisms - and I'll choose to talk about fungi. This is not to say that the arguments don't apply to the rest, they certainly do; but it is easier to channel this sort of thinking if we have a particular life style in mind.

Most fungi produce spores that result from a non-sexual process. It might be called asexual reproduction or vegetative reproduction. Whatever it's called, it's just a matter of fungal cells breaking up in some organized way. This makes little parcels of living material that might be protected in some way (like a wall that prevents them drying out) and usually are adapted to being dispersed (like having long thin processes which help dispersal in water currents). We have probably all seen mouldy food or mildewed clothing covered in a green, black or brown powder. The powder is made up of the spores of the mould and even small quantities of substrate can generate vast numbers of spores. These spores are extremely effective in dispersing the organism. They are in the air all around us, in the waters of streams, rivers and reservoirs, and among the dust that accumulates on surfaces in houses, cars and yards. If one of these spores lands on the surface of a potential source of food for the fungus, then the spore will germinate and a new fungus colony will grow. The genes of this 'daughter colony' will be the same as the genes of the 'parent colony' *unless* a mutation has occurred in one or more of the genes, either when the spore was first produced or while it was being dispersed. A mutation will change the function of a gene, usually making it work less well. But that's a value judgement. Literally a life and death value judgement, because if the mutation causes a really adverse change then the organism will be crippled and might die. Something which is unsuited to the present environment will be killed-off. This is evolutionary selection in operation. By selecting against genes which have a poor performance, evolution ensures that the genes which remain in the living population are among the best that have been tried so far in the present environment. That's why most mutations are 'bad' - the 'good' ones have occurred before and have been selected to operate in the present environment. I keep emphasizing the 'present environment' because circumstances can change and the genes that are 'good' now, say in middling temperature and humidity, may not be equally good if the temperature got hotter or it got a whole lot wetter. That's natural selection: make the genes work *in* the conditions where the organism lives. Mutation keeps generating variants. If the conditions don't change the mutations are most likely to lose. But if the conditions change some of the mutations might be winners. The rate at which mutation occurs is usually rather low. A representative rate to keep in mind is around one in a million; there are higher rates, there are lower rates, but one in a million is a fair average. So if a fungus produces about a million spores, there's a fair chance that at least one carries a mutation.

Look again at that mouldy food or mildewed cloth: there are tens, hundreds, thousands of millions of spores. And tomorrow there'll be millions more, and the day after. Such colossal numbers of asexual spores are routinely produced that you might expect that there are sufficient of them for mutation alone to provide all the variation which evolution might need for its operation. We can see this point now with any moldy material, but remember that we are searching or an argument which might have operated more than a billion years ago, and this point will have been just as valid then. Turn the clock back to a time when the highest organism alive was a primitive single-celled plant/animal/fungus creature. It may have been primitive, but the population must have been numbered in many billions. Let's assume that it was so primitive that it took a week to duplicate itself. That sounds pretty wimpish for an organism that is already, presumably, competing pretty well with all the bacteria that already existed then, but we can work with it. Imagine we have one such wimp on January first, we'll have two on January seventh, four on the fourteenth, eight on January twenty-first. By the end of June we'll have thirty-three million, and there'll be two thousand million million of these little wimps by the end of their first year. And, though they may not know it, they've got a thousand million years ahead of them to keep on doing the same old thing. This may not be a very likely scenario, these guys are wimps, so I guess a few will die, but it makes the point that simple duplication can make enormous numbers of the simplest organisms really quite quickly. And there is an awful lot of time for it to happen. So you can easily see that even if mutation is only a one-in-a-million thing every possible mutation in every possible gene will occur in the population at some time. But we know that the ancestors of these organisms (the bacteria) were already investing some effort in sexual reproduction. And we also know that the descendants of these organisms (present day plants, animals and fungi) invest enormous resources of time and effort in their much more complex sexual rituals (and many animals have abandoned asexual reproduction altogether). Clearly, sex must have distinct selective advantage because it has emerged alongside, and in some cases to supplant, asexual reproduction. Importantly, the selective advantage must be one which applies to the most primitive organisms as well as to those of the present day.

The crucial contrast between sexual and asexual reproduction is that sexual reproduction brings together genetic material from different individuals. In asexual reproduction, only the genes of the one individual are multiplied as it replicates and divides. There must be something about 'bringing together genetic material from different individuals' that provides immediate evolutionary advantage to the organism in which it happens. It doesn't have to be much of an advantage - *any* advantage will give it an edge in the battle for favorable selection. If the two individuals whose genetic material is brought together are different in some way then their differences can be rearranged during the sexual process. So the sexual mechanism enables new combinations of characters to be created in the next generation for selection. This is the most usual 'explanation' for sex, namely that it promotes gene variability through what is called 'out-crossing' and if that is put together with the expectation that variability is needed for the species to evolve to deal with competitors and environmental changes then sex is seen as an all round good thing to do. It's got to be admitted that plenty of evidence exists to show that out-crossing certainly does promote variability, and that asexual organisms change only very slowly with in time. Both of these lines of evidence seem to support of the view that variability in the population enables the organism to change in order to survive ecological and environmental challenges.

The problem with such a line of argument, though, is that it is a 'group selectionist' interpretation. It argues that variation generated in *individual* organisms is advantageous because it benefits the *group* or population to which the individual belongs. This goes against the current fashion in evolutionary thought because current theory emphasizes, instead, that selection acts on individuals. It follows, therefore, that any feature which is argued to be advantageous in selection must be advantageous because it benefits either the individual itself or its immediate offspring. On those grounds this idea does not adequately explain why sex is an advantage to the individual organism. Another idea is that since mutations are mostly damaging, simply bringing together genes from different individuals in the prelude to sexual reproduction will ensure that any mutation-damaged gene in one set will be masked by its non-mutant counterpart in the genes of the other parent. This would, potentially, be of immediate advantage to the individual which contains the two sets of genes, but the real advantage seems to be that damaged DNA caused by mutation or faulty duplication in one set of genes can be repaired by comparison with the normal one provided by the other parent. That seems to be the really crucial advantage of the sexual process. An opportunity to repair damage, providing immediate advantage to the individual and long term advantage to its offspring. It's fun as well. Oddly enough, 'fun' doesn't appear to have been rated very highly in terms of evolutionary significance.

Before any advantage can be gained, though, the genes from two individual organisms must be brought together. So the first step in any sexual cycle is a cell fusion, or at least a cell-to-cell contact. The process involves breakdown of two walls before the two separate cells can unite, but even before that stage, the cells must be able to recognize each other. Sex starts with those steps and evolution of the mechanisms which control recognition and union between two separate cells was a cardinal operation in the whole procedure. For the individual cell (and remember, it was at that stage of evolution that the evolution started) the problem is how to regulate cell unions so that the genetic advantages can be realized without hazard. There are contradictory requirements. Maximizing the advantage of sexual reproduction requires that the genes are as different as possible. On the other hand, safety for the cell requires that if cell contents are to mingle they must be as similar as possible. The danger at the cell level is that cell fusion carries the risk of exposure to contamination with alien genetic information from things like viruses or plasmids. They may not be damaging to the cell that contains them (because it's adapted to their presence), but when it fuses with another cell the second parent may suffer badly.

Protection against alien DNA is provided by a particular family of genes that only allow fusion with cells that have similar or identical genes in their corresponding copy of the family. These are called vegetative compatibility genes. They determine whether two cells are sufficiently compatible to fuse. In complex organisms like animals and plants gene families of this sort exist but they have been increased greatly in number an have now branched out to include features which determine gender - they make males express male characters, and females express female characters. In simple organisms like fungi the vegetative compatibility genes are the closest thing they've got to a self/non-self recognition system. When the colony edges of two fungi grow to meet each other the leading hyphal cells may mingle without interacting, or hyphal fusions may occur between their branches. If the colonies involved are not compatible (that is, if they have different vegetative compatibility genes) the cells which first fused are killed. Vegetative compatibility so prevents successful fusion except between fungi that are sufficiently closely related to belong to the same family group of vegetative compatibility genes. In fungi it is these genes that determine individuality. These are the genes that say 'I am me'. That's important in evolution because selection operates on individuals. When the vegetative compatibility genes do allow individuals to exchange genes, there are other genes (called mating or breeding systems) which then come in to play to regulate sexual exchange. Primarily, these genes prevent mating between genetically identical individuals. Between them, these families of genes achieve the require balance between similarity and difference; the ideal compromise. We know about this from our own experience because we have genes that make males into males and females into females. We have two sexes. Fungi seem to have gone overboard in this direction because their mating type system can generate hundreds, even thousands of different sexes. It's not entirely clear what the strategy for this peculiarity is; maybe there's more going on here than we realize.

The outcome of sex is the rearrangement of the genes that were brought together into new combinations that are 'packaged' into the offspring spores, which can be distributed into the environment. Many fungi produce fruiting bodies in which spores are produced and which both protect and distribute the spores. These are the truffles, puffballs, toadstools and mushrooms. These are composed of different tissues, so fungi can quite reasonably be considered to be multicellular organisms. Like animals and plants, they are able to produce tissues comprised of cells that have different functions. Fungal developmental biology is alive and well, but we need to know much more about it. Tissue patterns in mushrooms are established very early in development. Mushroom 'embryology' suggests that processes known to occur during animal embryo development have their analogues during mushroom development. Unfortunately, although all of these processes are well researched in animals (and, increasingly, in plants too) that other great Kingdom, Fungi, is being largely ignored. So while the occurrence of these processes during mushroom development may be inferred from indirect observations there are very few specific examples of research aimed directly at understanding how fungal multicellular structures develop. Fungi are simple - they are not primitive. They have several complex, sophisticated, and highly evolved relationships. They produce some remarkable structures. It all goes to show what can be achieved with simple tools.