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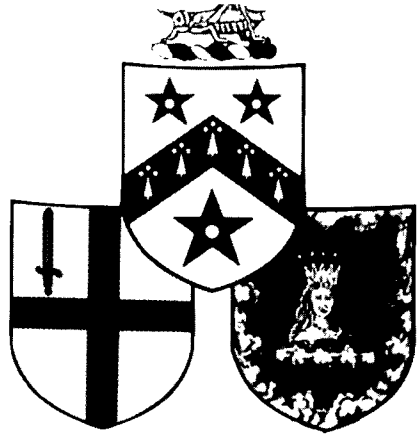

DOLLY, CARROTS AND SEX

A Lecture by

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Dolly, Carrots and Sex

Professor Hilary Rose and Professor Steven Rose

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First, an apology: so far the two Roses have given these lectures jointly, and we had planned to do so for this one, but Hilary has been called away to give a keynote speech at the EU in Brussels on women and science (she points out that it indicates just how few women in science there are that she has had to give up her share of this lecture) and has with great regret left me to give a biologist's viewpoint on today's theme alone. She apologises and it is a pity as we had planned to begin the discussion with a dialogue between us on sex and sexuality – maybe I'm slightly relieved that this now has to go by the board. Anyhow, today you will have to put up with a one-eyed view of our theme, though I will try to hint at the issues that Hilary would have raised. But it very important to recognise that this is not the version of the lecture that we would have given had we both been here.

Genetic technology, as we have seen in previous lectures, to say nothing of even the most casual reading of the daily papers, is moving at extraordinary speed, yet no single advance has generated more alarm and debate than the prospect, ever apparently nearer, of cloning humans – that is, of taking a body cell from an adult, and by appropriate manipulations, provoking it into dividing and growing so as eventually to form a fully competent individual genetically identical to the person from whom the original cell was taken. The current furore was of course generated by the report, back in 1997, by Ian Wilmut and his colleagues at the Roslin Institute in Edinburgh, that they had achieved what biologists had previously thought impossible, and cloned a sheep, the famous Dolly. The rush of publicity, headlines, protests and moral panics and concerns was astonishing even to those of us hardened to genetic alarms. Here are some examples

(transparencies)

Scientists like Richard Dawkins announced that they would be pleased to be cloned. Legislators rushed to devise controls over the new techniques, President Clinton announced that no federal money would be put into human cloning – though in the US you can't legislate nationally against private endeavours – and the delightfully named, though scientifically entirely unqualified Dr Dick Seed (a physicist) announced that he was going to set up a private institute to enable humans to be cloned. Another US company has offered to store the DNA of your dead pet – dog or cat – against the time when it too can be cloned. Since 1997 other mammals, including cows and mice, have been cloned, in the US and Japan, and commercial companies have rushed to patent the techniques. Roslin itself, at the time of Wilmut's report a partially private company and partially owned by the Ministry of Agriculture) is now largely bought out by a UK registered company a substantial part of whose research base is in the US.

Despite all this, the genetic and developmental problems involved in cloning are not simple, so before discussing the technology and its social context, origins and consequences, I want to backtrack and consider some of the biological issues that lie behind the headlines.

Reproducing without sex

The title of this lecture includes the word sex, so let me begin by unpicking some of the –less obvious - reasons why sex is a problem for biologists. It may sound surprising but the large majority of living forms do without sex at all. They reproduce simply by dividing and budding off. Bacteria for instance, simply unwind their DNA, copy it, dispatch the two copies to the two halves

of their single cell, and then divide in half. The whole process takes approximately 20 minutes, and where there was a single bacterium, there are now two separate but genetically identical individuals. Each is a clone of the other, though of course mutations can and do occur- you only have to think of the rise of antibiotic resistance to realise that. The process seems simple and reliable.

Bacteria don't have nuclei and obviously the business is a little more complicated for single celled organisms whose DNA is concentrated in the nucleus, as it is necessary first to duplicated the DNA and then to split the nucleus in two before dividing the cell. But of course this is exactly what happens in mitosis – in cell division and multiplication in multi-celled organisms – as we mentioned in previous lectures.

(transparency)

This is how organisms such as amoeba reproduce for instance. And it is worth pondering the fact that in such asexually reproducing organisms, there is in a sense no death – the organism, constantly dividing, is in a certain manner immortal – sex, death and individuality are indissolubly linked biologically as well as in poetry!

Now it is still relatively easy to see how single celled creatures can simply bud off new copies of themselves, but it seems intuitively more complicated for a multi-cellular organism. Of course we can't simply split ourselves down the middle. But asexual reproduction is possible for many if not most plants, for instance. Any gardener knows that you can take a cutting – a bit of a branch or twig for instance, dip it into hormone rooting powder, stick it into the ground, and with a bit of luck away it goes into a full grown bush or tree. Try to eradicate buttercups from your lawn and you will find that they have put out underground runners, and at intervals along the runner a new buttercup pops up through the grass. Cut the connection, the runner, between the original and the offspring buttercup and the latter will live perfectly happily, a clone of its parent.

What happens all the time in nature can be copied in the laboratory. Take a sliver of carrot, put it into an appropriate medium, at a decent temperature with all the foodstuffs it needs in solution around it, and its cells will begin to divide. Soon you will have in your test-tube a tiny but perfectly formed miniature full carrot plant, and once it is big enough you can plant it out – a clone indistinguishable from its parent.

So why bother with sex?

So why bother with sex at all? I don't mean for the pleasure – and pain – it generates for all of us in our own lives, but in terms simply of how reproduction occurs. Wouldn't it be simpler just to bud off like a carrot or amoeba than to have to go to the trouble of bringing two individuals together, and inventing the biological mechanisms to enable them to share their genes? Well, it is that sharing which is the precise point. Just because an asexually generated individual is the exact copy or clone of its parent, barring mutations, the opportunity for genetic diversity, of therefore generating new individuals containing a slightly different genetic mix, is lost. And genetic diversity provides a population of individuals with differing characteristics, and therefore able in a sense to experiment with different ways of living, and different ways of responding to environmental changes. The consequence is that evolutionary change is greatly speeded, as there is much more variation on which natural selection can operate, more genetic flux to drive internal processes within organisms, and so on. There is no doubt that the invention of sex, whenever it originally occurred during the evolution of life on earth, greatly speeded up subsequent evolutionary change.

This is of course why even plants which can reproduce asexually also reproduce sexually too; in this sense buttercups have the best of both worlds. And interestingly even bacteria have a mechanism which is essentially analogous to sex. There are circumstances in which two individual bacteria of the same species will come alongside each other, and one will inject copies of its DNA into the other – a process called conjugation.

(transparency)

So, for the biologist, that is essentially what sex is about. But there are still mysteries. In principle it would seem that if all we were after was genetic mixing, there would be a case for us all being hermaphrodites, like worms. And indeed there are many hermaphrodite species, and some, like slipper limpets, which begin as males but finish their lives as females. The variety of gene sharing mechanisms that have been invented during evolutionary history is astonishing. However one feature seems to be pretty constant. By and large in the overwhelming majority of living forms, and in sexual terms –I'm not talking about the complexities of human sexuality – there are only two sexes, even where as in hermaphrodites one individual is both male and female. One can think in principle of gene sharing processes involving more than two sexes, but they don't seem to occur in nature. Human technologies of course can now overcome this barrier.

There is a further mystery, which I'll hint at and not expand on here. Sexual reproduction in animal species is an asymmetric business. Females produce eggs, which are relatively large and also relatively few in number. Eggs are packages containing not merely DNA but also energy supplies, enzymes, mitochondria and much else. Males produce relatively speaking vast numbers of sperm which are little more than packets of DNA. In mammals this asymmetry is greatly enhanced as females have to carry the growing embryo till birth. So females can produce over their lifetime only a tiny number of offspring, whereas males can generate enough sperm to impregnate a vast number of females. No, I'm not drawing the macho conclusion here, but the reverse. Logically, a species could get on very well with a largely female population and only a tiny number of males. Why bother, you may well ask, with all this excess? (Feminists might well agree, considering all the trouble we men generate!). The evolutionary biologist John Maynard Smith has spent a good part of his research life trying to answer this question. To cut a long story short, a mathematical analysis shows that there is a sort of evolutionary trade-off, which means that in a population in which females greatly outnumber males the best chance of propagating your genes comes from producing male offspring, and vice-versa, and as a consequence, in the long term a roughly equal balance of males and females is generated (in fact, because males tend to be more fragile than females for all sorts of complex reasons, at birth in humans males outnumber females, but as we die sooner and more frequently as the population ages the sex ratio reverses).

But still, why can't we clone?

But even if that explains why sex, why two sexes, why roughly equal numbers of males and females, the question still remains. Why can't we also reproduce asexually, like plants? Why can't we clone? Of course its easy to see why mobile organisms like animals can't put out runners, but there are more fundamental reasons why, until 1997, it was thought by biologists that cloning from an adult mammal was impossible. The reasons lie in the nature of developmental processes. Development begins with a single fertilised egg and ends with the trillion cells of the human body. In all multi-cellular organisms, different cells are specialised for different functions – liver, muscle, bone, nerve. In humans for instance there are said to be some 250 different cell types. Each cell type has a different structure, expresses a slightly different range of enzymes and other proteins, and so forth. Yet each cell contains an entire copy of the organism's DNA – its genome. The genome, as we've said in earlier lectures, includes within its three billion base pairs of DNA some 100,000 genes. The number of different proteins present in any particular cell is not easy to estimate, but in general may come to 10,000 or so – the highest number is believed to be in nerve cells, which can express some 30,000 different proteins. So at best only 30% of the genome is actively engaged in any adult cell, and probably in most cases it is no more than 10%. At fertilisation, the egg can express all the hundred thousand or so genes it contains – it is said to be totipotent. The process of development is a steady reduction in totipotency, as cells become more specialised, they can express a smaller and smaller proportion of the genes. The remainder don't of course disappear; they simply become shut off and inactive. There seemed to be no way of reversing this process and activating the genes once more.

That's why, of course, you can't regrow an amputated limb, unlike a plant which seems to have almost unlimited powers of regeneration – plant cells remain totipotent throughout life. So do some

animals. Cut a worm in half, and each half will regenerate the lost portion. And indeed non-mammalian vertebrates can show some powers of regeneration – everyone knows for instance that a lizard will shed its tail if threatened, but can regrow another later. So cloning adult mammals ought to be impossible, but cloning some forms of animal life might work.

Cloning

So what would – what does – cloning involve? A clone means simply an identical copy of some other individual. The word is now used in a variety of senses. For instance, making multiple copies of a particular strand of DNA is called cloning the DNA. A group of bacteria all containing exactly similar DNAs is called a clone. It is becoming common nowadays to talk of spare part surgery in which skin or liver or other tissue is grown for grafting into an injured or sick person, and in which the skin or liver cells are clones of those the person possesses to start with. Outside biology if one company copies another's product almost exactly (for instance a computer) one even speaks metaphorically of it being a clone. But here we are talking primarily of making an exact genetic copy of an individual. In this sense, identical twins, or triplets, are clones of one another (sometimes not quite, for complex developmental reasons, but leave that to one side).

So cloning involves extracting the genome from a cell of the individual you wish to clone, and placing it in an environment in which it will express itself. In practice, as the genome is present as the DNA inside the nucleus, and the only environment in which it will develop is that provided by a living cell, this means, first removing the nucleus from the cell destined to act as host, extracting the nucleus from a cell of the individual you wish to clone, and inserting it into the now empty host cell. Obviously, this requires at the least some very precise micromanipulation, but in principle given a microscope, a pipette and some very fine tweezers it isn't too difficult. Even I can do it. The fancy bit is ensuring that the resulting cell with its foreign nucleus will then develop.

This was first successfully done by John Gurdon, in Cambridge, back in the late sixties and early seventies, with frogs. It's easy to see why. Frogs like other amphibia don't entirely lose totipotency as they develop, and as fertilisation is outside the body and the eggs develop into tadpoles comfortably in laboratory dishes, no special provisions are called for. Even so Gurdon's was a major breakthrough. But the possibility of mammalian cloning had to wait until procedures for *in vitro* fertilisation were developed, as obviously the host eggs have to be removed from the carrier, their nuclei replaced by that of the donor, and then reinserted into the carry mother to develop until the infant can be delivered at term. By the 1980s these procedures had become well established. So it was possible to contemplate at least the possibility of mammalian cloning. The first experiments were done as might be expected, using as donors embryonic cells which had not yet lost totipotency. Indeed the first sheep clone was made in 1979, by Steen Willadsen in Cambridge, simply by taking a two cell embryo and splitting it. Even so there were many failures, and reports of success - for instance by Karl Illmensee, a German embryologist, in 1981, who claimed to have cloned mice - were rapidly discounted as other laboratories couldn't replicate them. Thus the conventional biological wisdom through until the appearance of Dolly in 1997 was that it was impossible.

Ian Wilmutt and Keith Campbell, the principal players in the cloning story, have described how they did it in a book co-written with the science writer Colin Tudge – it is a fine account and well worth reading *(The Second Creation, Headline Books, 2000)* and for reasons of time, I'm not going to go into many technical details here. The first step to Dolly was the cloning of two sheep (Morag and Megan) from embryonic cell donors in 1995. Dolly, as I've said, followed in 1997. The trick in both cases lay not so much in micromanipulating and obtaining the donor nucleus. That is relatively straightforward. Nor in obtaining the carry cell and maintaining it in culture in a dish until the transfer had been complete, and eventually reimplanting into the womb. What it depended on was the state of that recipient cell when the donor nucleus was transplanted. You may remember from previous lectures me describing the cell cycle through which all cells go between cell divisions. It was only during the research that led up to Dolly – cloned from the mammary gland cells from an adult donor - that the significance of this became clear. There are a number of sequential phases in the cycle, and it turns out that only if the transplant is made at the correct phase of that cycle is it

possible to recover totipotency. Even now the reasons for this aren't quite clear but the practical consequences are profound. And of course, as anyone who has experience of IVF knows only too well, the ratio of successes to failures is still very low. Only a small percentage of the re-implanted cells turned out to be functional.

So far as is known, Dolly is a perfectly normal sheep, though there remain questions about whether she is ageing faster than would be expected, because of the state of the chromosomes in the transplanted nuclei. But it is important to remember that although she is a nuclear clone of the donor, the nuclei had to be transplanted into a recipient cell which contained all rest of the cellular apparatus required for cell growth and division, including of course the mitochondria, which you will remember also contain their own DNA. So don't forget in all this debate that there are clones and clones!

Why bother to clone?

Obviously cloning Dolly was a considerable technical feat, with very interesting scientific implications. But why did they bother to do it? Because, the argument goes, cloning domestic animals may be of commercial value. For instance, rather than go through the now traditional AI methods for cattle breeding one could produce cloned herds of good milk or meat yielders. Or – and this seems to be where the main commercial thrust is coming – one could produce cloned flocks or herds of genetically engineered sheep or cows that secreted medically important proteins in their milk.

We've not discussed this year the genetic engineering techniques that are potentially available – we'll come on to those in next year's series, so I'll just make a couple of points in this context. It used to be argued that one could genetically engineer bacteria, for instance to produce human insulin, and indeed just such a form of insulin, humulin, was produced by Eli Lilley back in the 1980s, but it seems to have fallen from grace, as people suffering from diabetes found it easier and safer to use the pig insulin, which is similar to human insulin, extracted from slaughtered animals. One reason for this is apparently that the protein synthetic mechanisms present in bacteria are unsuited to producing the soluble forms of desired human proteins as they differ slightly from the protein processing mechanisms utilised by multicellular organisms. So the production of expensive medically utilised proteins, insulin, hormones, clotting factors and so on in milk seems to be regarded as a potentially commercially viable proposition. I confess to having some doubts about it, but the prospect has been enough to excite major investment from biotech companies in the US, Japan and Europe, including of course PPL which owns a significant proportion of the Roslin patents on the methods that produced Dolly. The practicalities of course lie well into the future.

Human cloning?

Cloners such as Wilmutt insist that human cloning is outside their consideration, and there is no reason it seems to me not to believe them. But they also make clear that the techniques used for Dolly are in principle possible for humans too, and again there is no reason to doubt this. Of course the technical problems are substantial – it must not be forgotten that the failure rate in the production of Dolly was high and even if it is reducible it will always be there, so few women would be readily prepared to act as carry mothers for such a cloned offspring. However, that is not of course the moral or social point. And I am now going to trespass rather cautiously onto the ground that Hilary would have covered so as to raise some of the themes that I hope will provoke discussion.

The idea of cloning humans has been around in science fiction for a long time – long before the techniques were even potentially available. Everyone seems to remember *The Boys from Brazil*, and the fantasies of cloned armies of Hitlers or at least Hitler Youth. As far back as the 1930s geneticists like JBS Haldane and Herman Muller fantasised about cloning. Muller wanted to clone Einsteins and Lenins – typically as Hilary would have emphasised were she here it was only men to be cloned for their brains; the women candidates were all chosen for their beauty (Monroe, Bardot etcetera). Back in 1978 a supposedly factual account was written by David Rorvik of the

cloning of a man – a booOk called *In His Image*. It made headlines but was finally recognised as fiction. Now what was fiction is potentially fact – with Dolly came a rush of moralists and philosophers, ethicists and politicians to pontificate on what might be the implications of human cloning. Legislation began to be framed in Europe banning any such attempt, whilst despite the Clinton posturing companies began to be set up in the US and Japan to do precisely that. Nonetheless loopholes in the legislation allow experimental human clones to be produced – provided that they remain in the petri dish, are destroyed after a short period of growth and of course not re-implanted and allowed to come to term.

Ethicists tended to argue that cloning would be an assault on human dignity, whereas many geneticists asked whether there was any essential difference between a clone and a twin. They – we – were also quick to point out that having identical (nuclear) DNA does not mean that we would be identical people – we would have been brought up in different environmental contexts, technological, social and personal so would have very different life histories. Even identical twins aren't identical people of course. The clone of an adult would be born twenty or more years after its nucleus was donated, in a very different context. Arguments seemingly in favour of cloning contemplated the possibility that if a beloved partner or child was dying one might want to 'recreate' them by cloning. More macabrely the prospect of cloning for spare body parts has been argued – that one might want to generate the possibility of a clone so that if one suffered from liver or heart failure or whatever one would have a spare part bank on which to draw for transplants. Again, ethicists raised the objection that this was to treat a human individual as a means and not an end.

To this wealth of debate, Hilary I know would want to add some further points, and I will just hint at these. First, that although if a market researcher asks a simple question about what people feel about genetic engineering and cloning people casual responses are often in favour, more detailed probing especially when people are actually faced with real and not theoretical choices, reveals a much more complex and hesitant picture. The moral and personal concerns are very real – think of the current debate about opting in versus opting out of being an organ donor after death, where people are being asked to make real not hypothetical decisions and you get a flavour of it.

Second, and this I suspect would be one of the most important points she would want to emphasise, it simply isn't adequate to consider the issue as a purely technical one. We already live in a world in which what we have hitherto regarded as a 'natural' relationship of a child with its parents – mother and father, occasionally adopted or step-parents has been transformed, by IVF, AID, surrogacy and so forth. The idea of 'motherhood' becomes more and more problematic. And we see the increasing concern by adoptive children to discover their 'genetic' as well as their adoptive parents – especially of course mothers. Who are the 'parents' of a cloned child? How should such a child avoid growing up with the strong belief that it really had been produced as a means and not an end??

Gloomily, I suppose I would predict that somewhere some time in the next decades, someone, perhaps in Japan, is going to try human cloning whatever ethical concerns and legality. And despite the inevitable failures it is likely that someone will succeed. I am sufficiently sanguine to believe that the example will neither win a Nobel prize nor become widely followed; the traditional ways of making babies and of enjoying what Hilary calls the 'genetic joke' of enjoying their similarities and differences to their parents, are likely to prevail for reasons that we all know and understand. But that does not mean that we should not draw ethical lines in the sand beyond which we must endeavour to prevent the headlong rush of biotechnology to take us. And for both of us, I believe, human cloning represents a step too far across that line.