Why Are Cloned Cats Not Identical, Implications For Pet Cloning And Public Perception

By
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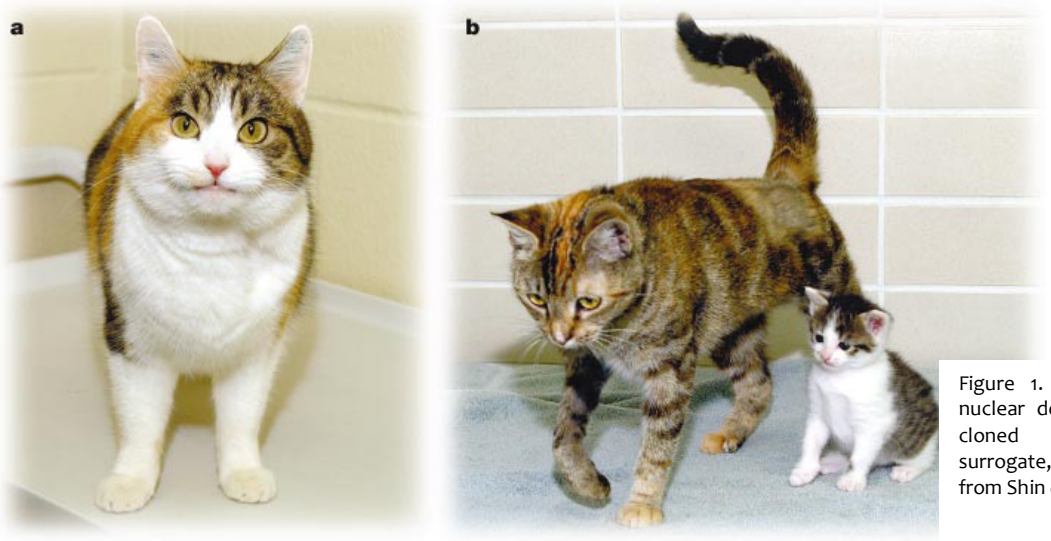

Figure 1. showing (a) nuclear donor and (b) cloned kitten with surrogate, reprinted from Shin *et al.* (2002).

Introduction

Cloning is a breeding technique used to produce offspring via asexual reproduction. This means that only one parent is needed and the offspring; termed a clone, is very nearly genetically identical to the original, (Winter *et al.,* 2002). The technique began to emerge in 1952, however since the cloning of the first mammal (Dolly the Sheep) in 1996, there has been increasing interest in cloning pet animals, (Westhusin, 2000).

Genetically identical offspring are found throughout the natural world. In non humans this occurs via asexual reproduction and in humans it occurs through the production of twins. However, in the laboratory a clone is produced via a technique called somatic cell nuclear transfer (SCNT).

There has been much discussion over the possible benefits and disadvantages of cloning. One benefit is that it could be used to create a clone of a recently deceased pet, which could help the grieving process of the owner. However even though the clone will be genetically identical, some animals (especially cats) are unlikely to have the same markings .

The first aim of the investigation was to look at the genetics of cat coat colour and explain the mechanisms that cause the differences. The second aim was to look at the public perception of cloning by conducting a survey. The aims of the survey were to identify whether the public had heard about cloning, to identify how many would be prepared to clone their pet and the main reasons for their views. The final aim of the investigation was to use the results of the survey to investigate whether pet cloning is likely to have a commercial future.

Cloning Techniques

The method used to clone animals is termed somatic cell nuclear transfer (SCNT). This involves the transfer of genetic material from a donor cell, to an unfertilised egg that has had the nucleus removed (Suk *et al.,* 2007). Although the technique has been used to clone many species it is a very inefficient technique, for every 1000 embryos created, on average only 3-10 result in live births,

(Shin *et al., 2002*). This is due to the complexity of the technique and the effect of many external factors such as the species and age of the donor, tissue type, oxygen concentration, medium of the culture and genetic abnormalities. Another major cause of inefficiency is during activation. During natural fertilisation the sperm causes an increase in calcium in the oocyte plasma, indicating fertilisation has occurred. This does not occur during SCNT and therefore the cell has to be activated artificially. There are many different activation techniques and efficiency is variable. Recently a new technique has been developed by Polejaeva *et al. (2000)* that eliminates the need for artificial activation, and therefore aims to greatly increase the efficiency.

On 22nd December 2001 the first cloned cat was born. She was produced by a team of researchers at Texas A&M College of Veterinary Medicine from a donor called Rainbow and was named 'carbon copy' or C.C., (Fairbanks, 2004). Although C.C and Rainbow were genetically identical, they were phenotypically different in terms of coat colour and markings. Rainbow was a calico cat with patches of white, black and orange fur. C.C however did not have any orange markings and was predominantly white with tabby patches as illustrated in figure 2. This is due to a phenomenon called x-inactivation described later on.

Figure 2: C.C showing a lack of orange fur, reprinted from BBC: http://news.bbc.co.uk/1/hi/sci/tech/1820749.stm

The genetics of cat coat colour

In the feline, there are eight basic coat colours; black, chocolate, cinnamon, blue, lilac, fawn, red and cream, produced through the interactions of several genes. Coat colour is produced by pigments called melanins that originate from specialist melanocyte cells. Melanin is deposited in the hair shaft and causes colour. (Long, 2006).

There are three basic genes that give rise to all the different cat coat colours, these are; the colour gene, the orange making gene and the colour density gene.

The colour making gene controls the basic colour of the coat and can give a black, brown or light brown coat. The orange making gene can either give an orange coat or a non orange coat. The colour density gene controls the distribution of pigment throughout the hair and can give a dense or a dilute colour. Figure 3 illustrates the coat colours.

		Dense	Dilute
Colour making genes;	Black	black 	Blue 
	Brown	Chocolate 	Lilac 
	Light brown	Cinnamon 	Fawn 
Orange making gene; phaemelanin	Orange	Red/orange 	Cream 

Figure 3: illustrating the eight different cat coat colours.

X inactivation

Rainbow was a calico cat with patches of white, orange and black fur. C.C however was white with a grey tabby pattern, this difference in colour is due to a process called X inactivation.
The sex of animals is determined by their chromosomes. This involves females having two X chromosomes whilst males have one X and one Y. This causes a problem because the X and the Y chromosomes are very different. The Y chromosome is small and does not contain many genes. In comparison the X chromosome is much

larger with many genes. This means that females could express each gene twice as much as the male causing a genetic imbalance. In order to cope with this, many animals have evolved mechanisms of dosage compensation, (Klug *et al.*, 2007).

In mammals including cats the dosage compensation is called X inactivation. This involved the genes on one of the X chromosomes being silenced so they are not expressed.

Rainbow would have been heterozygous for the genes encoding coat colour, one X chromosome would contain the gene encoding the orange making gene, and the other would encode the colour making gene. During development of the foetus all the cells come from the one original cell (the fertilised egg) that splits. It first splits into 2 cells, then 4 then 16 etc, X inactivation occurs at the 63 cells stage. At this point each cell would have one chromosome inactivated, therefore each cell would either contain the orange or colour making gene, not both. All the progeny cells would then originate from these 63 cells and each would have the same inactivation as the original. When daughter cells are produced they tend to stay in the areas of the original cell, forming patches of colour. Figure 2 illustrates how the process of X inactivation produces calico coloured cats such as Rainbow.

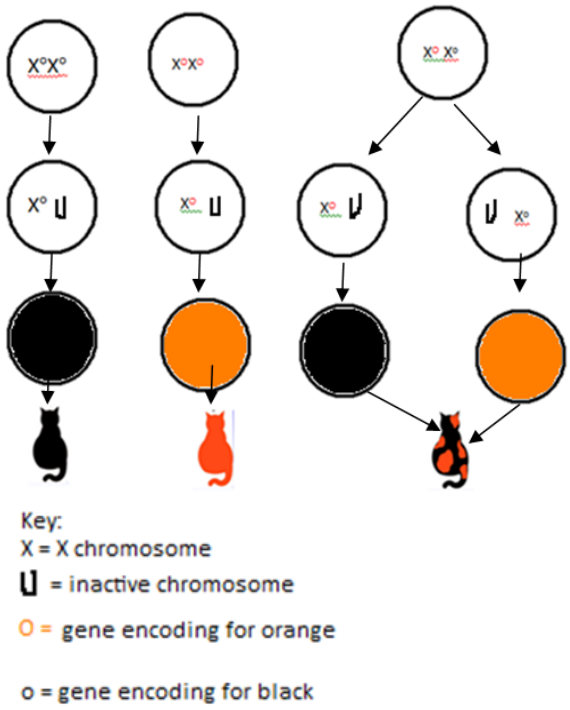

Figure 2. Showing how through X inactivation a heterozygous cat for the orange making gene will be calico in colour, adapted from Kremples, (2010).

C.C had a different colour coat to Rainbow because the genetic information used to clone her was taken from just one cell. This cell would have already undergone permanent X inactivation and would have had the orange making gene inactivated. With regards to cloning X-inactivation would affect all female animals that were heterozygous for coat colour.

The Survey

In order to investigate the opinions of the public an online survey was created and distributed. In total there were 142 respondents to the survey. When asked whether they had heard of pet cloning 59% of respondents stated they had. This was lower than expected as it was hypothesised that this would be higher due to media coverage. When asked what characteristics they thought a clone would have, the response was as shown in figure 3, with the majority of people believing the clone would have the same markings as the original animal.

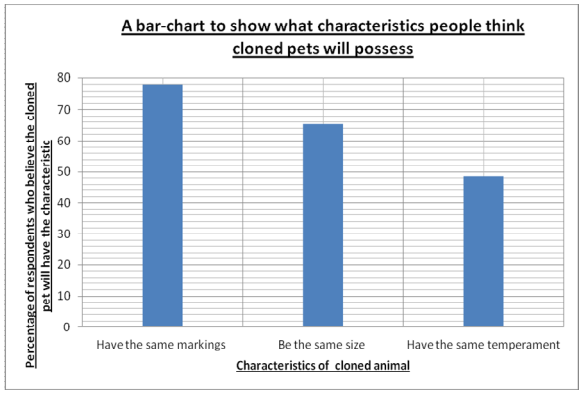

Figure 3: A bar chart showing the characteristics that people believe a cloned pet will have.

When asked if they would clone a pet, a resounding 90% of respondents said no, with the perceived suffering to the animal being the most important reason against. The 10% stating yes, chose the opportunity to save endangered species or breeds as the most important reason. When asked any further reasons for their views, several people stated that they would not clone a pet because they would rather have the variety of adopting a new one.

The future of pet cloning

As shown by the survey the use of cloning for companion animals owned by the general public is unlikely to be successful due to limited number of people wanting a cloned pet. The company currently offering companion animal cloning is RNL Bio, a South Korean company specialising in canines. RNL Bio illustrate that the market for pet cloning is very niche as they have only cloned 4 canines for the public.

However there are alternative uses in the companion animal sector. One future use would be for breeders, as they could clone an animal of exception genetic value to quickly increase the value of their breeding stock. The technique could also be of use for working animals such as guide dogs and trained sniffer dogs. Figure 4 shows cloned drug sniffer dogs. If a dog showed an exceptional talent for sniffing then it could be cloned to give a litter of puppies all with the same ability, which could be of great benefit to society.

Figure 4 showing cloned drug sniffer dogs, reprinted from Hsu, (2009)

Conclusions

Cloned animals are not guaranteed to be identical due to the epigenetic phenomena of X inactivation. Public opinion of companion animal cloning is not very positive with the majority of people not choosing to clone a pet. With regards to the future of companion animal cloning it is unlikely that cloning will become popular or mainstream with the general public. However it is likely that there will be a niche market for cloning companies, composed of breeders and owners of working animals such as guide dogs and sniffer dogs.

Further Reading –
Cat coat colour:
http://www.tenset.co.uk/catgen/catsofadifferentcolor.html

Cell Biology: A cat cloned by nuclear transplantation:
http://www.nature.com/nature/journal/v415/n6874/full/nature723.html?lang=en

Cloned drug sniffing dogs:
http://www.popsci.com/scitech/article/2009-07/were-clones-and-were-reporting-duty

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Author Profile -

Emma is 21 years old and graduated from the School of Biosciences in 2011 with a first class degree, BSc Animal Science. Emma is currently studying Graduate Entry Nursing at the University Of Nottingham.