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The McLaren Effect - a personal view

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England in the swinging sixties was an irresistible draw: the Beatles, Twiggy, Mary Quant, bobbies on bicycles, music, style and all the excitement of a post-war society shaking off memories of ration books and the lean '50s. It also contained the bud of what was to become a great flower of reproductive and developmental biology research in the next decades. Not that I anticipated anything like that, moving as I did to England as a graduate student in the late sixties. I knew of the great scientific traditions; I knew the great universities; the DNA story. Even Darwin seemed close at hand when one stepped off the London train into the coal-blackened, yellow-brick back streets of Cambridge, or strolled through the ancient college courts. I studied genetics at Cambridge, reveling in the ghostly presence of R.A. Fisher. There, I was a minor oddity. There weren't too many American students about at the time, especially in the depths of an East Anglian winter. But I could ride a bike and I quickly adapted to the lack of central heating (after all, my rural upbringing prepared me for that). I eventually realized that American English was a foreign language, learned to sip sherry at 11 AM on Sunday mornings, and tried hard not to let my teeth chatter at late-evening summer garden parties.

By the early seventies, I had joined the reproductive physiology group of Robert Edwards and was working under Richard Gardner, investigating early genetic influences on mammalian development and reproduction. It was around then that I became aware of a somewhat mythical figure working somewhere in the Scottish highlands who had published the seminal work that allowed much of our experimentation on embryos to be done. In the 1950's, Anne McLaren, along with Donald Michie, had published a series of studies on the transfer of mouse eggs to uterine foster mothers (McLaren and Michie, 1956; McLaren and Michie, 1959), and with John Biggers had produced adult mice by embryo transfer following the culture of preimplantation embryos *in vitro.* That landmark paper reported the first adult mice produced by embryo transfer in which part of the preimplantation stages of development occurred *in vitro* (Fig. 1) (McLaren and Biggers, 1958). These studies paved the way for all manner of manipulations on mammalian embryos and laid the groundwork for evaluating the effects of experimental perturbations. Embryos could be removed from the mother, subjected to experimental procedures, and then returned to the reproductive tracts of foster mothers, where development could continue. The value of Anne's studies was not simply showing that it could be done, but in defining the parameters by careful analysis of the confounding factors.

It is hard now to imagine a time when experimental mice were obtained from a place called "The Mousery", or when embryo transfer techniques were far from routine. Anne's experiments established the value of a day of asynchrony between the transferred embryo and the uterus, and compared the use of pregnant and pseudopregnant foster mothers (both of them work). Extrapolating from these early experiments, in which the success rates reached as high as 36%, McLaren and Michie predicted that implantation rates as high 50% might be achievable with embryo

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Fig. 1. The first adult mice produced by embryo transfer in which part of the preimplantation stages of development occurred *in vitro*. *The albino mice were cultured from the 8-cell to the blastocyst stage (from Biggers, 1987).*

transfer, a goal long since surpassed thanks to subsequent technical improvements and to the careful groundwork laid by these early studies. McLaren also tried to establish whether a law of diminishing returns applied with respect to the number of embryos implanting in the uterus, but failed to find it using embryo transfer, probably because the overall success rate was low. Not accepting this as the final word, however, she established, in a footnote to the research, that there was indeed an upper limit of embryo number above which fetal mortality sharply increased. But in order to show this, Anne had to use a different method of increasing embryo numbers, namely administration of gonadotropins rather than embryo transfer (McLaren and Michie, 1959)!

This thorough, scholarly approach to research, with more than a little innovation thrown in has been a characteristic of Anne McLaren's research throughout her career, and it has ensured that her publications have a long and permanently useful shelf-life. I occasionally find myself quoting arcane trivia from old works of Anne's that are still the final word on a topic of interest. E.g. Did you know that transmigration of mouse embryos from one uterine horn to the other is a vanishingly rare event? Or that monozygotic twinning is exceedingly rare in mice (McLaren, 1995)? And who could forget the profound conclusions of the modestly titled "Factors affecting the time of formation of the mouse blastocoele", where Anne once and for all showed that blastocoele formation takes place on some innate schedule that does not depend on cell number or on the number of cell divisions since fertilization (Smith and McLaren, 1977)?

This mythical figure had actually returned to London in the mid-seventies as head of the MRC Mammalian Development Unit at University College, based in Wolfson House just north of the Euston Road. Although more than a little in awe of her, I quickly learned that the doors to Wolfson House were always open and inside was one of the major hothouses for the ideas and excitement that was a part of that flowering of British reproductive and developmental biology. When I ventured down from Cambridge or Oxford, I met there a group of scientists whose careers she fostered and who have since dispersed around the globe as leaders in the fields of developmental and reproductive biology of mammals.

Anne was always interested and impossibly knowledgeable about everything to do with mammalian embryos and reproduction, and even had a precocious interest in the possibility of genetic transformation in mammalian embryos (Snow and McLaren, 1974). But it is not hard to pick out the main threads of interest that have occupied the bulk of her original research throughout her career: germ cells and sex determination. One of my favorites among her publications is the monograph "Germ Cells and Soma: A New Look at an Old Problem" (McLaren, 1981), which was adapted from a series of lectures given at Yale University. As the title hints, it is a refreshing, experiment-based look at the age-old problem of which came first, the chicken or the egg, or in this case, the mouse or the germ cell.

On the other hand, the aspect of her work that has affected my own research most directly has to do with the concept of embryonic stem cells. Her interest in germ cells is only one aspect of this more general interest in stem cells, and her pioneering explorations of mammalian chimeras have been central to an understanding of the nature of the developmental potential of embryonic cells. In the early 1970s, we were exploring the limits of plasticity of early embryonic cells and defining their developmental potential under different experimental conditions. One of the concepts that grew out of this work was that embryonic development involved a progression through different states in which certain populations of cells acquired limited stem cell characteristics for defined periods

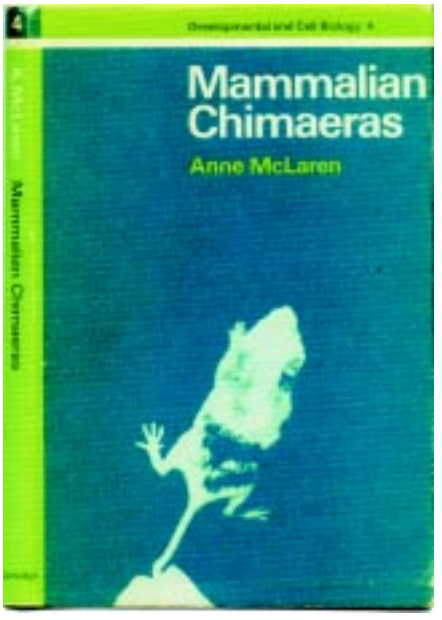

Fig. 2. Cover of the influential monograph published in 1976 from the author's well-thumbed copy. *(McLaren, 1976; with permission from Cambridge University Press).*

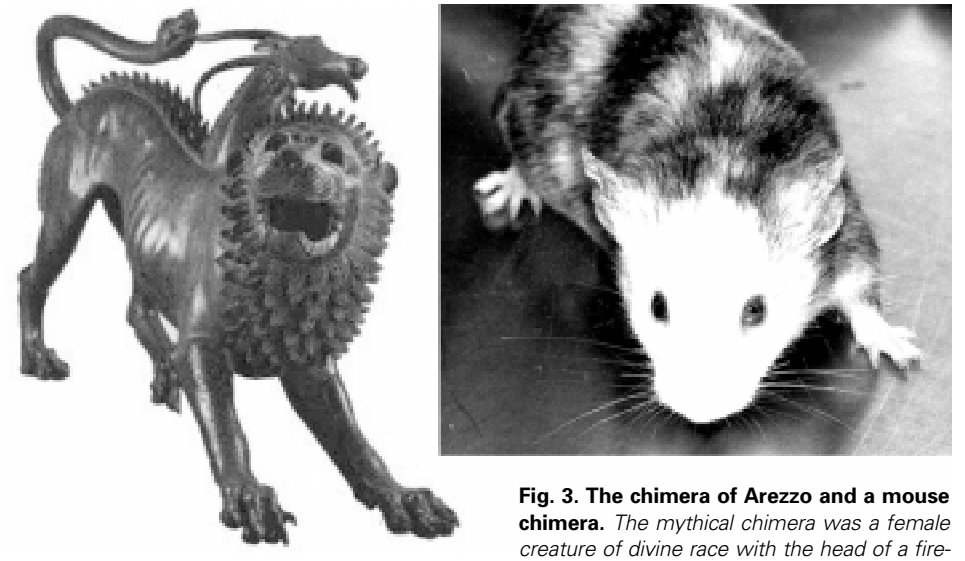

Fig. 3. The chimera of Arezzo and a mouse chimera. *The mythical chimera was a female creature of divine race with the head of a fire-breathing lion, the middle part a goat and the tail a serpent. The experimental mouse chimera is all mouse but is the product of two combined zygotes differing for pigmentation genes.*

during embryogenesis, and that the stem cell potential of different cell populations might be experimentally manipulated, or even captured (Papaioannou *et al.*, 1978). These lineage-restricted stem cell populations are much in vogue today as stem cell research has become a hot biomedical commodity. Much of Anne's work contributed directly to the development of the basic concepts that provide the foundation of embryonic stem cell research.

Anne McLaren's early explorations of size regulation in embryos touched upon the idea that the embryo was in some way capable of monitoring its own cell number and could either make up a deficit or trim down an excess (McLaren, 1972; Buehr and McLaren, 1974; Tsunoda and McLaren, 1983), a concept that relies on the inherent flexibility of stem cell populations. She had an early interest in the differentiation of trophoblast giant cells (Sherman *et al.*, 1972; Ansell *et al.*, 1974) from cells that are now considered to be trophoblast stem cells. Her large body of work on primordial germ cells, from their appearance in the embryo, their determination, their differentiation and their potential (work reviewed elsewhere in this volume) has at its core the concept of germ cells as stem cells, and as such has contributed greatly to an understanding of stem cell biology in general, and understanding of the ultimate stem cell, the germ cell, in particular.

But the work that I most often think of in association with Anne – the book my hand often reaches for on the bookshelf, is the monograph "Mammalian Chimeras" (Fig. 2)(McLaren, 1976). It was a definitive work in 1976 and has aged remarkably well. Mammalian chimeras were first produced in the early sixties (Tarkowski, 1963; Mintz, 1964), and captured the imagination of developmental biologists. The experimental techniques were embraced as a means of rendering the mammalian embryo amenable to new types of experimentation previously reserved for lower organisms. Anne's monograph was a review of the methods and applications through the mid-seventies, but offered the reader much more than a simple how-to book or review of the literature. Anne made sense of these monsters (Fig. 3), defined the terms, showed the limitations and made clear their promise.

The work was concerned with two types of experimental study for which chimeras are uniquely suitable: the first in the field of experimental embryology for the tracing of the origin and fate of tissues and cell lineages, and the second in the field of developmental genetics in order to analyze how genetically different cells collaborate to form an adult animal. Broadly speaking, these same two areas encompass most chimera research today, and the issues discussed in the book are as relevant now as they were then. Various examples were explored, such as pigmentation patterns, with respect to the interaction of genetically different cell populations, e.g. the melanocyte and hair follicle, coexisting during development and differentiation, and of course, because it was Anne writing, there is a clear description of the issues inherent in a chromosomally mixed-sex chimera with respect to phenotypic sex determination and the fate of the germ cells. She pointed out the differences between chimeras and mosaics, which both contain two or more genetically distinct cell populations, defining chimeras as being derived from more than one zygote and mosaics being derived from only one. There is a clear description of the difference between cell clones and patches of cells in a chimera, leaving no excuse for any subsequent confusion about the relationship between the number of patches in an adult chimera and the number of cell clones. Her discussion of the mathematical models and how they are affected by developmental and genetic heterogeneity should be required reading for anyone tempted to reconstruct embryonic events from cell distributions in adult chimeras. There is a short paragraph in the book detailing the characteristics of an ideal marker to distinguish one cell component from the other in a chimera. This deceptively simple list states that the marker should be cell-localized, cell-autonomous, stable, ubiquitous, easy to detect and available in several different variants. I would add to this list that the ideal marker should also be developmentally neutral, a requirement made clear in other parts of the monograph. The paragraph ends with the succinct statement, "No such marker exists." Now there is the crux of the matter and a challenge if ever I saw one! If ideal markers do not exist, how can experiments with chimeras be meaningful? Anne's lucid discussions make clear all the limitations that the lack of any of these criteria would present and point out the need for the development of markers close to this ideal. Twenty five years later, with green fluorescent proteins, we may at last have a marker that closely approximates her requirements. The book closes with a wish-list of research areas that Anne would pursue given a chimera factory and an army of workers. It is a list of research areas that have seen enormous progress in the decades since, much of it from Anne's lab and much of it from the countless scientists she inspired.

Although I have never had the great good fortune to work directly with Anne on a scientific experiment, our paths have crossed many times over the years at meetings, at the Cold Spring Harbor mouse course, and through the International Society of Differentiation.

The encounters have always been stimulating, and sometimes a little daunting when, with a twinkle in her eye, Anne unleashed her formidable intellect, always tempered however, with her unbounded generosity. Anne McLaren continues to be an inspiring mentor and a valued colleague. I salute her.

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