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The introduction of *Xenopus laevis* into developmental biology: of empire, pregnancy testing and ribosomal genes

JOHN B. GURDON[1]* and NICK HOPWOOD[2]

[1]Wellcome CRC Institute and [2]Department of History and Philosophy of Science, University of Cambridge, Cambridge, United Kingdom

Introduction

The ever greater concentration of biomedical research on fewer and fewer species is a striking general feature of the postwar era. In developmental biology, the South African clawed frog *Xenopus laevis* is, with *Drosophila*, the mouse, the chick, *C. elegans* and the zebrafish, one of only half-a-dozen 'model' organisms used for the vast majority of research. How did a species which occurs naturally only in Southern and Central Africa rise to such international prominence? Developmental biologists routinely answer this question by listing the main reasons currently assembled for using *Xenopus*: ease of maintenance of a wholly aquatic vertebrate in the laboratory; exceptional resistance to disease; a life cycle that among Amphibia is relatively short; large numbers and size of eggs suitable for microsurgery; and above all its year-round reproductive response to commercial hormone preparations compared to the limited breeding seasons of other amphibians (Kay and Peng, 1991; Tinsley and Kobel, 1996). Another kind of answer is historical, and this is what we offer here.

From the 1880s and the early work of Wilhelm Roux and others in Germany, experimental embryologists favoured Amphibia (Beetschen, 1996; Nieuwkoop, 1996), but they used the local European and North American species, initially mostly of the frog *Rana* (Maienschein, 1991). By the early twentieth century urodeles were preferred; Hans Spemann (Hamburger, 1988; Fäßler, 1997) experimented mainly on species of the newt *Triton* (now *Triturus*) and Ross Harrison on the axolotl *Amblystoma*. Following Spemann in the 1930s, even Joseph Needham and colleagues' biochemical analysis of the organizer used newts (Haraway, 1976). Only during and after World War II did *Xenopus* begin its rise to dominance. But developmental biologists in Europe and North America did not choose an African frog after a detached and comprehensive survey of world fauna. It turns out that the introduction of *Xenopus* into developmental biology laboratories was more fortuitous, and is more interesting. Its eventual embryological exploitation was made possible by specific histories of empire and of endocrinology which happen to have centred to a substantial extent in Britain; demand was ensured by a reorientation of embryology towards biochemical methods. Here we trace the scientific domestication of the South African clawed frog, focusing especially on how, between the 1930s and the early 1960s, it was introduced into European and North American laboratories, where not only developmental biologists but also converts from biochemistry took it up.

Natural history, anatomy and laboratory culture

Xenopus laevis was first described by a French naturalist at the beginning of the nineteenth century; at the turn of the twentieth a British zoologist first reported culturing its embryos in the laboratory. European men of science took advantage of imperial exploration to define and domesticate this exotic species. They were interested primarily in its natural history and in comparing the distinctive anatomy to that of other Amphibia.

***Address for reprints:** Wellcome CRC Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, United Kingdom. FAX: 44 1223 334185. e-mail: j.gurdon@welc.cam.ac.uk

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François Marie Daudin's *Histoire naturelle des rainettes, des grenouilles, et des crapauds* ('Natural History of Tree-frogs, Frogs and Toads'; 1802/03) contained the briefest description of what he called *Bufo laevis*, or 'Crapaud lisse' ('smooth toad'). Based on a single preserved specimen of unknown provenance in the Museum of Natural History in Paris, his drawing (Fig. 1) is just sufficient for a modern zoologist to recognise it as *Xenopus* from the lateral line system and dorsal eyes. But, analysing a specimen from South Africa, the Museum's great comparative anatomist, Georges Cuvier (1829, p. 107), was critical of Daudin's efforts, not least his failure to show claws, and pointedly renamed the animal *Dactylethra* (or 'finger thing'), a translation of Cuvier's 'dé à coudre' (thimble).

In the following decades, research on the taxonomy and anatomy of what appeared neither a typical frog nor a typical toad continued sporadically in the scientific centres of empire, especially London's museums and zoo, as and when specimens were received from Africa. Albert Günther (1858), an assistant at the British Museum, refined the classification of this tongueless anuran; his superior, Keeper of Zoology John Edward Gray (1864), published the first description and figure of a larva, based on fixed specimens sent via Liverpool from Lagos, Nigeria. William Kitchen Parker, Hunterian professor of comparative anatomy at the Royal College of Surgeons and a man 'once known to speak for four hours continuously on the lower jawbone of the raven without saying anything that was other than valuable' (Prosser, 1895-96, p. 292), used larvae from the same bottle as Gray to include *Dactylethra* in two truly massive descriptions of the development of the batrachian skull (Parker, 1876, 1881). By studying lower vertebrates he amassed evidence against Richard Owen's vertebral theory of the skull and in favour of the views of his friend and Owen's arch-enemy T.H. Huxley. In a note from the South African Cape, J.M. Leslie (1890) gave the earliest account of the breeding habits in swamps and ponds of what he called *Xenopus laevis*, a name proposed by Wagler (1827), and one which has stuck (see Deuchar, 1975). Leslie noted that the natural spawning season in South Africa is spring (August). Around this time living specimens began to be imported, and Frank

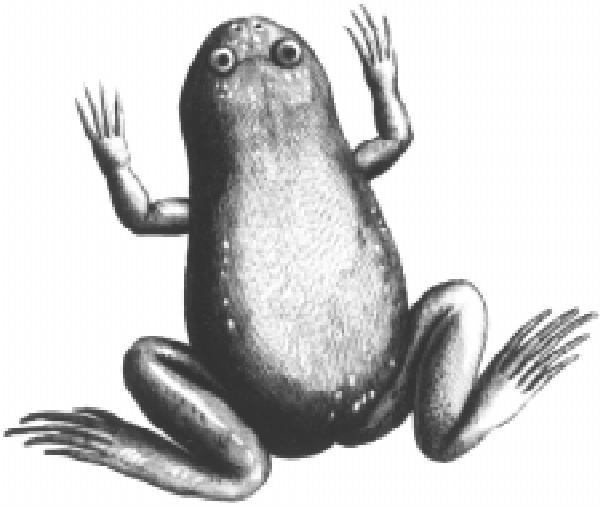

Fig. 1. The first published picture of *Bufo laevis,* from Daudin (1802/03). *The species was eventually re-named* Xenopus laevis. *Reproduced by permission of the Syndics of Cambridge University Library.*

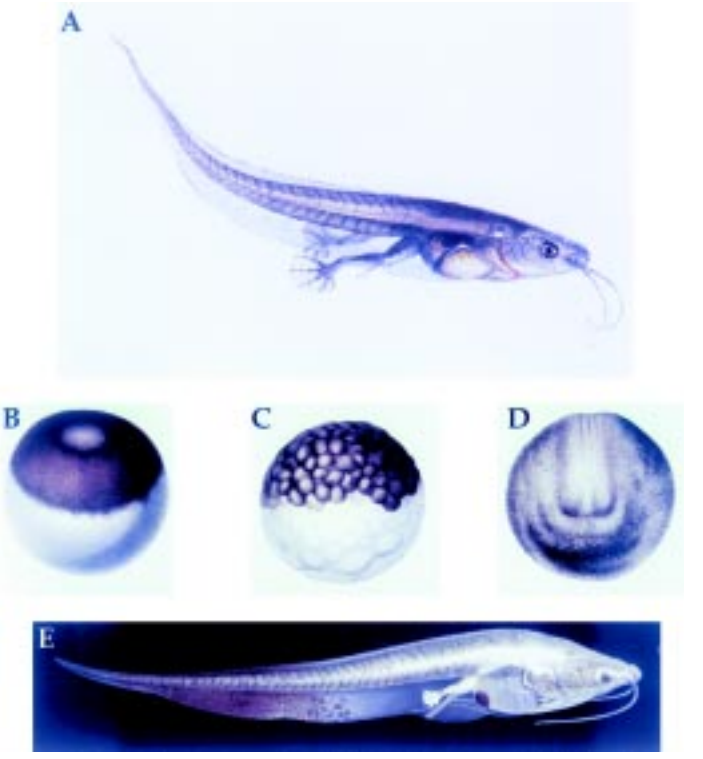

Fig. 2. The first published pictures of *Xenopus laevis* larvae and embryos. (A) *Tadpole of* Dactylethra *(subsequently* Xenopus laevis*), from Gray (1864).* **(B-E)** *A.K. Maxwell's drawings of eggs and embryos of* Xenopus laevis*, from Bles (1905).* **(B)** *Unfertilized egg;* **(C)** *mid-blastula;* **(D)** *neurula;* **(E)** *feeding tadpole. Reproduced by permission of the Syndics of Cambridge University Library.*

Evers Beddard, prosector of the Zoological Society of London, reported (1894) the natural spawning in the Gardens of the Society of some animals newly arrived from Zanzibar. This first account of *Xenopus* breeding outside Africa showed drawings of larvae of 5 mm and up (Fig. 2A).

Meanwhile, in the hands of the German zoologist Ernst Haeckel and the Cambridge morphologist Francis Maitland Balfour, Darwin's theory of evolution had greatly stimulated comparative embryology, not least of colonial fauna (MacLeod, 1994). A couple of years after Beddard, Edward J. Bles, a zoologist formerly of Owens College in Manchester and then in Cambridge, began an extended study of *Xenopus* development. Obtaining four adults from a dealer in 1896, he maintained them for two years on a diet of earthworms and strips of liver, and then arranged a space in a corner of the tropical lily tank in the Cambridge University Botanic Garden (Bles, 1901). In February 1899 he achieved a natural spawning, at a temperature of 22-24°C, and noted the black nuptial pads on the forearms of the males. In 1905, now an assistant (i.e. lecturer) in the Department of Zoology at Glasgow University, Bles published a fine article, which not only contains A.K. Maxwell's exquisitely drawn figures from the unfertilised egg right through to the late feeding larva (Fig. 2B-E), but also meticulously describes conditions for the ovulation, fertilisation and rearing of *Xenopus* in the laboratory. Bles kept the animals in a bell-jar over a Bunsen burner (Fig. 3), a set-up designed by John Samuel Budgett, a 'Balfour Student' at Cambridge (Shipley, 1907; MacLeod, 1994). Bles

brought the *Xenopus* to 22°C several days before coupling, and changed the water just before egg-laying. He found that it helped to allow them to hibernate before attempting to induce egg-laying; apparently, the same female lived for a decade and spawned spontaneously for at least three consecutive years.

Endocrinology and the *Xenopus* pregnancy test

During the early twentieth century, *Xenopus* continued to be imported occasionally for research, and increasingly also for hobby aquaria in Europe. At first it was only among the scientific colonists in South Africa that *Xenopus* was bound up in a more general shift from comparative work, which might require only a few museum specimens, to anatomical and, increasingly, physiological studies which demanded large numbers of animals of the same species (Clarke, 1987). In South Africa the 'Plathander', 'Platanna', or 'Platie' as *Xenopus* was known for short, was a readily available substitute for that 'old martyr of science', the frog (Holmes, 1993). *Xenopus* was used both as a 'type' animal in school and university teaching, and by the 1920s also in physiological research (Dreyer, 1913, p. 341; Zwarenstein *et al.*, 1946, Preface and pp. 2-5; Deuchar, 1975, p. 2). But only from the 1930s did it become a regular inhabitant of European and North American laboratories. The establishment of laboratory colonies of *Xenopus* outside Africa depended on its introduction into endocrinological research and its adoption as the bioassay of choice in the early diagnosis of pregnancy. This depended in turn on the peripatetic career of the left-wing British biologist Lancelot Hogben (Wells, 1978; Werskey, 1978; Hogben and Hogben, 1998).

Hogben (Fig. 4A) was born in 1895 into the poor family of a Methodist evangelist. His mother, grateful for his safe delivery two months premature, pledged that Lancelot would become a medical missionary. But the scientific pursuits she had intended as preparation for this career actually led him to militant atheism and socialism. He won a scholarship to Trinity College, Cambridge, where he studied physiology under such luminaries as Lord Adrian and Sir Joseph Barcroft, and decided to pursue biological research rather than medical practice. In 1917 Hogben married the mathematician Enid Charles, and during the 1920s they had four children. But unlike other socialist scientists with whom in the 1930s they would be linked —J.B.S. Haldane, J.D. Bernal and Joseph Needham— he had no private means with which to support the family, and was forced in search of a higher salary to take a succession of academic jobs. After lecturing at Birkbeck College and then Imperial College, London, he moved to the University of Edinburgh in 1922, initially as deputy to F.A.E. Crew in the newly established Animal Breeding Research Department (later the Institute of Animal Genetics; see Hogben, 1974), and then as a senior lecturer in the Physiology Department. Having moved to McGill University in Canada, he was tempted to South Africa by a well-paid professorship of zoology at the University of Cape Town, and there he moved in 1927.

Most of Hogben's experimental work was in the new field of comparative endocrinology. While in Edinburgh, he had begun studying the physiology of the pituitary gland, using primarily hypophysectomy and the provision of pituitary extracts to investigate the hormonal control of skin colour change in frogs. Once in Cape Town, Hogben took advantage of the local fauna. Following the example of South African physiologists, notably W.A. Jolly, he continued the work, conducted until then on European amphibia, with *Xenopus laevis*. Such 'a godsend' (Hogben and Hogben, 1998, p. 101) did he find it that he named his house after the animal. The Communist printer and editor Eddie Roux recalled that, with the 'brilliant and outrageous' Hogben as host, 'Parties at Xenopus were rarely formal' (quoted in Hogben and Hogben, 1998, p. 215).

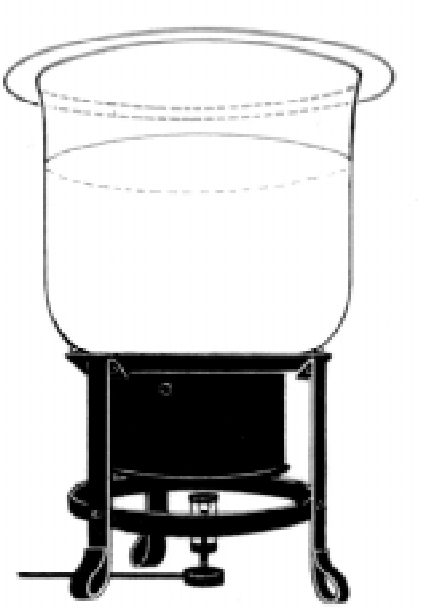

Fig. 3. The 'Tropical Aquarium' devised by J.S. Budgett and used by E.J. Bles (1905) to obtain spawning of *Xenopus laevis*. *A bell-jar of 20 inches diameter is supported on a tripod in contact with a galvanised iron tank heated by a Zeiss micro-burner. Reproduced by permission of the Syndics of Cambridge University Library.*

With David Slome and Enid Charles, Hogben initiated a programme to investigate various physiological changes following removal of the pituitary. On 17 March 1930, he reported to the Royal Society of South Africa that hypophysectomised female *Xenopus* suffered ovarian involution, whilst both implantation of glands and injection of ox anterior pituitary extracts induced ovulation (Hogben, 1930). Removal of the anterior lobe also prevented the animals' characteristic secretion of slime in response to handling. Hogben presented this work as demonstrating more definitively than had been possible in mammals the effect of the pituitary on the ovaries, and generalising it to all land vertebrates (Hogben *et al.*, 1931; on the mammalian studies, see Oudshoorn, 1994). He inspired H. Zwarenstein of the Department of Physiology in Cape Town to come and learn his method of hypophysectomising *Xenopus*, and Zwarenstein and his student H.A. Shapiro began a series of studies following up issues raised by the research of Hogben and his group (Shapiro and Zwarenstein, 1933).

Hogben's communication (1930) would later be taken to have shown in principle that *Xenopus* might be used as an indicator of the presence of gonadotrophins in the urine of pregnant women, but neither this nor the full report (Hogben *et al.*, 1931) mentioned pregnancy testing. He appears initially to have had other priorities, and it was at the outset far from clear that it would prove possible to make *Xenopus* the test animal of choice. But with pregnancy diagnosis a prominent early product of the reproductive sciences (Clarke, 1998), these endocrinologists can hardly have been unaware of the possibility of clinical application. The first reliable laboratory pregnancy test had just been invented in Berlin in 1928 by the gynaecologist Bernhard Zondek and the chemist Selman

Fig. 4. The *Xenopus* pregnancy text. (A) *Lancelot Hogben in 1952, from Hogben and Hogben (1998). Reproduced by permission of The Merlin Press Ltd, Rendlesham, Suffolk, England.* **(B)** *Test jar, from Elkan (1938). Reproduced by permission of the Syndics of Cambridge University Library.*

Aschheim (Oakley, 1984, pp. 96-98), and was then very widely discussed. It involved injecting five immature female mice twice a day for three days with morning urine, and then killing them to see if the ovaries were enlarged and congested. The later Friedman test, which used rabbits, gave quicker results, but demanded young does of which the history was rigorously known. In 1929, Hogben's friend Crew opened a Pregnancy Diagnosis Station associated with his Edinburgh Institute (Crew, 1929; Johnstone, 1929), which by 1935 was performing on mice and rabbits for hospitals and general practitioners about 6000 tests a year (Crew, 1936).

By 1930, perhaps because he was disenchanted with racism in South Africa and concerned about the worsening political climate, Hogben was easily persuaded to take a new chair of social biology at the London School of Economics. There he became a leading critic of the class bias of the eugenics movement and, drawing on his experience in South Africa, a prominent campaigner against scientific racism (Dubow, 1995, pp. 191-195). To continue research in reproductive physiology, he imported *Xenopus* and set up a colony in the basement of his laboratory, a building he described as 'like a delapidated early-nineteenth-century Baptist chapel' (Hogben and Hogben, 1998, p. 121). Hogben recruited Charles Bellerby, who had experience with pituitary extracts, to attempt to establish ovulation of *Xenopus* as a reliable bioassay. This did not simply follow from Hogben's preliminary work; they had to confirm lack of spontaneous ovulation and determine conditions for a reproducible response. The major threat to the test, however, was Zwarenstein and Shapiro's report (1933) of ovarian atrophy in unoperated animals kept in captivity. Bellerby (1933) showed that if instead of keeping the frogs in a cold underground room, he housed them in warm and well lit surroundings he could eliminate the 'captivity effect' and achieve reliability of testing close to 100% (see further Alexander and Bellerby, 1935). (*Rana*, incidentally, was found to be wholly unsuitable.)

In a preliminary report to the Royal Society of South Africa in October 1933, Shapiro and Zwarenstein announced that in the previous month they had successfully used *Xenopus* in 35 pregnancy tests. The following spring *Nature* carried an excerpt from this report (Shapiro and Zwarenstein, 1933) and short papers from both Bellerby (1934) and Shapiro and Zwarenstein (1934) describing this new rapid diagnosis of early pregnancy. Priority for the *Xenopus* test became publicly contested between Hogben and Zwarenstein when in response to Crew's (1939) attachment of Hogben's name to the test, the South African group insisted that they had independently performed it first (Gunn, 1939a,b). Hogben (1939, 1946a,b) claimed that their work derived from his own in South Africa, where he had left members of his laboratory (inconclusively) pursuing a test, and from Bellerby's, about which he said Zwarenstein had learned on a visit to London. He further accused Zwarenstein of delaying things by the 'exploit in defective animal husbandry' (Hogben, 1939, p. 39) which produced the so-called 'captivity effect', suggesting that the test required freshly caught animals and so would be useless outside South Africa. The dispute was never resolved (Shapiro and Zwarenstein, 1946; Zwarenstein, 1985).

Shortly after arriving back in Britain, Hogben had sent Crew some *Xenopus* and encouraged him to investigate their suitability for pregnancy testing (Hogben, 1939). Initially, the Edinburgh Station had difficulty maintaining the frogs, and was perhaps put off by the 'captivity effect', but by 1937 Crew was keen enough to import 1500 animals from the Cape. He kept *Xenopus* by thirties in galvanised metal tanks, transferring frogs overnight into individual glass jars with perforated platforms for the test itself (Fig. 4B). He and others compared *Xenopus*, mouse and rabbit results, and concluded that 'the Hogben test' was quickest and for most purposes the best (Crew, 1939; Landgrebe, 1939). Just one injection of urine containing gonadotrophic hormone into the dorsal lymph sac induced egg-laying 8-12 h later. The Edinburgh laboratory carried out tens of thousands of tests over the next two decades. Other British laboratories offered a similar service, and after the War the frogs were also available for consultation in the basement of the Family Planning Association clinic in Sloane Street, London (Oakley, 1984, p. 97).

Crew encouraged New York gynaecologist Abner Weisman's interest in importing *Xenopus* for pregnancy testing into the United States, and Weisman proved a tireless campaigner for the frog (Weisman and Coates, 1944). Other enthusiasts ensured that by the end of the War there were *Xenopus* colonies in laboratories and clinics all over the world. Breeding in captivity was still regarded as relatively difficult. Shapiro (1935) had established that crude hormone preparations could be used to obtain fertilised eggs and embryos throughout the year. He injected an acid extract of sheep's anterior pituitary or of pregnancy urine into *Xenopus* females and males to induce coupling and production of fertilised eggs outside the normal season, and reared the tadpoles for several months. Landgrebe and Purser (1941) raised adult frogs. Other reports followed (Gasche, 1943; Aronson, 1944), but impor-

Fig. 5. Pieter Nieuwkoop (left) in about 1993. *(Photograph kindly supplied by the Hubrecht Laboratory, Utrecht, The Netherlands).* **Michaïl Fischberg (right) in about 1960.** *(Photographs from the collection of J.B. Gurdon).*

tation from suppliers in South Africa continued to be the rule. Hormone preparations increasingly were obtained commercially, such as 'Pregnyl' from Organon (Oudshoorn, 1994). In the 1960s the Hogben test was replaced by immunological methods, but by this time *Xenopus* was firmly established in biological research.

Xenopus and developmental biology

Pregnancy testing had made *Xenopus* a regular laboratory animal (see e.g. Elkan, 1947), and during and after World War II biological and biomedical scientists in many countries exploited its ready availability (Zwarenstein *et al.,* 1946; Zwarenstein and Burgers, 1955). With thousands of induced *Xenopus* ovulations a month, embryologists moved to break the seasonal rhythm of their research —for 40 years the newt spawning season had deprived Spemann of spring (Mangold, 1942, pp. 390-391). Developmental biologists put *Xenopus* into service for traditional microsurgery, and especially for the biochemical studies of development which demanded large quantities of equivalent biological material and were around this time becoming the cutting edge of the field.

The Utrecht zoologist Pieter D. Nieuwkoop (1917–1996; Fig. 5A), from 1953 to 1984 Director of the Hubrecht Laboratory (Gerhart, 1997), played a key role in making *Xenopus* an effective tool in embryology. Already during the War, he and J.C. van de Kamer (1946) assessed the advantages (and some disadvantages) of *Xenopus laevis* for microsurgery. They highlighted ease of decapsulation of early stages compared to other anurans, favourable tissue consistency for cutting, ready separation of germ layers and excellent survival after operations. They did note that smaller eggs and more rapid development than the commonly used urodeles presented the microsurgeon with difficulties, but concluded that it was 'of utmost importance' to 'have an experimentally easily accessible anuran species at our disposal' (p. 118). Nieuwkoop showed that presumptive mesoderm cells are internal even before gastrulation begins (Nieuwkoop and Florschütz, 1950), and would later stress the difficulty of following the consequently atypical gastrulation and neurulation (Nieuwkoop, 1996).

Since its foundation in 1917 the Hubrecht Laboratory had had an international mission to make vertebrate embryos available to embryologists. Nieuwkoop was keen to revive this tradition, which included producing Normal Tables (Nieuwkoop, 1961), standards of development which play an important role in domesticating animals for the embryological laboratory (Hopwood, 2000). He had shown *Xenopus* to be suitable for experimental work, and was also interested in comparative studies of the 'rather aberrant development of this systematically somewhat isolated species' (Nieuwkoop and Faber, 1956, p. 1). The Greifswald anatomist Karl Peter (1931) had completed Bles's developmental series from the British zoologist's material, and shortly after the introduction of the *Xenopus* pregnancy test into North America, Paul Weisz (1945) of McGill University had produced a brief series of normal stages. But in the early 1950s Nieuwkoop extended his preliminary work on the early development (Nieuwkoop and Florschütz, 1950) by organizing with Job Faber an international collaboration to create the lavish and very substantial *Normal Table of* Xenopus laevis *(Daudin)* (Nieuwkoop and Faber, 1956), which soon became definitive and has been much reprinted since.

By the early 1950s, developmental biological work on *Xenopus* was already appearing from laboratories in various countries, especially Britain. Some of the first experimental studies were by David Newth (1948, 1949), then working in London. In Edinburgh, C.H. Waddington had taken over Crew's pregnancy diagnosis operation, and biochemically oriented studies on *Xenopus* soon began to appear from the Institute of Animal Genetics (Deuchar, 1956; Curtis, 1957, 1958). Both Elizabeth Deuchar (1975, p.v) and Adam Curtis (letter to J.B.G., 21 October 1996) recalled the limited breeding season of the local newts as the main reason for turning to the Pregnancy Diagnosis Laboratory for X*enopus*, which Curtis

(1960) continued to use for cortical grafting at University College London.

The widespread availability of *Xenopus* in laboratories and the ability reliably to obtain eggs in all seasons may sufficiently explain its increasing employment in developmental biology. However, its dominance over other amphibia was ensured by scientists with primarily biochemical, cellular, and/or genetic interests who in the 1960s were increasingly entering the field (Oppenheimer, 1966). Particularly important in attracting such people was the Oxford laboratory of Michaïl Fischberg (1918–1988; Fig. 5B). Born in St Petersburg, he was brought up by an aunt in Switzerland and studied zoology in Zürich, where he remained for a doctorate on newt heteroploidy under the supervision of Ernst Hadorn (Gloor and Gurdon, 1989). Coming to Britain in 1948, he took a post in Waddington's institute and experimented on mouse embryos. Appointed to a lectureship in the Department of Zoology in Oxford in 1951, he established a breeding colony of *Xenopus*. Ten years later he left for Geneva, where he and his group concentrated on collecting new mutations in *Xenopus laevis* and on describing new species collected on expeditions to Africa (see Tinsley and Kobel, 1996). In Oxford, Fischberg had the wisdom to isolate a 1-nucleolated frog that had turned up by chance in his laboratory (Elsdale *et al*., 1958); he and colleagues took advantage of the relatively short life cycle and convenient laboratory maintenance of *Xenopus* to generate the homozygous O-nu from the heterozygous 1-nu originally discovered. This provided an invaluable genetic marker which was immediately deployed in nuclear transplantation (Fischberg *et al*., 1958; Gurdon *et al*., 1958), and was also used for ingenious germ-cell transplantations (Blackler and Fischberg, 1961). Especially the serial nuclear transfer work required an animal that could be made to breed almost on demand.

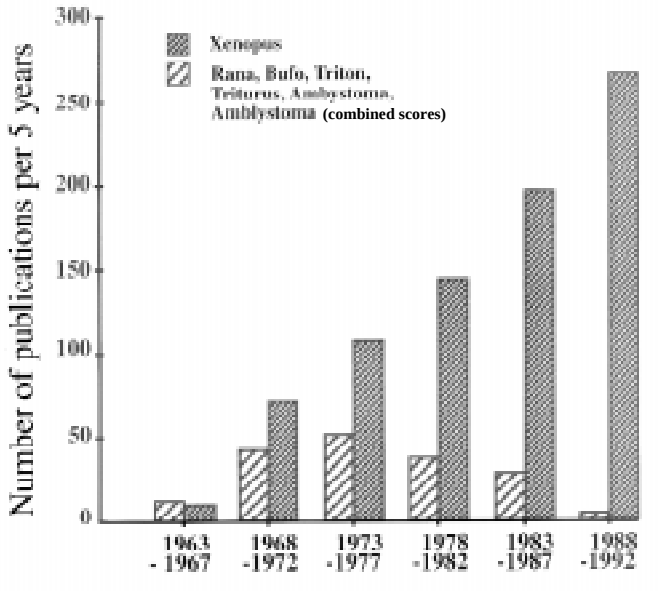

Fig. 6. Increase in developmental biology publications on *Xenopus* compared with other Amphibia. *Data were retrieved from Advanced Medline Search by scoring those titles in* Journal of Embryology and experimental Morphology *(subsequently Development) and* Developmental Biology *that include* Xenopus *or (for other Amphibia)* Rana, Bufo, Triturus (Triton*), or* Amblystoma (Ambystoma).

It was above all this mutation which attracted biochemists, luring them to use *Xenopus* to study gene activity in early development. Far from Nieuwkoop's continuing interest in the diversity of vertebrate embryogenesis, for them *Xenopus* was a convenient species they could use throughout the year to establish principles they expected would be universal. When still a medical student at the University of Chicago, Donald D. Brown had chosen embryology, of which he then knew only that "it was a field so primitive", in his view, "that no modern research was being done in it. And yet it had this huge, incredible problem —how an egg develops into a multicelled organism" (Brown, 1988). He took degrees in medicine and biochemistry, and spent a year at the Pasteur Institute, before starting biochemical work on *Rana pipiens* at the Carnegie Institution's Department of Embryology in Baltimore. In 1962 he met Fischberg's student John Gurdon, who was travelling around the United States in search of a job, and they began to collaborate on the biochemistry of the O-nu mutant. Gurdon, having gained a position in Oxford, sent material to Baltimore. Brown and Gurdon (1964) showed that O-nu embryos fail to synthesize ribosomal RNA, and so established that such a mutation could be used for molecular analysis of gene function.

The mutant also provided material for the DPhil. thesis which Hugh Wallace did with Fischberg, before moving to Edinburgh, where he collaborated with Max Birnstiel (until that time a plant biochemist) to show by direct gene separation on caesium chloride gradients that it lacked ribosomal genes (Wallace and Birnstiel, 1966). By this time, Brown had switched entirely from *Rana* to *Xenopus* (e.g. Brown and Littna, 1964), and this stimulated Igor Dawid, a newly appointed member of the Baltimore Department, who had previously been in yeast biochemistry, to produce a major paper on *Xenopus* mitochondrial DNA (Dawid, 1965). Gurdon, Brown, Dawid and others had already established independent laboratories in the early 1960s, and the suitability of *Xenopus* for the combination of experimental embryological methods with molecular and genetic techniques was by then very clear. Use of *Xenopus* as a laboratory organism rose dramatically from that time (Fig. 6).

Conclusion

Xenopus laevis was made known to nineteenth-century science through European anatomical and natural historical investigations of imperial fauna. Used by South African scientists, but only sporadically in Europe, Hogben's endocrinological research introduced *Xenopus* into the physiological mainstream. Historians of biology stress that laboratory animals are not just found, they are made -often literally by constructing stocks, but also by establishing the material and social conditions for successful husbandry, and by matching the characteristics of an animal to tasks it might perform well (Clause, 1993; Kohler, 1994; Keller, 1996; de Chadarevian, 1998). In the case of *Xenopus* and embryology, quite a bit of the work of domestication had already been done before developmental biologists appeared on the scene. Its adoption depended above all on endocrinologists' having shown how to maintain the animal in captivity and, by injecting commercial mammalian hormone, to make it lay eggs. In the 1940s and '50s medical demand for pregnancy testing made *Xenopus* very widely available in European and North American laboratories, and it needed relatively little further adaptation for developmental biol-

ogy. Microsurgery and embryo culture, already perfected for other amphibians, were extended to *Xenopus*. It proved less convenient than urodeles for certain embryological manipulations, but these were not then a priority. Wild-type frogs could be bred or imported directly from South African farms, and maintaining the anucleolate mutant in the laboratory worked well. But *Xenopus* claimed the mantle of experimental embryology once oriented primarily towards urodeles because it became generally obtainable just before the field reoriented around cellular and biochemical approaches which intensified demand for large quantities of embryos all year round. The O-nu mutant notwithstanding, *Xenopus laevis* has not become an effective genetic organism, and the future may see increasing interest in *Xenopus tropicalis*, as a diploid rather than tetraploid species suitable for transgenesis (Amaya *et al.*, 1998). Molecular methods may open up study of amphibian diversity (Malacinski and Duhon, 1996; Elinson, 1997), but *Xenopus* will surely retain an important place in twenty-first-century developmental biology. Whereas once the occasional specimen had been valued as an 'aberrant' amphibian, in the 1950s and '60s the eggs from hundreds of frogs were used to initiate the molecular analysis of early animal development.

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