Ursula Mittwoch – pioneering geneticist who solved the riddle of sexes

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Picture a tidy, petite, middle-aged lady in Florence in the 1970s, brandishing calipers and marching insouciantly up to a renaissance sculpture to make her measurements. What on earth did she want to know, you may be wondering. Scrotal asymmetry is the answer. Did the great artists, going back to the ancient Greeks, know that the right testis is slightly larger than the left? They did know some surprising things – the right ball is higher than the left, for example – but this particular gem eluded them. It didn't elude Professor Ursula Mittwoch. She saw it as the exception that proved the rule – not just any old rule, but one of the deepest and most mysterious rules in all of life, the distinction between the sexes. Male development is determined by a higher metabolic rate, she realized, and the elusive role of sex-determining genes was betrayed by intersexual states: true hermaphrodites have an ovary and a testis, and in two thirds of cases the testis develops on the right-hand side (in humans, at least). In men generally, the right-hand testis is around 5–10 per cent larger. What kind of genes would arrange that?

Ursula Mittwoch was an unusual geneticist who forged her reputation working with Lionel Penrose on chromosomal disorders in the early days of cytogenetics, yet who declared later that she "*didn't much like chromosomes*". She became Professor of Genetics at UCL, yet often seemed suspicious of genes. In truth, she blazed her own luminous path through the second half of the 20th century. Her views were at odds with genetic determinism in its widest sense, for she was more interested in the subtle interplay between genes and environment – 'genetic indeterminism'. Despite more than 200 publications, including 14 *Nature* papers through the 1960s alone, many as a single author, her work perhaps did not receive the recognition it deserved, at least among geneticists; but her bold insights into the sexes were appreciated by the media and she wrote regularly (often with a waspish sense of humour) for magazines like *New Scientist*. In fact, her thinking was decades ahead of its time, more in tune with the renewed interest in epigenetics of recent years. Today, when so many people rebel against a binary definition of sexes, Ursula's work shows that sex determination is far from a simple switch governed by Mendelian genes but reflects a 'threshold dichotomy' – in effect, a quantitative rather than a qualitative difference. To be sure, our genes stack the quantitative odds towards one sex or another, yet the spectrum in between leaves plenty of scope for intersexuality.

Ursula was born in Germany in 1924. Her father was professor of oriental languages at what is now Humboldt University, but was forced to retire in 1933, the document being signed by both Hitler and Göring. Her mother had qualified in medicine but did not practice. The family remained in Germany until the horrors of 'Kristallnacht' in 1938 persuaded them to flee to London, where they arrived in April 1939. Ursula attended schools in Brighton and London, broken by a 9-week spell, separated from her parents, interned on the Isle of Man (having just turned 16) when Germany invaded Holland, Belgium and France in April 1940. By the time she left school at the age of 19 in 1943, wartime regulations meant she was too old to apply to university. Instead she worked at the John Innes Horticultural Institution, then near Wimbledon, as technical assistant to Kenneth Mather. Experiments "*assessing the yield of tomatoes grown out of doors were probably most germane to the war effort*", she wrote (Mittwoch, 1995), but her work ranged from breeding primrose and snapdragons to the genetics of bristle number in *Drosophila*, with much time spent computing on a noisy Monroe calculating machine. She greatly enjoyed her time at John Innes, her daughter Caroline Springer recalls, who attributed her green fingers ("*she could grow any plant from seed and revive any dying plant*") and her skill in planning experiments to her time at John Innes.

During these years, Ursula attended evening classes in botany and chemistry, eventually graduating after the war in 1947. Seeing a chance to make up for her 'lost education' (having left school at 14 in Germany), she applied to UCL to do a PhD in genetics, with mycology as second choice. She seemed little daunted by her interview with JBS Haldane, Lionel Penrose and Hans Kalmus, recalling to Peter Harper (Harper, 2004), *"I rather felt they were all talking at cross-purposes, but anyway Penrose said yes he had got room. I mean, he had only just started up, but I think neither he nor Kalmus knew anything about PhD students. And Haldane said, 'well Miss Mittwoch first has to graduate,' which I don't think occurred to them particularly."* She became the first PhD student in the old Galton Laboratory, was given a desk and a waste paper basket, and told 'well, do research.' She had 'very little supervision', apart from a few short courses on fungal genetics, though one suspects she would not readily have brooked being told what to do.

Clearly Mittwoch had impressed Lionel Penrose, who asked her to stay on after completing her PhD. She confided to Harper that Hans Grünberg advised her against it. *"He said, 'you know the professor doesn't know anything about chromosomes.' But I thought, if Professor Penrose asks me to stay on then he must think I am good, which was a mistake to think that, I think, but he obviously thought I would be useful."* And so it was that she began her research on Down syndrome in the early 1950s,

first showing it was not a haploid condition, as had been thought until then (Mittwoch, 1954), before going on to study other chromosomal anomalies, notably Klinefelter (XXY) and Turner (single X) syndromes. These in turn prompted her to think about gene dosage, especially in relation to the sex chromosomes. She became interested in Barr bodies (inactivated X chromosomes, condensed as heterochromatin in cells with multiple X chromosomes during early development) as well as 'drumsticks', the tell-tale inactivated X in polymorphonuclear leukocytes, which could be identified in normal female blood films, as well as in those with atypical numbers of sex chromosomes, such as XXX, XXY, XXXY.

Ursula Mittwoch, front centre in black, at the age of 26 in 1950, beneath the Portico of UCL

Most notably, in light of her later research, Ursula began to think about turnover, and especially the rate of change in markers of the cell cycle over time. She initially found that alkaline phosphatase levels were raised in both Down and Klinefelter syndromes, suggesting there may be disturbance in leukocyte turnover in both syndromes (Weber et al., 1965). Feulgen-stained nuclei indicated that DNA synthesis was a little slower in people with Down syndrome. With David Wilkie and David Kirk, she found that anti-mitochondrial drugs, notably chloramphenicol (which inhibits mitochondrial protein synthesis) and chlorimipramine (an inhibitor of complex III activity) slowed the mitotic cycle. They concluded that synthesis of the respiratory chain is a prerequisite for normal cell division, in fibroblasts at least (Mittwoch et al., 1974). All this led to the idea that growth rates mattered, and

that mitochondria can determine growth rates, setting the stage for her great insights into sex determination.

But the key insights did not materialise immediately. First the problem had to become more explicit. The necessary perspective developed while writing a book on sex chromosomes, published in 1967 (Mittwoch, 1967). *"There were lots of discoveries about sex chromatin and X inactivation, and all the sex chromosomes in different animals. But I felt that in a book on sex chromosomes, one should also say something about the role of sex chromosomes in sex determination … I spent many hours in the library, and I saw what had been written wasn't very convincing."* By then, her thinking had been shaped by the quantitative approach to genetics of the old Galton laboratory. One example that clearly impressed her was Penrose's observation that the total ridge count on fingerprints depended on the number of X or Y chromosomes – the more sex chromosomes, the fewer the ridges, despite the genes involved apparently not being on the sex chromosomes at all. For Ursula, genes often acted obliquely and the best way to measure their combined effects was through quantitative measurements.

These ideas on gene dosage, quantitative cytogenetics and growth rates were the foment that led to Ursula's classic *Nature* paper in 1969, entitled 'Do genes determine sex?' (Mittwoch, 1969). When asked years later by Harper which work gave her the most satisfaction, she wondered *"whether this is the best paper, the best work, I have ever done"* (Harper, 2004). It certainly set the agenda for her next three decades of research. The hypothesis was fully formed, if still lacking direct evidence. She postulated that the Y chromosome induces a burst of mitoses at a critical time and place in development, pushing the indifferent gonad to develop into a testis. Failing this burst of mitoses, the gonad would become an ovary, explaining why the testis differentiates before the ovary. Drawing on Grünberg, she wrote: *"It would seem that the sex difference is an outstanding example of quasi-continuous variation: that is, a difference with an obvious quantitative basis and a threshold effect which usually gives rise to two separate classes, but where in a small proportion of cases intermediate types appear."* Citing EB Wilson, she ended by observing that nature offers a series of experiments carried out on a grand scale for testing the basis of heredity. That wider perspective became an inexhaustible fount of inspiration.

How Ursula set about unravelling nature's 'experiments' illustrates the clarity of her quantitative thinking. Still suspicious that genes on the Y chromosome alone determine sex, she turned to the lateral asymmetry found in birds and mammals. In birds, where females are the heterogametic sex,

the ovaries usually develop only on the left-hand side, whereas the right-hand gonad becomes a nonfunctioning testis. She observed that this relationship was mirrored in human hermaphrodites who have an ovary and a testis – again, the ovary is more likely to develop on the left-hand-side, and the testis on the right, regardless of the chromosomal constitution, a conundrum she later encapsulated vividly as 'a genotype-phenotype mismatch in need of attention'. Some gonadal tumours are also more likely to develop on the right side. In another classic *Nature* paper from 1975, with David Kirk, she asked whether mammalian gonads exhibit an inherent but hitherto undetected asymmetry (Mittwoch and Kirk, 1975). The answer was unambiguous in both sexes, with the difference even more pronounced in females. When judged by fresh weight, total protein or total DNA content, not only was the right ball bigger, but the right-hand ovary was up to a fifth larger during female development.

Ursula Mittwoch in 1964, at the outset of her work on sex determination

These observations prompted a short comment from Chris McManus on his own measurements of gonadal asymmetries in renaissance sculptures (McManus, 1976), a study that earned him an Ig Nobel prize in 2002. In the flurry of predictable banter that followed, little mention was made of the profound insight that had underpinned the original study – at some critical stage of development, growth rates are faster on the right-hand side, which drives male differentiation by way of a threshold effect, regardless of any sex-determining genes. The same sex chromosomes are present or absent on both sides of the body. This difference in gonadal growth rate is not reflected in organs developing later such as kidneys (Mittwoch and Mahadevaiah 1980). Far from being pointless knowledge, Ursula was thinking widely across nature and linked sex-determination with growth rates more generally, most obviously with temperature-dependent sex determination in amphibians and reptiles. Her thinking made sense of the outrageous cacophony of different mechanisms of sex determination across life, ranging from strict environmental cues, such as temperature, to non-homologous chromosomal systems such as stacks of X and Y chromosomes in platypus, ZW chromosomes in birds and some insects, and the feminization of toads by pesticides. Unlike the deep conservation of genes involved in specifying body plans (notably Hox or Pax genes) there seemed neither rhyme nor reason to shaping sexual body plans. Offering a unifying principle, Ursula saw the sex chromosomes as an outstanding example of Waddington's concept of 'genetic assimilation', where phenotypic effects originally induced by environmental stimuli (temperature in this case) are taken over by the genetic material, so that "*differences in chromosomal constitutions may be traced back to differences in rates of cell proliferation*" Mittwoch, 1971).

But why would males grow faster? Is it always so? Males are clearly not always larger; for example, in many birds of prey, the female is larger. The answer turned out to be subtle. Ursula had a simple case for eutherian mammals: in the womb males develop in a 'sea of female hormones'. If they are not to succumb to these feminizing surroundings, males need to develop testes and start producing sex hormones such as testosterone swiftly. Marsupials were then an interesting test case for Ursula, as they escape from the sea of hormones much earlier in development. She found that males do still develop faster in marsupials – but the difference is much less marked than in eutherians (Mittwoch et al, 1993). And birds were even more interesting. Insulated by the shell, Ursula argued, chick embryos are not subject to the same developmental constraints to become male; instead, the growth of the ovaries duly outstrips the testes. Yet characteristically, she did not take this finding at face value, and made meticulous quantitative measurements of growth rates. For the first week of incubation, she found that the testes still grow faster than the ovaries; only after that does ovarian development outstrip testicular (Mittwoch et al., 1971). The reason was still far from clear, but by

the 1980s Ursula was beginning to wonder if "*the initial dominance of the male gonad is a widespread phenomenon in the sexual development of vertebrates*." She took a step closer to an answer in 1987, discovering a maternal effect on testis size in mice born from crosses between inbred lines, in a paper with Suzan Hunt that is remarkable for its acknowledgement of two distinguished statistical geneticists, Cedric Smith and Nick Barton, for their maximum likelihood analyses. *"It is tempting to speculate that the maternal effect might be due to a difference in mitochondria,"* they wrote (Hunt and Mittwoch, 1987), noting that another UCL colleague, David Wilkie, had recently marshalled a case that mitochondria can drive the growth of cancer cells, by modulating nuclear genes. Here was the nub of an idea: males initially grow faster, and maternally inherited mitochondria influence growth rate.

By her retirement in 1989, Ursula Mittwoch had enjoyed a long and distinguished career. She had made arguably the most coherent explanation of sex determination, perhaps the most emblematic problem in all biology. While scientifically something of a loner, rarely collaborating with more than one or two others at once, she had worked with some of the most illustrious biologists of the 20th century. She was supported throughout by her husband, Bernard Springer, who met Ursula soon after she had completed her PhD. A surveyor rather than a scientist, he always admired her work, reading all her papers and books, and sometimes even typing them out. His support was vital for Ursula continuing to work while raising a child. Their daughter Caroline Springer, now Director of Drug Discovery at the CRUK Manchester Institute, recalls frequent dinner parties with UCL colleagues, especially the more junior ones. Her mother was "*a great cook, marrying fresh ingredients with traditional German and Italian cooking*." She went out of her way to help others. Caroline has fond memories of the brilliant but tormented geneticist George Price, who stayed with the family for a while after he lost his flat, and would chat over the various texts for English O' level with her. Ursula later kept his suicide from her daughter. A university friend of Caroline's, whose father had refused to pay his student grant, practically lived with them for three years. A former technician recalls the much-valued opportunity Ursula gave her to study for a PhD, allowing her to stay in the field of sex chromosomes biology. Their family life was enriched by many pets, Caroline recalls. "*She was brilliant with animals. I grew up with multiple cats. My parents started with one, but others came in through the cat flap and refused to leave no matter how many times my father returned them to our neighbours. We also had rabbits, guinea pigs, terrapins, silkworms and stick insects. The latter used to escape and hide in the green Liberty print curtains, landing on our heads when we drew them or pulled them open too quickly*."

But none of this could replace the science. Just when Ursula was starting to grapple with a universal explanation for the sexes, linked with growth rates, she had been legally obliged to retire. She railed in *New Scientist* that "*discrimination is alive and well and living in the laboratory"*; prejudice against age. The argument that it is necessary to make room the next generation had previously been used to discriminate against women and foreigners, she observed (in that case taking jobs from working-class men). She didn't even deign to comment on that other hoary old chestnut, that younger people are more productive, perhaps because she was about to embark on an extraordinarily productive retirement. Granted a little lab space by Queen Mary and Westfield College (before later returning to UCL as Professor Emeritus), Ursula published more than 40 papers in the first two decades of her 'retirement'. Some were among her most important.

One wonders if Ursula was goaded by the identification of the famous SRY (sex-determining region Y) gene on the Y chromosome in the early 1990s. Ostensibly responsible for male sex determination, and taught in most textbooks, this gene was the apotheosis of the molecular-biology approach that Ursula had long criticised. She came out with guns blazing in a 1992 paper, listing six observed facts in the abstract alone that ran counter to the hypothesis that a gene with dominant effect (SRY) was responsible for testis development (Mittwoch, 1992). With a pleasing knack of capturing complex ideas in a few well-chosen words (for which she prided herself, having learnt English only from the age of 16) she later framed this as 'Sex is a threshold dichotomy mimicking a single gene effect.' In yet another *Nature* comment from 1990, she wrote that *"The Y chromosome functions to minimize the chance element in XY individuals and to maximize the probability of their developing as males … I believe that all the Y chromosomal DNA sequences concerned with 'testis determination' act by enhancing the growth of the relevant gonadal cells, thus making the probability of testis development a near certainty"* (Mittwoch, 1990). The idea certainly appealed to evolutionary biologists. Laurence Hurst used her thinking as the basis for papers on the Y as an 'attractor for selfish growth factors' which did not need to evolve a means to ensure correct parent-dependent expression rules (Hurst, 1994). Andrew Pomiankowski pointed out that imprinting of the paternal X chromosome (switched off in daughters before random X-inactivation) would also tend to promote male growth relative to females (Iwasa and Pomiankowski, 1999). All of this was a far cry from the simplistic notion of a dominant SRY gene determining male development. Yet none of it could quite explain *why* males grow faster.

That crucial insight came from Ursula herself, in what was probably her most important paper for a modern audience, published in 2004 when she was eighty (Mittwoch, 2004). Once again, her title

nailed the argument: *'The elusive action of sex-determining genes: mitochondria to the rescue?'* This was the second paper from a lifetime's work that Ursula singled out to Peter Harper (Harper, 2004). While it might sound like a simple extension of her work on growth rate, it actually provided the key insight. Mitochondria are needed for growth, but are inherited from the mother. Males do not face the same constraints during development, as their mitochondria are a genetic dead end. That gives rise to an evolutionary tension. As Ursula put it, *"Male sex is determined by nuclear genes inherited from the father regulating the activity of maternally derived mitochondria."* This framing is far from the Mendelian paradigm that guided the crowning glories of molecular biology, but resonates with more recent developments in epigenetics, systems biology and evolutionary biology. While Ursula's body of work might now seem a touch dated, involving quantitative measurements of weight, DNA and protein content, she uniquely saw beyond the Mendelian paradigm. Her insights into one of the deepest questions in biology were essentially correct and deserve celebrating. More than that, her brave and honest tenacity in seeking the truth, always polite, precise and reasoned in the face of an establishment that was mostly looking the other way, is an example to us all. She was an exemplary scientist.

Ursula Mittwoch being presented with the painting Mitochondria in Action by Odra Noel, to celebrate her 90th birthday. With (from left) Profs John Allen, Sue Povey, Dallas Swallow and the author, in the Housman Room at UCL in 2014

Like the best scientists, Ursula's ideas reached beyond science, linking myths and history with deep human preoccupations. Commenting on the timing of 'ensoulment' – the gradual 'becoming' of a

human person – Ursula called attention to the 'exotic error' of Aristotle and Thomas Aquinas: the notion that ensoulment occurs gradually during embryogenesis, and is complete at 40 days in male embryos, compared with 90 days in female embryos (Mittwoch, 1995). '*The apparent bizarreness of this idea'*, she said, '*owes less to embryology than to the extreme reluctance of most present-day molecular biologists to take on board quantitative, as opposed to histological and biochemical aspects of development, and to consider the importance of time*'. The faster metabolic rate of human males could explain why men have a shorter life expectancy than women, Ursula observed wryly, so '*even if female ensoulment is delayed for 7 weeks, women would seem to be amply recompensed by the expectation that the reverse process of the soul's departure will be delayed by at least 5 years*.' In her own case, we can be grateful it was delayed much longer than that, for Ursula died in 2021 at the age of 97. She had been active, sociable, and intellectually engaged to the end. Even at the age of 97 with her failing memory, Caroline recalls, she read textbooks on brain wiring and Mendel. Few lives were better spent.

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References

Harper, P. (2004). Interview with Professor Ursula Mittwoch, 2004. Available here: https://genmedhist.eshg.org/interviews/recorded-interviews/.

Hunt, S.E., Mittwoch, U. (1987). Y-chromosomal and other factors in the development of testis size in mice. Genetic Research, **50**, 205– 211.

Hurst, L. (1994). Embryonic growth and the evolution of the mammalian Y chromosome. I. The Y as an attractor for selfish growth factors. *Heredity*, **73**, 223– 232.

Iwasa, Y., Pomiankowski, A. (1999). Sex specific X chromosome expression caused by genomic imprinting. *Journal of Theoretical Biology*, **197**, 487– 95.

McManus, I.C. (1976). Scrotal asymmetry in man and in ancient sculpture. *Nature*, **259**, 426.

Mittwoch, U. (1954). The chromosome compliment in a Mongolian imbecile. *Annals of Eugenics*, **17**, 37.

Mittwoch, U. (1967). *Sex Chromosomes*, New York, Academic Press.

Mittwoch, U. (1969). Do genes determine sex? *Nature*, **221**. 446– 448.

Mittwoch, U., Kirk, D.J. (1975). Superior growth of the right gonad in human foetuses. *Nature*, **257**, 791– 792.

Mittwoch, U. (1971). Sex determination in birds and mammals. *Nature*, **231**, 432– 434.

Mittwoch, U. (1990), Sex, growth and chance. *Nature*, **344**, 389– 390.

Mittwoch, U. (1992). Sex determination and sex reversal: genotype, phenotype, dogma and semantics. *Human Genetics*, **89**, 467– 479.

Mittwoch, U., Burgess, A.M.C., Baker, P.J. (1993) Male Sexual development in 'a sea of oestrogen'. *Lancet*, **342**, 123– 124.

Mittwoch, U. (1995). Living history – biography. *American Journal of Medical Genetics*, **55**, 3– 11.

Mittwoch, U. (1995). Female ensoulment: late but durable. *Nature*, **373**, 379.

Mittwoch, U. (2004). The elusive action of sex-determining genes: *mitochondria to the rescue? Journal of Theoretical Biology*, **228**, 359– 365.

Mittwoch, U., Mahadevaiah, S. (1980). Comparison of development of human fetal gonads and kidneys. *Journal of Reproduction and Fertility*, **58**, 463– 479.

Mittwoch, U., Kirk, D.J., Wilkie, D. (1974). Effects of mitochondrial inhibition by chloramphenicol on the mitotic cycle in human cell cultures. *Journal of Medical Genetics*, **11**, 260– 266.

Mittwoch, U., Narayanan, T.L., Delhanty, J.D.A., Smith, C.A.B. (1971). Gonadal growth in chick embryos. *Nature New Biology*, **231**, 197– 200.

Weber, W.W., Mittwoch, U., Delhanty, J.D.A. (1965). Leucocyte alkaline phosphatase in *Klinefelter's syndrome*, *Journal of Medical Genetics*, **2**, 112– 115.