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PERSPECTIVE AND REFLECTION ARTICLE

The life and times of Ferruccio Ritossa

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Fig. 1 "Hen That Started Thinking," Self-Portrait, Bronze, Ferruccio Ritossa

Se hai la foto di un evento e ogni tanto la vai a sbirciare,
dopo un po' vedrai che l'evento fotografato,
se qualche volta va a spasso nel tuo cervello,
si modifica
e qualche volta se ne è addirittura andato…….

If you have the picture of an event
and from time to time you glance at it,
after a while you will notice that the photographed event,
if it sometimes goes for a stroll in your brain,
it changes
and sometimes it has even gone away........

Abstract Ferruccio Ritossa wrote these lines only a few months before he died, as a preface to a book he wanted to write and that, unfortunately, we will never be able to read. It was to be the story of his life, an amazing story indeed. With this article, we want to take a picture of Ferruccio's life, a mosaic of events, facts, ideas, hopes, and memories linked in a way that they will not go away, even after "a stroll in our brain."

Ferruccio Ritossa died this year on January 9th at the age of 77. He was born on February 26th, 1936, in Pinguente, a small town in Istria. This region had a complex history in the XX century: part of the Austro-Hungarian Empire until WWI, an Italian province in the Interwar period, controlled by Yugoslavia after WWII, and now in Slovenia. Ferruccio's geographical origins strongly affected his early years. After 1943 and the fall of the Fascist regime, thousands of families chose or were forced to leave their homes in Istria. Many Italians (estimates vary greatly, from a few hundred to tens of thousands)—mostly outspoken fascists and military officers, but also ordinary people, including women, children, and elderly people—were murdered or died in concentration camps. Their corpses were thrown in the so-called foibe, deep sinkholes (up to 200-m deep) typical of the north-east Italian-Yugoslavian area. Ferruccio's father, a butcher, was one of the

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victims; he was killed when Ferruccio was 8 years old. We can only imagine the impact of these events on Ferruccio's personality and development. In the first page of his unfinished book, he writes the following:

> *'Lo dico qui e adesso*, *e non lo dirò più: io so che la cattiveria dell'uomo cresce piano*, *piano*, *e non ci sono scherzi e magari non è apparente'*, 'I am going to say it here and now, and I will never say it again: I know that the evil of man grows slowly, very slowly, and there is no joking about it and perhaps it is not even perceptible'.

A few months before his father died, Ferruccio's mother, a school teacher, decided to move with the three children to Italy, first to Trieste and later to Monfalcone. A few years later, they made their way to Pesaro on the eastern shore of Italy. For some years, the family lived in an orphanage, where the mother taught the pupils in the institution and the three boys lived in the boarding school. Thanks to the efforts of their strong mother and the opportunities offered by the charity, all the boys managed eventually to get a proper education, reaching the university level. "I have always been poor and studious," Ferruccio defines himself in his book.

Ferruccio proved to be a brilliant student, and in his early 20s, he graduated in Agricultural Sciences from the University of Bologna (1958). He recalled that genetics at the time was not a fully established part of the curriculum: the double helix structure of the DNA had just been discovered. However, only a faint echo of this discovery was heard in Bologna, for that faculty was mainly oriented to practical study and did not emphasize the theoretical and biophysical approaches developed abroad. As a matter of fact, Italy was still struggling to close the gap with other countries, and only a few geneticists were fully aware of what was happening in the USA or in the UK (Capocci and Corbellini 2002; Cassata 2013). Italian genetics, well through the 1940s, was mostly applied genetics, the study of useful variations in plant and animal breeding (Volpone 2008). Physical, chemical, and mathematical studies of evolution and genetics by means of the Drosophila model were mostly unheard of within the Italian life science landscape and even more so in a "technical" curriculum. Ferruccio recalled in a personal conversation: "DNA simply wasn't there. Maybe it was there for the really good ones, surely it was not there for the student." Yet, the young Ferruccio became acquainted with the chromosomal theory of heredity and was willing to work on the Drosophila polytene chromosomes for his final dissertation. However, no fruit flies were available in the laboratories of Bologna at the time, so Ritossa searched for the same giant chromosomes in bees: plenty of them were around for research purposes because of the economic interest in honey production. This early interest in genetics led Ritossa to the encounter that changed his life and career when he met Adriano Buzzati-Traverso, 20 years his senior. Buzzati-Traverso was one of a handful of genetics professors in 1950s Italy. Before WWII, he travelled to the USA, getting in touch with population genetics and Drosophila research, whereas a stretch of time spent in Berlin working with Nikolaj Timofeef-Ressovsky acquainted him with biophysics and radiogenetics. After WWII, he made a huge effort to introduce in Italy the new approach to the study of living matter—molecular biology and genetics, exploiting his international connections and with a strict cooperation with several physicists. In the early 1950s, he managed to create a Drosophila-type culture collection at the University of Pavia (where he had been professor of Genetics since 1948). He let statistics and physics sneak into the life science degree curriculum, making that university the main center for the study of the "new" genetics in Italy.

The help of Italian physicists (an important and respected scientific community, both nationally and internationally, and well-endowed with governmental funds) and international networking were central for further developing Buzzati-Traverso's plans. At the end of the 1950s, with funding coming from the Italian nuclear energy agency and the Rockefeller Foundation (plus a series of Italian companies and banks), he managed to set up a 2-year course on the "biological effects of ionizing radiations"—a sort of postgraduate school in molecular biology. Ferruccio Ritossa applied to join this special opportunity in the second year of the course (1959–1961), after a friend brought it to his attention. The courses in Pavia were something completely new with respect to the Italian landscape: the focus was on biophysics and the emerging molecular biology, and it comprised a large amount of laboratory work in the second year, with close tutoring for students, several international guests lecturing on novel methods and techniques, and the possibility to spend a semester abroad to improve English and expose the students to the frontiers of research. As a matter of fact, the courses provided the backbone for the later development of genetics in Italy, as nearly all the university professors in genetics in the next decades were part of this group of students.

As Ritossa himself recalled, "the staff were terrific," and greatly helped the young students in choosing their own research. As a matter of fact, Ferruccio turned to Drosophila as a second choice: he originally wanted to work on plants, but he didn't fare well with the plant genetics professor, whose approach was more old-fashioned biometrics than genetics. He thus turned towards Drosophila, Buzzati's favorite subject, and whose research was definitely closer to Ferruccio's interest in the new approach to life sciences.

The evolution of the Pavia courses was the creation of the International Laboratory of Genetics and Biophysics (ILGB) in Naples, a research center at the forefront of genetics and molecular biology for nearly a decade. Buzzati founded the new institution and brought in some of the most promising students from the courses in Pavia: Ritossa was among them.

And, this is why the seminal paper (Ritossa 1962) about the heat shock response written in early 1962 bore the ILGB institutional affiliation. Yet, the breakthrough actually happened in Pavia. Ritossa already wrote about the fortuitous context of the discovery (Ritossa 1996). His work, initially aimed at discerning if the polytene chromosomes in Drosophila were producing DNA or RNA, ended up by the intervention of fate in the hand of some unknown collaborator in unveiling the first known environmental stress acting directly on gene activity. The chromosomal "puff" was at the time used to reveal the genome regions with the most intense transcription, and Ritossa managed to produce a stable "new puffing pattern"—that is, induce activation of specific DNA regions—just by switching temperature during larval development. The same effect was also obtained by chemical shock, applying 2-4 dinitrophenol and salicilate to salivary gland cells whose metabolic activity was maintained under oil drops, a method devised by Ferruccio himself. With the benefit of hindsight, the results showed the existence of a widespread mechanism of response to environmental stress, whether thermally or chemically induced. As we know, *Nature* refused the very short paper submitted by Ritossa, for a lack of biological interest (Ritossa 2009), and finally, it was published in a lesser known Swiss journal. This and the sense we now have of a paper well ahead of its time may explain why the original article received little attention.

The effect of temperature on Drosophila development, heat shock, was not completely unknown: in 1961, a study was published detailing how temperature could affect pupae and how this thermal shock could actually lower the sensitivity of pupae to further exposure to high temperature (Milkman 1961).The author suggested that a higher production of proteins was caused by the heat shock, in order to compensate for their thermal denaturation. However, the study was only performed at the organismal level, and it did not concern chromosomes nor chromosomal puffs. Ritossa, working on chromosomes and with a very different approach, was influenced by Szent-Gyorgy's book "Bioenergetics," (Szent-Gyorgy 1957) and thought that the heat shock response was primarily connected to energy production (Ritossa 1996). It should be mentioned how Ferruccio described his first interpretation of the discovery of the heat shock response: "It did not matter if this interpretation was true or false, it was a working link between imagination and reality, like love" (Ritossa 1996). Recent studies have shown links among metabolic pathways involved in exercise, the heat shock response and oxidative energy production (reviewed by Hooper et al. 2014), so Ritossa's intuition that the heat shock response and bioenergetics are related now has experimental support.

Ritossa's finding was only fully appreciated years later. His original publication was often overlooked, and other later papers were cited as the reference. As a matter of fact, now, the 1962 *Experientia* paper is not even listed in PubMed. *Experientia* ended publication in 1994 and now continues as a Springer journal with the new name *Cellular and Molecular Life Sciences*. Fortunately, Ferruccio's paper has a very modern digital object identifier (doi:10.1007/BF02172188) to locate it in SpringerLink. In fact, temperature-induced puffs were for the next decade primarily a method to use and not an object to study. Only in the mid-1970s was the real biological meaning of the heat shock-induced protein production unearthed—a defense mechanism for coping with stress, and a very widespread one. The discovery of the heat shock phenomenon thus paved the way for a glorious research field, whose significance cannot be overstated and one that goes directly to the molecular heart of biology.

In the early years of ILGB, Ferruccio was definitely a bright star in the molecular biology universe. He spent a large amount of time in the USA, working with Robert C. (Jack) Van Borstel, researcher at the Oak Ridge National Laboratory and a visiting scientist in Pavia at the end of the 1950s. Subsequently, he helped to define the association of DNA with RNA together with Sol Spiegelman and Kim Atwood at the University of Illinois (Ritossa and Atwood 1966; Ritossa et al. 1966; Ritossa and Spiegelman 1965). A brilliant result of this exciting time was the discovery of the phenomenon of ribosomal DNA (rDNA) magnification, the inheritable increase of rDNA in Drosophila *bobbed* mutants (Ritossa 1968, Ritossa et al. 1971). Ferruccio, already oriented to the political Left, had the chance to taste life in the USA: it was not his cup of tea, and he refused several job offers. In Naples, however, he was part of the most "American" enterprise in Italian science, Buzzati-Traverso's ILGB. The Laboratory was meant to be a European Cold Spring Harbor, and several top notch scientists visited between 1962 and 1969. Similarly, it attracted researchers from all over the world for the courses in DNA-RNA hybridization (Sol Spiegelman was obviously the star of the faculty) and many other events held in the attractive venue of Naples. No ties with the "old" academic world of the university, no teaching duties, richly endowed by the European Atomic Energy Agency (EURATOM) and the National Research Council, with an unusual administrative autonomy and freedom (so that stipends could vary among staff members—and usually were higher with respect to other Italian research institutions), the ILGB was a sort of "Ivory Tower" in the landscape of Italian life sciences.

Ferruccio along with several other colleagues soon left the ILGB, after the harsh political turmoil in spring 1969, becoming a university professor at the University of Bari. He published several outstanding papers during this period, despite being somewhat isolated from the international scientific context (Caggese et al. 1979; Palumbo et al. 1973; Ritossa 1973).

After 15 years in Bari, Ferruccio moved to the University of Bologna, where he spent the last few years of his scientific career: art was now his most important passion (as were his grandchildren), and the university was not stimulating him anymore. However, until his early retirement in 1996 at the age of 60, he was an inspiring teacher to many young students, always pushing them not to be narrow-minded and to add something personal to their activity: “Put something of yours in what you write!” was his suggestion.

After leaving the University of Bologna, Ferruccio moved to a very quiet place in the Italian Apennine Mountains near the charming village of Dozza. He lived in a farm, growing fruit trees, and started to sculpt and, from time to time, to write poems. He became well known as a sculptor in Italy. His sculptures are fascinating: no matter whether he used alabaster, bronze, Brazilian marble, or olive wood, he was able to transfer feelings and ideas into the inert material, always full of symbols and irony, as in what he called his self-portrait, “the hen that started thinking” (Fig. 1). Many sculptures reveal the scientific imprint (Fig. 2).

It should be mentioned that recently, in 2011 and 2012, Ferruccio started thinking about going back to work in the lab on a project concerning “deciphering locus 93D,”, something that had been in his mind for several years. He visited Gabriella Santoro and Antonio Rossi at the University of Rome Tor Vergata and the CNR IFT Institute during several trips to Rome to plan the experimental work. Unfortunately, his health did not allow Ferruccio to pursue his new goal.

Ferruccio Ritossa’s career mirrors the trajectory of Italian science, broadly speaking. He was part of the “boom” of Italian genetics in the 1950–1960s, when a handful of scientists introduced novel concepts and approaches, mostly coming from Anglo-American sources. These scientists broke traditional disciplinary boundaries embedded in the Italian academic environment, coupling biology with other research fields aimed at investigating the physical nature of living organisms. The ILGB experience was probably the most ambitious enterprise in this effort. Ritossa was part of this

Fig. 2 The life and times of Ferruccio Ritossa: the Mosaic

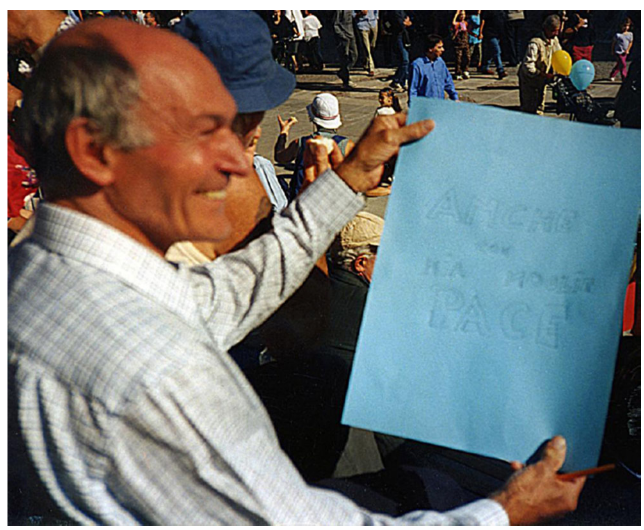
process, as a member of the first generation of molecular biologists educated in Italy, and integral to the ILGB since its inception. His outstanding result obtained in 1961–1962—the heat shock response—was one of the earliest and most relevant Italian contributions to molecular biology, even though its importance was unearthed only years later.

What happened to Ferruccio and the heat shock response in the ensuing time period? After the discovery in Pavia and research in Naples, Ferruccio moved to the USA. As mentioned before, he worked at Oak Ridge National Laboratory in Tennessee and at the University of Illinois at Urbana with Professor Sol Spiegelman. There, Ferruccio helped to develop the methods that would be needed to study the molecular biology of heat shock genes and proteins. He was better known internationally for this latter work on mechanisms and methods of nucleic acid hybridization than for the discovery of the heat shock response. That would start to change in 1982. During the decade following the discovery, a few labs continued the work of trying to understand the changes in Drosophila brought about by heat shock. The genomic response to heat was widely considered to be a "curiosity" of Drosophila biology, and indeed even among Drosophila biologists, research on the response was considered a "backwater" and quite possibly a laboratory artifact. Little did we know that these few dedicated researchers were tending the flame for what was to come. Unraveling the significance of the response and the mechanisms involved would have to await the development of the tools, initially in *Escherichia coli* and its phages, of the new fields of Molecular Biology and its love child Molecular Genetics.

Ferruccio's choice of Drosophila for his study was fortuitous. In his words, he thought of Drosophila as "an organism somehow between bacteria and man." Indeed, Drosophila with its large inventory of classical genetic tools and mutants was soon to become the favored eukaryotic organism of the Molecular Biology revolution of the 1960s and 1970s. The products of the heat shock puffs, the Drosophila heat shock proteins, were reported in 1974 (Tissieres et al. 1974), and Drosophila heat shock genes were among the first eukaryotic genes to be subjected to the then new methods of genetic cloning and nucleotide sequencing. Ferruccio's heat shock puffs became prime models for studies of eukaryotic gene regulation and chromosomal organization. In the summer of 1982, investigators from numerous countries gathered at Cold Spring Laboratory for the first international meeting titled "Heat Shock: from Bacteria to Man." The heat shock response was shown to be universal among known organisms (Schlesinger et al. 1982), allowing the power of bacterial and yeast genetics and biochemistry to join Drosophila and other organisms in the study of the heat shock response. Ferruccio reappeared in the field briefly as a participant, poster presenter, and session chair at this meeting.

In the spring of 2013, a celebration of the life of Ferruccio Ritossa was held in Rome. It was titled "The Art of Living with Stress: A Lesson from Ferruccio Ritossa," and the meeting, organized largely by Professor M. Gabriella Santoro, was in the Ara Pacis museum, a monument to peace. Again, Ferruccio's work, this time as both a scientist and a sculptor, brought colleagues together from all over the world in peace and harmony for the crowning event of a year of celebrating the 50th anniversary of the discovery of the heat shock response. The participants shared stories of the early days of Ferruccio's career, original poetry, music, and updates on the science that continues to flow from Ferruccio's pioneering discovery. As part of the anniversary recognition, Professor Ritossa had received the medallion of the Cell Stress Society International, an award for career achievement, in his hometown of Dozza, in October of 2010. Subsequently, a session of the Fifth International CSSI Congress, held in Québec in 2011, was organized to commemorate Ferruccio and his discovery. An article (De Maio et al 2012) published in Cell Stress and Chaperones reflecting on this session has a title that says it all "Ferruccio Ritossa's scientific legacy 50 years after his discovery of the heat shock response: a new view of biology, a new society and a new journal."

Thank you, Ferruccio



Ferruccio Ritossa, 1936–2014

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