Articles on Founders of Systems and Evolutionary Biology

Denis Noble's work on systems biology and evolutionary biology has depended on a deep understanding of how these fields have originated and developed. This collection brings together his historical work forming this important background.

William Harvey (1578-1657)

Auffray, C & Noble, D. 2009. Origins of Systems Biology in William Harvey's Masterpiece on the Movement of the Heart and the Blood in Animals. *International Journal of Molecular Sciences*. 10, 1658-1669.

William Harvey used quantitative calculation to prove the circulation of the blood and to predict the existence of capillaries. He can be seen as the first to use mathematics to help in understanding a high-level function in the body.

Claude Bernard (1813-1878)

Noble, D. 2008. Claude Bernard, the first Systems Biologist, and the Future of Physiology. *Experimental Physiology*. 93, 16-26.

Claude Bernard introduced the concept of the maintenance of the 'internal environment, of organisms, which requires the physiological organisation of control processes.

Gregor Mendel (1822-1884)

Auffray, C & Noble, D. 2022. Gregor Mendel at the source of genetics and systems biology. Celebrating the relevance of Gregor Mendel's experiments on the development of hybrid plants on the occasion of his bicentenary. *Biological Journal of the Linnean Society*. XX 1-17.

Gregor Mendel worked in a context of strong interest in hybridisation in Brno, he was not simply a lone monk working in isolation. His work is a foundation stone of the genetics of evolutionary biology.

Conrad Waddington (1905-1975)

Noble, D. 2015. Conrad Waddington and the Origin of Epigenetics. *Journal of Experimental Biology*. 218, 816-818.

Waddington's idea of epigenetics arose from his concept of canalisation of development, together with experiments in which he showed the genetic assimilation of epigenetic changes in DNA.

Julian Huxley (1887-1975)

Noble, D. & Noble, R. 2023. How Purposive Agency Became Banned from Evolutionary Biology. In *Evolution "On Purpose".Teleonomy in Living Systems.* (Eds Corning, P. Et al) 221-236.

This article pinpoints the date of the hardening of the Modern Synthesis as 1963, when Huxley edited the second edition of his book, *Evolution: The Modern Synthesis.* The trigger was the Central Dogma of molecular biology.

Richard Dawkins (1941-now)

Noble, D. & Noble, R. 2022. Origins and Demise of Selfish Gene Theory. *Theoretical Biology Forum.* 115. 29-43.

This articles traces the development of Selfish Gene theory from its beginnings in the work of George Williams (1966) book *Adaptation and Natural Selection* to Dawkins' *The Selfish Gene* (1976, 2016) and to the demise of Selfish Gene Theory through the demonstrations that:

1. DNA is not a *self*-replicator
2. The replicator is not separate from its vehicle, the living cell
3. The discovery of Natural Genetic Engineering
4. The discovery of Darwin's Gemmules as extracellular vesicles
5. The demise of the Weismann Barrier.

John Maynard Smith (1920-2004)

Noble, D. & Noble, R. 2023. Maynard Smith (in preparation)

Planned:
J B S Haldane (1892-1964),
R A Fisher (1890-1962) ,
Sewall Wright (1889-1988),
Francis Galton (1822-1922).
William D Hamilton (1936-2000)
George R Price (1922-1975)
George Williams (1926-2010)

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Communication

Origins of Systems Biology in William Harvey's Masterpiece on the Movement of the Heart and the Blood in Animals

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Abstract: In this article we continue our exploration of the historical roots of systems biology by considering the work of William Harvey. Central arguments in his work on the movement of the heart and the circulation of the blood can be shown to presage the concepts and methods of integrative systems biology. These include: (a) the analysis of the level of biological organization at which a function (e.g. cardiac rhythm) can be said to occur; (b) the use of quantitative mathematical modelling to generate testable hypotheses and deduce a fundamental physiological principle (the circulation of the blood) and (c) the iterative submission of his predictions to an experimental test. This article is the result of a tri-lingual study: as Harvey's masterpiece was published in Latin in 1628, we have checked the original edition and compared it with and between the English and French translations, some of which are given as notes to inform the reader of differences in interpretation.

Keywords: William Harvey; heart rhythm; circulation of the blood; mathematical deduction: experimental verification; systems biology.

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1. Introduction

In recent articles, we have both drawn attention to some of the historical antecedents of modern systems biology, notably in articles referring to Claude Bernard's *Introduction à l'étude de la Médicine Expérimentale* [1] and to Gregor Mendel's *Versuche über Pflanzen-Hybriden*, [2] both published in 1865. The first is considered as the founder of modern experimental medicine, while the second laid the ground for the development of genetics. We argued that both approached and unraveled the functioning of the biological systems they were studying through a highly relevant combination of experiment and modelling which is the hallmark of systems biology. In this article we draw attention to the very important historical antecedent represented by the work of William Harvey (1578-1657). While there may be no generally accepted and simple definition of systems biology, many good expressions of its main features can be found in review articles and books ([3-18] see also the paper by Saks *et al*. in this issue [19]), and it is almost universal to refer in some way to the concept of level and to the role of mathematics, whether they are combined in data-driven (bottom-up) or top-down (model-driven) approaches, or the middle-out (question-driven) research strategy that we favor. These two features appear prominently in the masterpiece of William Harvey, *Exercitatio anatomica de motu cordis et sanguinis in animalibus* published in Latin in Frankfurt in 1628 (translated into modern English by Gweneth Whitteridge [20], and into French by Charles Richet [21]), where he reported his experimental work demonstrating the circulation of blood in animals. Identifying historical precedents for modern biological ideas and methods is important. A subject that neglects its roots fails to benefit from the insights and problems of our predecessors. It is also somewhat humbling to realize that, however enthusiastic we may be about the modern systems approach to biology, the approach is not entirely new. Moreover, claiming such antecedents as Harvey, Bernard and Mendel serves to encourage other biologists to view systems biology in a more favourable light.

2. Results and Discussion

2.1. Identifying the biological level at which rhythm is generated and integrated

Harvey first describes an experiment in which he seeks a lower level than the organ for the origin of the rhythmic activity of the heart. He writes:

> "The heart of an eel and of certain other fish and animals, having been taken out of the body, beats without auricles. Furthermore, if you cut it in pieces, you will see the separate pieces each contract and relax, so that in them the very body of the heart beats and leaps after the auricles have ceased to move."

Harvey could not, in his day, take this dissection further down to discover that the rhythmic mechanism was integrated at the level of individual cells (see [22], chapter 5), since the cell theory was formulated by Matthias Schleiden (1804-1881) and Theodor Schwann (1810-1882) two centuries later based on observations with the microscope introduced in practice in the life sciences by Anton van

Leeuwenhoek (1632-1723) only after Harvey's death. However, he was the first to realise that rhythmicity was a property of the smallest structures he could discern.

2.2. Demonstration of the circulation of the blood through a systems approach

The discovery for which Harvey is best-known is, of course, the circulation of the blood. Already in 1616, in his lecture notes *Prelectiones Anatomiae Universalis* [23], he wrote:

> "So it is proved that a continual movement of the blood in a circle is caused by the beat of the heart."

It is perhaps less well-known that this was the result of a careful series of observations and calculations subjected to an iterative process of modelling and experimental validation which has already all the features of a systems biology inquiry which typically comprises the following steps: formulate a general or specific question; define the components of a biological system and collect previous relevant datasets; integrate them to formulate an initial model of the system and generate testable predictions and hypotheses; systematically perturb the components of the system experimentally or through simulation, and study the results; compare the responses observed to those predicted by the model; refine the model so that its prediction fit best to the experimental observations; conceive and test new experimental perturbations to distinguish between the multiple competing hypotheses; iterate the process until a suitable response to the initial question is obtained [9, 13]. In what follows, we examine how William Harvey goes through this multi-step process to address the general and fundamental question of the significance of the movements of the heart and the blood for the understanding of life and disease in animals.

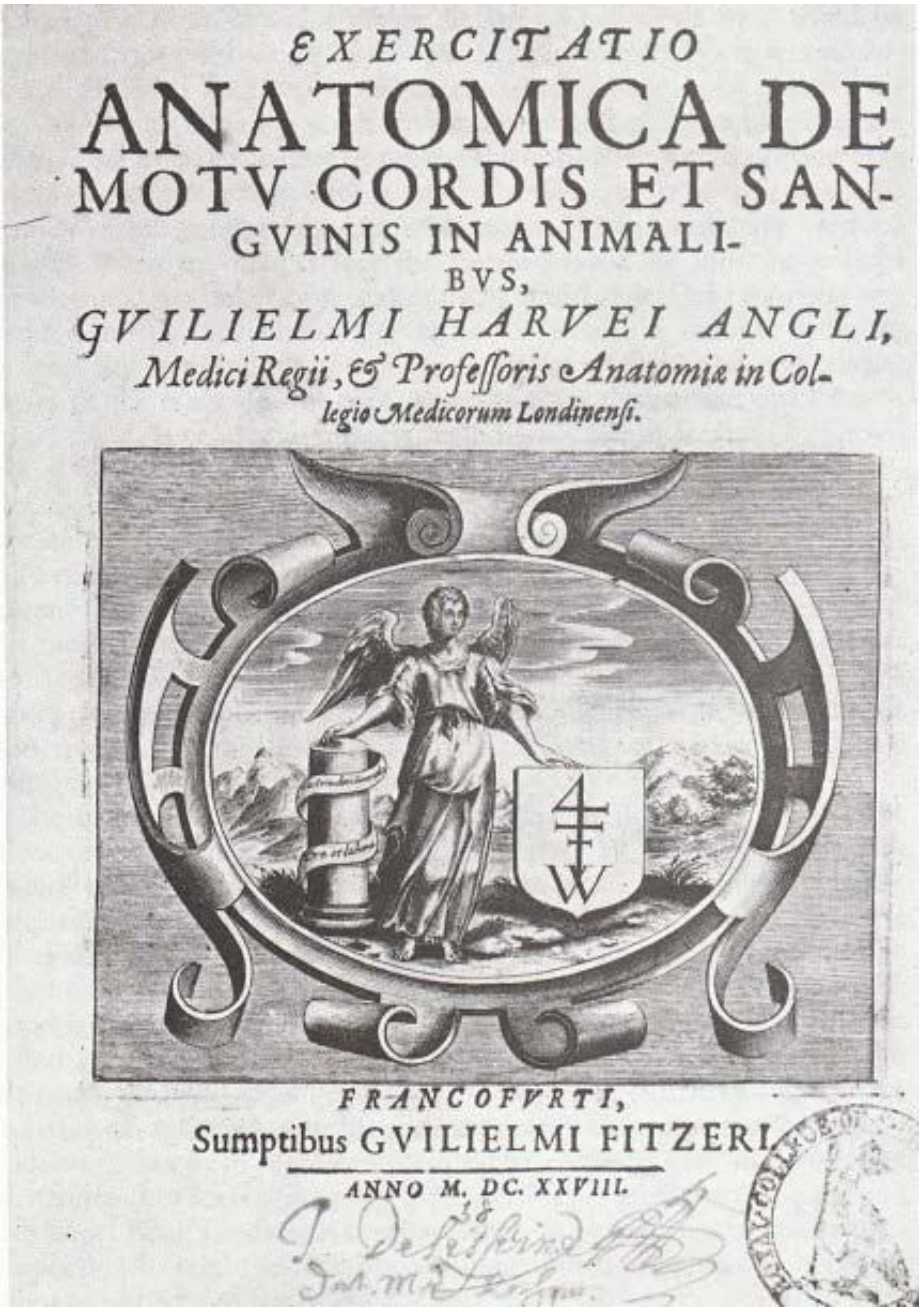
2.2.1. Critical assessment of previous data

The introduction and the first seven chapters of his book are devoted to a critical assessment of previous work by the Greek philosophers and naturalists considered as the fathers of medicine: Hippocrates of Cos (460 BC-370 BC), Aristotle (384 BC- 322 BC), Eristratus of Chios (304 BC- 250 BC), and Galen of Pergamum (129-200), the founder of the medical practice in use until Harvey's time.

As a result, he was able to assemble in a coherent manner a wealth of relevant information gathered by his predecessors, identifying uncertainties and contradictions in the description of the movement of the heart and the blood, and dismissing factual errors of observation or interpretation. While he cites them explicitly and extensively, he does not refer to the work of the Arabic polymath Ibn al-Nafis (1213-1288) or of Miguel Servet (1511-1553) who both described the pulmonary circulation independently, but remained unknown to him. Their observations were most likely conveyed by his immediate predecessors the anatomists Andreas Vesalius (1514-1564), who had been in contact with Servet, and Realdo Colombo (1510-1559) as they both worked for some time in Padua where Harvey obtained his medical degree in 1602. Details on the life of Harvey can be found in Keynes [24] and Whitteridge [25], while his anatomical lectures have been completely translated in a bilingual (Latin and English) edition [23].

While in Padua, Harvey was influenced by Girolamo Fabrizi d'Acquapendente or Fabricius (1537-1619) his teacher at the Faculty of medicine, and became fascinated by the valves of the veins (already known to Erasistratus). He showed that these could pass the blood only in one direction. From which it followed that the blood that was taken out to the limbs and organs by the arteries had to return via the veins. The existence of the valves ensured that it could not be just an ebb and flow of fluid, as had been taught since antiquity.

Figure 1. Title page of *Exercitatio anatomica de motu cordis et sanguinis in animalibus*, 1628 [20].



2.2.2. Formulation of a model and derivation of testable hypotheses

In chapter eight of *Exercitatio anatomica de motu cordis et sanguinis in animalibus* (*Decopia sanguinis transeuntis per cor e venis in arteria, et de circulari motu sanguinis* [26]), Harvey presents his model: the circular movement of blood (*de circolari motu sanguinis*) depends on the movements and pulsations of the heart. The model is based on a quantitative evaluation of the amount of blood passing through the heart, the veins and the arteries, and the disposition of the valves in the heart and the blood vessels.

In chapter nine (*Esse sanguinis circuitum ex primo supposito confirmato* [27]), he derives three hypotheses (*suppositio*) from his model, which he intends to demonstrate through experiments: in a brief period of time, the totality of the blood in the organism passes 1) from the veins into the arteries; 2) from the arteries in all body parts; and 3) from the body parts to the heart through the veins. He states:

> "These proved, I think it will be clear that the blood circulates, passing away from the heart to the extremities and then returning back to the heart, thus moving in a circle"[28]

2.2.3. Quantitative assessment of experimental parameters

He then proceeds with a numerical calculation, based on a quantitative estimation of the parameters and of their variation: the volume of blood in the heart, the volume of blood ejected from the left ventricle into the aorta at each contraction, the number of contractions in half an hour or a day. He writes:

> "Then we may suppose in man that a single heart beat would force out either a half ounce, three drams, or even one dram of blood, which because the valvular block could not flow back that way into the heart. The heart makes more than a thousand beats in half an hour, in some two, three, or even four thousand. Multiplying by the drams, there will be in half an hour either 3,000 drams, 2,000 drams, five hundred ounces, or some other such proportionate amount of blood forced into the arteries by the heart, but always a greater quantity than is present in the whole body."[29]

and concludes:

> "On this assumption of the passage of blood, made as a basis for argument, and from the estimation of the pulse rate, it is apparent that the entire quantity of blood passes from the veins to the arteries through the heart, and likewise through the lungs."[30]

and

> "But suppose even the smallest amount of blood be transmitted through the lungs and heart at a single beat, a greater quantity would eventually be pumped into the arteries and the body than could be furnished by the food consumed, unless by constantly making a circuit and returning." [31]

2.2.4. Submission of the mathematical predictions to experimental tests

Without the mathematics, the conclusion would not have been reached. But Harvey went even further. From the prediction of his calculation he proceeds to the key experiment. The calculation predicts that the body should empty itself of blood in half an hour if the blood is prevented from circulating:

> "This is also clearly to be seen by any who watch the dissection of living creatures, for not only if the great artery be cut, but, as Galen proves, even in man himself, if any artery even the smallest be cut, in the space of about half an hour, the whole mass of blood will be drained out of the whole body…"

This is the iteration between theory and experiment that is essential to success of a systems approach today as it was already in Harvey's time.

He summarizes at the beginning of chapter ten (*Primum suppositum decopia pertranseuntis sanguinis e venis in arterias, et esse sanguinis circuitum ab obiectionibus vindicatur, et experimentis ulterius confirmatur* [32]):

> "Whether the matter be referred to calculation or to experiment and dissection, the important proposition has been established that blood is continually poured into the arteries in a greater amount than can be supplied by the food. Since it all flows past in so short a time, it must be made to flow in a circle."[33]

He then proceeds in a similar manner in chapters eleven (*Secundum suppositum confirmatur* [34]) and twelve (*Esse sanguinis circuitum ex secundo supposito confirmato* [35]) to demonstrate the second hypothesis, on the basis of observations and numerical calculations performed during experiments using ligatures and compressions, discussing their consequences in terms of medical practice:

> "If these things are so, we may very readily compute the amount of blood and come to some conclusion on its circular motion."[36]

In chapter thirteen (*Tertium suppositum confirmatur, et esse sanguinis circuitum ex tertio supposito* [37]), Harvey endeavours to prove the third hypothesis:

> "This proposition will be perfectly clear from a consideration of the valves found in the venous cavities, from their functions, and from experiments demonstrable with them."

He bases his argument on a series of experiments in which he details the consequences of ligatures and compressions exerted on arm veins, as illustrated in anatomic schemas, and supported once again by a numerical calculation:

> "By careful reckoning, of course, the quantity of blood forced up beyond the valve by a single compression may be estimated, and this multiplied by a thousand gives so much blood transmitted in this way through a single portion of the veins in a relatively short time, that without doubt you will be very easily convinced by the quickness of its passage of the circulation of the blood."[38]

He concludes briefly in chapter fourteen (*Conclusio demonstrationis de sanguinis circuitu* [39]) on the demonstration of the circulation of blood, ending the first iteration of a typical systems biology approach:

> "It must therefore be concluded that the blood in the animal body moves around in a circle continuously, and that the action or function of the heart is to accomplish this by pumping. This is the only reason for the motion and beat of the heart."[40]

2.2.5. Refinement of the model through further observations

The last three chapters are the beginning of a second iteration, intended to confirm circulation on the basis of compatible physiological observations (chapter fifteen: *Sanguinis circuitus rationibus verisimilibus confirmatur* [41]), the consequences for the treatment of diseases (chapter sixteen: *Sanguinis circuitus ex consequentibus probatur* [42]):

> "Assuming the truth of this proposition there are certain consequences which are useful in coaxing belief a posteriori. Although some of them may seem to be clouded in considerable doubt, a reasonable case may easily be made of them."[43]

and finally a number of anatomical observations on the structure and development of the heart in diverse animals (chapter seventeen: *Confirmatur sanguinis motus, et circuitus ex apparentibus Corde, et ex iis, quaex dissectione Anatomicapatent* [44]).

2.3. Circulation, circuit and capillaries

While the central notion of circular movement is explicit from Chapter eight on, it is worth pointing that Harvey made a clear distinction between the anatomical structures and the action taking place within them (the movement of the heart and the circulation of the blood). This is apparent in his repeated use in eight of the last nine chapter headings of the Latin word *circuitus*, which has been translated rather loosely as "circulation" in both the English and French versions. As an outstanding anatomist, he was well aware that in order to allow "circulation" of the blood, the "circuit" had to be closed at the juncture between the arteries and the veins. It is therefore worth pointing to his reference to "*venis capillaribus in paruas ramifications*" (chapter fifteen), and "*ultimae diuisiones capillares, arteriolae videantur*" (chapter seventeen) which were wrongly translated as "tiny veins" and "terminal arteries" [20], giving the impression he had missed this important notion. The existence and role of capillaries in the circulation would be demonstrated only later in 1661 by the Italian histologist Marcello Malpighi (1628-1694) when he examined blood vessels in frogs using the then recently invented microscope. Malpighi is also famous for giving his name to a number of anatomical structures in animals and insects, and was the first to report his experimental findings in scientific articles including a method section, as has become the routine practice in modern science ever since.

3. Conclusions: Harvey and the Conceptual and Ethical Foundations of Modern Science

Harvey's achievements are all the more remarkable since they were performed when the basic concepts and methods that would form the bases for the development of modern science were just being established. Francis Bacon (1561-1626) published his *Novum Organum* in 1620, and René Descartes (1596-1650) *Discours de la Méthode* in 1637, the same year when he introduced the algebraic notation using Latin letters. It is therefore not surprising that all of Harvey's calculations are expressed literally. Despite these limitations, he was very much aware of the conceptual and practical aspects of his experiments, which were known to Descartes himself, as is shown in his responses to the criticisms of Jean Riolan Fils, of Paris, the chief medical doctor of Louis XIII's mother (Harvey himself was the doctor of two kings of England, James I and Charles I). Riolan, one of Harvey's severest critics on the circulation of the blood wrote in his *Encheiridium anatomicum et patholigicum*:

> "That this circulatory movement may be more easily and more conveniently maintained, William Harvey, Englishman, Royal Physician, and author and discoverer of this movement of the blood, and John Waleus, professor of Leyden, who defends and vigorously upholds it, believe the blood to be taken through the lungs from the right to the left ventricle of the heart and deny its passage through the septum of the heart, and so they believe that in one or two hours all the blood passes through the heart and through the whole body. This I do not admit."

Riolan was trying, valiantly but vainly, to reconcile strict Galenic teaching with Harvey's observations. "The resulting inconsistencies and contradictions Harvey was not slow to point out" [25]. In his *Exercitationes duae anatomicae de circulatione sanguinis* (Two anatomical exercises concerning the circulation of blood) [21, 24], published in 1649 in response to Riolan, Harvey states:

> "There is no science that derives only from a priori ideas, and there is no solid and certain knowledge that does not taken its origin from our sense organs" (first dissertation) [45].
>
> "But it is our senses, not accepted theories, dissection and not the dreams of imagination, that should teach us what is true or false (second dissertation) [46]
>
> "A man remarkable for his brilliant genius, René Descartes, who I thank for the complimentary reference that he has made of me" (second dissertation) [47].
>
> "But I think it a thing unworthy of a Philosopher and a searcher of the truth, to return bad words for bad words; and I think I shall do better and more advised, if with the light of true and evident observations I shall wipe away those symptoms of incivility" (second dissertation) [48].

Only two years later, using the same approach as for the study of circulation, he published *Exercitationes de Generatione Animalium* [49] which contributed to the foundation of modern embryology. It would be interesting to speculate on why some of the important features of the systems approach, particularly the use of mathematics and modeling, became neglected until recently. Factors that may have played a part include: the sheer difficulty of applying mathematics in biology; the lack of suitable means for solving the problems, which became tractable only after the invention of the

digital computer in the second half of the 20th century; the rise of a positivist (reductionist) bias in biology from the 19th century onwards (many leading physiological journals actually excluded mathematical biology); and, most recently, the rise of molecular biology, with a tendency to avoid theory (except, very significantly, the central dogma of molecular biology). A full treatment of these and other factors would require a detailed historical analysis and will be the subject of a further article.

In any event, almost four centuries after he published his masterpiece, the concepts and experimental principles that were laid out by William Harvey are some of the pillars on which several branches of natural and engineering sciences have been flourishing. This common origin should facilitate the cross-fertilization of biology, following the quantitative footsteps of physics and engineering, thus enabling the extension of physiology into integrative systems biology.

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26. Amount of blood passing through the heart from the veins to the arteries, and the circular motion of the blood [20]; De la quantité de sang qui passe par le coeur, des veines dans les artères, et du mouvement circulaire du sang [21].
27. The circulation of the blood is proved by a prime consideration [20]; Démonstration de la circulation du sang par la confirmation de la première hypothèse [21].
28. « Je dis qu'alors évidemment le sang circule, qu'il est chassé du cœur aux extrémités, et qu'il revient des extrémités au cœur, et ainsi de suite, accomplissant un mouvement circulaire. » [21].
29. The Apothecaries of Troy weight is used: 3 scruples equal 1 dram; 8 drams equal 1 ounce; 12 ounces equal 1 pound. This was in general use in Europe (Note from Leake, C.D. in the tercentennial edition of Harvey's *Exercitatio anatomica de motu cordis et sanguinis in animalibus*, Springfield and Baltimore: Thomas, C.C., ed., 1928).
30. « Ainsi en supputant la quantité de sang que le cœur envoie à chaque contraction et en comptant ces contractions, on voit que toute la masse du sang passe des veines dans les artères par le coeur et aussi par les poumons. » [21].

31. « Mais, quelque petite que soit la quantité de sang qui passe par le cœur et les poumons, il y en a néanmoins bien trop pour que les aliments ingérés y puissent suffire, à moins que le sang ne revienne par les mêmes trajets. » [21].
32. The first proposition, concerning the amount of blood passing from veins to arteries, during the circulation of the blood, is freed from objections, and confirmed by experiments [20]; La première hypothèse sur la circulation du sang, fondée sur la quantité de sang qui passe des veines dans les artères, est confirmée par des expériences ; et les objections qu'on lui avaient opposées sont réfutées [21].
33. « Jusqu'ici le calcul, les expériences, les dissections ont confirmé notre première hypothèse, que le sang passe continuellement dans les artères, et en trop grande quantité pour que les aliments y puissent suffire, en sorte que comme la totalité du sang passe en très peu de temps par le même endroit, le sang doit nécessairement revenir par les mêmes voies et accomplit un véritable circuit. » [21].
34. The second proposition is proven [20]; Confirmation de la seconde hypothèse [21].
35. That there is a circulation of the blood follows from the proof of the second proposition [20]; La confirmation de la seconde hypothèse démontre la circulation du sang [21].
36. « Maintenant calculons la quantité de sang qui passe par les veines, et démontrons à l'aide de calculs le mouvement circulaire du sang. » [21].
37. The third proposition is proven, and the circulation of the blood is demonstrated from it [20]; Confirmation de la troisième hypothèse, qui démontre la circulation du sang [21].
38. « Calculez maintenant combien de sang vous aurez arrêté en mettant le doigt au-dessus de la valvule, et multipliez cette quantité par milliers ; vous verrez alors quelle grande quantité de sang passe ainsi dans cette petite portion de veine, en un temps aussi court, et je crois que vous serez bien convaincu de la circulation du sang et de la rapidité de son mouvement. » [21].
39. Conclusion on the demonstration of the circulation of blood [20]; Conclusion de la démonstration de la circulation du sang [21].
40. « Il faut donc nécessairement conclure que chez les animaux le sang est animé d'un mouvement circulaire qui l'emporte dans une agitation perpétuelle, et que c'est là le rôle, c'est là la fonction du cœur, dont la contraction est la cause unique de tous ces mouvements. » [21].
41. The circulation of blood is confirmed by plausible methods [20]; La circulation du sang confirmée par les vraisemblances.
42. The circulation of the blood is supported by its implications [20]; La circulation du sang prouvée par les implications qu'elle entraîne [21].
43. « Il y a encore des problèmes qui sont comme la conséquence de la vérité de la circulation. Ils ne sont point inutiles pour y faire croire et leur démonstration est comme un argument a posteriori. » [21].
44. The motion and circulation of the blood is established by what is displayed in the heart and elsewhere by anatomical investigation [20]; Confirmation du mouvement et de la circulation du sang par ce que nous voyons dans le Coeur, et par les observations anatomiques [21].
45. « Il n'y a pas de science qui ne dérive d'une idée a priori, et il n'y a pas de connaissance solide et sûre qui ne tire son origine des sens. » [21].

46. « Or ce sont nos sens et non les théories admises, la dissection et non les rêves de l'imagination qui doivent nous apprendre si elles sont vraies ou fausses.» [21].
47. « Un homme remarquable par son brillant génie, René Descartes, que je remercie de la mention élogieuse qu'il a fait de moi. » [21].
48. « Pour moi, je trouve que répondre à des injures par des injures est une action indigne d'un philosophe qui cherche la vérité, et qu'il vaut mieux confondre ces méchants par la lumière de l'observation et de la vérité.» [21].
49. Whitteridge, G. *Disputations touching the Generation of Animals;* Blackwell: Oxford, U.K., 1981.

Experimental Physiology – *Paton Lecture*

Claude Bernard, the first systems biologist, and the future of physiology

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The first systems analysis of the functioning of an organism was Claude Bernard's concept of the constancy of the internal environment (*le milieu intérieur*), since it implied the existence of control processes to achieve this. He can be regarded, therefore, as the first systems biologist. The new vogue for systems biology today is an important development, since it is time to complement reductionist molecular biology by integrative approaches. Claude Bernard foresaw that this would require the application of mathematics to biology. This aspect of Claude Bernard's work has been neglected by physiologists, which is why we are not as ready to contribute to the development of systems biology as we should be. In this paper, I outline some general principles that could form the basis of systems biology as a truly multilevel approach from a physiologist's standpoint. We need the insights obtained from higher-level analysis in order to succeed even at the lower levels. The reason is that higher levels in biological systems impose boundary conditions on the lower levels. Without understanding those conditions and their effects, we will be seriously restricted in understanding the logic of living systems. The principles outlined are illustrated with examples from various aspects of physiology and biochemistry. Applying and developing these principles should form a major part of the future of physiology.

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Historical introduction

Claude Bernard was Sir William Paton's great physiological hero. When the Physiological Society celebrated its centenary in 1976, Bill contributed a paper to the historical part of the meeting concerning one of Bernard's experiments on curare and drawing attention to the important role his ideas played in the foundation of the Society in 1876 (Paton, 1976). The reasons for his admiration of Claude Bernard are not hard to find. Bernard was a superb experimentalist, as the history of his work on digestion shows (Holmes, 1974). He also displayed his skills in many other areas of physiology and he laid out the principles of his science in his highly influential *Introduction à l'étude de la Médecine Expérimentale* (Bernard, 1865, 1984), in which he revealed himself to be a great thinker as well as a great experimentalist. The theoretical problem he addressed is one that is very relevant both to my claim that he was the first systems biologist and to the challenge that physiology faces today.

What was Claude Bernard's problem? It was that the chemists had created 'organic' molecules. This was a major development, since people had thought since Lémery's *Cours de Chymie* (published in 1675) that there were three completely separate classes of compounds: mineral, vegetable and animal. The first break in this idea came from the work of Lavoisier (1784), who showed that all compounds from vegetable and animal sources always contained at least carbon and hydrogen, and frequently nitrogen and phosphorus. This work bridged the vegetable–animal chemical boundary, but it left intact the boundary between the living and non-living. In fact, Berzelius (1815) even proposed that organic compounds were produced by laws different from inorganic compounds; the idea that there was a specific vital force that could not operate outside living systems. In 1828, however, Wöhler succeeded in creating urea from ammonium cyanate. The distinction between organic and non-organic origins was further weakened by Kolbe who, in 1845, synthesized acetic acid from its elements. Many

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other discoveries of this kind (Finar, 1964) led to the idea that life itself could be reduced to chemistry and physics.

This was the challenge that physiologists such as Claude Bernard faced. His answer was precise. Neither vitalism nor chemical reductionism characterized living organisms. To the challenge that 'There are . . . chemists and physicists who . . . try to absorb physiology and reduce it to simple physico-chemical phenomena', Bernard responded, 'Organic individual compounds, though well defined in their properties, are still not active elements in physiological phenomena. They are only passive elements in the organism.' The reason, he explained, is that 'The living organism does not really exist in the *milieu extérieur* but in the liquid *milieu intérieur* . . .. a complex organism should be looked upon as an assemblage of simple organisms . . .. that live in the liquid *milieu intérieur*.'

His response to vitalism was equally robust: 'Many physicians . . .. assume a vital force in opposition to physico-chemical forces. I propose therefore to prove that the science of vital phenomena must have the same foundations as the science of the phenomena of inorganic bodies, and that there is no difference between the principles of biological science and those of physico-chemical science.'

By 'principles' here Bernard meant the laws governing the behaviour of the components. The control of the *milieu intérieur* meant not that the individual molecules did anything different from what they would do in non-living systems, but rather that the *ensemble* behaves in a controlled way, the controls being those that maintain the constancy of the internal environment. How could that be formalized? Could there be a theoretical physiology? Physical scientists had long since used mathematics to formalize their theories. Could that also be done in physiology? Bernard's answer to this question was 'yes, but not yet.' He cautioned, 'The most useful path for physiology and medicine to follow now is to seek to discover new facts instead of trying to reduce to equations the facts which science already possesses.' I believe that this view has been in part responsible for the broadly antitheoretical stance of British and American Physiology. It is important, therefore, to recognize that it represents only half of Bernard's views on the matter. For the emphasis in that statement should be on the word *now*. He also wrote that it was necessary to 'fix numerically the relations' between the components. He continued: 'This application of mathematics to natural phenomena is the aim of all science, because the expression of the laws of phenomena should always be mathematical.' His caution, therefore, was purely practical and temporal. In 1865 he saw, correctly of course, that physiology simply did not have enough data to make much mathematical application worthwhile *at that time*. But he clearly foresaw that the day would come when there would be sufficient data and that mathematical analysis would then become necessary.

The problem physiology faces today both resembles that faced by Bernard and differs from it. We face a new form of reductionism: that of genetic determinism, exemplified by the idea that there is a genetic program, what Jacob and Monod called '*le programme génétique*' (Monod & Jacob, 1961; Jacob, 1970). This challenge strongly resembles that of 'reducing life to physics and chemistry', the chemical being DNA. The major difference from Bernard's day is that we now have more facts than we can handle. There is a data explosion at all levels of biology. The situation is almost the reverse of that in Bernard's time. I have no doubt, therefore, that if he were alive today he would be championing his 'application of mathematics to natural phenomena.' I will illustrate why this is necessary and how it can be achieved by outlining some principles of systems biology from a physiologist's viewpoint. The principles are derived from my book on systems biology, *The Music of Life* (Noble, 2006), but their arrangement as a set of 10 was first presented by Noble (2007).

The principles of systems biology

First principle: biological functionality is multilevel. I start with this principle because it is obviously true, all the other principles can be shown to follow from it, and it is therefore the basis on which a physiological understanding of the phenomenon of life must be based. It is also a more general statement of the insight contained in Claude Bernard's idea of the constancy of the internal environment. That functionality is attributable to the organism as a whole and it controls all the other levels. This is the main reason why I describe Bernard as the first systems biologist. It is hard to think of a more important overall systems property than the one Bernard first identified.

Yet, the language of modern reductionist biology often seems to deny this obvious truth. The enticing metaphor of the 'book of life' made the genome into the modern equivalent of the 'embryo-homunculus', the old idea that each fertilized egg contains within it a complete organism in miniature (Mayr, 1982; p. 106). That the miniature is conceived as a digital 'map' or 'genetic program' does not avoid the error to which I am drawing attention, which is the idea that the living organism is simply the unfolding of an already-existing program, fine-tuned by its interaction with its environment, to be sure, but in all essentials, already there in principle as a kind of zipped-up organism. In its strongest form, this view of life leads to gene-selectionism and to gene-determinism: 'They [genes] created us body and mind' (Dawkins, 1976).

Dawkins himself does not really believe that. In a more recent book, he entitles one chapter 'Genes aren't us' (Dawkins, 2003) and, even in *The Selfish Gene*, the bold, simple message of the early chapters is qualified at the

end. My reservations, however, go much further than his. For, in truth, the stretches of DNA that we now call genes do nothing on their own. They are simply databases used by the organism as a whole. This is the reason for replacing the metaphor of the 'selfish' gene by genes as 'prisoners' (Noble, 2006; chapter 1). As Maynard Smith & Szathmáry (1999) express it, 'Co-ordinated replication prevents competition between genes within a compartment, and forces co-operation on them. They are all in the same boat.' From the viewpoint of the organism, genes as DNA molecules are therefore captured entities, no longer having a life of their own independent of the organism.

Second principle: transmission of information is not one way. The central dogma of molecular biology (Crick, 1970) is that information flows from DNA to RNA, from RNA to proteins, which can then form protein networks, and so on up through the biological levels to that of the whole organism. Information does not flow the other way. This is the dogma that is thought to safeguard modern neo-Darwinian theory from the spectre of 'Lamarckism', the inheritance of acquired characteristics. Applied to all the levels, this view is illustrated in Fig. 1. It encourages the bottom-up view of systems biology, the idea that if we knew enough about genes and proteins we could reconstruct all the other levels. Bioinformatics alone would be sufficient.

There are two respects in which the dogma is at least incomplete. The first is that it defines the relevant information uniquely in terms of the DNA code, the sequence of C, G, A, T bases. But the most that this information can tell us is *which* protein will be made. It does not tell us *how much* of each protein will be made. Yet, this is one of the most important characteristics of any living cell. Consider the speed of conduction of a nerve or muscle impulse, which depends on the density of rapidly activated sodium channels: the larger the density, the greater the ionic current and the faster the conduction. But this relationship applies only up to a certain optimum density, since the channel gating also contributes to the cell capacitance, which itself slows conduction, so there is a point beyond which adding more channel proteins is counter-productive (Hodgkin, 1975; Jack *et al.* 1975; p. 432). A feedback mechanism must therefore operate between the electrical properties of the nerve and the expression levels of the sodium channel protein. We now refer to such feedback mechanisms in the nervous system, which take many forms, as electro-transcription coupling (e.g. Deisseroth *et al.* 2003).

Similar processes must occur in the heart (e.g. Bers & Guo, 2005) and all the other organs. One of the lessons I have learnt from many attempts to model cardiac electrophysiology (Noble, 2002) is that, during the slow phases of repolarization and pacemaker activity, the ionic currents are so finely balanced that it is inconceivable that nature arrives at the correct expression and activity levels without some kind of feedback control. We don't yet know what that control might be, but we can say that it must exist. Nature cannot be as fragile as our computer models are! Robustness is an essential feature of successful biological systems.

There is nothing new in the idea that such feedback control of gene expression must exist. It is, after all, the basis of cell differentiation. All nucleated cells in the body contain exactly the same genome (with the exception of course of the germ cells, with only half the DNA). Yet the expression pattern of a cardiac cell is completely different from, say, a hepatic or bone cell. Moreover, whatever is determining those expression levels is accurately inherited during cell division. This cellular inheritance process is robust; it depends on some form of gene marking. It is this information on relative gene expression levels that is critical in determining each cell type.

By what principle could we possibly say that this is not relevant information? In the processes of differentiation and growth it is just as relevant as the raw DNA sequences. Yet, it is clear that this information *does* travel 'the other way'. The genes are told by the cells and tissues what to do, how frequently they should be transcribed and when to stop. There is 'downward causation' (Noble, 2006; chapter 4) from those higher levels that determines how the genome is 'played' in each cell (Fig. 2). Moreover, the possible number of combinations that could arise from so many gene components is so large (Feytmans *et al.* 2005) that there wouldn't be enough material in the whole universe for nature to have tried more than a small fraction

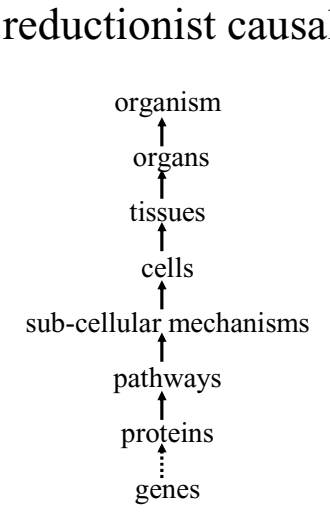


Figure 1. The reductionist 'bottom-up' causal chain (reproduced with permission from Noble, 2006)
This begins with the central dogma that information flows from DNA to proteins (bottom dotted arrow), never the other way, and extends the same concept through all the higher levels.

of the possible combinations even over the billions of years of evolution (Noble, 2006; chapter 2).

So the dogma is at least incomplete. But I also think it is incorrect in several important ways. Sure, protein sequences are not back-translated to form DNA sequences. In this limited original form, as formulated by Crick (1970), the central dogma is correct. But there is growing evidence from work on plants and microbes that environmental factors *do* change the genome, particularly by gene transfer (Goldenfeld & Woese, 2007). We cannot, therefore, use the original central dogma to exclude information transfer *into* the genome, determined by the organism and its environment.

Moreover, the DNA code itself is marked by the organism. This is the focus of the rapidly growing field of epigenetics (Qiu, 2006). At least two such mechanisms are now known at the molecular level: methylation of cytosine bases and control by interaction with the tails of histones around which the DNA is wound. Both of these processes modulate gene expression. The terminological question then arises: do we regard this as a form of code-modification? Is a cytosine, the C of the code, a kind of C* when it is methylated? That is a matter of definition of code, and one which I will deal with in the next section, but what is certain is that it is relevant information determining levels of gene expression, and that this information does flow against the direction of the central dogma. In fact, a form of inheritance of acquired characteristics (those of specific cell types) is rampant within all multicellular organisms with very different specialized cell types (Noble, 2006; chapter 7). At the least we have to say that, during the lifetime of the individual organism, transmission of information is far from being one way.

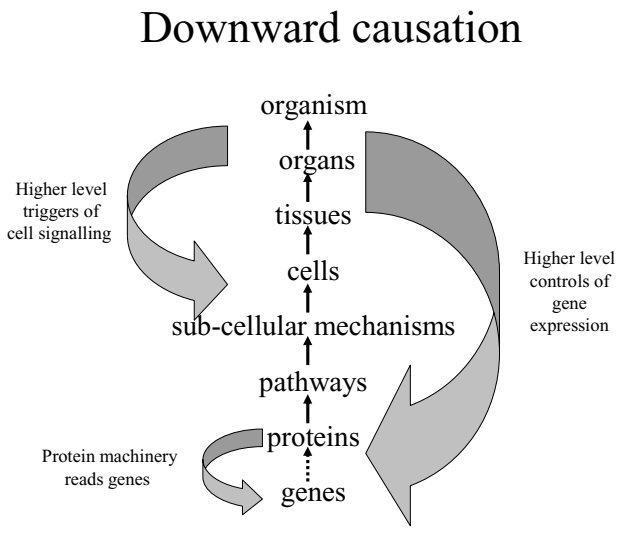


Figure 2. Figure 1 has been completed by adding the downward forms of causation, such as higher levels triggering cell signalling and gene expression
Note the downward-pointing arrow connecting from proteins to genes to indicate that it is protein machinery that reads and interprets gene coding. Loops of interacting downward and upward causation can be built between all levels of biological organization. Reproduced with permission from Noble (2006).

Third principle: DNA is not the sole transmitter of inheritance. The defenders of the original version of the central dogma would argue that, while my conclusions regarding the second principle are correct, what happens when information is transmitted to the next generation through the germ-line nevertheless involves wiping the slate clean of epigenetic effects. Methylation of cytosine bases and other forms of genome marking are removed. The genome is reset so that 'Lamarckism' is impossible.

But this is to put the matter the wrong way round. We need to explain *why* the genome (usually) reverts to an unmarked state. We don't explain that by appealing to the central dogma, for that dogma is simply a restatement of the same idea. We are in danger of circular logic here. Later, I will suggest a plausible reason why, at least most of the time, the resetting is complete, or nearly so. In order to do that, we first need to analyse the idea that genetics, as originally understood, is just about DNA.

This is not the original biological meaning of 'gene'. The concept of a gene has changed (Kitcher, 1982; Mayr, 1982; Dupré, 1993; Pichot, 1999). Its original biological meaning was an inheritable phenotype characteristic, such as eye/hair/skin colour, body shape and weight, number of legs/arms, to which we could perhaps add more complex traits like intelligence, personality, sexuality, etc. Genes, as originally conceived, are not just the same as stretches of DNA unless we subscribe to the view that the inheritance of all such characteristics is attributable entirely to DNA sequences. That is clearly false, since the egg cell is also inherited, together with any epigenetic characteristics transmitted by sperm (Anway *et al.* 2005), perhaps via RNA in addition to its DNA, and all the epigenetic influences of the mother and environment. Of course, the latter (environment) begins to be about 'nurture' rather than 'nature', but one of my points is that this distinction is fuzzy. The proteins that initiate gene transcription in the egg cell and impose an expression pattern on the genome are initially from the mother, and other such influences continue throughout development in the womb. Where we draw the line between nature and nurture is not at all obvious. There is an almost seamless transition from one to the other. 'Lamarckism', the inheritance of acquired characteristics, lurks in this fuzzy crack to a degree yet to be defined (Jablonka & Lamb, 1995, 2005). As the evolutionary geneticist Maynard Smith says, 'It [Lamarckism] is not so obviously false as is sometimes made out' (Maynard Smith, 1998).

Inheritance of the egg cell is important for two reasons. First, it is the egg cell DNA-reading machinery (a set of around 100 proteins and the associated cellular ribosome architecture) that enables the DNA to be used as a

template to make more proteins. Second, the set of other cellular elements, mitochondria, endoplasmic reticulum, microtubules, nuclear and other membranes, and a host of chemicals arranged specifically in cellular compartments, is also inherited. Most of this is not coded for by DNA sequences. Lipids certainly are not so coded. But they are absolutely essential to all the cell architecture. There would be no cells, nuclei, mitochondria, endoplasmic reticulum, ribosomes and all the other cellular machinery and compartments without the lipids. The specific details of all this cellular machinery matter. We can't make any old DNA do its thing in any old egg cell. Most attempts at interspecies cloning simply don't work. Invariably, a block occurs at an early stage in development. The only successful case so far is that of a wild ox (*Bos javanicus*) cloned in a domestic cow egg. The chances are that it will work only in very closely related species. The egg cell information is therefore also species specific.

Could epigenetic inheritance and its exclusion from the germ cell line be a requirement of multicellular harmony? The exact number of cell types in a human is debatable. It is partly a question of definition. A project that seeks to model all the cell types in the body, the Human Physiome Project (Crampin *et al.* 2004), estimates that there are around 200, all with completely different gene expression patterns. There would be even more if one took account of finer variations, such as those that occur in various regions of the heart and which are thought to protect the heart against fatal arrhythmias.

The precise number is not too important. The important fact is that it is large and that the range of patterns of gene expression is therefore also large and varied. Their patterns must also be harmonious in the context of the organism as a whole. They are all in the same boat; they sink or swim together. Disturbing their harmony would have serious consequences. It was arrived at after more than 2 billion years of experimentation.

Each cell type is so complex that the great majority of genes are expressed in many cell types. So it makes sense that all the cells in the body have the same gene complement, and that the coding for cell type is transmitted by gene marking, rather than by gene complement. I think that this gives the clue to the purpose of re-setting in germ-line inheritance. Consider what would happen if germ-line inheritance reflected adaptive changes in individual cell types. Given that all cell types derive ultimately from the fused germ-line cells, what would the effect be? Clearly, it would be to alter the patterns of expression in nearly all the cell types. There would be no way to transmit an improvement in, say, heart function to the next generation via gene marking of the germ cells without *also* influencing the gene expression patterns in many other types of cell in the body. And of course there is no guarantee that what is beneficial for a heart cell will be so in, say, a bone cell or a liver cell. On the contrary, the chances are that an adaptation beneficial in one cell type would be likely to be deleterious in another.

Much better, therefore, to let the genetic influences of natural selection be exerted on undifferentiated cells, leaving the process of differentiation to deal with the fine-tuning required to code for the pattern of gene expression appropriate to each type of cell. If this explanation is correct, we would not necessarily expect it to be 100% effective. It is conceivable that some germ-line changes in gene expression patterns might be so beneficial for the organism as a whole, despite deleterious effects on a few cell lines, that the result would favour selection. This could explain the few cases where germ-line 'Lamarckian' inheritance seems to have occurred. It also motivates the search for other cases. The prediction would be that it will occur in multicellular species only when beneficial to overall intercellular harmony. It might be more likely to occur in simpler species. That makes sense in terms of the few examples that we have so far found (Maynard Smith, 1998). Notice that, in contrast to the central dogma, this explanation is a systems level explanation.

Finally, in this section, I will comment on the concept of code. Applied to DNA, this is clearly metaphorical. It is also a useful metaphor, but we should beware of its limitations. One of these is to imply that only information that is coded is important, as in talk of the genome as the 'book of life'. The rest of cellular inheritance is not so coded; in fact, it is not even digital. The reason is very simple. The rest of the cellular machinery doesn't need to 'code for' or get 'translated into' anything else for the simple reason that it 'represents' itself; cells divide to form more cells, to form more cells, and so on. In this sense, germ-line cells are just as 'immortal' as DNA but a lot of this information is transmitted directly without having to be encoded. We should beware of thinking that only digitally 'coded' information is what matters in genetic inheritance.

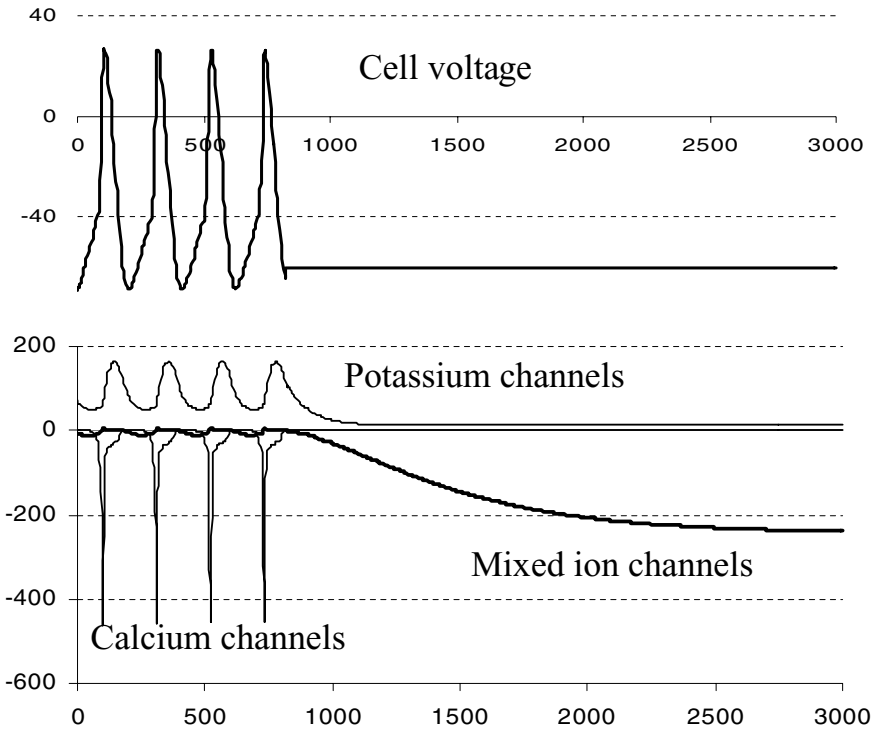
Fourth principle: the theory of biological relativity; there is no privileged level of causality. A fundamental property of systems involving multiple levels between which there are feedback control mechanisms is that there is no privileged level of causality. Consider, as an example, the cardiac pacemaker mechanism. This depends on ionic current generated by a number of protein channels carrying sodium, calcium, potassium and other ions. The activation, de-activation and inactivation of these channels proceed in a rhythmic fashion in synchrony with the pacemaker frequency. We might therefore be tempted to say that their oscillations generate that of the overall cell electrical potential, i.e. the higher-level functionality. But this is not the case. The kinetics of these channels varies with the electrical potential. There is therefore feedback between the higher-level property, the cell potential, and

the lower level property, the channel kinetics (Noble, 2006; chapter 5). This form of feedback was originally identified by Alan Hodgkin working on the nerve impulse, so it is sometimes called the Hodgkin cycle. If we remove the feedback, e.g. by holding the potential constant, as in a voltage clamp experiment, the channels no longer oscillate (Fig. 3). The oscillation is therefore a property of the system as a whole, not of the individual channels or even of a set of channels unless they are arranged in a particular way in the right kind of cell.

Nor can we establish any priority in causality by asking which comes first, the channel kinetics or the cell potential. This fact is also evident in the differential equations we use to model such a process. The physical laws represented in the equations themselves, and the initial and boundary conditions, operate *at the same time* (i.e. during every integration step, however infinitesimal), not sequentially.

It is simply a prejudice that inclines us to give some causal priority to lower-level, molecular events. The concept of level in biology is itself metaphorical. There is no literal sense in which genes and proteins lie *underneath* cells, tissues and organs. It is a convenient form of biological classification to refer to different levels, and we would find it very hard to do without the concept (Fig. 4). But we should not be fooled by the metaphor into thinking that 'high' and 'low' here have their normal meanings. From the metaphor itself, we can derive no justification for referring to one level of causality as privileged over others. That would be a misuse of the metaphor of level.

One of the aims of my book, *The Music of Life* (Noble, 2006), is to explore the limitations of biological metaphors. This is a form of linguistic analysis that is rarely applied in science, though a notable exception is Steven J. Gould's monumental work on the theory of evolution



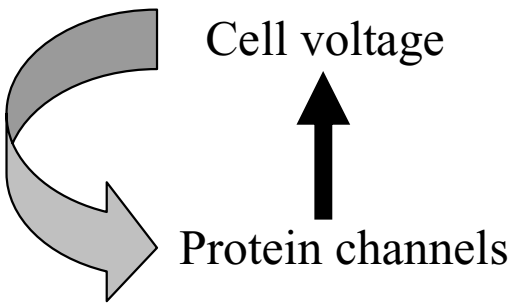


Figure 3. Computer model of pacemaker rhythm in the heart (reproduced with permission from Noble & Noble, 1984)
For the first four beats, the model is allowed to run normally and generates rhythm closely similar to a real heart. Then the feedback from cell voltage to protein channels is interrupted. All the protein channel oscillations then cease. They slowly change to steady, constant values. The diagram shows the causal loop involved. Protein channels carry current that changes the cell voltage (upward arrow), while the cell voltage changes the protein channels (downward arrow). In the simulation, this downward arrow was broken at 800 ms.

(Gould, 2002), in which he analyses the arguments for the multiplicity of levels at which natural selection operates.

These points can be generalized to any biological function. The only sense in which a particular level might be said to be privileged is that, in the case of each function, there is a level at which the function is integrated, and it is one of our jobs as biological scientists to determine what that level may be.

The idea that there is no privileged level of causality has a much wider range of applications than purely biological ones (Dupré, 1993; Cartwright, 1999; Keller, 2002), though the idea is rarely expressed in this bold, relativistic form. I use the word 'relativity' in formulating the principle because it shares certain features with theories of scale relativity proposed by some theoretical physicists, in particular the idea that there is no privileged scale, which is at the foundation of the theory of scale relativity (Nottale, 1993). There is an obvious correlation between scale and level, since lower and higher levels in any system operate at different scales. For this reason, some have proposed the application of the scale relativity theory framework and its associated mathematical tools to tackle the challenge of multiscale integration in systems biology (Nottale, 2000; Auffray & Nottale, 2008; Nottale & Auffray, 2008). But it is too early to judge whether this can provide a firm basis to a fully fledged theory of systems biology. Although the theory of scale relativity has already delivered a number of predictions in the realm of astrophysics which have been validated by subsequent observations, it still has to establish fully its position within theoretical physics. Nor is it possible yet to decide which principles are specific to systems biology and which are of general importance beyond the boundaries of biology.

Fifth principle: gene ontology will fail without higher-level insight. Genes, as defined by molecular genetics to be the coding regions of DNA, code for proteins. Biological function then arises as a consequence of multiple interactions between different proteins in the context of the rest of the cell machinery. Each function therefore depends on many genes, while many genes play roles in multiple functions. What then does it mean to give genes names in terms of functions? The only unambiguous labelling of genes is in terms of the proteins for which they code. Thus, the gene for the sodium–calcium exchange protein is usually referred to as *ncx*. Ion channel genes are also often labelled in this way, as in the case of sodium channel genes being labelled *scn*.

This approach, however, naturally appears unsatisfactory from the viewpoint of a geneticist, since the original question in genetics was not which proteins are coded for by which stretches of DNA [in fact, early ideas on where the genetic information might be found (Schrödinger, 1944) favoured the proteins], but rather what is responsible for higher-level phenotype characteristics. There is no one-to-one correspondence between genes or proteins and higher-level biological functions. Thus, there is no 'pacemaker' gene. Cardiac rhythm depends on many proteins interacting within the context of feedback from the cell electrical potential.

Let's do a thought experiment. Suppose we could knock out the gene responsible for L-type calcium channels and still have a living organism (perhaps because a secondary pacemaker takes over and keeps the organism viable – and something else would have to kick-in to enable excitation–contraction coupling, and so on throughout the body because L-type calcium channels are ubiquitous!). Since

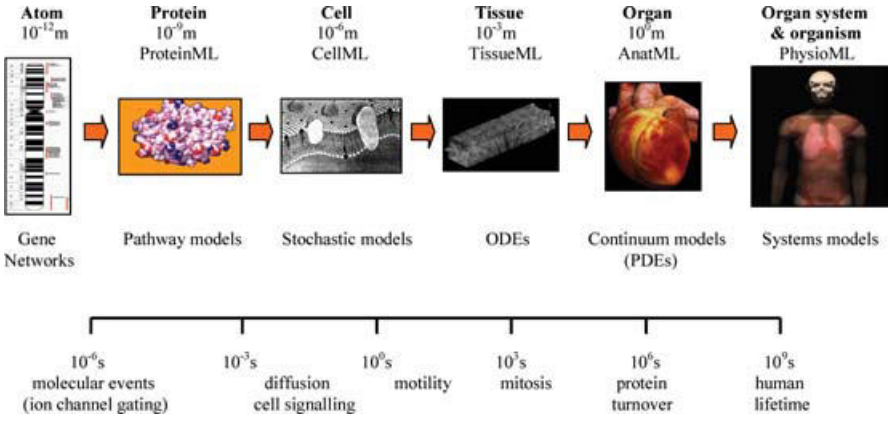


Figure 4. Spatial (top) and temporal (bottom) scales encompassed by the Human Physiome Project
The types of mathematical model appropriate to each spatial scale are also indicated. The last two images on the right in this figure, and all subsequent anatomical images, are from anatomically based models developed by the Auckland Bioengineering group. The tissue image is a three-dimensional confocal microscopy reconstruction of a transmural segment of rat heart by the Auckland group led by Peter Hunter (Hunter *et al.* 2002). Abbreviations: ML, markup language; ODE, ordinary differential equations; PDE, partial differential equations. Reproduced with Permission from Hunter *et al.* (2002).

L-type calcium current is necessary for the upstroke of the action potential in the SA node of most species, we would find that we had abolished normal pacemaker rhythm. Do we then call the gene for L-type calcium channels the 'pacemaker' gene? The reason why this is unsatisfactory, even misleading, to a systems-level biologist is obvious. Yet it is the process by which we label many genes with high-level functions. The steadily growing list of 'cancer genes' have been identified in this way, by determining which mutations (including deletions) change the probability of cancer occurring. We can be fairly sure though that this characteristic is not why they were selected during the evolutionary process. In this sense, there are no 'cancer genes'. As the Gene Ontology (GO) Consortium (http://geneontology.org/) puts it, 'oncogenesis is not a valid GO term because causing cancer is not the normal function of any gene'.

Another good example of this approach is the discovery of what are called clock genes, involved in circadian rhythm. Mutations in a single gene (now called the *period* gene) are sufficient to abolish the circadian period of fruit flies (Konopka & Benzer, 1971). This discovery of the first 'clock gene' was a landmark, since it was the first time that a single gene had been identified as playing such a key role in a high-level biological rhythm. The expression levels of this gene are clearly part of the rhythm generator. They vary (in a daily cycle) in advance of the variations in the protein for which they code. The reason is that the protein is involved in a negative feedback loop with the gene that codes for it (Hardin *et al.* 1990). The idea is very simple. The protein levels build up in the cell as the *period* gene is read to produce more protein. The protein then diffuses into the nucleus, where it inhibits further production of itself by binding to the promoter part of the gene sequence. With a time delay, the protein production falls off and the inhibition is removed so that the whole cycle can start again. So, we not only have a single gene capable of regulating the biological clockwork that generates circadian rhythm, it is itself a key component in the feedback loop that forms the rhythm generator.

However, such rhythmic mechanisms do not work in isolation. There has to be some connection with light-sensitive receptors (including the eyes). Only then will the mechanism lock on to a proper 24 h cycle rather than free-running at say 23 or 25 h. In the mouse, for example, many other factors play a role. Moreover, the clock gene itself is involved in other functions. That is why Foster and Kreitzman have written 'What we call a clock gene may have an important function within the system, but it could be involved in other systems as well. Without a complete picture of all the components and their interactions, it is impossible to tell what is part of an oscillator generating rhythmicity, what is part of an input, and what is part of an output. In a phrase, it ain't that simple!' (Foster & Kreitzman, 2004).

Indeed not. The *period* gene has also been found to be implicated in embryonic development as the adult fly is formed over several days, and it is deeply involved in coding for the male love songs generated by wing-beat oscillations which are specific to each of around 5000 species of fruit fly and ensure that courtship is with the right species. Perhaps it should be renamed the 'fruit fly love gene'!

The point is obvious. We should not be misled by gene ontology. The first function a gene is found to be involved in is rarely, if ever, the only one and may not even be the most important one. Gene ontology will require higher-level insight to be successful in its mission. Moreover, current methods of relating genotype to phenotype suffer from a major methodological limitation: by determining the effects of *changes* (mutations) in the genome, we can say little *a priori* on the direct causal relations between wild-type genes and the phenotype. They reveal simply the *differences* produced as a result of the *change* in genotype. All the causal effects *common* to both the wild-type and the mutated gene are hidden. What is observed may be just the tip of the iceberg.

Gene ontology in its fullest sense, as originally conceived by geneticists to relate genes to high-level features, is therefore very difficult and subject to many traps for the unwary. This would explain why projects such as the GO Consortium are more limited in their scope. Thus, GO assigns three categories to a gene, namely molecular function, biological process and cellular component, which are not intended to deal with higher-level function. It specifically excludes protein domains or structural features, protein–protein interactions, anatomical or histological features above the level of cellular components, including cell types, and it excludes the environment, evolution and expression. In other words, it excludes virtually all of what we classically understand by physiology and most aspects of evolutionary biology.

Sixth principle: there is no genetic program. No genetic programs? Surely, they are all over the place! They are the crown jewels of the molecular genetic revolution, invented by none other than the famous French Nobel Prize winners, Monod and Jacob (Monod & Jacob, 1961; Jacob, 1970). Their enticing idea was born during the early days of electronic computing, when computers were fed with paper tape or punched cards coded with sequences of instructions. Those instructions were clearly separate from the machine itself that performed the operations. They dictated those operations. Moreover, the coding is digital. The analogy with the digital code of DNA is obvious. So, are the DNA sequences comparable to the instructions of a computer program?

An important feature of such computer programs is that the program is separate from the activities of the machine that it controls. Originally, the separation was

physically complete, with the program on the tape or cards only loaded temporarily into the machine. Nowadays, the programs are stored within the memory of the machine, and the strict distinction between the program, the data and the processes controlled may be breaking down. Perhaps computers are becoming more like living systems, but in any case the concept of a genetic program was born in the days when programs were separate, identifiable sets of instructions.

So, what do we find when we look for genetic programs in an organism? We find no genetic programs! There are no sequences of instructions in the genome that could possibly play a role similar to that of a computer program. The reason is very simple. A database, used by the system as a whole, is not a program. To find anything comparable to a program we have to extend our search well beyond the genome itself. Thus, as we have seen above, the sequence of events that generates circadian rhythm includes the *period* gene, but it necessarily also includes the protein for which it codes, the cell in which its concentration changes and the nuclear membrane across which it is transported with the correct speed to effect its inhibition of transcription. This is a gene–protein–lipid–cell network, not simply a gene network. The nomenclature matters. Calling it a gene network fuels the misconception of genetic determinism. In the generation of a 24 h rhythm, none of these events in the feedback loop is privileged over any other. Remove any of them, not just the gene, and you no longer have circadian rhythm.

Moreover, it would be strange to call this network of interactions a program. The network of interactions *is itself the circadian rhythm process*. As Enrico Coen, the distinguished plant geneticist, put it, 'Organisms are not simply manufactured according to a set of instructions. There is no easy way to separate instructions from the process of carrying them out, to distinguish plan from execution' (Coen, 1999). In short, the concept of a program here is completely redundant. It adds nothing to what a systems approach to such processes can reveal.

Seventh principle: there are no programs at any other level. I have introduced the analogy of the genome as a database and the metaphor of 'genes as prisoners' in order to provoke the change in mindset that is necessary for a fully systems approach to biology to be appreciated. The higher levels of the organism 'use the database' and 'play the genome' to produce functionality. If the genome can be likened to a huge pipe organ (Noble, 2006; chapter 2), then it seems correct to ask who is the player, who was the composer? If we can't find the program of life at the level of the genome, at what level do we find it? The answer is 'nowhere'!

We should view all such metaphors simply as ladders of understanding. Once we have used them we can, as it were, throw them away. This way of thinking can seem strange to some scientists for whom there must be just one correct answer to any scientific question. I explore this important issue in *The Music of Life* by analysing the 'selfish gene' and 'prisoner gene' metaphors linguistically to reveal that no conceivable experiment could decide which is correct (Noble, 2006; chapter 1). They highlight totally different aspects of the properties of genes. This philosophy is applied throughout the book as it answers questions like 'where is the program of life?' The conclusion is simply that there are no such programs at any level. At all levels, the concept of a program is redundant since, as with the circadian rhythm network, the networks of events that might be interpreted as programs are themselves the functions we are seeking to understand. Thus, there is no program for the heart's pacemaker separate from the pacemaker network itself.

While causality operates within and between all levels of biological systems, there are certain levels at which so many functions are integrated that we can refer to them as important levels of abstraction. Sydney Brenner wrote, 'I believe very strongly that the fundamental unit, the correct level of abstraction, is the cell and not the genome' (unpublished Lecture, Columbia University, 2003). He is correct, since the development of the eukaryotic cell was a fundamental stage in evolutionary development, doubtless requiring at least a billion years to be achieved. To systems physiologists though there are other important levels of abstraction, including whole organs and systems.

Eighth principle: there are no programs in the brain. In his book *The Astonishing Hypothesis*, Francis Crick proclaimed, 'You, your joys and your sorrows, your memories and your ambitions, your sense of personal identity and free will, are in fact no more than the behaviour of a vast assembly of nerve cells and their associated molecules' (Crick, 1994). This is a variation of the idea that in some sense or other, the mind is just a function of the brain. The pancreas secretes insulin, endocrine glands secrete hormones ... and the brain 'secretes' consciousness! All that's left is to find out how and where in the brain that happens. In one of his last statements, Crick has even hinted at where that may be: 'I think the secret of consciousness lies in the claustrum' (Francis Crick, 2004, quoted by V. S. Ramachanran, in *The Astonishing Francis Crick*, Edge, 18 October, 2004, http://www.edge.org/3rd_culture/crick04/crick04_index.html). This structure is a thin layer of nerve cells in the brain. It is very small and it has many connections to other parts of the brain, but the details are of no importance to the argument. The choice of brain location for the 'secret of consciousness' varies greatly according to the author. Descartes even thought that it was in the pineal gland. The mistake is always the same, which is to think that in some way or other the brain is a kind of performance space in which the world of perceptions is reconstructed

inside our heads and presented to us as a kind of Cartesian theatre. But that way of looking at the brain leaves open the question: where is the 'I', the conscious self that sees these reconstructions? Must that be another part of the brain that views these representations of the outside world?

We are faced here with a mistake similar to that of imagining that there must be programs in the genomes, cells, tissues and organs of the body. There are no such programs, even in the brain. The activity of the brain and of the rest of the body simply *is* the activity of the person, the self. Once again, the concept of a program is superfluous. When a guitarist plays the strings of his guitar at an automatic speed that comes from frequent practice, there is no separate program that is making him carry out this activity. The patterns and processes in his nervous system and the associated activities of the rest of his body simply *are* him playing the guitar. Similarly, when we deliberate intentionally, there is no nervous network 'forcing' us to a particular deliberation. The nervous networks, the chemistry of our bodies, together with all their interactions within the social context in which any intentional deliberation makes sense, *are* us acting intentionally. Looking for something in addition to those processes is a mistake.

Ninth principle: the self is not an object. In brief, the mind is not a separate object competing for activity and influence with the molecules of the body. Thinking in that way was originally the mistake of the dualists, such as Sherrington and Eccles, led by the philosophy of Descartes. Modern biologists have abandoned the separate substance idea, but many still cling to a materialist version of the same mistake (Bennett & Hacker, 2003), based on the idea that somewhere in the brain the self is to be found as some neuronal process. The reason why that level of integration is too low is that the brain, and the rest of our bodies which are essential for attributes such as consciousness to make sense (Noble, 2006; chapter 9), are tools (back to the database idea again) in an integrative process that occurs at a higher level involving social interactions. We cannot attribute the concept of self-ness to ourselves without also doing so to others (Strawson, 1959). Contrary to Crick's view, therefore, our selves are indeed much 'more than the behaviour of a vast assembly of nerve cells and their associated molecules' precisely because the social interactions are essential even to understanding what something like an intention might be. I analyse an example of this point in much more detail in chapter 9 of *The Music of Life*. This philosophical point is easier to understand when we take a systems view of biology, since it is in many ways an extension of that view to the highest level of integration in the organism.

Conclusions

Tenth principle: there are many more to be discovered; a genuine 'theory of biology' does not yet exist. Well, of course, choosing just 10 principles was too limiting. This last one points the way to many others of whose existence we have only vague ideas. We do not yet have a genuine theory of biology. The Theory of Evolution is not a theory in the sense in which I am using the term. It is more an historical account, itself standing in need of explanation. We don't even know yet whether it consists of events that are difficult, if not impossible, to analyse fully from a scientific perspective, or whether it was a process that would have homed in to the organisms we have, regardless of the conditions. My own suspicion is that it is most unlikely that, if we could turn the clock right back and let the process run again, we would end up with anything like the range of species we have today on earth (Gould, 2002).

But, whichever side of this particular debate you may prefer, the search for general principles that could form the basis of a genuine theory of biology is an important aim of systems biology. Can we identify the logic by which the organisms we find today have succeeded in the competition for survival? In searching for that logic, we should not restrict ourselves to the lower levels. Much of the logic of living systems is to be found at the higher levels, since these are often the levels at which selection has operated (Keller, 1999; Gould, 2002) and determined whether organisms live or die. This is the level at which physiology works. Physiology therefore has a major contribution to make to systems biology.

In conclusion, I return to the theme with which this article began. Claude Bernard's concept of the constancy of the internal environment was the first example of multilevel functionality. It was critical in defining physiology as a subject distinct from the applications of physics and chemistry. The challenge we face today resembles that faced by Bernard in the mid-nineteenth century, but the chemistry involved is that of the molecule DNA. The answer though should be much the same. Higher-level control cannot be reduced to lower-level databases like the genome. A major part of the future of physiology surely lies in returning to our roots. Higher-level systems biology is, I suggest, classical physiology by another name.

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Gregor Mendel at the source of genetics and systems biology

Celebrating the relevance of Gregor Mendel's experiments on the development of hybrid plants on the occasion of his bicentenary

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Gregor Mendel is generally presented as the 'ignored and solitary founder of genetics'. This Moravian friar would have worked in strict isolation on the heredity of peas in the garden of his monastery, and his experiments would have been ignored by his contemporaries, before being 'rediscovered' independently by three botanists in 1900, 34 years after their publication. Historians have contributed to replace the genesis of Mendel's work in the context of his time, questioning the mythical image that prevailed in academic circles and the public perception. This paper recalls that Mendel benefitted from a very favourable context for the development of his experiments at St Thomas Monastery in Brno and was not isolated from the scientific community of his time. Although the notions on which his work was based were already present in scientific publications, this does not diminish the importance of Mendel's contribution to the development of modern biology. We provide a detailed analysis of the results of his experiments on the development of hybrid plants that he presented in two lectures at the Brno Society of Natural History in 1865, demonstrating that beyond his major contribution to the foundation of genetics, Mendel was one of the pioneers of systems biology.

ADDITIONAL KEYWORDS: bicentenary celebration – genetics – Gregor Mendel – systems biology.

GREGOR MENDEL, A MORAVIAN FRIAR IN THE BIRTHPLACE OF GENETICS

BRNO, THE ECONOMIC AND ACADEMIC CAPITAL OF MORAVIA IN THE 19TH CENTURY

According to his autobiography, his family relatives and informed biographers, Johann Mendel was born on 22 July 1822 (Iltis, 1954; Schindler, 1965; Van der Pas, 1972; Klein & Klein, 2013) in the village of Heinzendorf, located in Silesia close to the north border of Moravia, a province that was part of the Austro-Hungarian Empire, the capital of which, Brno, is close to the Austerlitz battlefield. As Vítězslav Orel indicates in his magisterial biography of Mendel (Orel, 1996), it is in Brno, as early as 1819, that the first empirical laws of genetics were published. They were formulated by Count Festetics at the request of the naturalist Christian Carl André, in response to the concerns of sheep farmers, who were eager to find ways to improve the quality of wool of their animals and had formed an Association for the Promotion of Agriculture (Orel & Wood, 1998; Poczai *et al.*, 2014). The term 'genetics' appears here for the first time, well before the beginning of the 20th century. In this context, the question of inheritance was widely debated in Brno during the first half of the 19th century, notably by Cyrill František Napp, the abbot of the Augustinian monastery of the city. Napp chaired the local Association of Pomology created by André to address the issue of improving fruit trees through artificial pollination. This association was publishing the results obtained in Germany and England, among them the work of Thomas Andrew Knight, the President of the London Horticultural Society. Knight had initiated experiments on the hybridization of annual plants as early as the end of the 18th century and described in peas the phenomenon of dominance, the uniform appearance of the hybrids and the assortment of parental characters in the offspring.

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Saint Thomas Monastery, a horticultural research and education organization

In 1854, Napp had installed an experimental greenhouse and a botanical garden within the monastery (Orel, 1996), whose members had the duty, by Imperial decree, to be actively involved in teaching at the secondary and higher education establishments of the city. Johann entered the monastery in 1843 under the name of Gregor and became a priest in 1847, allocated on emergency to the service of the Altbrünn parish after three of the priests died from an epidemic of infectious disease that Mendel also most probably contracted (Nivet, 2004).

Gregor Mendel had already been identified by Napp as gifted for the sciences. Several monks of the monastery participated in the insurrection of 1848 against the imperial power. Among them was Matouš František Klácel, a professor of philosophy and polemist, who petitioned, with the support of Mendel, for the right of the monks to choose freely between pastoral service and the study and teaching of the sciences (Nivet, 2006); Klácel was indeed very influential to the young Mendel's fate (Peaslee & Orel, 2007). Gregor Mendel was nominated in 1849 as a professor by Imperial decree. According to Nivet, Napp used these circumstances astutely to free Mendel from his parish duties and sent him to Vienna to follow a university course for 2 years, with the aim of qualifying for higher education teaching (Nivet, 2004).

In Vienna, Gregor Mendel was introduced to the cell theory and the experimental method by the botanist Franz Unger and the physicist Christian Doppler. Following the ideas of Unger, who was in favour of the concept of a particulate inheritance, Mendel stepped away from the dominating idea of inheritance by blending, according to which fluids coming from the parents would mix in their progeny, which was endorsed by his contemporary, Charles Darwin, who frequently refers to blended characters in his book on *The Variation of Animals and Plants under Domestication* (Darwin, 1868).

In 1856, when Gregor Mendel began his experiments on hybrid plants, he had acquired an excellent level of university training. Moreover, he worked in a favourable environment, in terms of both theoretical discussions and material conditions: he benefitted from half a century of active debates within the Brno learning associations and from the experimental infrastructures of the monastery. Furthermore, he was actively encouraged by his superior Napp who, as soon as 1836, had formulated the question, 'what is transmitted, and how is it transmitted?', as a subject for fundamental research on inheritance that had to be resolved using the experimental method (Serre, 1984; Orel, 2009).

Johann Mendel, aspiring botanist and researcher

According to one of his first biographers (Iltis, 1924), the young Johann Mendel had demonstrated since his early years in a peasant family an excellent aptitude for observation and had been introduced to botany and agronomy by his parents, his schoolmaster and the village priest, Schreiber, himself a correspondent of the Brno Agricultural Society. All had encouraged him to pursue his studies because of his brilliant results in the unique class of the village school. He joined the monastery in Brno in 1843 after completing a course at the Philosophical Institute in Olmütz, where he studied physics, mathematics and logic (Orel, 1996; Klein & Klein, 2013). The modest resources of his family were insufficient to support the full curriculum of higher education. In his early years living in a pleasant rural environment, he was identified as a gifted young boy by his schoolmasters, but owing to an accidental fall of his father at the farm, he had on several occasions to interrupt his curriculum to take care of the chores (Klein, 2000; Klein *et al.*, 2009; Nivet, 2020).

Johann Mendel had therefore the right profile articulated prophetically in 1820 by G. Hempel, a member of the Agriculture Society in Brno, to describe the new type of natural scientist that would have to emerge in order to explain the laws of hybridization of sexual plants: 'able to conduct demanding experiments; a researcher having a profound knowledge of botany and acute observation capabilities, who could, with a tireless and relentless patience, capture the subtleties of these experiments, firmly take them under control, and provide a clear explanation' (Orel, 1996).

The legendary discoveries of a wrongly unknown genius

Thus, Gregor Mendel is not the 'unknown genius' described in the historical textbooks, who would have discovered the laws of heredity alone and without any help, guided only by his passion for peas (Blanc, 1984). As a matter of fact, most of the notions and knowledge on which Mendel's work relies can be found in the scientific literature of his time. It is known that he had access to it through the books available in the excellent library of the monastery (Figs 1, 2). He was in no way isolated from the scientific and industrial community of his time, as testified by his numerous trips to congress and exhibits in Austria, England, France, Germany and Italy, as he had affiliations to learned societies.

Knight had proposed the pea as a particularly well-adapted experimental model for the study hybridization and had described the phenomenon of dominance, the uniform appearance of the hybrids,

Figure 1. The rich main library at St Thomas Monastery, Brno.

Figure 2. Portrait of Mendel (left) on the door of his private library (right) at St Thomas Monastery.

and the assortment of the parental characters in the offspring. These notions had been reinforced by other breeders, such as the British J. Goss and A. Seton, who worked on the same peas of the genus *Pisum* studied by Mendel, or the French A. Sageret working on the melon, when Mendel was still a schoolboy. Moreover, the German Gaertner had published in 1849 a monumental treatise, in which he described the artificial pollination method he had used no less than 10 000 times in 700 species of plants, including, in particular, its use to study the first four characters that Mendel included in his study. Mendel knew this work well, and refers to it in his report, because it was the topic of lessons given by F. Diebl at the Institute of Philosophy in Brno that Mendel attended (Orel & Wood, 1998). It is therefore clear that Mendel is not the discoverer of these notions, nor the inventor of the hybridization methods or the choice of *Pisum* as experimental model. One has to look elsewhere for what made his work an inescapable masterpiece for biology.

Back to the source: *Versuche über Pflanzen-Hybriden* by Gregor Mendel

In this perspective, it is appropriate to refer to the small number of Mendel's original publications and, in particular, to his paper, *Versuche über Pflanzen-Hybriden*, written in German, his native language. It was published in 1866 by the Society of Natural History of Brünn (the German name of Brno), on the basis of the two lectures presented by Mendel on 8 February and 8 March 1865, in which he reported the results of > 8 years of experimental work (Mendel, 1866; Bateson, 1902; Naudin *et al.*, 1990). The two lectures were well attended and echoed in the local press (Zhang *et al.*, 2017).

The first impression that comes to mind on reading this text is its modern and clear style, although there has been, since the 'rediscovery' of his work, a wealth of never-ending interpretations and speculations on what Mendel really discovered (Sturtevant & Lewis, 2001) or thought he had uncovered (Hartl & Orel, 1992; Orel & Wood, 2000). The reality of this rediscovery and its interpretation are subject to contradictory debates. In 1936, the mathematician and geneticist Ronald Fischer suggested that Mendel had, voluntarily or not, biased his observations, while refuting the view put forward by Bateson, who conjectured that Mendel's paper was not a literal description of his work but a reconstruction (Fisher, 1936). Taking broader views, Jan Sapp has described 'the nine lives of Mendel' and the posthumous controversies that his work triggered and continue to feed (Sapp, 1990; Olby, 1997), while John Porteous, after showing that it is still not possible to account fully for Mendel's observations, made the case for a rational use of Mendelian genetics that takes into account the discoveries of the last century dominated by the development of molecular biology (Porteous, 2004a, b).

A question of vocabulary: factors, elements and genes

The second striking fact when reading Mendel's paper is the vocabulary that he uses or, more precisely, the words he does not use. There is no gene or genetics in sight and only one explicit but indirect mention of heredity. Indeed, the term 'gene' was introduced only in 1909 (Johannsen, 1909), simplifying the terminology of Hugo de Vries, one of the 'rediscoverers' of Mendel's laws, who used the term 'pangenes' to designate the elementary units of heredity, in reference to Charles Darwin's theory of pangenesis, in which he referred to gemmules, probably the equivalent of the exosomes that are the focus of intense research nowadays (Noble, 2020). Even more surprising, the term 'factor', taught to students as representing the gene for Mendel, appears only twice, with two distinct meanings: the first, 'it remains more than probable that there is a factor in action for the variability of cultivated plants, which hitherto has received little attention', probably refers to the fact that the different forms of the characters are transmitted separately (the law of independent assortment), whereas the second, 'We must then treat it as necessary that the very same factors combine in the production of constant forms in the hybrid plant', could designate the elementary units of heredity. Indeed, Mendel uses the term 'element' and not 'factor' on ten occasions in his conclusions, clearly indicating his standing in the framework of a particulate concept of heredity, in contrast to Darwin, who supported the blending hypothesis of heredity (Mayr, 1982).

Sexual reproduction and 'development' at the heart of Mendel's interests

Hartl & Orel (1992) follow Mayr in using the argument of pedagogic repetition to discern in Mendel's text the main idea he wants to convey: 'The law of combination of the differing characters, by which the development of hybrids results, finds its foundation and explanation accordingly in the conclusive principle that hybrids produce germ and pollen cells corresponding in equal number to all constant forms that arise from the combination of the characters united through fertilization'. This idea is repeated six times in different forms. This vision contrasts, they say, 'with the traditional presentation according to which Mendel discovered that hereditary characters are determined by cellular elements, now called genes, that exist in pairs, are submitted to independent segregation

and assortment, and persist unchanged through the successive generations of hereditary transmission' (Hartl & Orel, 1992).

This point of view insists on the fact that Mendel mostly endeavoured to establish explicitly the equal contribution of both sexes to the generation of offspring, with the underlying mechanism remaining implicit, because it was not accessible through his experimental means: chromosomes and nuclein were described after 1865, and their roles understood much later. Iris Sandler insists on the fact that Mendel uses, in different forms throughout his exposé, not less than 50 times the German term *Entwicklung*, usually translated as 'development', in the broad sense that prevails in the 19th century (Sandler, 2000), when the term 'evolution' was not yet widely used (Abbott & Fairbanks, 2016; Fairbanks, 2020). To her, this term is the central element of Mendel's thought, reflecting his interest in the study of a biological process that includes the transmission of characters and their manifestation throughout the life of an organism and a species.

In order to convince ourselves of the relevance of this view, let us follow Gregor Mendel step by step in the different sections of his exposé. In the sections that follow, we use one of the most recent English translations of Mendel's report because it takes into account the contextual relationships of Mendel's and Darwin's parallel and independent works and thoughts (Abbott & Fairbanks, 2016; Fairbanks & Abbott, 2016; Fairbanks, 2020); for another see Müller-Wille & Hall (2016).

GREGOR MENDEL'S METHOD: AN OBSERVATION, AN OBJECTIVE, AN EXPERIMENTAL PLAN

Introductory remarks

Whatever importance is given to what was formulated by Mendel implicitly or explicitly, his text is one of the foundations of modern biology because of the methodology he uses and the mathematical formalism he introduces, as was the case for William Harvey, the founder of physiology in the 17th century (Auffray & Noble, 2009). Even in his preliminary remarks, he indicated clearly the initial observations on which he founded his study:

> The striking regularity with which the same hybrid forms reappeared whenever fertilisation took place between the same species was the stimulus for further experiments, whose objective was to follow the development of hybrids in their progeny.

The starting point is the observation of a regularity, in the manner of the meteorologist Mendel also was, registering daily for many years the climate parameters in Brno and publishing in one of his nine papers in this field a careful description of a tornado. Indeed, Mendel published twice as many papers on meteorology as on biology, a fact that has been recognized by naming the Czech polar station after his name ('Mendel Polar Station', 2022). Mendel then precisely states the problem he proposes to study and resolve (i.e. the search for a law governing the development of hybrids):

> That a generally standard law for the formation and development of hybrids has not yet been successfully given is no wonder to anyone who knows the extent of the subject and who realizes the difficulties with which experiments of this kind must struggle. A final determination will result only when detailed experiments on the most diverse plant families are available.

Referring to the breeders of the 18th and 19th centuries, such as Koelreuter, Gaertner, Herbert, Lecocq and Wichura, cited nominally, Mendel mentions the difficulties they encountered and introduces as a means to overcome them the objective of establishing the numerical ratios that exist between the hybrid forms in the successive generations. In other words, his objective is to establish a mathematical formalism, a model of the phenomenon of his study, the development of the hybrids:

> Anyone who surveys the work in this area will be convinced that among the numerous experiments, none have been carried out in the extent and manner that would make it possible to determine the number of the various forms in which the progeny of hybrids appear, so that one could, with confidence, arrange these forms into the individual generations and determine their relative numerical relationships. Some courage is certainly required to undertake such an extensive work; nevertheless, it seems to be the only proper means to finally reach resolution of a question regarding the evolutionary history of organic forms, the importance of which must not be underestimated.

Notably, Mendel tackles the development of organisms in a global manner, without referring explicitly to the underlying questions of heredity and evolution. He concludes his preliminary remarks by introducing the notion of an experimental plan:

> Whether the plan by which the individual experiments were arranged and carried out corresponds to the given objective may be determined through a benevolent judgment.

Such a concise and modest statement can only trigger admiration for what constitutes one of Mendel's major contributions to biology. While following Mendel step by step in the exposé of his motives, his hypotheses, his results, and the practical and theoretical means he employs, one will see that he is not only at the origin of genetics, which should be considered as a by-product of his work developed by his successors, the geneticists, but he is, first and foremost, through his global approach to the development of organisms and the back-and-forth move between mathematical modelling to generate hypotheses and experimental exploration of these hypotheses, one of the founders of systems biology, together with William Harvey and Claude Bernard (Noble, 2008; Auffray & Noble, 2009). His research is integrative in nature and conducted iteratively to study and model biological systems as complex systems in interaction with their environment. His methodology should therefore be considered as a forerunner of the systems approaches that have become pervasive in biology, physiology and medicine since the beginning of the 21st century (Auffray *et al.*, 2020).

Selection of the experimental plants

Gregor Mendel introduces his experimental plan by a sentence that would merit being posted at the entrance of all biological laboratories:

> The worth and validity of any experiment are determined by the suitability of the materials as well as by their effective application.

He then defines the necessary and sufficient conditions to ensure that the observations made during his experiments would be protected from perturbations that would render their interpretation difficult or even impossible. He chooses from the outset to study individual characters that are easy to distinguish in the hybrids and to follow in their offspring, and he identifies uncontrolled pollination and fertility defects as potential obstacles to the discernment of the laws of development of hybrids:

> The experimental plants must necessarily
> 1. Possess constantly differing characters.
> 2. At the time of flowering, their hybrids must be protected from the action of all pollen from other individuals or be easily protected.
> 3. The hybrids and their progeny in the succeeding generations must not suffer any noticeable disturbance in fertility.

Mendel pays particular attention to the role of pollinating insects. He then clarifies what he means by detailed experiments, which he underlined already in his preliminary remarks, by pointing to the necessity of an exhaustive description, insisting that all individuals must be observed:

> To recognise the relationships of the hybrid forms to one another and to their original parents, it appears to be necessary that every member that develops in the series in every single generation be subjected to observation.

This concern about exhaustivity, in concordance with the fourth precept of Descartes' *Méthode*, explains why Mendel's approach could have been considered as strictly analytical and reductionist (Serre, 1984). However, in his project to study the development of the hybrids as a process, Mendel always pays attention to the context, the dynamic relationships and the conditions that determine the stable or unstable behaviour of the hybrids. He retains from his observations only what is pertinent in relationship to the question driving his study. From this point of view, he is more Cartesian than the early 20th century geneticists who developed his work further while 'reducing' it to the sole question of heredity, when Mendel dealt also with evolution and development. Indeed, he avoids 'carefully precipitation and prevention', and he divides the difficulties in how many parts 'that would be necessary to better resolve them'. In other words, he works out what is necessary and sufficient to resolve his question of interest, without considering other facts that are not indispensable in the framework of the hypotheses he formulates. Thus, he verifies the adequacy of peas to his experimental plan, as the reading of his predecessors had suggested to him, and retains only the convenient varieties, probably because of their very distinct characters and robustness:

> Experiments made with several members of this family (*Leguminosae*) led to the conclusion that the genus *Pisum* sufficiently meets the necessary requirements. Several completely independent forms of this genus possess uniform characters that are easily and certainly distinguishable, and they give rise to perfectly fertile hybrid progeny when reciprocally crossed. … From these, 22 (out of 34) were selected for cross-fertilisation and were cultivated annually throughout the duration of the experiments.

Finally, he considers that his work stands outside the debate on the question of the definition of species and varieties, and thus of the position of the hybrids in evolution, a question that sparked debates after the publications by Darwin and Wallace of their theories on the role of natural selection, which was the subject of the lecture given by Makowsky in January 1865, just before those by Mendel (Fairbanks, 2020):

> The systematic classification is difficult and uncertain. … In any case, these systematic ranks are completely unimportant for the experiments described here.

Mendel thus develops a methodological but not an ontological reductionism; he intends to focus on a precise problem: to clarify the remarkable regularity he has observed in the development of the offspring of the hybrids.

W. Focke referred ~15 times to Mendel in his treatise on plant hybridization published in 1881, without completely understanding its meaning, because he mentions the work on *Pisum* only once. For him, Mendel's work represented an isolated series, poorly significant when compared with the voluminous contributions of Koelreuter, Gaertner and Wichura, with studies that dealt with no less than 98 plant varieties … none of which had been organized with the rigor and precision of Mendel. Nonetheless, it is this single mention that would later draw the attention of the 'rediscoverers', first of all Carl Correns, who attributed 'the laws of heredity' to Mendel. This was an astute manner on his part to circumvent the priority battle that could have developed with Hugo de Vries, who had, like him, conducted experiments similar to those of Mendel, or with Erich von Tschermak-Seysenegg.

One is inclined to think that, without their late knowledge of Mendel's work, all three would have encountered difficulties in making complete sense of their own results. Charles Darwin himself had conducted during 11 years crosses between self-fertilizing plants, which he published in 1876 (Darwin, 1876; Ruse, 2010), without discerning or understanding the meaning of the regularity on which Mendel focused: in one of his crosses, Darwin obtained a ratio of 3.6:1 between the second-generation characters, close to Mendel's 3:1 ratio. Although he received an advance copy of Fockes's 1881 book, Darwin passed it to a colleague without reading it, shortly before he died in 1882 (Sclater, 2006); this was a missed opportunity for two great minds to meet.

Arrangement and order of the experiments

In the following section, Mendel progressively accounts for the details of his experiments, while constantly referring to his aim and to the results of his predecessors on which he relies, particularly the uniformity of the first-generation hybrids:

> If two plants that are constantly different in one or more characters are united through fertilisation, the characters in common are transmitted unchanged to the hybrids and their progeny, as numerous experiments have shown; each pair of differing characters, however, unites in the hybrid to form a new character that generally is subject to variation in the progeny. To observe these variations for each pair of differing characters and to ascertain a law according to which they occur in succeeding generations was the objective of the experiment. This experiment, therefore, breaks up into just as many individual experiments as there are constantly differing characters in the experimental plants.
>
> … the individual experiments, which had to be limited to characters that appear clearly and decidedly in the plants. A successful result would finally show whether they all are observed as portraying identical behaviour in hybrid union …

After the rediscovery of Mendel's work, the reality of his experiments was questioned, on the pretext that the results were 'too good to be true'. William Bateson, who was the first translator of Mendel's work into English and was his active promoter (Bateson, 1902), thought that Mendel had not really conducted the experiments as reported in his paper, because he could not have had access to pure varieties for the seven characters he studied. According to him, Mendel would have proceeded through a reconstruction from experiments in which multiple characters would have co-existed in the same plants. This is curious for the person who proposed to designate the new discipline born from Mendel's discovery by the term 'Genetics'. Contemporary botanists have, on the contrary, demonstrated that Mendel had access to the biological material necessary (Fairbanks & Rytting, 2001; Kemp, 2002). Ronald Fisher, in his 1936 paper that had a resounding echo (Fisher, 1936), showed that Bateson wanted principally to attribute to the Darwinists the blackout on Mendel's work that he was fighting. It is troubling that this pioneer of statistics and the theory of experimental plans had considered it appropriate to suppose that Mendel had, voluntarily or through an excessively zealous assistant, biased his results to set them in concordance with those expected from his theory; these suppositions have now been dismissed (Franklin *et al.*, 2008). Fisher's position is probably explained by his endorsement of the school of thought that rejected the Darwinian interpretation of Mendel's work.

Mendel continues:

> Of a larger number of plants of the same kind, only the most vigorous were selected for fertilisation. Feeble specimens always yield uncertain results …
>
> Further, in all experiments reciprocal crosses were undertaken in this manner: One of the two

kinds that served as seed plants for a number of fertilisations was used as the pollen plant for the other.

For each experiment a number of potted plants were placed in a glasshouse during the flowering period. They served as a control for the main garden experiment in case of possible disturbance by insects.

With more than 10 000 carefully examined plants, the case of such undoubted interference occurred only a few times.

One recognizes here Mendel's concern to preserve his experiments from any undesirable interference, whether internal (the fecundity of the hybrids) or external (the intervention of insects), by taking the necessary controlling measures. Importantly, he introduces systematic cross-fertilization, clearly indicating his interest in measuring precisely the contribution of both sexes to the characters of the hybrid offspring. This is an essential point in Mendel's observations and his working hypothesis, because the idea that had prevailed since antiquity was that of a female matrix stimulated, without a material contribution by the male; an Aristotelian concept reformulated in different forms by the preformationists of Mendel's time.

Mendel has thus been able to take the best advantage of the knowledge and techniques he learned from his masters, in order to design an experimental plan suitable to reach his objective: to provide an explanation for the remarkable regularity during the development of hybrid plants. In contrast to his predecessors, he built his experiments on solid foundations, focusing on what was necessary and sufficient to reach his goal.

GREGOR MENDEL'S SYSTEMIC EXPERIMENTATION: CONTEXTUALIZATION, RELATEDNESS, CONDITIONALITY, PERTINENCE

During the 8 February 1865 session of the Natural History Society in Brno, after summarizing his motives and his method, already discussed in previous sessions, Mendel reports the results he has obtained, insisting on the numerical ratios observed between the different forms of the hybrids during the successive generations. Mendel thus formulates a first mathematical model that conforms to the precepts of systems biology. Indeed, although his approach is based on the Cartesian precepts of objectivity, division, causality and exhaustivity that characterize analytical reductionism, he completes it by adhering to the systemic precepts of contextualization, relatedness, conditionality and pertinence (Auffray *et al.*, 2003).

THE FORM OF THE HYBRIDS

In the first section of his paper dealing with the experimental results, Mendel reports that he first verified, through what we would today call 'pilot experiments', that his experimental model does not fit with the idea of heredity by mixing that predominates, including in Darwin's writings: 'when two commingled breeds exist at first in equal numbers, the whole will sooner or later become intimately blended' (Darwin, 1868). For him, the uniformity of the hybrids prevails as evidence. He then defines the notion of dominance by introducing the appropriate vocabulary:

> The experiments conducted with ornamental plants in past years already produced evidence that hybrids, as a rule, do not represent the precise intermediate form between the original parents.
>
> In the following discussion those characters that are transmitted wholly or nearly unchanged in the hybrid association, that themselves represent the hybrid characters, are defined as dominant, and those that become latent in the association are defined as recessive.

He then summarizes what is one of his major contributions: the equal contribution of the two sexes to the characters of the hybrids, transmitted by the sexual cells during pollination, and underlines the advantage of studying the seed characters that can be observed very rapidly:

> Further, it has been shown through all the experiments that it is completely unimportant whether the dominant character belongs to the seed plant or to the pollen plant; the hybrid form remains exactly the same in both cases.
>
> The hybrid forms of the seed shape and albumen develop directly after artificial fertilisation simply through the action of the pollen from another individual.

One can only recognize here Mendel's extraordinary capacity for synthesis and concision, as he is able to summarize in the same sentence his theoretical and practical views.

THE FIRST GENERATION OF THE HYBRIDS

By careful study of the fate of the different forms of the seven characters examined in the offspring of the hybrids, Mendel confirms the reappearance of the recessive forms, a fact established by his predecessors. This is the disjunction phenomenon that geneticists designated as 'Mendel's first law'. However, Mendel's main contribution is his report of a ratio of 3:1 between the hybrid forms as a general rule observed in all crossings, independently of the contribution of the two sexes:

> In this generation, along with the dominant characters, the recessive characters reappear in their full individuality and do so in the determinate and pronounced average ratio of 3:1, so that of every four plants from this generation, three produce the dominant and one the recessive character. This applies without exception for all characters included in the experiment.
>
> Because the hybrids produced from reciprocal crosses acquired a wholly similar form and because no appreciable variation appeared in their further development, the results for each experiment could be combined.

Mendel lingers on the first two experiments concerning the form (round or wrinkled) and the colour (yellow or green) of the seeds, for which he reports ratios of 2.96:1 and 3.01:1, respectively. He also provides a numerical table of the features of ten out of 250 experimental plants, thus illustrating the variability of the results. He then generalizes his observations to all seven characters, reporting ratios varying between 2.85:1 and 3.15:1:

> As in the individual pods, the distribution of characters varied similarly among individual plants.
>
> These two experiments are important for ascertaining the mean ratios because they produce especially meaningful averages with a smaller number of experimental plants.
>
> If the results of all experiments are summarised, there is an average ratio between the number of forms with dominant and recessive characters of 2.98:1 or 3:1.

Mendel presents his results as a mathematician, as for his meteorological observations, using numerical values and tables (Kemp, 2002). By doing this, he differs from his contemporary naturalists, whose treatise are richly illustrated by descriptive plates. In his paper, there is not a single representation of his plants or a scheme of the pollination procedures. Instead, he insists on the quantification and measurement of variability using the statistical methods he learned from his physicist teachers, which he uses in a very innovative manner for a naturalist.

A controversy was initiated by readers of Ronald Fisher's paper (Fisher, 1936), leading to a statement that Mendel's results were 'too good to be true' from a statistical point of view. Without going into the details of Fischer's paper (whose intention was to support Mendel's work), it is worth pointing out that he first considers that the variations observed in the individual experiments discussed here are within the expected norm, before noting a deviation he considers as abnormal in the following experiments (Fairbanks & Rytting, 2001; Rédei & Kang, 2001). Several authors have endorsed a view opposite to that of Fisher, denying the existence of a bias (Pilgrim, 1984, 1986; Corcos & Monaghan, 1985, 1993). Alfred Sturtevant, Thomas Morgan's collaborator who established the first genetic maps in *Drosophila*, compared in his history of genetics the seven experimental series in which crosses of hybrid peas with yellow and green seeds were performed between 1896 and 1924 and collected by Johannsen, with that of Mendel (Sturtevant & Lewis, 2001). Tschermak, who counted half the number of seeds compared with Mendel, obtained results closer to the ideal proportion, whereas Lock, who counted four times fewer, reported the ratio with the largest deviation, and Darbishire, who counted 18 times more, obtained a ratio within average. It can therefore be concluded that Mendel's experiments are clearly coherent between themselves and with established statistical laws, which has been confirmed by recent works (Franklin *et al.*, 2008; Pires & Branco, 2010; Radick, 2015, 2022). In recent years, Gregor Mendel's experimental design and methodology for data recording, analysis and mathematical modelling have inspired positive comments from scientists in many fields, praising his logical empiricism (Cohn, 2003; Birchler, 2015; Opitz & Bianchi, 2015; De Castro, 2016; Deichmann, 2019; Huminiecki, 2020; Berger, 2022; Mittelsten Scheid, 2022).

Let us come back to Mendel's exposé, as he now introduces a contextual distinction and a prediction:

> The dominant character can have a double signification here, namely that of the original parental character or that of the hybrid character.
>
> An original parental character must be transmitted unchanged to all progeny, whereas the hybrid character must follow the same behaviour as observed in the first generation.

Mendel tells us that the dominant characters should not be endowed with an absolute value and that it is necessary to take into account the context in which they manifest themselves, a necessary condition in order to predict in a pertinent manner their transmission to the offspring. In doing so, Mendel acts as a precursor of systems biology by enforcing the systemic precepts: he understands the transmission of the dominant character in relationship to the environment in which it evolves, which is different in the pure and hybrid lines, and not only as an isolated object with particular properties. His quest is not about the structure of the gene, but rather about its behaviour in the different contexts in which it can manifest itself, principally in the successive generations.

The second generation of the hybrids

On the basis of his model of the behaviour of the characters in the course of generations, he formulates the hypotheses that form the basis for the design of a second series of observations for the characters studied:

> Those forms that preserve the recessive character in the first generation do not vary in the second generation in relation to that character; they remain constant in their progeny. This is not the case for those that possess the dominant character in the first generation. Of these two-thirds yield progeny that carry the dominant and recessive character in the ratio 3:1 and thus show the same behaviour as the hybrid forms; only one-third remains constant with the dominant character.
>
> The ratio 3:1, which results in the distribution of the dominant and recessive characters in the first generation, resolves then for all experiments into the ratio 2:1:1, if one simultaneously distinguishes the dominant character in its signification as a hybrid character and as an original parental character. Because the members of the first generation arise directly from the seeds of the hybrids, it now becomes apparent that the hybrids from each pair of differing characters form seeds, of which one-half again develops the hybrid form, whereas the other yields plants that remain constant and produce in equal parts the dominant and the recessive character.

This is the first part of his text that Mendel underlines extensively, in order to highlight the importance he attributes to this step in his work. He thus implements the systemic precepts of contextualization, relatedness and conditionality (Auffray *et al.*, 2003), by showing his interest first and foremost about the dynamic relationship between the characters and the rules they follow in different contexts, and not only about their elementary causality. It is this systemic approach that leads him to the pertinent conclusion that the observed relationships result from the production of hybrid and pure seeds in equal numbers.

The subsequent generations of the hybrids

After indicating that he has verified his conclusion by following the fate of the characters during four to six generations, depending on the character, Mendel changes gear by introducing an algebraic formula to express his model of the development of the hybrids:

> If A represents one of the two constant characters, for example the dominant, a the recessive, and Aa the hybrid form in which the two are united, then the expression A + 2 Aa + a shows the developmental* series for the progeny of the hybrids of each pair of divergent characters.

This formula triggered various interpretations, because Mendel does not display pairs of characters (AA and aa) in each of the terms corresponding to the pure forms, as geneticists would do later. In fact, at this stage of reasoning, in his logic he has only to care about the latent co-existence of the characters: he thus introduces the minimal hypothesis required to explain his observations, without attempting to explore exhaustively the underlying mechanisms that were inaccessible to his experiments, thus complying with the systemic precept of pertinence (Auffray *et al.*, 2003).

*As pointed out by Iris Sandler (Sandler, 2000), Mendel used the German term *Entwicklungsreihe*, whereas the initial English and French translations bypassed the term ‘development’.

The progeny of the hybrids in which several different characters are combined

In order to test the validity of his model for the serial development of the hybrids in equiprobable combinations, Mendel the undertakes a more complex series of experiments, in which he combines the differential characters two by tow or three by three. We refer here only to his principal conclusions that were interpreted by the geneticists as Mendel’s second law of the independent assortment of the characters:

> There is, then, no doubt that for all of the characters admitted into the experiments the following sentence is valid: The progeny of hybrids in which several essentially differing characters are united represent the terms of a combination series in which the developmental* (polynomial) series for each pair of differing characters are combined. Simultaneously it thus is shown that the behaviour of each pair of differing characters in hybrid association is independent of other differences between the two original parental plants.

Given that Mendel had studied seven characters in a species that has seven chromosomes, some considered he had been lucky, because his results would have been different if the characters had been linked on the same chromosomes. It is now established that Mendel could not have detected a linkage of the characters he studied because they are spread on five of the seven chromosomes in *Pisum*, with two on each of chromosomes 1 and 4, but too far away to appear as linked. Furthermore, certain biases in the distributions reported can be attributed to the fact that Mendel could not conduct all possible experiments

and reported in his paper only a fraction of the results obtained (Fairbanks & Rytting, 2001). Mendel then generalizes the role of random combinations of the characters as a principle applicable to any character:

> Simultaneously, factual evidence is produced that constant characters occurring in different forms of a plant genus can, through repeated artificial fertilisation, occur in all possible combinations according to the rules of combination.
>
> If we endeavour to summarise the results, we find that for those differing characters that admit easy and certain differentiation of the experimental plants, we observe completely identical behaviour in hybrid union. One-half of the progeny of the hybrids for each pair of differing characters is also hybrid, whereas the other half is constant in equal proportions for the characters of the seed and pollen plants.
>
> The perfect identity shown by all characters tested in the experiment fully permits and justifies the assumption that the same behaviour applies to other characters that appear less sharply in the plants and thus could not be included in the individual experiments.

At this stage, Mendel has reached his first goal: to provide a law for the development of the hybrids corresponding to the 'remarkable regularity' observed initially and enabling 'the establishment of numerical ratios existing between these forms'. He relied for this on a proven experimental model and the notions of uniformity of the hybrids, of disjunction and independent segregation of the characters established by his predecessors. His original contribution consists of the link he established between the precise numerical ratios observed in the diverse combinations of the characters in the offspring of the hybrids, on the one hand, and the random combination of the reproductive cells, on the other hand. His explanation is based on the experimental demonstration of the existence of an equal contribution of the two sexes, in contradiction to the views prevailing since antiquity.

Mendel's next step is to validate his hypothesis through a new series of experiments.

GREGOR MENDEL'S MODEL: THE GENERAL EQUATION OF THE DEVELOPMENT OF HYBRIDS

At the end of the 8 February 1865 session of the Society of Natural History in Brno, Gregor Mendel has finished his exposé of the fate of the differential characters during the successive generations of the hybrids, thus providing an experimental proof that the regularity observed was not the fruit of his imagination. Moreover, he has given this regularity a numerical value grounded on statistical analysis and has placed it in relationship to the behaviour of the reproductive cells during fertilization.

During his next exposé during the 8 March 1865 session, Mendel reports his efforts to generalize his model, with the results of the experiments conducted in order to test it.

Contrary to the legend, Mendel's exposés were presented to numerous and interested audiences, despite the unusual character of his presentation, as testified by the reports published in the local press. However, the understanding of the significance of his observations and thesis was beyond reach for even the most educated of his contemporaries. Nevertheless, continuing his investigations, Mendel presents the first general equation of biology that describes the development of hybrids.

The fertilizing cells of the hybrids

Mendel initiates a second iteration of his systemic approach, with the goal of testing the hypothesis he has derived from the model developed from his first series of experiments. For this, he designs a new experimental plan to perform reciprocal crosses of hybrids for two differential characters with the original pure lines, with the hybrid providing the male cells in the first cross and the female cells in the second cross:

> The results of the initial experiments led to further experiments whose success appeared capable of throwing light on the nature of the germ and pollen cells of the hybrids.
>
> We must then treat it as necessary that the very same factors* combine in the production of constant forms in the hybrid plant. Because the different constant forms are produced in one plant, even in one flower of the plant, it appears logical to assume that in the ovaries of the hybrids as many germ cells (germinal vesicles) and in the anthers as many pollen cells form as there are possible constant combination forms and that these germ and pollen cells correspond to the individual forms in their internal nature.

*This is the only occurrence in Mendel's main text of the term 'factor' to designate the entities involved in the transmission of the characters, which is nowadays interpreted and used abusively as a reference to the gene in its modern sense.

> In fact, it can be shown theoretically that this assumption would be thoroughly ample to account for the development of the hybrids in individual generations, if one were simultaneously allowed to assume that the different kinds of germ and pollen

cells are, on average, formed in equal numbers in the hybrid.

Further, if the individual forms of the germ and pollen cells of the hybrid were formed on average in equal numbers, then in each experiment the four previously stated combinations necessarily would be equal in their numerical relationships.

Also, this assumption is limited in that the formation of the different germ and pollen cells merely approaches equality in numbers and not that every individual hybrid reaches such numbers with mathematical precision.

Here again, Mendel demonstrates his ability to delineate the conditions that are necessary and sufficient in his experiments and to predict the results that are pertinent with regard to his working hypothesis. Indeed, the five experiments he conducted produced an equal distribution of the four possible forms in the offspring of the crosses, in conformity with the prediction:

> The yield corresponds to these requirements perfectly. … In all of the experiments, then, all forms appeared as this assumption required and, in fact, in nearly the same numbers. … The proposed theory finds ample confirmation in this experiment as well. … All combinations possible through the union of different characters appeared as expected and in nearly equal numbers.

Mendel then attempts to refine his model and to test his hypothesis repeatedly, before completing the formulation of his law for the development of the hybrids through a general equation:

> Thus, through experimental means the assumption is justified that pea hybrids form germ and pollen cells that, according to their nature, correspond in equal numbers to all the constant forms that arise from the combination of characters united through fertilisation.
>
> The simplest case is offered by the developmental series for each pair of differing characters. It is known that this series is defined by the expression A + 2Aa + a, in which A and a signify the forms with constant differing characters and Aa signifies the hybrid form of both. It includes four individuals among the three different classes. In their formation, pollen and germ cells of the forms A and a occur in equal proportions on average in fertilisation, and thus each form appears twice, since four individuals are formed. Therefore, participating in fertilization are the pollen cells, A + A + a + a; and the germ cells, A + A + a + a. It is a matter of chance which of the two kinds of pollen unites with each individual germ cell.

Given that Mendel cannot distinguish the pure line characters through observations or experiments, he introduces the minimal hypothesis that the co-existence of the similar pure characters in a hybrid is equivalent to a fusion, whereas he makes the necessary and sufficient distinction for the differential characters. In other words, he does not yet perform a complete distinction between the notions of genotype and phenotype introduced later by Johannsen and Bateson that the geneticists would use later. However, in order to account for the mode of intervention of the sexual cells in the development of the hybrids, he includes in his model the pairing of the different forms of the characters as a fraction, which leads him to the general equation for the development of the hybrids:

> The result of fertilisations can be clearly illustrated if the designations for united germ and pollen cells are shown as fractions, with the pollen cells above the line, the germ cells below. Thus, in this case: A/A + A/a + a/A + a/a. In the first and fourth classes the germ and pollen cells are the same, so the products of their association must be constant, A and a. With the second and third classes, however, once again a union of the two differing original parental characters takes place, and hence the forms that appear from this fertilisation are completely identical to the hybrid from which they are derived. Consequently, a repeated hybridization takes place. This accounts for the striking phenomenon that the hybrids are able, like the two original parental forms, to produce progeny that are identical to themselves; A/a and a/A both produce the same combination Aa, because, as alluded to earlier, it makes no difference for the result of fertilization which of the two characters belongs to the pollen or germ cells. Thus A/A + A/a + a/A + a/a = A + 2Aa + a. This is the average course for the self-fertilisation of hybrids when two differing characters are united in them.

The formulation of the law for the development of the hybrids in the form of an algebraic equation is the crowning of Mendel's systemic approach: after two iterations, he has placed in apposition his mathematical model (the development of a binomial series) with the reality he has been able to perceive through his experiments (the development of the hybrids), thanks to a pertinent experimental plan. This is a revolutionary action that brings biology into the era of quantification through mathematics, as William Harvey did earlier for physiology (Auffray & Noble, 2009). The geneticists would later be the first to step through this opening when they realized the immense consequences of Mendel's method and model. They would be followed and joined by the evolutionary

and developmental biologists only after the quarrels over precedence and authority were overcome at the beginning of the 20th century. Mendel ends this long section with a new formulation of his law for the development of the hybrids:

> The law of combination of the differing characters, by which the development of hybrids results, finds its foundation and explanation accordingly in the conclusive principle that hybrids produce germ and pollen cells corresponding in equal number to all constant forms that arise from the combination of the characters united through fertilisation.

In other words, Mendel claims to have found the explanation for the regularity in the appearance of the different forms of the hybrids in the course of successive generations in the equal contribution of the two sexes during fertilization.

CONCLUDING REMARKS

In this last part of his paper, Mendel continues the generalization of his model by extending it to other cases and refining it. After a section reporting preliminary studies on other plants (beans of the genus *Phaseolus*), which lead him to generalize the law established in *Pisum*, Mendel attempts to explain the phenomenon he observed, by using no less than ten times the term 'elements' (notably, a plural that we have highlighted in bold in Mendel's text whenever he uses *Elemente*). This is the section that refers to the notion of the gene in its modern sense of an elementary substrate of heredity. Mendel refers explicitly to the most recent and still controversial results of his time about the cell theory in the physiology of fertilization and to the particulate vision of heredity:

> According to the view of famous physiologists, in phanerogams, for the purpose of reproduction, one germ cell and one pollen cell unite into a single cell* that is able to develop into an independent organism through the uptake of matter and the formation of new cells. This development takes place according to a constant law that is founded in the material nature and arrangement of the **elements**, which succeeds in a viable union in the cell.

This is where Mendel introduces a footnote, marked by an asterisk, in which the first mention of elements appears. It is possible that this corresponds to an addition following his presentations and the initial versions of his manuscript, reflecting the evolution of Mendel's thought and his will to consolidate the validity of his model further, without ignoring its limits:

> *With *Pisum* it is shown without doubt that there must be a complete union of the **elements** of both fertilising cells for the formation of the new embryo. How could one otherwise explain that among the progeny of hybrids both original forms reappear in equal number and with all their peculiarities? If the influence of the germ cell on the pollen cell were only external, if it were given only the role of a nurse, then the result of every artificial fertilisation could be only that the developed hybrid was exclusively like the pollen plant or was very similar to it. In no manner have experiments until now confirmed that. Fundamental evidence for the complete union of the contents of both cells lies in the universally confirmed experience that it is unimportant for the form of the hybrid which of the original forms was the seed or the pollen plant.

In the next three paragraphs of the concluding remarks, Mendel uses the term 'elements' eight times, suggesting that it might have been, like his footnote, a late addition:

> If the reproductive cells are the same and if they accord to the foundational cell of the mother plant, then the development of the new individual will be governed by the same law that applies to the mother plant. If there is a successful union of a germ cell with a dissimilar pollen cell, we must assume that between the **elements** of both cells that determine their reciprocal differences, there is some sort of counterbalance. The intervening cell that arises becomes the foundation of the hybrid organism whose development necessarily follows another law than for the two original parents. If the balance is assumed to be complete in the sense that the hybrid embryo is formed from similar cells in which the differences are completely and permanently connected, then it can be further concluded that the hybrid, like every other autonomous plant species, will remain constant in its progeny. The reproductive cells that are formed in the ovaries and the anthers are the same and are identical to the underlying intervening cell.
>
> In relation to those hybrids whose progeny are variable, one might perhaps assume that there is an intervention between the differing **elements** of the germ and pollen cells so that the formation of a cell as the foundation of the hybrid becomes possible; however, the counterbalance of opposing **elements** is only temporary and does not extend beyond the life of the hybrid plant. Because no changes are perceptible in the general appearance of the plant throughout the vegetative period, we must further infer that the differing **elements**

> succeed in emerging from their compulsory association only during development of the reproductive cells. In the formation of these cells, all existing **elements** act in a completely free and uniform arrangement in which only the differing ones reciprocally segregate themselves. In this manner the production of as many germ and pollen cells would be allowed as there are combinations of formative **elements**.
>
> This attempted ascription of the essential distinction of either a permanent or a temporary association of the differing cell **elements** in the development of the hybrids can, of course, be of value only as a hypothesis for which a wide scope of interpretation is possible given the dearth of reliable data. Some justification for the stated view lies in the evidence given for *Pisum* that the behaviour of each pair of differing characters in hybrid union is independent of the other differences between the two original plants and, further, that the hybrid produces as many types of germ and pollen cells as there are possible constant combination forms. The distinctive characters of two plants can ultimately rest only on differences in the nature and grouping of the **elements** that are present in their foundational cells in living interaction.

Mendel reaches here the limit of what his experimental plan and model allow him to capture in the development of the hybrids. The convoluted formulation of his argument shows that he does not manage, in the framework of his hypotheses, to account through a common mechanism for the differences between the combinations of elements associated with constant and variable characters. However, he opens the way for his successors who, empowered by the indispensable supplementary knowledge, will take charge of developing genetics in the 20th century, verifying and confirming his hypotheses and thus responding to his invitation to repeat his main experiments in order to validate his laws.

> The validity of the set of laws suggested for *Pisum* requires additional confirmation and thus a repetition of at least the more important experiments would be desirable, for instance the one concerning the nature of the hybrid fertilising cells.

The main reason why Mendel's invitation was not followed by his colleagues is most probably that his conception and his method were not immediately intelligible for his contemporaries. The endorsement of the dominant theory of heredity by mixing by the Swiss naturalist Carl von Nägeli (and Darwin) explains Mendel's failure to convince him, despite extensive attempts in correspondence, to reproduce his experiments. Nägeli was principally a specialist in hawkweed of the genus *Hieracium*, multicoloured flowering plants endowed with a partly asexual mode of reproduction that does not allow a direct application of Mendel's experimental plan. Indeed, Mendel reported in a second paper, presented at the Society of Natural History in Brno in 1869, and in his correspondence with Nägeli the extensive and detailed results he had obtained in *Hieracium* (Nogler, 2006; van Dijk & Ellis, 2016), before shifting his attention to bees.

Having taken the charge of abbot of the monastery after the death of Napp in 1868, Mendel was increasingly absorbed by many administrative duties and could no longer spend the time required to develop his research further. It would take until the beginning of the 20th century and the rise of genetics for better-prepared minds to demonstrate the fecundity of Mendel's work.

Gregor Mendel died in 1884 from a crisis of uraemia resulting from the chronic nephritis he suffered, without encountering in his lifetime the recognition he would have deserved (Cox, 1999; Allen, 2003). We still have a number of lessons to draw from the work of a man who was a systemic precursor of genetics and developmental biology (Auffray, 2002, 2004) and from the pertinent manner in which his experiments have been conceived, conducted, exposed, ignored, rediscovered, criticized, exploited and revisited. Gregor Mendel has been praised as a 'man of God and science' (Tan & Brown, 2006), remembered by family relatives as 'a human, a catholic priest, an Augustinian monk, and abbot' (Richter, 2015). Others have debated whether Mendel was forgotten or ignored in his lifetime, to conclude that he was both ignored and forgotten, then rediscovered (Kessel, 2002; Keynes, 2002; Keynes & Cox, 2008). In an attempt to set the record straight, Fairbanks has recently endeavoured at 'demystifying the mythical Mendel' through a comprehensive biographical review (Fairbanks, 2022). The French naturalist and writer Jean Rostand summarized Mendel's life and scientific achievements beautifully in an eloquent portrait (Rostand, 1979):

> It would require, in order to conduct such a long-term study, the marvellous, tireless patience of Mendel, who, alone, without help, with no collaboration, performs several hundred artificial pollinations, and examines no less than ten thousand plants. … In the end it required independence, dedication, solitude. … Mendel in the cloister silence has duration in his favour. He does not fear to engage into experiments that will take him years to complete. It is for his pleasure that he cultivates his peas, and even though they might not provide him with truths, he is satisfied

Figure 3. Left, reproduction of Gregor Mendel pea cultures near the Mendel Museum in Brno. Right, Charles Auffray standing at the footstep of Mendel's statue near the Mendel Museum (photograph by Dr Manlio Vinciguerra, Masaryk University).

> to see them grow and flourish. His time is not precious, avariciously counted. He has no book to write, no masters to flatter, no reputation to sustain, no intrigues to conduct, no application to prepare. … For this priest, there is only his peas, after God.

The myth and the legend are still alive 200 years after Mendel's birth. As physiologists and geneticists and systems biologists, we follow in the footsteps of Mendel who, together with William Harvey and Claude Bernard, pioneered the development of systems biology and physiology (Noble, 2008; Auffray & Noble, 2009). Let us celebrate him during the 2022 Mendel.Brno festival (Mendel.Brno – Mendel opted for Brno, available at: https://mendel.brno.cz/en/, 2022; Eckardt *et al.*, 2022), together with the geneticists from around the world gathering at the International Mendel genetics conference (Mendel Genetics Conference, available at: https://www.mendel22.cz/about-conference/, 2022) and make good use of his contributions for the benefit of future generations.

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DATA AVAILABILITY

This paper is an updated and expanded version of a two-part online essay in French by C.A., posted in 2005 at the Observatory of Genetics in Montréal, Canada (now closed) and translated into English on the occasion of Gregor Mendel's bicentenary. An overview

for the general public was broadcast in French by Radio Prague on 8 February 2015 on the occasion of the 150th anniversary of his two 1865 lectures in Brno (Auffray, 2015).

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Conrad Waddington and the origin of epigenetics

Denis Noble discusses Conrad Waddington's classic paper, 'The genetic assimilation of the bithorax phenotype', published in *Evolution* in 1956.

In 1956, the British developmental biologist, Conrad Waddington, published a paper in the journal *Evolution* (Waddington, 1956) in which he succeeded in demonstrating the inheritance of a characteristic acquired in a population in response to an environmental stimulus. Much earlier, in 1890, August Weismann had tried and failed to achieve this. He amputated the tails of five successive generations of mice and showed absolutely no evidence for an effect on subsequent generations. Weismann's discovery that the effects of an environmental stimulus (tail amputation) cannot be transmitted to subsequent generations, together with his assumption that genetic change is random, formed the foundations of the Modern Synthesis (Neo-Darwinism) of our understanding of genetic inheritance.

Waddington's approach, however, was much more subtle and more likely to be successful because he realised that the way to test for the inheritance of acquired characteristics is first to discover what forms of developmental plasticity already exist in a population, or that the population could be persuaded to demonstrate with a little nudging from the environment. By exploiting plasticity that already existed he was much more likely to mimic a path that evolution itself could have taken.

He used the word 'canalised' for this kind of persuasion since he represented the developmental process as a series of 'decisions' that could be represented as 'valleys' and 'forks' in a developmental landscape (Fig. 1). He knew from his developmental studies that embryo fruit flies could be persuaded to show different thorax and wing structures, simply by changing the environmental temperature or by a chemical stimulus. In his landscape diagram, this could be represented as a small manipulation in slope that would lead to one channel in the landscape being favoured over another, so that the adult could show a different phenotype starting from the same genotype.

The next step in his experiment was to select for and breed from the animals that displayed the new characteristic. Exposed to the same environmental stimulus, these gave rise to progeny with an even higher proportion of adults displaying the new character. After a relatively small number of generations, he found that he could then breed from the animals and obtain robust inheritance of the new character even without applying the environmental stimulus. The characteristic had therefore become locked into the genetics of the animal. He called this process genetic assimilation. What he had succeeded in showing was that an acquired characteristic could first be inherited as what we would now call 'soft' inheritance, and that it could then be assimilated into becoming standard 'hard' genetic inheritance. Today, we call 'soft' inheritance epigenetic inheritance, and of course, we know many more mechanisms by which the same genome can be controlled to produce different epigenetic effects.

What was happening at the gene level in Waddington's experiments? A standard Neo-Darwinist explanation might be that some mutations occurred. That is possible, but extremely unlikely on the time scale of the experiment, which was only a few generations. Moreover, random mutations would occur in individuals, not in a whole group. Single small mutations would have taken very many generations to spread through whole populations, and many such mutations would have been required.

But I think there is a much simpler explanation. Recall that the experiment exploited plasticity that is already present in the population. That strongly suggests that all the alleles (gene variants) necessary for the inheritance of the characteristic were already present in the population, but not initially in any particular individuals in the correct combination. The experiment simply brings them together. This is a modification of the pattern of the genome in response to the environmental change, but not in a way that requires any new mutations. I came to this conclusion before reading Waddington's (1957) book, *The Strategy of the Genes*. But it is in fact one of Waddington's own ideas! He writes 'There is no … reason which would prevent us from imagining that all the genes which eventually make up the assimilated genotype were already present in the population before the selection began, and only required bringing together' (p. 176). Not only does he clearly see this possibility, he also tests it. He continues (p. 178) 'Attempts to carry out genetic assimilation starting from inbred lines have remained quite unsuccessful. This provides further evidence that the process depends on the utilisation of genetic variability in the foundation stock with which the experiment begins'. His text could not be clearer.

Orthodox Neo-Darwinists dismissed Waddington's findings as merely an example of the evolution of phenotype plasticity. That is what you will find in many of the biology textbooks even today (e.g. Arthur, 2010). I think that Waddington showed more than that. Of course, plasticity can evolve, and that itself could be by a Neo-Darwinist or any other mechanism. But Waddington was not simply showing the evolution of plasticity in general; he was showing how it could be exploited to enable a particular acquired characteristic in response to an environmental change to be inherited and be assimilated into the genome. Moreover, he departed from the strict Neo-Darwinist view by showing that this could happen even if no new mutations occur (Fig. 2).

Epigenetics means 'above genetics' and it was originally conceived by

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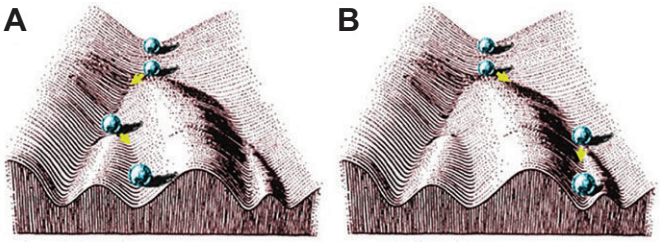

Fig. 1. Waddington's developmental landscape diagram. The landscape itself and the ball at the top are from his original diagram. The subsequent positions of the ball have been added to illustrate his point that development can be canalised to follow different routes (A and B). The plasticity to enable this to happen already exists in the wild population of organisms (modified diagram by K. Mitchell).

Waddington himself to describe the existence of mechanisms of inheritance in addition to (over and above) standard genetics (Bard, 2008). Waddington regarded himself as a Darwinist since Darwin also, in *The Origin of Species*, included the inheritance of acquired characteristics. But significantly, Waddington was not a Neo-Darwinist since Neo-Darwinism, following Weismann, specifically excludes such inheritance. Waddington was a profound thinker about biology, and much else too. *The Strategy of the Genes* is a masterly account of the many reasons why he dissented from Neo-Darwinism, and it has stood the test of time. It was reprinted over half a century later, in 2014. He did not describe himself as a Lamarckian, but by revealing mechanisms of inheritance of acquired characteristics, I think he should be regarded as such. The reason he did not do so is that Lamarck could not have conceived of the processes that Waddington revealed. Incidentally, it is also true to say that Lamarck did not invent the idea of the inheritance of acquired characteristics. But, whether historically correct or not, we are stuck today with the term 'Lamarckian' for inheritance of a characteristic acquired through an environmental influence.

Waddington's concepts of plasticity and epigenetics have been very influential in my own thinking about experiments on cardiac rhythm. We found that the heart's pacemaker is very robust, so much so that protein mechanisms normally responsible for a large part of the rhythm could be completely blocked or deleted (Noble et al., 1992). Only very small changes in rhythm occur, because other mechanisms come into play to ensure that pacemaker activity continues. The relation between individual genes and the phenotype is therefore mediated through networks of interactions that can buffer individual gene variation, just as Waddington envisaged in his diagrams of epigenetic effects and canalisation. This is one of the reasons why I became interested in evolutionary biology many years ago, and why I have also explored ways in which evolutionary theory can be integrated with recent discoveries in molecular and physiological biology (Noble et al., 2014).

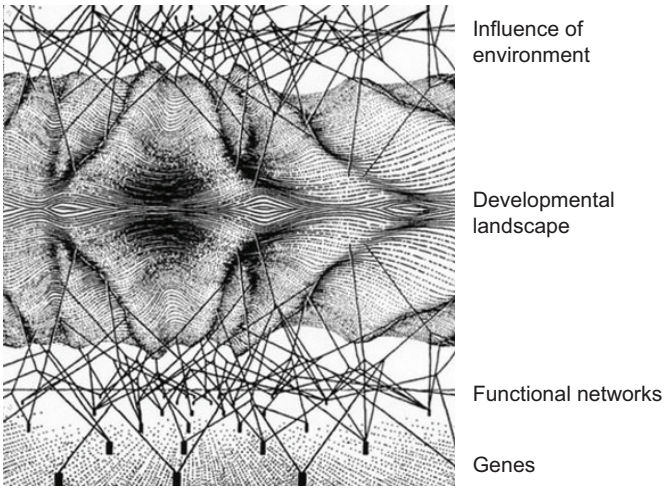

Fig. 2. Waddington's diagram to show how the developmental landscape relates to individual genes (bottom pegs) through networks of interactions in the organism. Since he also showed the influence of the external environment on canalisation of development, I have extended the diagram by adding the top part to represent the environmental influences. It is the combination of these influences that can lead to an evolutionary change without mutations (modified from Waddington, 1957).

Waddington's concepts are also highly relevant to biologists interested in the ways in which organisms adapt to their environment, and to comparative biologists interested in how this varies between species. Many of the ways in which modern epigenetics plays an essential role in these fields have been described in a special issue of this journal (see overview by Knight, 2015). The discovery of epigenetic marking of DNA and its associated chromatin proteins has opened up new vistas for experimental biology.

I conclude this article with a warning: if you are inspired to try to repeat Waddington's 1956 experiment, do remember that you will fail if you try to do it on a cloned laboratory population. The mechanism depends on using a wild population with natural genetic diversity. In this respect it resembles a phenomenon first noted by James Baldwin (1896). This is that individuals in a population with the 'correct' allele combinations could choose a new environment and so permanently change the evolutionary development in that environment. It resembles Waddington's idea, as he himself recognised, because it does not require new mutations. More recently, Karl Popper, the great logician of science, also noted the possible importance of genetic assimilation without mutations in evolutionary theory (Niemann, 2014; Noble, 2014). Popper and Waddington had both taken part in discussions on evolutionary biology during the 1930s and 1940s when the field of molecular biology was still developing (Niemann, 2014).

While celebrating the recent rapid rise in epigenetics research (see Hoppeler, 2015; Knight, 2015), let's also celebrate the father of epigenetics, Conrad Waddington, who opened our eyes to the rich opportunities of adaptation through epigenetic regulation.

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ORIGINS AND DEMISE OF SELFISH GENE THEORY

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Contents: 1. Introduction: the First Debate. 2. The Duality of Williams and Dawkins is a Philosophical Idea. 3. The original Modern Synthesis was more open than Its Current Popularizations. 4. The Hardening of 20th Century Views on the Central Dogma and the Weismann Barrier. 5. 21st Century Deconstruction of the Central Dogma and the Weismann Barrier. 6. The 40th Anniversary Edition of *The Selfish Gene:* The Last Stand? 7. The Ethics of Germ-line Modification. 7.1. Gene Therapy. 7.2. Diagnosis and Informed Consent. 7.3. The Non-dualistic Nature of Organisms and Their Interactions with the Environment. 8. The End of Unnecessary Dualism in Biology. 9. Conclusion.

Keywords: Selfish Gene, Central Dogma, Weismann Barrier, Self-replication, One-way Causation.

Abstract: *The idea of The Selfish Gene, first published in 1976, grew out of the Modern Synthesis of evolutionary biology formulated by Julian Huxley in 1942, and more specifically from George Williams' Adaptation and Natural Selection in 1966. It presents a severely narrowed down version of Huxley's synthesis, which developed in the 1960s following the formulation of the Central Dogma of molecular biology by Francis Crick. The idea rests on three assumptions: the isolation of the genome from any influences by the soma and its development in interaction with the environment (the Weismann Barrier), one-way causation from DNA to proteins (The Central Dogma), and the auto-replication of DNA (Schrödinger's aperiodic crystal). All three of these assumptions have now been shown to be incorrect. The 'replicator' (DNA) is not independent of the 'vehicle', the organism itself, so that The Selfish Gene can no longer be regarded as a valid scientific hypothesis.*

1. Introduction: the First Debate

When his book *The Selfish Gene* appeared in 1976 (1), Richard Dawkins took part in a debate at the Graduate Centre of Balliol College in Oxford University. One of us (DN) had arranged the event in the charming 16th century Manor House at the core of the Graduate Centre. The other author (RN) travelled from Edinburgh, where he worked on the somatosensory system. There was a specific reason for our keen interest in what Richard Dawkins would say. Both of us had studied Zoology and Comparative Anatomy as undergraduates. DN had been a medical science student at UCL in the 1950s under J Z Young, the renowned anatomist and comparative zoologist (2, 3). RN was a Zoology student at Manchester

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University in the 1970s, where one of his tutors was the gene-centred behaviourist Robin Baker, author of *Sperm Wars: Infidelity, Sexual Conflict and Other Bedroom Battles* (4).

The two-decade difference is significant because, as our article will show, it spans the period when standard evolutionary biology hardened into the Selfish Gene version. Moreover, RN recalls opposing his zoology tutors on the genetic basis of behaviour, particularly its fixed algorithmic nature. Thus, we both encountered and argued about the selfish gene idea before Dawkins' book appeared.

That is not surprising since gene-centrism entered biological research well before *The Selfish Gene*. Julian Huxley's *Evolution. The Modern Synthesis* (5, 6), first published in 1942, provided the general neo-Darwinist background, which rapidly became the standard school teaching of evolution (7). George Williams in 1966 (9) then clearly laid out the specific ideas of Selfish Gene theory in his book, *Adaptation and Natural Selection*.

Williams described a form of mechanistic duality,[1] a clear separation between a replicator (genes) and its vehicle (organism), in Williams' words, "genic selection and organic adaptation" (9, p. 124).[2] The selection acted entirely on genes as the replicator, thus stripping the organism of agency in its evolutionary destiny. Organisms became seen as passive bystanders to the results of blind natural selection in developing their structure and function. Crucially, no adaptations created by the organism could pass to the next generation, as if by some principle of prohibition. Thus, while Dawkins did not invent this dualist idea, his *The Selfish Gene* gave it a powerful voice through his colourful writing.

The 1976 Holywell Manor debate involved Dawkins and two philosophers, Anthony Kenny, author of *The Metaphysics of Mind* (10)) and Charles Taylor, author of *The Explanation of Behaviour* (11). Kenny questioned the dualism by putting the question what interprets the replicator: "if all I knew about the English language was its alphabet, surely I would not be able to understand Shakespeare?" Dawkins did not even attempt to address the problem. He replied: "I am not a philosopher. I am a scientist, I am only interested in truth", a mantra he has used to sidestep philosophical questions repeatedly over many years.[3]

2. The Duality of Williams and Dawkins is a Philosophical Idea

Yet, the duality of Williams and Dawkins is primarily a *philosophical* idea, just as was the 'ghost in the machine' dualism of Descartes. Thus, it requires justification. The

[1] We have followed Gould (8, p. 615), in using the term "duality" to distinguish mechanistic duality from Cartesian "dualism", but, as our article shows, mechanistic duality shares some problems with Cartesian dualism.

[2] The full quote is "We must always bear in mind that group selection and biotic adaptation are more onerous principles than genic selection and organic adaptation."

[3] Precisely that statement was repeated in the debate between Rowan Williams (then Archbishop of Canterbury) and Richard Dawkins, chaired by Anthony Kenny in Oxford in 2012: https://www.youtube.com/watch?v=bow4nnh1Wv0.

question this article addresses is how such a successful book, selling in millions, could have been allowed to sidestep the central philosophical question about any form of dualism: why. Just as Descartes' soul-body separation is unnecessary, so too is that of the vehicle-replicator. There is no need for an organiser (director) within the organiser (enterprise).

The problematic philosophical questions about the mechanistic duality of *The Selfish Gene* were either missed or appeared to be irrelevant. The extraordinary achievements in molecular biology, not least the structure of DNA, gave the sense of solid ground: the 'code' or 'secret' of life appeared to be laid bare. All that was necessary was to unravel it, to open its pages like a book. But three other ideas were erroneously taken as established scientific facts: the one-way causation interpretation of the Central Dogma of molecular biology, the Weismann Barrier, and DNA replicating like a crystal. The first contended that the DNA was read-only and unchangeable by the organism. Thus, it was "sealed off from the outside world", as Dawkins expressed it (1, p. 21). The second was assumed to protect the germ-line cells from carrying any information from the soma in addition to the genes, while the third made it appear that DNA could alone "create us body and mind" (1) and so be the "secret of life" (12). None of this had any empirical foundation. All of it was assumption. It simply had to be so for the gene-centric view to hold.

So, the duality of *The Selfish Gene* is the complete separation between the replicator and its vehicle, with strict one-way causation from replicator to vehicle, presented as scientific truth, almost beyond question. It was, of course, still important to know how organisms can *interpret* their DNA for their development and physiological function. The new discipline of genomics took off, slowly at first, but with the idea that somehow science could unravel the 'genetic blueprint', decipher and understand it. By the turn of the century, this had become a political strategy for health. It is the prevailing viewpoint. Yet, this is another fallacy. It isn't how the cell or organism interacts with the genome. Cells use and control the genome; they don't simply wait for instructions from it. Without the cell, the genome can issue no instructions. It is functionally an integral part of the cell and subservient to its needs. The genome is a slave to the cell, not its master. That was the point of Kenny's question about the alphabet, words and Shakespeare, but this question could not be relevant to a gene-centred view of evolution. Thus, science reduced organisms to the transient disposable 'vehicles' for their 'immortal' genes. Physiology became mainly irrelevant in evolutionary theory. This viewpoint differed from Charles Darwin's, an honorary member of The Physiological Society at its foundation in 1876, while T H Huxley was a leading light in its early days.

So, what of the other pillar of the central dogma, the Weismann barrier? It, too, has fallen in the light of empirical evidence. Physiologists are always sceptical about barriers. They tend to be mutable, like the Blood-Brain barrier, which has selective and changeable permeability. Boundaries in physiology are functional and rarely fixed. So it is for the limitations on transmission to the germ cells. They do not isolate the germ-line cells from RNAs, DNAs, proteins, and many other molecules passing across the 'barrier' from the soma. If a barrier exists, it is functional and selective. The assumptions required for the dualistic separation of replicator and or-

ganism are incorrect. So how was such an edifice created, and how did it gain hold of the science? To answer that, we need to consider its history.

3. The original Modern Synthesis was more open than its current popularizations

Julian Huxley's 1942 book (5) pulled together the early 20th-century work on evolutionary biology, creating the Modern Synthesis viewpoint. It was an extraordinary work of scholarship, providing in almost 600 pages a valuable resource for early 20th century discoveries in evolutionary biology. Compared to the rigid *Selfish Gene* viewpoint, it was also a remarkably open view of evolutionary biology. Shapiro & Noble (13) have documented many discoveries neglected or downplayed in widely used *modern* textbooks and popularisations, creating a closed view of biological systems and closing down a much-needed dialogue and further study. Ideas that science should have rigorously tested empirically became a corpus of self-evident beliefs. Yet 80 years ago, Huxley was open to many of those discoveries. We will list just a few he anticipated:

1. He was critical of Weismann's reliance entirely on natural selection since "mutation alone has been shown to be incapable of producing directional change." (6, p. 29).
2. Nor was he fully convinced of the validity of the Weismann Barrier: "the distinction between soma and germplasm is not always so sharp as Weismann supposed." (6, p. 29).
3. He anticipated Gould (14) on punctuated evolution: "abrupt changes of large extent do play a part in certain kinds of evolution in certain kinds of plants." (6, p. 38).
4. He acknowledged the role of hybridization in species evolution (6, p. 147).
5. He acknowledged that the "mutation rate is increased by sudden environmental changes." (6, p. 137)
6. He anticipated the work of Barbara McClintock (15, 16) and James Shapiro (17, 18) in acknowledging chromosome rearrangement in response to environmental stress (6, p. 137).
7. Remarkably, he anticipated the relative failure of genome-wide sequence studies, in showing very small correlations, even to the extent of formulating the polygenic theory of genomics – phenotype correlations when he wrote "every character is dependent on a very large (possible all) of the genes in the hereditary constitution: but some of these genes exert marked differential effects upon the visible appearance." (6, p. 19)

This range of openness to multiple processes in evolution is extraordinary. But there were two key areas where Huxley's mind appeared closed. The first was that inexplicably perhaps, he did not follow up on his doubts about Weismann. The second was that he followed Wallace in rejecting Darwin's interpretation of sexual and other forms of social selection. Any purpose was to be rigorously excluded from

scientific explanations of evolution, so expunging agency or any direction in evolutionary change.

4. The Hardening of 20TH Century Views on the Central Dogma and the Weismann Barrier

In retrospect, there could have been a progressive and constructive development of the Modern Synthesis towards a resolution of the conflict between purposive and non-purposive interpretations of evolution. Had Huxley followed his instincts on Weismann and sided with Darwin against Wallace on sexual selection, the late 20th century moves in the opposite direction might never have happened. So, what prevented Huxley from leading the way on such a progressive and constructive development?

The answer to that question is relevant to the date of the appearance of *The Selfish Gene* in 1976. By then, the interpretations of the significant discoveries of molecular biology had merged into what became viewed as a robust empirical vindication of George Williams' ideas in his 1966 book.

Huxley himself provides the historical clue in his Introduction to the second edition of his 1942 book, published in 1963, just 5 years after Crick (19) had formulated the Central Dogma of Molecular Biology as the one-way process DNA → RNA → protein.

No doubt enthused by Crick's formulation, Huxley wrote:

> I have left to the end the most important scientific event of our times – the discovery by Watson and Crick that the deoxyribonucleic acids – DNA for short – are the true physical basis for life, and provide the mechanism of heredity and evolution. Their chemical structure, combining two elongated linear sequences in a linked double spiral or bihelix, *makes them self-reproducing*, and ensures that they can act as a code, providing an immense amount of genetical "information," together with occasional variations of information (mutations) which also reproduce themselves. Linear constructions of DNA are, of course, the primary structures in the genetic organelles we call chromosomes. (6, p. 614)

Just three years later, Williams (9) published *Adaptation and Natural Selection*, which by his (Dawkins') own account was the most significant influence on his writing of *The Selfish Gene*.

The emphasis in the quoted passage is ours since it is the smoking gun in this story. There are three critical problems, not least of which is the assumption that DNA is a 'code' containing information. This assumption is now engrained in mythology and rarely challenged. So, let's consider what it means. A code is a system of words, letters, figures, or symbols used to represent others, especially secrets. Or, it might be like a code in computing, a set of assembly code, or instructions. DNA is none of this. Remarkable as it is, it is a tool enabling cells to make and do things. It is not a set of instructions. Of course, when we represent DNA or the genome on our pages or a screen, we use letters of the alphabet. In that sense, we are representing DNA in a code. But the DNA itself is not a code. It is not a representation of something, and it certainly is not an instruction manual. The metaphor of DNA as a code or blueprint feeds into the 'ghost' in the machine duality, some-

thing unique that controls and directs life. Yet, life itself is definitively that special something, with an agency to make and do things, for which it uses DNA. Life, as we know it on earth, depends on much that doesn't require DNA.

The point about DNA as a code might be regarded as semantic or a linguistic convenience, but Huxley's passage contains another fundamental problem: a matter of empirical observation; the concept of DNA as a replicator. Whilst DNAs are replicated most wonderfully, they do not do so without help from the cell. They are not self-replicators. A sufficiently exact replication would be impossible with an error rate of around 1 in 10,000 base pairs. Only small RNA viruses can survive that copying error rate, and even then, they rapidly mutate, as we observe during viral pandemics. We now know that cells use an army of proof-correcting troops to reduce the error rate to just 1 in 10 billion. Such a faithful replication is dependent on this error correction (20, 21). Huxley could not have known in 1963 that genomes the length of the human genome would not work without the activity of the living cell. Nor could Schrödinger when he first proposed that the genetic molecule could be a self-replicating crystal in his 1942 book *What is Life?* (22). But the idea of DNA as the self-replicator persisted and became the key to the replicator-vehicle separation, the mechanistic duality.

It is packaged as information in a code that gets passed on in the germ cells.

Another significant push toward the gene-centred view also appeared in Huxley's introduction to the 2nd edition of the Modern Synthesis:

> I must, however, draw attention to the outstanding event in this field, namely the dethronement of the proteins from their biological pre-eminence. It used to be held that life was based on proteins. Today, we know that DNA is the basis of life and its evolution, and that proteins, though essential for its operation, owe their production to the activities of DNA.
>
> (6, p. 607)

This interpretation did not merely 'dethrone' proteins; it left the organism bereft of agency, for proteins are how such agents do things; how we move, talk, feel, and think. DNA does none of this.

But since highly accurate DNA replication depends on the organised activity of the cellular error-correcting proteins, there has to be a two-way interaction between the 'replicator' and its 'vehicle'. Furthermore, the cell controls gene expression. This interaction or integrative function removes the duality. Causation runs both ways, not one-way. That is the real thrust of Kenny's question to Dawkins. We can only ascribe meaning, purpose, and other social attributes at the level of organisation at which such ascription makes sense. A letter is significant in a word; a word has meaning in a sentence, a paragraph, an idea, or a view. Life can be selfish; DNA cannot. This fact is fundamental to understanding the misuse of "selfish" as a metaphor in *The Selfish Gene*. It is precisely when a metaphor can easily elide into the literal sense from which it derives that care must be taken not to confuse the metaphorical and literal uses. Dawkins regularly confuses them with statements like "we are born selfish" Dawkins (1, p. 3). Dawkins (23) defends his usage of the word as being technical in biology, rather like the use of "spin" and "charm"

in particle physics. But that technical usage in physics is so far from common language that the likelihood of elision into thinking that a particle can really have charm is utterly remote. By contrast, the idea that genes cause selfish behaviour is now so embedded in our culture that it has become the modern version of the theological concept of original sin.

It is the physiological organisation of the living cell, which is a higher-level activity of life, which is necessary for faithful replication. It is also the Trojan Horse at the heart of the Central Dogma. Vehicle and replicator are one. DNA does not 'swarm' within us, controlling us; we use it and maintain it. We can be selfish or selfless in different instances using the same genes or using the same genes to create a piece of music or paint a picture. Nor is a gene selfish because it persists in a 'gene pool'; it might just as readily stay because we are capable of selflessness. The 'gene pool' is us.

Still, the gene-centric view was not fully closed. In 1963, Huxley would not have known what we now know about the process of DNA replication, nor that the way the cell controls its expression and maintains it also provides a way for the cell to alter it. Nevertheless, he was on the right track about Lamarckian forms of inheritance, for he also acknowledged the significant work of Conrad Waddington (24) in showing how genetic assimilation (incorporation) could form the basis of a Lamarckian form of inheritance:

> Meanwhile in Britain, Waddington (1957, 1960) has made a notable contribution to evolutionary theory by his discovery that Lamarckian inheritance may be simulated by a purely neo-Darwinian mechanism. This is called genetic assimilation. It operates through the natural selection of genes which dispose the developing organism to become modified in reaction to some environmental stimulus. (6, p. 580)

It is worth noting that Huxley was not entirely correct. Waddington did not *simulate* the Lamarckian process; he experimentally *reproduced* it since he did the equivalent of the social selection of variants showing the inheritable variation by actively *choosing* the variants to breed from in each generation. It was a strictly Lamarckian process since it was the inheritance of a characteristic acquired through artificial (*i.e.* human) selection. Therefore, it was a model for what animals do through sexual and other forms of social selection (choice). Organisms are active in the natural selection process.

Nevertheless, the quotation shows just how open Huxley was to what came later. It is a historical tragedy that, just when Huxley could have extended his synthesis even more openly, he was thrown off course by what seemed to be irrefutable evidence for the more rigid and closed version of his 1942 Synthesis. This is what led to the frequently quoted mantra that "the Weismann Barrier is now buttressed by the Central Dogma of Molecular Biology".[1] Sadly, Waddington for his sin was excluded from the modern synthesis circle (25).

[1] see *e.g.* "The dogma is a modern version of the Weismann barrier (after August Weismann). This is the principle that hereditary information moves only from genes to body cells, and never in reverse. Hereditary information moves only from germline cells to somatic cells" https://simple.wikipedia.org/wiki/Central_dogma_of_molecular_biology (accessed 18 August 2021).

Before we leave the analysis of why the hardening of the synthesis happened during the 1960s and 1970s, it is important to note two other developments that influenced Huxley in 1963 and greatly encouraged rigidity.

First, many leading scientists still thought that eugenics was a natural consequence of the Modern Synthesis. Huxley wrote:

> It is also clear that, in so far as immediate threats to human progress are overcome, such as over-population, atomic war, and over-exploitation of natural resources, eugenic improvement will become an increasingly important goal of evolving man. (6, p. 587)

Supporters of the Modern Synthesis now avoid reference to how it led to eugenics in the 1930s and 1940s. Sadly, some of the originators of the synthesis were its advocates, however much the holocaust was later denounced. Nevertheless, ethical problems persist in proposals for editing the human germ-line and concepts of 'good' or 'bad' genes. We will return to this question later.

The second development also relates to an outcome of the Second World War. The Soviet Union became the champion of a complete travesty of Lamarck's ideas by generously supporting the work of Lysenko. Huxley makes this clear:

> Only in the U.S.S.R has Lamarckism found favour. Here, under the influence of Lysenko, the peculiar brand of Lamarckism called Michurism was given official sanction, and extravagant and ill-founded claims were made on its behalf, while neo-Mendelian genetics, which everywhere else was advancing in a spectacular way, was officially condemned as bourgeois or capitalist "Morganist-Mendelist" and Soviet geneticists were exiled or lost their jobs. (6, p. 580)

No wonder then that Waddington did not describe himself as a Lamarckian in 1957. It took until 1972-3, when Waddington gave some of the Gifford Lectures in Edinburgh (26, p. 127), for him to admit that his 1957 work demonstrated a Lamarckian process.

This history demonstrates just how much political philosophy influences thought and scholarship. It is not enough to ascribe 'truth' to ideas under a carapace of science.

5. 21ST Century Deconstruction of the Central Dogma and the Weismann Barrier

There were philosophical challenges to *The Selfish Gene* during the 20th century, and those are well-documented in Gould's last magnum opus *The Structure of Evolutionary Theory* (27, chapter 8). These were significant challenges as the extensive influence of the selfish-gene concept in many fields is attributed to the colourful language and use of metaphors for which there has been an assumption of truth. In this section, we will focus on factual errors.

The Music of Life (28) began this process by demonstrating the lack of factual content in the central statement of *The Selfish Gene*:

> Now they swarm in huge colonies, safe inside gigantic lumbering robots, sealed off from the outside world, communicating with it by tortuous indirect routes, manipulating it by

remote control. They are in you and me; they created us body and mind; and their preservation is the ultimate rationale for our existence. (1, p. 21)

The absence of factual content was shown by simply reversing the meaning of each sub-clause to read:

Now they are trapped in huge colonies, locked inside highly intelligent beings, molded by the outside world, communicating with it by complex processes, through which, blindly, as if by magic, function emerges. They are in you and me; we are the system that allows their code to be read; and their preservation is totally dependent on the joy we experience in reproducing ourselves. We are the ultimate rationale for their existence. (28, p. 12)

Except for the trivially true factual statement "they are in you and me", which is the same, no experimental test could distinguish between the diametrically opposing phrases. Dawkins admitted as much in *The Extended Phenotype* (23) when he wrote "I doubt that there is any experiment that could prove my claim". Yet, we can trace a dogmatic gene-centric position back to Weismann, who made the same claim of certainty for his Barrier concept. He wrote, "We accept it….simply because we must, because it is the only plausible explanation that we can conceive." Remarkably, he also admitted that it was not possible to observe the process in detail, so there could be no experimental proof, but continued:

It does not matter whether I am able to do so or not, or whether I could do it well or ill; once it is established that natural selection is the only principle which has to be considered, it necessarily follows that the facts can be correctly explained by natural selection. (29)

Huxley was not alone in criticising Weismann. One of the strong supporters of The Modern Synthesis, John Maynard Smith, wrote in 1998:

it is not clear why he thought it [Weismann's claim that the germ line is independent of the soma] was true. (30)

The absence of empirical evidence is also in Julian Huxley's work. Noble (28) shows that the selfish gene idea is not a physiologically testable hypothesis since the characterisation of the central entity in *The Selfish Gene* as what persists is not independent of the only test of the theory, which is the frequency of occurrence in the gene pool. As Dawkins says "Genes can be counted and their frequency is the measure of their success." (31, p. 346). How else could persistence be measured other than by measuring such frequencies?

In 2011 one of us also noted that

accurate replication of DNA is itself a system property of the cell as a whole, not just of DNA. DNA on its own is an extremely poor replicator. (32, p. 1012)

Speculation about necessary, dogmatic positions, independent of factual evidence, is a *philosophical* position requiring justification. Claims by scientists to deny being philosophers are then self-defeating.

6. The 40TH Anniversary Edition of *The Selfish Gene:* The Last Stand?

2016 was the 40th anniversary of the original publication of *The Selfish Gene* in 1976. As a result, a reprint (31) was issued together with an extensive Epilogue. There are several highly significant facts about this reprint.

First, the original 1976 version is reprinted in its entirety with no revisions. As Dawkins himself comments

> So many exciting things are fast happening in the world of genomics, it would seem almost inevitable – even tantalizing – that a book with the word 'gene' in it would, forty years on, need drastic revision if not outright discarding. (31, p. 345)

Indeed so. So why are there no revisions? Dawkins' answer is

> This might indeed be so, were it not that 'gene' in this book is used in a special sense, tailored to evolution rather than embryology. (31, p. 345)

Precisely so. Dawkins' 'gene' is constructed in such a way that, as we have shown, his thesis apparently has no empirical content.

It is a clever and beguiling tale. It comes equipped with a central character, the gene, selfishly enslaving the organism for its end to maintain its existence. Its objective is a fairy-tale land called the gene pool. But what is this gene? It is whatever it is that makes the story definitively true. It is whatever is inherited, both cause and effect. But it is founded on an illusion. R*egardless of the definition of a gene*, it all depends critically on whether the Weismann Barrier exists, whether DNA self-replicates "like a crystal", and whether the Central Dogma keeps the genome isolated. All three of those assumptions are false. The organism awakes and can make decisions. It can even write a book called "The Selfish Gene".

As a prelude to the quotations above, Dawkins muses

> In some ways I would quite like to find ways to recant the central message of *The Selfish Gene*. (31, p. 345)

We conclude that Richard Dawkins can now rest in peace: the way to recant is to acknowledge that the three foundation stones of the book have gone. They are presumed rules that are now seen to be broken. Furthermore, the facts that have removed those cornerstones inevitably present ethical problems since we can no longer assume that the germ-line is "sealed off from the outside world." Dawkins (1, p. 21).

7. The Ethics of Germ-line Modification

The ideas of the modern synthesis have been adopted and taken for granted in a wide variety of fields, ranging from economics (33) to sociology (34), and, notably, the implications for clinical medicine.

The gene-centred view insidiously affects dialogue in ethics. It has entered our culture as 'truth', a given, and it invades our language. Phrases such as "It is in our

DNA" are now loosely used, often without question of its real meaning or significance. We speak of genetics as a golden bullet for health and well-being (35). Governments provide considerable resources to unravelling its mystery; for several decades study of organisms suffered from this approach. Systems physiology was relatively starved of the resources it needed unless it fitted the modern synthesis.

Had the germ-line really been "sealed off from the outside world" in a way that prevents changing the genome through the actions of organisms or through the deliberate editing of germ-line genomes by clinical intervention, then the ethical problems created by Selfish Gene theory would have at least have been limited in scope in clinical practice. The demise of the theory creates at least three sets of ethical issues arising from the fact that genomes can be edited both by organisms themselves and by us as humans with genetic engineering of the germ-line and diagnosing potential genetic influences in embryos.

7.1. *Gene Therapy*

One of us has already highlighted the ethical dilemma for germ-line gene therapy in humans:

> The major concern with germ-line therapy remains the potential unseen and long- term consequences. We know very little of the way in which mutations might produce both harms and benefits. Genes that might be harmful in one set of circumstances might confer an advantage in another. The classic example is the higher resistance to malaria for heterozygote carriers of the sickle-cell gene mutation. Balanced selection maintains more than one variant of a gene in the population as a result of both the harms and benefits they confer in different circumstances. Another classic example of this in biology would be *Biston betularia*, the peppered moth, which has both dark and white polymorphic states that confer selective advantage or disadvantage in relation to the background. To manipulate the germ-line with insufficient knowledge of long- term consequences would be a high-risk strategy (36).

The possibility of germ-line therapy is still a high-risk strategy and we suspect it will remain so. The polygenic or even omnigenic nature of most diseases makes it impossible to predict all the possible consequences of editing the germ-line since this would involve indefinitely long trans-generational effects. We still know only a modest amount about the many factors involved in the environmental and social impacts on health and disease (37, 38), any of which could also influence the germ-line since it is not protected by a fixed barrier but by a functional and selective process.

7.2. *Diagnosis and Informed Consent*

This is true not only for Germ-line therapy. Even just pre-implantation genetic diagnosis on embryos, which could be used to advise parents of potential genetic risks, opens a Pandora's box of problems, largely concerning the fact that in most cases even the clinical practitioner would not know how best to advise parents:

> The extension of preimplantation genetic diagnosis raises practical ethical issues involving relative burdens, duty of care, freedom of choice, distributive justice, and informed consent. This paper argues for caution in advocating reproductive methods that are costly, have limited chances of success, and for which the long-term outcome is unknown (39).

7.3. *The Non-dualistic Nature of Organisms and Their Interactions with the Environment*

Organisms are necessarily open systems. They mesh with their environment, including other organisms with which they interact, in a multitude of ways. It is strictly impossible to unravel the multitude of interactions between nature and nurture, or indeed any other dualist approach. When causality takes the form of If X and Y then Z we cannot assume that the effects of X & Y can be added linearly. Physiological regulatory networks (often called gene regulatory networks) are adept at managing even when major genetic components are missing. In this sense organisms are robust. This is another reason why it is difficult to predict the outcome of gene therapy.

8. The End of Unnecessary Dualism in Biology

Dualism is a repeating problem in the history of biology. Descartes famously adopted this approach by inventing the 'ghost in the machine', a separate immaterial soul that was thought to be responsible for agency and will in humans. Animals were not thought to be anything more than automata.

The Williams-Dawkins duality is not itself immaterial. It is formulated in a material sense by hiving off a small part of the physical properties of an organism as the centre of organisation from which the whole organism develops. Descartes had the same idea, as he made clear in his *Treatise on the Fetus*:

> If one had a proper knowledge of all the parts of the semen of some species of animal in particular, for example of man, one might be able to deduce the whole form and configuration of each of its members from this alone, by means of entirely mathematical and certain arguments, the complete figure and the conformation of its members.[1]

Furthermore, by making the duality material, the Williams-Dawkins duality leads yet again to a form of Cartesian immaterial soul restricted to human beings:

> Let us understand what our selfish genes are up to, because we may then at least have the chance to upset their designs, *something which no other species has ever aspired to*.
>
> (1, p. 3)

There is no explanation of how this upsetting of physical processes could happen through the immaterial will of humans, nor why the ability is ascribed only to one species. The muddle becomes even worse when immaterial attributes such as 'selfish' and 'immortal' become ascribed to bits of DNA. All such attributions are simply meaningless if interpreted literally; and very misleading when interpreted metaphorically, because the metaphorical and literal meanings are so close as to be easily confused.

[1] The French text reads «Si on connoissoit quelles sont toutes les parties de la semence de quelque espèce d'Animal en particulier, par exemple de l'homme, on pourroit déduire de la seul, par des raisons entierement Mathematiques et certaines, toute la figure et conformation de ses membres;» (*de la formation du fœtus*, para LXVI p. 146; https://archive.org/stream/lhommeetlaformat00desc#page/146/mode/2up).

There *are* immaterial factors that influence the behaviour of organisms. But those immaterial factors are necessarily social properties of whole organisms in their interactions with other organisms. The ways in which that happens require a multi-level analysis of biological organisation (40, 41). Meaning and purpose, including selfishness, can only be ascribed at levels of organisation at which they are appropriate.

9. Conclusion

The Selfish Gene is the best selling science book of the 20th century. But as this paper has shown, *The Selfish Gene* got cause and effect backwards, assigning agency to natural selection instead of the organism itself (41, 42, 43, 44). It embraced the greatest errors of the Modern Synthesis (20, 21) while downplaying much of what it got right (5). It crowned the gene king of biology, even though genes are only servants of the cell (28). Gene-centric duality caused genomics to promise (45, 46)[1] far more than it could or ever can deliver since it cannot distinguish between correlation and functional causation (47). That failure has been at great cost to health care by promising miracle genetic cures that have not met the greatest challenge to health services for ageing populations caused by complex multi-factorial diseases that cannot be reduced to genetic causation (48).

Thus did *The Selfish Gene* turn Neo-Darwinism into a pop religion with its own dogmas, dressed up as science, but without the gold standard of a scientific hypothesis: an empirical test independent of the central assumption of the theory (32). To challenge its rigid dogmas was considered heresy, so that many science careers were lost by those who questioned it. Their discoveries were ignored or, at best, sidelined (49). These misunderstandings have set back treatments in cancer and infectious diseases by many decades (13, 49, 50). This is why *The Selfish Gene* is one of the greatest mistakes in the history of science.

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[1] Collins (45) claimed that human genome sequencing would lead to "previously unimaginable insights, and from there to the common good [including] a new understanding of genetic contributions to human disease and the development of rational strategies for minimizing or preventing disease phenotypes altogether."

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