

## Essay

# Oswald Avery, DNA, and the transformation of biology

Seventy years ago, Oswald Avery and his colleagues from the Rockefeller Institute published the first evidence that genes are made of DNA. Their discovery was received with a mixture of enthusiasm, suspicion and perplexity. In this article, I trace the reasons for these different responses, and show how we need to revise our usual explanations of what finally convinced everyone that the Avery group was right.

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On 1 February 1944, the *Journal of Experimental Medicine* published one of the breakthrough discoveries of the 20th century: Oswald Avery (1877–1955), together with his colleagues Colin MacLeod (1909–1972) and Maclyn McCarty (1911–2005), reported that the transformation of pneumococcus bacteria from one type to another occurred through the action of a ‘transforming principle’ that they identified as being composed of ‘sodium desoxyribonucleate’ or DNA [1]. The implication of the Avery lab’s discovery, although it was not stated this clearly, was that genes are made of DNA, not of protein as most people had thought. This conclusion was soon reinforced by two additional papers from the group [2,3].

Transformation had been discovered in London in 1928 by Fred Griffith, who reported the amazing finding that a pneumococcal strain could adopt the form of another strain even if the donor strain was dead. Avery began working on the chemical nature of the transforming principle in 1934, when Colin MacLeod joined his lab [4,5]. Progress was slow, and the pair were distracted by the discovery of sulfonamide antibiotics and MacLeod’s need to get some publications on his CV. In 1940, they returned to the topic and soon showed that the transforming principle was a white precipitate. After MacLeod left in summer 1941 to work on war-related topics, Maclyn McCarty joined the lab; although he carried out much of the biochemical work that followed, the driving force behind the project was Avery. By 1942 they had shown that the transforming principle was active at 1 part per 100,000,000 and that it was affected by enzymes that attacked DNA.

A year later, Avery and McCarty finished their first article, which they co-signed with MacLeod. Their

identification of the transforming principle as DNA was based on several strands of evidence: chemical composition; inactivation of the extract by enzymes or temperatures that affect DNA; no effect of enzymes that digest proteins; absence of immune reactions typical of those produced by proteins; and responses to centrifugation, electrophoresis and UV light that were all identical to those of DNA. Every result converged on the same conclusion: the transforming principle was composed of DNA. And yet in retrospect the conclusion to their article looks strangely muted:

“If the results of the present study on the chemical nature of the transforming principle are confirmed, then nucleic acids must be regarded as possessing biological specificity the chemical basis of which is as yet undetermined.”

Despite his reticence in print, in private Avery was clear about what his group’s discovery implied. Shortly before the first article appeared, the Australian immunologist Macfarlane Burnet visited Avery’s lab. Burnet told his fiancée that Avery “has just made an extremely exciting discovery which, put rather crudely, is nothing less than the isolation of a pure gene in the form of desoxyribonucleic acid” [6]. Responses to the publication of the Avery group’s article were immediate and positive, despite the dislocation caused by the war [7–10]. One report in *Nature* proclaimed “the genetic implications of this work are considerable” [11], while another suggested that “slight differences in molecular configuration” of different forms of DNA might explain differences in biological activity [12].

There was also official recognition: in October 1944, Avery was awarded the Gold Medal of the New York Academy of Medicine, primarily for his decades of work on pneumococcal

bacteria, but also for his 1944 paper and its “very far-reaching implications” [13]. In 1945, Avery received the Copley Medal from the Royal Society; the citation stated that he had shown that the gene “appears to be nucleic acid of the desoxyribose type” [14]. Pioneer geneticist Herman Muller was critical of Avery’s interpretation but nevertheless described the work of Avery’s group as “remarkable”: “If this conclusion is accepted”, wrote Muller, “their finding is revolutionary” [15]. Biochemist Howard Mueller expressed astonishment and enthusiasm: “a polymer of a nucleic acid may be incorporated into a living, degraded cell, and will endow the cell with a property never previously possessed (...) When thus induced the function is permanent, and the nucleic acid itself is also reproduced in cell division. The importance of these observations can scarcely be overestimated” [16].

There were also less public expressions of amazement. On 20 January 1945, Joshua Lederberg, a brilliant 19 year old, sat down to read an article that had been handed to him by a fellow student [17]. The effect on Lederberg was electric. As he wrote in his diary:

“I had the evening all to myself, and particularly the excruciating pleasure of reading Avery ’43 [sic] on the desoxyribose nucleic acid responsible for type transformation in *Pneumococcus*. Terrific and unlimited in its implications... I can see real case for excitement in this stuff” [18].

In October 1944, William Astbury, who had used X-ray crystallography to study the structure of DNA, told a friend that he considered the Avery group’s finding to be “one of the most remarkable discoveries of our time”. Astbury continued: “I wish I had a thousand hands and labs with which to get down to the problem of proteins and nucleic acids. Jointly those hold the physico-chemical secret of life, and quite apart from the war, we are living in a heroic age, if only more people could see it” [19]. In Paris, André Boivin, the deputy director of the Institut Pasteur, was inspired to study transformation



Figure 1. Oswald T. Avery in 1944. (Image: courtesy of the Tennessee State Library and Archives, and the National Library of Medicine.)

in *Escherichia coli* and in November 1945 reported that in this species, too, the transforming agent was “a highly polymerised thymonucleic acid”. Boivin’s conclusion was explicit: “we should now look to the nucleic acid component of the giant nucleoprotein molecule that forms a gene, rather than to the protein part, to find the inductive properties of the gene” [20].

As Europe and America emerged from the Second World War, there was a wave of research on the structure and function of nucleic acids, partly impelled by Avery’s work. In the period 1945–47 more than 250 papers were published on nucleic acids and nucleoproteins [21]. Above all, there were four major international scientific conferences on nucleic acids — in Cambridge (1946), at Cold Spring Harbor (1947, 1948), and in Paris (1948). Nucleic acid structure and function was becoming one of the hottest scientific topics of the time. But despite the widespread enthusiasm for the Avery group’s finding, the idea that genes were made of DNA was not accepted by the whole of the scientific community.

The historian and microbiologist H. Vivian Wyatt [22] and the pioneer molecular geneticist Gunter Stent have both argued that Avery’s articles did not have the wide impact that might be expected because they did

not fit in with the dominant view that proteins were the hereditary material. According to Stent, Avery’s discovery was “premature”. [23] These broad explanations hide the historical reality of how the Avery group’s work was received, and obscure why some scientists rejected the implication of Avery’s work while so many others enthusiastically embraced it, and also mislead us as to why everyone eventually came to accept that genes are made of DNA.

#### Why Avery might have been wrong

There were two main reasons not to accept that the transforming principle was made of DNA. The major difficulty was that, as the Avery group was well aware, the DNA extracts he used contained trace quantities of protein that might produce the transforming effect. The main advocate of this argument was Avery’s Rockefeller Institute colleague Alfred Mirsky. In 1946, Mirsky and Arthur Pollister published a widely read article in which they pointed out that “there can be little doubt in the mind of anyone who has prepared nucleic acid that traces of protein probably remain in even the best preparations” and that “as much as 1 or 2 percent of protein could be present in a preparation of ‘pure, protein-free’ nucleic acid” [24]. This criticism ignored the varied experimental data in the Avery group’s papers, all of which suggested that DNA was the sole active component in their extracts.

This dispute was expressed publicly at the 1947 Cold Spring Harbor symposium (Avery was not present — he hated attending meetings and was on the verge of retiring). At the meeting, Boivin summarised his experimental data from *E. coli* and presented the big picture implications of the Avery group’s findings: “each gene can be traced back to a macromolecule of a special desoxyribonucleic acid. (...) Thus, this amazing fact of the organization of an infinite variety of cellular types and living species is reduced, in the last analysis, to innumerable modifications within the molecular structure of one single fundamental chemical substance, nucleic acid” [25].

In the discussion, Mirsky repeated his criticism that small amounts of protein could still be present in ‘pure’ DNA extracts. Boivin replied by underlining the varied kinds of

evidence that he and the Avery group had presented: “it seems to us that the burden of proof rests upon those who would postulate the existence of an active protein lodged in an inactive nucleic acid” [25]. The chemist Erwin Chargaff turned the tables on Mirsky, pugnaciously pointing out that there was no evidence that the nucleoproteins Mirsky had spent his life studying were actually present in cells; it was quite possible that an extraneous protein had bound to the DNA while the two substances were being isolated. Chargaff went on to outline a research programme that would preoccupy many scientists over the coming decade:

“If, as we may take for granted on the basis of the very convincing work of Avery and his associates, certain bacterial nucleic acids of the desoxypentose type are endowed with a specific biological activity, a quest for the chemical or physical causes of these specificities appears appropriate, though it may remain completely speculative for the time being. (...) Differences in the proportions or the sequence of the several nucleotides forming the nucleic acid chain also could be responsible for specific effects” [26].

Chargaff’s final suggestion touched on the second obstacle to the immediate acceptance of the Avery group’s findings: given that DNA was essentially composed of four ‘bases’ it was unclear how it could produce the almost infinitely different effects produced by genes. It had been thought that the four bases were repeated in a constant, boring sequence, but in 1946 this had been challenged by the British chemist Masson Gulland, who wrote: “there is at present no indisputable evidence that any polynucleotide is composed largely, if at all, of uniform, structural tetranucleotides” [27]. Chargaff developed sophisticated techniques for measuring the exact proportion of the different bases and discovered that they were present in different proportions in different species — DNA was not ‘boring’, and both he and Gulland suggested that DNA molecules might differ in the sequence of bases. Gulland was tragically killed in a train accident in 1947; had he lived, the history of the study of DNA might have been very different. [28]

In response to Mirsky's criticisms, Avery's collaborator Hotchkiss reduced the amount of protein in the DNA extract to at most 0.2% — this was within the margin of error of a result of 0.0%, so it was quite possible that there was no protein at all in his samples [29]. Boivin reinforced the circumstantial evidence in favour of a genetic role for DNA by showing that diploid cells contained twice the amount of DNA (but not protein) as haploid cells. He concluded that "each gene can, in the final analysis, be considered as a macromolecule of DNA" [30]. Shortly before Boivin died in July 1949, doubts were raised about transformation in *E. coli* — his results could not be replicated and his original strains had been lost [31]. Despite — or perhaps because of — Boivin's bold statements and his prophetic visions of transferring genes between species, an air of disbelief accumulated around his fundamental discoveries. His findings were eventually confirmed in the 1970s, and his views on the nature of heredity and the future of biology also turned out to be true.

#### The phage group is nonplussed

Despite all this evidence, many biologists, and in particular many leading geneticists, were not inspired by the Avery group's discovery. This was not because they accepted Mirsky's criticisms, but because they simply did not 'get' Avery's finding. This was particularly true of Max Delbrück's informal 'phage group', which was pioneering the use of molecular techniques in biology through the study of bacteriophage viruses. Delbrück first heard of the Avery group's conclusion in 1943, eight months before publication. He later recalled his "total shock and surprise" at the news, but he did not start studying the role of DNA in bacteriophage, nor did any of his colleagues [32]. It appears that Delbrück was nonplussed by the suggestion that genes were made of DNA: "you really did not know what to do with it", he explained. [33].

The three key members of the phage group — Delbrück, Salvador Luria and Al Hershey — all later claimed that they were interested in genetics, not chemistry, and so simply did not realise the potential implications of Avery's discovery. Delbrück said, with typical robustness:

"And even when people began to believe it might be DNA, that wasn't really so fundamentally a new story, because it just meant that genetic specificity was carried by some goddamn other macromolecule, instead of proteins" [33].

Luria recalled:

"I don't think we attached great importance to whether the gene was protein or nucleic acid. The important thing for us was that the gene had the characteristics that it had to have" [33].

In 1994, Hershey explained that their focus was simply elsewhere: "as long as you're thinking about inheritance, who gives a damn what the substance is — it's irrelevant" [34].

With the easy wisdom of hindsight, this lack of interest looks somewhat short-sighted. Unlike Lederberg, Boivin and others, the phage group did not react positively to the Avery group's articles. This surprisingly diffident response from some of the key figures in molecular biology was one component of the failure of Avery's discovery to produce an immediate 'paradigm shift' in biology.

#### The tide turns

Despite the initial lack of interest from Delbrück and his colleagues, by the end of the 1940s there was growing support in favour of the hypothesis that DNA played a fundamental role in heredity. In summer 1950, Daniel Mazia summarised the experimental evidence showing that DNA fitted the key criteria for the hereditary material, whereas protein did not, and concluded: "DNA is the most likely candidate so far for the role of the material basis of heredity" [35]. Even members of the phage group began to pay attention. In 1951, John Northrop outlined the contrasting potential roles of proteins and DNA in viruses:

"The nucleic acid may be the essential, autocatalytic part of the molecule, as in the case of the transforming principle of the pneumococcus (...) and the protein portion may be necessary only to allow entrance to the host cell" [36].

This coincided with an idea that Roger Herriott described in a letter to Hershey:



Figure 2. Maclyn McCarty. (Image: courtesy of the History of Medicine Division at the National Library of Medicine.)

"I've been thinking — and perhaps you have, too — that the virus may act like a little hypodermic needle full of transforming principles; that the virus as such never enters the cell; that only the tail contacts the host and perhaps enzymatically cuts a small hole through the outer membrane and then the nucleic acid of the virus flows into the cell" [37].

Another phage group member, Thomas Anderson, later recalled:

"I remember in the summer of 1950 or 1951 hanging over the slide projector table with Hershey, and possibly Herriott, in Blackford Hall at the Cold Spring Harbor Laboratory, discussing the wildly comical possibility that only the viral DNA finds its way into the host cell, acting there like a transforming principle in altering the synthetic processes of the cell" [38].

It was in this context that Hershey, together with his technician, Martha Chase, began a series of experiments to identify the functions of protein and DNA in bacteriophage. Their 1952 paper in the *Journal of General Physiology* has since taken on an iconic quality [39]. Unlike the Avery group's 'premature' finding, claimed Gunter Stent, "the general impact of the Hershey-Chase experiment was immediate and dramatic" [23]. The reality is rather different.

Hershey and Chase first confirmed and extended previous reports that the

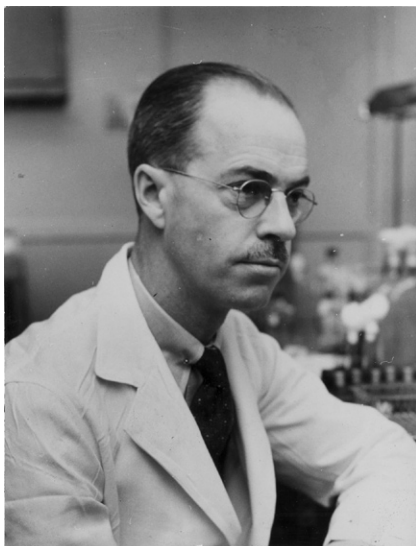


Figure 3. Colin MacLeod. (Image: courtesy of The Lillian and Clarence de la Chapelle Medical Archives at NYU.)

viral protein coat was not infectious and that it protected the DNA it contained. They next demonstrated that when the virus settled onto a bacterium, it ‘ejected’ (sic) DNA into the cell. Hershey and Chase did not show what the DNA did, nor could they be certain that no protein entered the bacterial cell. In a final set of experiments, infected bacterial cells were agitated in a Waring blender to shake off the protein-rich viral shells — they were able to remove up to 82% of the phage protein and yet viral reproduction continued. A separate experiment showed that up to 85% of phage DNA was transferred into the bacterial cell.

These results are now generally interpreted as showing that DNA is the genetic material, but strictly speaking they did no such thing [40]. Hershey and Chase faced a similar contamination problem to that encountered by Avery, but in spades. Hotchkiss had reduced the protein component in his DNA extracts to effectively zero (at most 0.02%), and still some people did not accept his findings; for Hershey and Chase, the corresponding protein contamination level in their DNA was around 20%! Furthermore, none of the experiments revealed the function of DNA in viruses. The paper concluded:

“The sulfur-containing protein of resting phage particles is confined to a protective coat that

is responsible for the adsorption to bacteria, and functions as an instrument for the injection of the phage DNA into the cell. This protein probably has no function in the growth of intracellular phage. The DNA has some function. Further chemical inferences should not be drawn from the experiments presented” [39].

Strikingly, Hershey and Chase did not cite any of Avery’s papers.

Hershey later admitted “I wasn’t too impressed by the results myself” [41]. When he presented his findings in a laboratory meeting, prior to publication, Hershey expressed surprise that protein apparently had no function in viral reproduction. [42] Hershey’s first public presentation of his results, at the June 1953 Cold Spring Harbor meeting, after the double-helix structure of DNA had been described, makes for surprising reading. Drawing very different conclusions from those that are attributed to these experiments today, Hershey made clear that he was certain that DNA could *not* be the sole hereditary molecule. After summarising his results he concluded:

“None of these, nor all together, forms a sufficient basis for scientific judgement concerning the genetic function of DNA. The evidence for this statement is that biologists (all of whom, being human, have an opinion) are about equally divided pro and con. My own guess is that DNA will not prove to be a unique determiner of genetic specificity, but that contributions to the question will be made in the future only by persons willing to entertain the contrary view” [43].

Hershey’s caution shows the rigour of his thinking, the power of the old ideas about the dominant role of proteins, and the difficulty of imagining how DNA could produce the multiple effects produced by genes. With the realisation that DNA could contain a genetic code, as first hypothesised by Watson and Crick in their second 1953 *Nature* paper, as well as having a structure that enabled replication through base-pairing, the role of DNA was gradually accepted, even in the absence of definitive experimental proof. By 1956, even Mirsky accepted that Avery’s interpretation was correct [44].

### Vindication

The final step was to generalise this view to the whole of life — even if DNA was the hereditary material in both bacteria and viruses, this did not mean that the same was necessarily true of multi-cellular organisms. What looks obvious to us now was still a matter of debate for a surprisingly long time. At a 1956 symposium on ‘The Chemical Basis of Heredity’, researchers discussed the lack of experimental evidence that could prove that DNA was the genetic material in all organisms. Steven Zamenhof, who had been an early supporter of Avery’s work, even had to accept that although “extensive evidence” suggested that the Avery’s transforming principle was composed of DNA, and “no evidence to the contrary had ever been presented”, there was still ‘no absolute proof’ [45]. In his key-note speech, George Beadle stated that “it is assumed as a working hypothesis that the primary genetic material is DNA rather than protein” [46].

That ‘working hypothesis’ applied to all organisms, even though there was no ‘absolute proof’. Confirmation of what everyone believed eventually arrived in the 1970s, when a series of experiments showed that DNA could transform cells from a wide variety of organisms, including mammals. These findings, even if they were the product of extreme experimental ingenuity, were not a surprise.

So even after the discovery of the double helix and following Hershey and Chase’s supposedly definitive experiment, our predecessors were still not absolutely convinced that all genes were made of DNA. Seen in this light, the apparent failure of Avery’s discovery to immediately transform biology looks less enigmatic. As Hershey later argued that the complex route from Avery’s 1944 discovery to the widespread acceptance that genes were made of DNA “shows that some redundancy of evidence was needed to be convincing and that diversity of experimental materials was often crucial to discovery” [37]. In the second half of the 1940s, there were plenty of scientists who enthusiastically embraced Avery’s discovery and began to explore its implications. Those who missed the boat were an influential section of the scientific community who

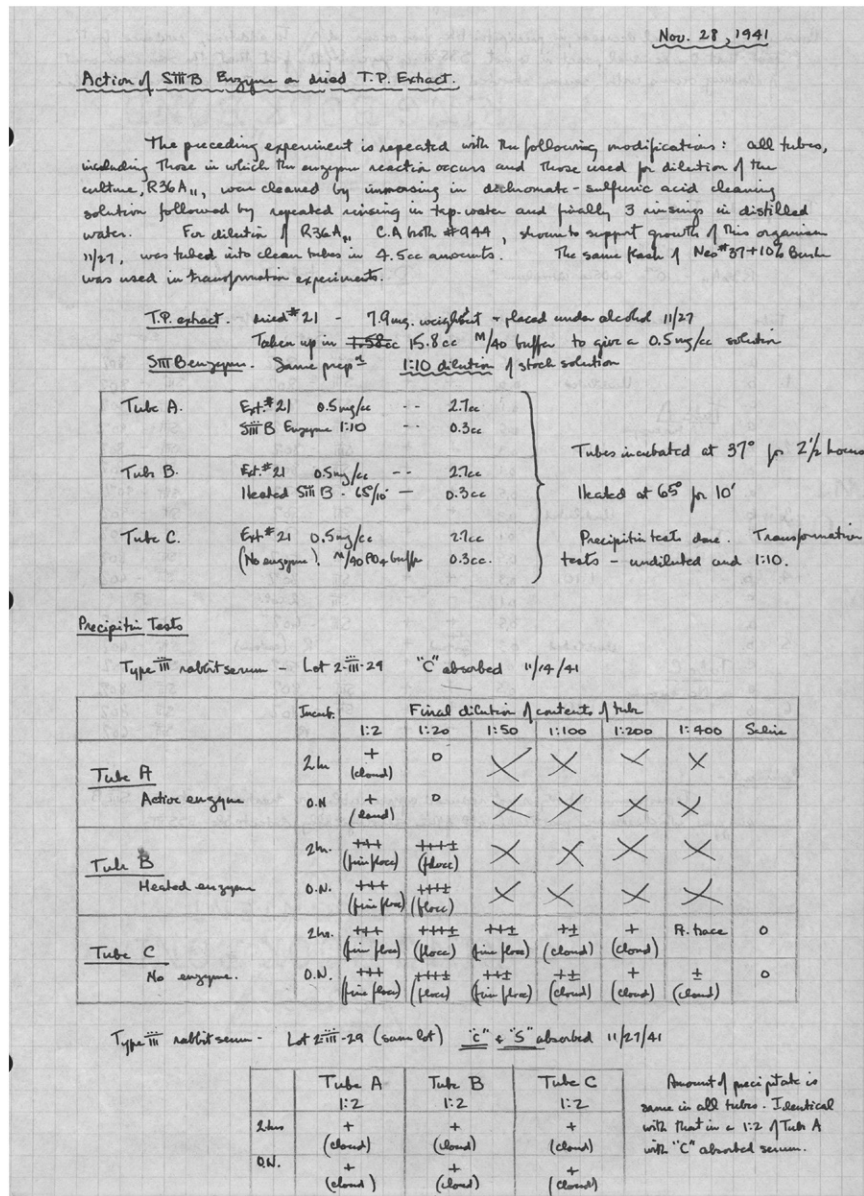


Figure 4. An extract from McCarty's laboratory notebook, 28 November 1941. The experiment described here is involves the action of the S III B enzyme on dried T[ransforming]. P[rinciple]. (Image: courtesy of the History of Medicine Division at the National Library of Medicine.)

were either overly-concerned with potential technical flaws, or were so focused on their own system that they could not immediately grasp the significance of a finding from outside of their field.

By the time Watson and Crick discovered the double helix structure of DNA in 1953, Avery had retired, and was living with his brother Roy in Nashville. It is not known what he thought of their breakthrough, or even if he heard of it. Two years later, Avery was dead, without ever receiving the

public recognition that he deserved. He was not given a Nobel Prize, and his brief obituary in the *New York Times* (21 February, 1955) merely mentioned his "studies of pneumonia". Avery, MacLeod and McCarty's 1944 article has now been cited nearly 2,000 times, with an average of around 40 citations per year over the last 20 years. Among the scientific community at least, the work of the Avery group is not forgotten, and the profound implications of their careful and precise experiments are widely

recognised as being of massive importance. It is no exaggeration to say that their discovery transformed the whole of modern biology.

**Acknowledgments**

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## Quick guides

### Trade-offs

Theodore Garland, Jr.

**How do organisms evolve as coordinated wholes?** As noted by Charles Darwin (1859) in *The Origin of Species*, “The whole organism is so tied together that when slight variations in one part occur, and are accumulated through natural selection, other parts become modified. This is a very important subject, most imperfectly understood.” Biologists have made major advances since then, and one of the primary conceptual tools used to understand how traits evolve in a correlated fashion is the idea of trade-offs. Indeed, the concept of trade-offs underpins much of the research in evolutionary organismal biology, physiology, behavioral ecology, and functional morphology, to name just a few fields.

**What is a trade-off?** In engineering and economics, trade-offs are familiar enough (e.g., money spent on rent is not available to buy food). In biology, a trade-off exists when one trait cannot increase without a decrease in another (or vice versa). Such a situation can be caused by a number of physical and biological mechanisms. One type of mechanism is described by the so-called ‘Y-model’, which states that for a given amount of resource (e.g., energy, space, time), it is impossible to increase two traits at once. A commonly cited example is a trade-off between the size and number of eggs that, for example, a fish, bird or turtle can produce in a given clutch. Depending on the organism, this trade-off can be caused by a limitation in the amount of energy available, the amount of time available to produce eggs or the amount of space available to hold eggs (e.g., inside the shell of a turtle). Similarly, time spent foraging may be time wasted with respect to finding a mate. Trade-offs also occur when characteristics that enhance one aspect of performance necessarily decrease another type of performance.

**What happens when functional demands conflict?** Having survived a decade of frigid winters in Wisconsin, I like to use the example of gloves versus mittens. Gloves are good for making snowballs and getting keys out of your pocket, but they do not keep your hands nearly as warm as mittens do. Moreover, you must remove the mittens to get the keys. Returning to biology, limbs can be ‘designed’ for speed, through lengthening and thinning of bone, but this will often reduce strength and make them more likely to break when in use. Hence, a predator that evolves to be a fast runner may have to trade-off its ability to subdue large or strong prey (e.g., cheetah versus lion).

**How do I recognize a trade-off?** Empirically, trade-offs usually are initially identified by comparing species or individuals within species, and testing for a negative relationship between two (or more) traits. A classic example is the trade-off between speed and stamina among species of animals (e.g., cats versus dogs) and among Olympic athletes (e.g., the best sprinters are not the best marathoners). These trade-offs in locomotor performance are based on variation in muscle fiber-type composition and other morphological and physiological characteristics, and possibly variation in motivation.

**Are trade-offs ubiquitous?** In some cases, expected trade-offs based on mathematical models or on basic biological principles are not found. This may occur because nature has more ‘degrees of freedom’ than assumed by simple conceptualizations that predict trade-offs. For one example, aside from changes in fiber-type composition, muscles can evolve to be larger, positions of origins and insertions can shift, legs can become longer, and gaits can evolve (including bipedality). As another example, animals may be able to acquire and process more food (e.g., by altering their preferred prey type), thus allowing them to secure more energy and increase both number and size of offspring. Another reason trade-offs may not occur is that ‘grade shifts’ can change the average values for multiple traits, or even the relationship between traits,