Concept

Genetic = Heritable (Genetic ≠ DNA)

Root Gorelick
Department of Biology and School of Mathematics and Statistics
Carleton University, Ottawa, Ontario, Canada
Root_Gorelick@carleton.ca

Manfred D. Laubichler
School of Life Sciences
Centers for Biology and Society and for Social Dynamics and Complexity
Arizona State University, Tempe, AZ, USA
Manfred.Laubichler@asu.edu

According to Varmuza (2003: 963),

> Classical neo-Darwinian theory is predicated on the notion that all heritable phenotypic change is mediated by alterations of the DNA sequence in genomes. However, evidence is accumulating that stably heritable phenotypes can also have an epigenetic basis, lending support to the long-discarded notion of inheritance of acquired traits.

Implicit in this, and many other writings, is the equivalence between the term "genetic" and those signals encoded by DNA sequences. How did we get to this peculiar definition of the term "genetic," a definition that seems to be held by most contemporary evolutionary biologists (Sarkar 1998)? Here we discuss the ramifications of this definition and how it has created a false dichotomy between neo-Darwinian and neo-Lamarckian evolution.

Before 1953: Genetic = Heritable

Genes were originally defined as operational units of heredity. The molecular structure of genes as well as the biochemical mechanisms underlying gene action were unknown when Johannsen (1909) first coined the term "gene," as well as in the 1800s when terms such as "pangene" were used to explain phenomena of heredity (Darwin 1868). These were very much black-box definitions of gene and genetic. But these operational definitions became the foundation upon which Fisher and Wright built their respective successful versions of population genetics. Furthermore, in the context of these definitions, there was also no clear distinction between what we today refer to as genetic and epigenetic. Instead, at the dawn of the 20th century, the principle dichotomy was between hard and soft inheritance. Hard inheritance referred to largely invariant effects, while soft inheritance referred to traits that were less stable between successive generations. This distinction became the basis for distinguishing neo-Darwinian and neo-Lamarckian evolution. Neo-Darwinism "may be defined as the Darwinian theory of evolution without recourse to any kind of soft inheritance" (Mayr 1942: 537).

However, we argue that the distinction between hard and soft inheritance is fuzzy. Morgan (1917) conceived of genes as a form of hard inheritance but still clearly recognized that mutation is natural and sufficiently common to supply the variation that is the fodder for natural selection. But, if mutations are common enough, the invariance of hard inheritance can evaporate. On the flip side, soft inheritance can be seen to correspond to a higher rate of mutations (or epimutations). Thus, Castle's (1914) concept of genes as a form of soft inheritance is not substantively different from Morgan's genes as a form of hard inheritance. Castle's and Morgan's notions of the gene primarily differed as a matter of degree, that is, *how heritable* is the trait?

Although Morgan's and Weisman's notions of hard inheritance of genes came to predominate biology for the next 90 years (Cook 1999), throughout the 1920s and 1930s, ideas that were more closely aligned with soft inheritance were still espoused by a few dominant figures in the field. Goldschmidt (1928) believed in the dynamic aspects of genes, with chromosomes as placeholders (Maienschein 1992). Schultz even provided a molecular mechanism underlying such malleable genes. Around 1937, before his pioneering work with Caspersson (Caspersson and Schultz 1940), Schultz thought that chromosomes were composed of information-coding proteins, with DNA heterogeneously distributed across the chromosome. Schultz thought that the more DNA present, the less likely the locus was to be expressed. "[C]oncentrations of DNA along the chromosome acted to block the expression of expression of the nearest gene" (Judson 1979: 235). Wright (1941)

May 30, 2008; revised and accepted June 2, 2008

and Dobzhansky (1937) held similar views on the composition of genes and the mechanism underlying their action. As we now know, with respect to regulatory loci and transposons, this is how cytosine methylation works: the more cytosine nucleotides that are methylated at a regulatory locus or transposon, the greater silencing of that gene or transposon. In other words, late 1930s ideas about the functional role of DNA sequences would be considered epigenetic phenomena from a modern perspective.

In retrospect, it is not surprising that Goldschmidt believed in a form of soft inheritance and the dynamic nature of genes. He studied position-effect variegation (Goldschmidt 1946), which is the consequence of a heritable form of protein that regulates gene expression. Position-effect variegation was also studied by Sturtevant (1925) and Jollos (1934). Another so-called heretic during the first half of the 20th century was Barbara McClintock, who was the first to investigate transposons (McClintock 1950). Although transposons are not themselves heritable proteins or epigenetic effects *per se*, their expression is largely controlled by the presence of an epigenetic effect, cytosine methylation (Gorelick 2003b). Thus the most prominent supporters of soft inheritance and advocates of a dynamic concept of the gene in the first half of the 20th century were people who studied heritable effects that were not directly encoded by DNA.

It was not until the 1940s and early 1950s that biologists realized that DNA is primarily contained in chromosomes (Caspersson and Schultz 1940; citing Brachet 1940) and that DNA is the primary information-coding portion of genes (Avery et al. 1944; Hershey and Chase 1952). Watson and Crick's (1953) elucidation of the structure of DNA was a watershed event in molecular genetics. However, these discoveries also largely foreclosed research into heritable molecular signals other than nucleotides. In light of Watson and Crick's 1953 paper, especially in conjunction with Hershey and Chase's paper the previous year, most biologists began equating genetic material with DNA nucleotides. This situation has not changed much, and currently there is still virtual unanimity that genes are strings of nucleic acids, either DNA or RNA, despite occasional assertions otherwise (e.g., "A gene is anything a competent biologist has chosen to call a gene"; Kitcher 1992: 131).

After 1953: Genetic = DNA

This emphasis on nucleotide sequences as the genetic material is also reflected in current definitions of the term "genetic" in the context of molecular biology. Heritable molecular signals other that nucleotides—such as cytosine methylation and chromatin formation—are no longer called genetic. Instead they are called epigenetic. This modern definition of "epigenetic" makes philological sense insofar as the proteins and methyl groups are literally attached to nucleotides ("epi-" is Greek for "on top of"). Yet this is a different definition of "epigenetic" than the one traditionally used in developmental biology.

In the early 20th century, the term "epigenetic" was used to describe all factors controlling gene expression and cell differentiation (Waddington 1940). Waddington used the term "epigenetic" as an amalgamation of epigenesis and genetics, where epigenesis refers to the gradual and progressive development of new structures (Hall 1992). It was not until after Waddington was writing his seminal book that biologists realized that DNA is primarily contained in chromosomes and that the information-coding portion of genes is DNA. Waddington thus used the term "epigenetic" to refer to any phenomena that affected development *within an individual* and, therefore implicitly, *within a generation* (although he also sometimes used the term "epigenetic" to refer to transgenerational phenomena; Waddington 1957). This is a far cry from the modern molecular evolutionary use of the term "epigenetic" to mean molecular signals attached to nucleotides that are heritable across generations. For that reason, when specifically talking about molecular signals such as cytosine methylation or chromatin formation, below, we will use the phrase "molecular epigenetic." We distinguish mitotic from meiotic (i.e., development from inheritance), as well as molecular versus nonmolecular epigenetic effects (cf. Müller and Olsson 2003).

In the second half of the 20th century, three lines of work brought heritable signals other than nucleotides to the fore of evolutionary biology. First was Crouse's (1960) discovery of genomic imprinting, in which she showed that the sex of an individual was determined by heterochromatic proteins on chromosomes and not by the underlying nucleotide sequence itself. Second was the discovery that cytosine methylation–controlled gene regulation (Holliday and Pugh 1975; Riggs 1975) and the subsequent realization that these methylation signatures were heritable ("This suggests a supplementary definition of epigenetics to include transmission from one generation to the next, other than the DNA sequence itself"; Holliday 1994: 454). Third was the discovery that the presence or absence of an acetyl group from histones can act in much the same way as cytosine methylation (Nan et al. 1998; Bird 2001). Each of these phenomena (cytosine methylation, histone modification) can be considered to be "molecular epigenetic signals" and appear to be related to each other. Furthermore, as these signals can affect gene regulation and development, they are also epigenetic in the sense of Waddington and later developmental biologists (mitotic inheritance and development).

Several biologists (e.g., Varmuza 2003) have speculated that molecular epigenetic signals could be responsible for chromosomal rearrangements and hence macroevolutionary changes in the sense of Goldschmidt (1940). This assertion is usually based on position-effect variegation, which, as far as we know, is unique to the fruit fly family *Drosophilidae*.

Table 1. Evolutionary effects of molecular epigenetic signals.

	Mechanisms	Consequences	Selected references
Cytosine methylation	• Gene silencing • Delayed replication • Repressed transposons • X-inactivation & genomic imprinting • Telomere degradation	• Developmental regulation • Genome defense • Disease susceptibility • Sex determination • Senescence	Holliday and Pugh (1975) Riggs (1975) Howard (1996) Yoder et al. (1997) Gorelick (2003b)
Chromatin formation and RNA interference	• Gene silencing • X-inactivation • Repressed transposons • Genomic imprinting	• Developmental regulation • Genome defense • Disease susceptibility • Sex determination	Crouse (1960) Ekwall et al. (1997) Spencer (2002) Prahald et al. (2003) Lippman and Martienssen (2004)
Environmental epigenetic control	• Removal of chromatin • Inhibition of maintenance methylation • Horizontal transfer of hormones, RNA (between species)	• Increased phenotypic plasticity • Developmental plasticity • Neo-Lamarckian effects • Coevolution	Jollos (1934) Agrawal et al. (1999) Relyea and Mills (2001) Hooper et al. (2001) Gilbert (2002)

However, there are other more common aspects of epigenetic inheritance systems, such as the virtually ubiquitous signals of cytosine methylation and chromatin formation and their environmental epigenetic control that also have evolutionary implications (see Table 1). Presence of cytosine methylation or heterochromatin on promoters is known to downregulate genes (Boyes and Bird 1992; Tate and Bird 1993), including those responsible for upstream stages of biochemical or developmental pathways (Gorelick 2003a). This can result in drastic phenotypic changes, possibly even including the origins of neoteny (Gorelick 2004b). Lack of cytosine methylation and heterochromatin can also cause large phenotypic changes because transposons can be inserted and chromosomal rearrangements occur at these loci (McClintock 1950; O'Neill et al. 1998). The underlying mechanism for these phenotypic changes is that cytosine methylation and heterochromatin occupy binding sites that otherwise would be used by enzymes that mediate recombination and transcription (Catcheside 1986; Hsieh et al. 1986; Rauth et al. 1986; Iguchi-Ariga and Schaffner 1989; Gorelick 2003a, 2003b).

A Nostalgic Definition of "Genetic"

In light of our brief historical sketch, the question arises why should molecular epigenetic signals of cytosine methylation and chromatin formation not be considered genetic? They are heritable. Why are other heritable signals, such as RNA-editing mechanisms, developmental modules, microtubule-organizing centers, standing waves of enzymes—considered to be epigenetic effects, and not genetic? In many ways, these questions closely resemble the debates 90 years ago regarding the problem of hard versus soft inheritance (Richards 2006; Pigliucci 2007). The molecular epigenetic signals listed above probably all have a lower degree of heritability than do DNA nucleotides (assuming here that the nucleotide sequence is the phenotype; Lewontin 1992). That is, epimutation rates are typically higher than point mutation rates of nucleotides. However, if degree of heritability becomes our basis for distinguishing epigenetic from genetic effects—as generally seems to have been done over most of the 20th century—then we would be obliged to consider RNA nucleotides to be epigenetic because RNA lacks the proof reading, mismatch repair, and other error-correcting enzymes present in DNA (Poole et al. 2000). Such distinctions between "epigenetic" and "genetic" seem at best strained and at worst confounding, especially when cytosine and histone methylation patterns are mediated by RNAi (Cao et al. 2003; Zilberman et al. 2003). We therefore propose to refer to all heritable signals as "genetic," regardless of their underlying molecular mechanism, thereby removing from use the molecular definition of epigenetic ("molecular epigenetic"). We propose, however, to retain the definition of "epigenetic" used by developmental biologists, that of so-called mitotic inheritance. Calling meiotic inheritance genetic and calling mitotic inheritance epigenetic is a clean dichotomy, at least for eukaryotes.

Referring to all heritable signals as "genetic" is not a new idea. It is largely how Fisher defined additive genetic variance of a population. Fisher's (1930) *magnum opus* preceded the molecular elucidation of chromosomes, hence he was compelled to take a black-box approach. Lush (1937) built on Fisher and Wright's work to define heritability, effectively establishing the continuum between soft and hard inheritance. However, as we have shown, referring to all heritable signals as "genetic" has not been in vogue with geneticists and molecular biologists since the publications of Hershey and Chase (1952) and Watson and Crick (1953). And even critics of such DNA-centered views of genes, such as developmental systems theorists, implicitly accept this distinction when they "emphasize the importance of extragenetic [i.e., non-nucleotide] forms of

inheritance [such as] cytoplasmic gradients [and] methylation patterns" (Gray 2001: 194).

It should now be clear why Goldschmidt espoused neo-Lamarckian evolution.

> The basic concept underlying this belief is that the genetic material itself is pliable, or "soft." For this theory it does not matter whether the genetic material changes slowly or fast, nor whether it changes directly or via "acquired characters": what matters is that the genetic material is believed not to be constant, not changeable, not "hard." (Mayr 1982: 687)

Early in his career, Goldschmidt studied sex determination in nematodes and lepidopterans. We now believe that sex is determined by the epigenetic signal of histone acetylation in the nematode *Caenorhabditis elegans* (Prahald et al. 2003), by heterochromatin in all Lepidoptera (Traut and Marec 1996), and by cytosine methylation in many of those taxa that have cytosine methylation and equal-length sex chromosomes (Gorelick 2003a; Jablonka 2004). Later in his career, Goldschmidt was studying the molecular epigenetic effect of position effect variegation, which is a form of chromatin formation. The associated proteins and their positions along a chromosome are more pliable than are DNA nucleotides. Most forms of chromatin formation and cytosine methylation are believed to be somewhat alterable by environmental perturbations, and their new configurations are probably highly heritable. The environmental perturbations, however, generally cause unpredictable changes in phenotype (McClintock 1984; Chong and Whitelaw 2004; Gorelick 2005; Long et al. 2006). A similar situation occurs with environmental perturbations altering DNA nucleotides in the germ line. Carcinogens and free radicals can cause point mutations of nucleotides in germ-cell genomes, with the new genome sequence being highly heritable. Likewise, environmental perturbations can cause highly heritable changes in cytosine methylation, usually due to deamination being uncoupled from remethylation (Gorelick 2004a; Pembrey et al. 2006; Pfeifer 2006; Walsh and Xu 2006). In this sense, there is no real distinction between soft and hard inheritance and no real distinction between molecular epigenetic and genetic signals. Consequently, there can be no real distinction between neo-Lamarckian and neo-Darwinian evolution (Gorelick 2006). Once the terms "gene" and "genetic" are extended to all heritable signals, and not just DNA nucleotides, then neo-Lamarckian evolution simply reflects a slightly larger influence of environmental perturbations and hence slightly lower heritability. The demarcation between neo-Lamarckian and neo-Darwinian evolution is no more real than the demarcation between gene and epigene or the demarcation between hard and soft inheritance, all of which depend on setting an arbitrary threshold for heritability.

Conclusion

We recommend that the term "genetic" be used interchangeably with the term "heritable." As mentioned above, the term "heritable" is sometimes used to describe mitotic inheritance, especially by cancer researchers and anyone studying prokaryotes. This muddling should be cleared up, and the term "heritable" only used to describe transmission of information via meiosis. Prokaryotes, viruses, viroids, prions, and obligately apomictic eukaryotes lack meiosis. However, viruses, viroids, and prions are genetic insofar as they can be passed on via meiosis from one eukaryotic cell to another. We also believe that there are few or no completely apomictic eukaryotes. The only real gap in our definition of genetic is with prokaryotes and viruses that affect them.

Narrow-sense heritability can be small (corresponding with soft inheritance or many molecular epigenetic effects) or large (corresponding with hard inheritance, especially of DNA nucleotides). Heritability is a quantitative genetic construct that depends on phenotype (often called "genotypic value") that takes values between 0 and 1 and does not depend upon the underlying molecular mechanisms causing the phenotypes. This is historically sensible in that Lush (1937) did not know the molecular basis of heredity when he initially defined heritability. "Genetic" should simply mean that narrow-sense heritability is greater than zero. Nonzero narrow-sense heritability and thus genetic effects can arise from transgenerational inheritance of phenotype that is due to any cellular machinery that gets passed from the micro- or megaspore mother cell to the surviving products of meiosis, including all of the following:

1. Nuclear DNA, organelle DNA, viral DNA, transposons
2. rRNA, tRNA, RNAi, viral RNA, ribosomes
3. Cytosine methylation, prions
4. Histones, including their phosporylation, acetylation, and methylation
5. Heterochromatic proteins, kinetochores, position effect variegation.

References

Agrawal AA, Laforsch C, Tollrian R (1999) Transgenerational induction of defences in animals and plants. Nature 401: 60–63.

Avery OT, MacLeod CM, McCarty M (1944) Studies on the chemical nature of the substance inducing transformation in pneumococcal types. Journal of Experimental Medicine 79: 137–158.

Bird AP (2001) Methylation talk between histones and DNA. Science 294: 2113–2115.

Boyes J, Bird A (1992) Repression of genes by DNA methylation depends on CpG density and promoter strength: Evidence for involvement of a methyl-CpG binding protein. EMBO Journal 11: 327–333.

Brachet J (1940) La détection histochimique des acides pentosenucléiques. Comptes Rendus des Séances de la Société Belge de Biologie 133: 88–90.

Cao XF, Aufsatz W, Zilberman D, Mette MF, Huang MS, Matzke M, Jacobsen SE (2003) Role of the DRM and CMT3 methyltransferases in RNA-directed DNA methylation. Current Biology 13: 2212–2217.

Caspersson T, Schultz J (1940) Nucleic acid metabolism of the chromosomes in relation to gene reproduction. Proceedings of the National Academy of Sciences of the USA 26: 507–515.

Castle WE (1914) Pure lines and selection. Journal of Heredity 5: 93–97.

Catcheside DG (1986) A restriction and modification model for the initiation and control of recombination in *Neurospora*. Genetical Research 47: 157–165.

Chong SY, Whitelaw E (2004) Epigenetic germline inheritance. Current Opinion in Genetics and Development 14: 692–696.

Cook GM (1999) Neo-Lamarckian experimentalism in America: Origins and consequences. Quarterly Review of Biology 74: 417–437.

Crouse HV (1960) The controlling element in sex chromosome behavior in *Sciara*. Genetics 45: 1429–1443.

Darwin CR (1868) The Variation of Animals and Plants Under Domestication. London: John Murray.

Dobzhansky TG (1937) Genetics and the Origin of Species. New York: Columbia University Press.

Ekwall K, Olsson T, Turner BM, Cranston G, Allshire RC (1997) Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. Cell 91: 1021–1032.

Fisher RA (1930) The Genetical Theory of Natural Selection. Oxford: Clarendon Press.

Gilbert SF (2002) The genome in its ecological context: Philosophical perspectives on interspecies epigenesis. In: From Epigenesis to Epigenetics: The Genome in Context (Van De Vijver G, Van Speybroeck L, De Waele D, eds). Annals of the New York Academy of Sciences 981: 202–218.

Goldschmidt RB (1928) The gene. Quarterly Review of Biology 3: 307–356.

Goldschmidt RB (1940) The Material Basis of Evolution. New Haven, CT: Yale University Press.

Goldschmidt RB (1946) Position effect and the theory of corpuscular gene. Experientia 2: 197–232, 250–256.

Gorelick R (2003a) Evolution of dioecy and sex chromosomes via methylation driving Muller's ratchet. Biological Journal of the Linnean Society 80: 353–368.

Gorelick R (2003b) Transposable elements suppress recombination in all meiotic eukaryotes, including automictic ancient asexuals: A reply to Schön and Martens. Journal of Natural History 37: 903–909.

Gorelick R (2004a) Neo-Lamarckian medicine. Medical Hypotheses 62: 299–303.

Gorelick R (2004b) Resolving the phylogenetic placement of *Blossfeldia liliputana* (Cactaceae): Reticulate evolution, chloroplast inheritance, and graft-chimeras. Bradleya 22: 9–14.

Gorelick R (2005) Environmentally-alterable additive genetic variance. Evolutionary Ecology Research 7: 371–379.

Gorelick R (2006) Evolutionary flatland. Biological Theory 1: 203–205.

Gray RD (2001) Selfish genes or developmental systems? In: Thinking About Evolution: Historical, Philosophical, and Political Perspectives (Singh RS, Krimbas CB, Paul DB, Beatty J, eds), 184–207. Cambridge: Cambridge University Press.

Hall BK (1992) Evolutionary Developmental Biology. London: Chapman and Hall.

Hershey AD, Chase M (1952) Independent functions of viral protein and nucleic acid in growth of bacteriophage. Journal of General Physiology 36: 39–56.

Holliday R (1994) Epigenetics: An overview. Developmental Genetics 15: 453–457.

Holliday R, Pugh JE (1975) DNA modification mechanisms and gene activity during development. Science 187: 226–232.

Hooper LV, Wong MH, Thelin A, Hansson L, Falk PC, Gordon JI (2001) Molecular analysis of commensal host-microbial relationships in the intestine. Science 291: 881–884.

Howard BH (1996) Replicative senescence: Considerations relating to the stability of heterochromatin domains. Experimental Gerontology 31: 281–293.

Hsieh P, Meyn S, Camerini-Otero D (1986) Partial purification and characterization of a recombinase from human cells. Cell 44: 885–894.

Iguchi-Ariga SMM, Schaffner W (1989) CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation. Genes and Development 3: 612–619.

Jablonka E (2004) The evolution of the peculiarities of mammalian sex chromosomes: An epigenetic view. BioEssays 26: 1327–1332.

Johannsen W (1909) Elemente der exakten Erblichkeitslehre. Jena, Germany: Gustav Fischer.

Jollos V (1934) Inherited changes produced by heat treatment in *Drosophila*. Genetica 16: 476–494.

Judson HF (1979) The Eighth Day of Creation. New York: Simon and Schuster.

Kitcher P (1992) Gene: current usage. In: Keywords in Evolutionary Biology (Keller EF, Lloyd EA, eds), 128–131. Cambridge, MA: Harvard University Press.

Lewontin RC (1992) Genotype and phenotype. In: Keywords in Evolutionary Biology (Keller EF, Lloyd EA, eds), 137–144. Cambridge, MA: Harvard University Press.

Lippman Z, Martienssen R (2004) The role of RNA interference in heterochromatic silencing. Nature 431: 364–370.

Long LK, Lin XY, Zhai JZ, Kou HP, Yang W, Liu B (2006) Heritable alteration in DNA methylation pattern occurred specifically at mobile elements in rice plants following hydrostatic pressurization. Biochemical and Biophysical Research Communications 340: 369–376.

Lush JL (1937) Animal Breeding Plans. Ames: Iowa State College Press.

Maienschein J (1992) Gene: historical perspectives. In: Keywords in Evolutionary Biology (Keller EF, Lloyd EA, eds), 122–127. Cambridge, MA: Harvard University Press.

Mayr E (1942) Systematics and the Origin of Species. New York: Columbia University Press.

Mayr E (1982) The Growth of Biological Thought: Diversity, Evolution, and Inheritance. Cambridge, MA: Belknap Press.

McClintock B (1950) The origin and behavior of mutable loci in maize. Proceedings of the National Academy of Sciences of the USA 36: 344–355.

McClintock B (1984) The significance of responses of the genome to challenge. Science 226: 792–801.

Morgan TH (1917) The theory of the gene. American Naturalist 51: 513–544.

Müller GB, Olsson L (2003) Epigenesis and epigenetics. In: Keywords and Concepts in Evolutionary Developmental Biology (Hall BK, Olson WM, eds), 114–123. Cambridge, MA: Harvard University Press.

Nan XS, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird AP (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393: 386–389.

O'Neill RJW, O'Neill MJ, Graves JAM (1998) Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. Nature 393: 68–72.

Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, Golding J (2006) Sex-specific, male-line transgenerational responses in humans. European Journal of Human Genetics 14: 159–166.

Pfeifer GP (2006) Mutagenesis at methylated CpG sequences. In: DNA Methylation: Basic Mechanisms (Doerfler W, Böhm P, eds), 259–281. Heidelberg, Germany: Springer.

Pigliucci M (2007) Do we need an extended evolutionary synthesis? Evolution 61: 2743–2749.

Poole A, Penny D, Sjöberg BM (2000) Methyl-RNA: An evolutionary bridge between RNA and DNA? Chemistry and Biology 7: R207–R216.

Prahald V, Pilgrim D, Goodwin EB (2003) Roles of mating and environment in *C. elegans* sex determination. Science 302: 1046–1049.

Rauth S, Song KY, Ayares D, Wallace L, Moore PD, Kucherlapati R (1986) Transfection and homologous recombination involving single-stranded DNA substrates in mammalian cells and nuclear extracts. Proceedings of the National Academy of Sciences of the USA 83: 5587–5591.

Relyea RA, Mills N (2001) Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). Proceedings of the National Academy of Sciences USA 98: 2491–2496.

Richards EJ (2006) Inherited epigenetic variation: Revisiting soft inheritance. Nature Reviews Genetics 7: 395–401.

Riggs AD (1975) X inactivation, differentiation, and DNA methylation. Cytogenetics and Cell Genetics 14: 9–11.

Sarkar S (1998) Genetics and Reductionism. Cambridge: Cambridge University Press.

Spencer HG (2002) The correlation between relatives on the supposition of genomic imprinting. Genetics 161: 411–417.

Sturtevant AH (1925) The effects of unequal cross-over at the bar-locus in *Drosophila*. Genetics 10: 117–147.

Tate PH, Bird AP (1993) Effects of DNA on DNA-binding proteins and gene expression. Current Opinion in Genetics and Development 3: 226–231.

Traut W, Marec F (1996) Sex chromatin in Lepidoptera. Quarterly Review of Biology 71: 239–256.

Varmuza S (2003) Epigenetics and the renaissance of heresy. Genome 46: 963–967.

Waddington CH (1940) Organizers and Genes. Cambridge: Cambridge University Press.

Waddington CH (1957) The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology. London: George Allen and Unwin.

Walsh CP, Xu GL (2006) Cytosine methylation and DNA repair. In: DNA Methylation: Basic Mechanisms (Doerfler W, Böhm P, eds), 283–315. Heidelberg, Germany: Springer.

Watson JD, Crick FHC (1953) Molecular structure of nucleic acids. Nature 171: 737–738.

Wright S (1941) The physiology of the gene. Physiological Reviews 2: 487–527.

Yoder JA, Walsh CP, Bestor TH (1997) Cytosine methylation and the ecology of intragenomic parasites. Trends in Genetics 13: 335–340.

Zilberman D, Cao XF, Jacobsen SE (2003) ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation. Science 299: 716–719.