Inheritance of acquired characteristics revisited

by Otto E. Landman

Can environmentally induced or acquired changes in organisms be transmitted to future generations? Does the inheritance of acquired characteristics—if it occurs at all—play a significant role in evolution? These questions were the subject of heated scientific and political controversy until as recently as the 1960s, when the decisive successes of classical genetics submerged this debate. If asked, most biologists today would say that inheritance of acquired characteristics never occurs. (Among 30 of the most widely used college textbooks of genetics published since 1962, none indicated that actual examples of inheritance of acquired characteristics had been found).

In a sharp departure from this near-universal skepticism, my recent review of pertinent literature of the last 50 years describes a substantial number of experimental systems that display inheritance of acquired characteristics (Landman 1991). In most of these systems, a brief transient change in environment calls forth particular new traits in all or most of the treated animals or cells, which persist when they are put back into the original environment. Subsequently, the new traits are transmitted heritably to all their offspring (Figure 1). Obviously, such behavior is contrary to that expected of Mendelian traits but, instead, is descriptive of the behavior expected of acquired traits.

Note that this new definition of inheritance of acquired characteristics focuses on the phenomonology of sudden, induced, and inherited massive change in a particular character. The examples cited in this article show that several fundamentally different mechanisms can give rise to this phenomenology. Each of these mechanisms is fully compatible with modern molecular biology.

Acquired heritable changes in traits, displayed in various experimental systems, can now be understood in terms of molecular genetics

The situation I describe is not often encountered in natural science: a near-consensus among scientific specialists on a viewpoint that is squarely contradicted by a substantial body of reliable experimental evidence. In the latter part of this article, I offer an account of how this anomalous situation developed. Briefly, the confrontation and vituperation generated by the Lysenko affair effectively silenced discussion of acquired inheritance. Lysenko's partisans on one side and many Western geneticists on the other came to believe that Mendelian inheritance and acquired inheritance are mutually exclusive as systems of inheritance. Only after the attitudes toward acquired inheritance had crystallized came the revolutionary advances in molecular biology necessary to understand the mechanisms underlying the various acquired-inheritance systems. Acquired heritable changes in traits can now be understood in terms of molecular genetic processes such as gene expression and horizontal gene transfer.

In this article, I introduce inheritance of acquired characteristics by briefly describing some representative examples. I give a fresh assessment of the role of the inheritance of acquired characteristics in evolution and recount some key episodes in the history of the changing concepts concerning acquired inheritance.

Description of some prototype systems

Induced inherited changes in traits so far described in different systems have three basic causes. They are stabilization of gene expression without any attendant change in nucleic acid sequence (extranucleic inheritance); alterations in DNA substituents such as methyl or glycosyl groups, without any attendant change in nucleic acid sequence (epinucleic inheritance); and removal or addition of foreign nucleic-acid-containing elements such as plasmids, viruses, or bacteria (nucleic inheritance). These three modes of inheritance were the first distinguished by J. Lederberg (1958).

Extranucleic inheritance. Examples of extranucleic inheritance of acquired characteristics systems include inheritance of the wall-less state in *Bacillus subtilis*, maintenance of the induced state in the *lac* operon of *Escherichia coli*, and cortical inheritance in ciliates (see box page 698). The molecular basis of the heritable persistence of the expressed characteristics is quite different in the three examples, although in none is there a change in the DNA sequences of the cell's genetic complement in either nucleus or cytoplasm.

In the *B. subtilis* system, inheritance of the wall-less state depends on a stabilized equilibrium between post-translational gene products: nascent cell wall and an enzyme, autolysin, that keeps destroying wall. In the *lac*

operon system, a transcription switch is permanently kept in the "on" position by a dilute extracellular supply of inducer boosted to a concentrated intracellular inducing level by permease activity acquired during an earlier induction episode. In cortical inheritance, messages emanating from the grafted-on cell fragments modify a feedback system that links cell cortex and morphogenetic genes.

Epinucleic inheritance. Inheritance mediated by substituents of DNA such as methyl or glucosyl groups has been called epinucleic inheritance. Methylation of the cytosine of CpG dinucleotides in DNA seems to play an important role in the regulation of transcription in many eukaryotic systems (Cedar 1988, Holliday 1987). Methylation leads to changes in local chromatin configuration that, in turn, alter the accessibility of genes to regulatory proteins. Most commonly, methylation inhibits gene expression, whereas demethylation leads to gene activation. Tissue-specific genes are nearly fully methylated in the germ cells, whereas the demethylation of these genes usually takes place only in the specific tissue of expression (Cedar 1988, Yisraeli et al. 1986).

Apart from the specific remethylation of the chromosomes in the germ line and the demethylation of particular genes in differentiated tissues, methylation patterns are fairly stably inherited (see box page 700). The agent responsible for this heritability is thought to be a maintenance methylase that, during replication, rapidly adds methyl groups to the nascent daughter strand at sites corresponding to methylated sites in the template strand (Holliday 1987). In analogy to inheritance of base sequences in replicating DNA, the inheritance of DNA methylation may thus be guided by a kind of enzyme-mediated complementarity (Figure 2).

Nucleic inheritance. Heritable changes in *Euglena* and fruit flies can be triggered by chemically or physically induced mass elimination of elements containing nucleic acid (see box page 702). These elements include double-stranded DNA and RNA and single-stranded RNA and range in size from that of bacteria to that of small viruses. Some of the elements are lodged in the nucleus, others in the cytoplasm. All the heritably transmitted nucleic acid elements susceptible to such selective curing must have been acquired earlier by their host organisms.

Heritable state I	Organisms exhibit trait S while replicating in environment A
	↓
Induction	Brief, nonlethal treatment (e.g., enviroment B) changes trait S to trait T in all (or a large fraction of) organisms
	↓
Heritable state II	Organisms exhibit trait T while replicating in environment A

Figure 1. Inheritance of acquired characteristics defined operationally and phenomenologically.

Acquisition of nucleic acid elements can be viewed as the reverse of curing. Several cases are known where the acquisition of particular nucleic acid–borne gene banks occurs with near 100% efficiency. In several of these cases, the elements acquired are plasmids—small circles of DNA commonly found in bacteria.

These cases of mass acquisition of particular nucleic acid elements clearly conform to our operational definition of inheritance of acquired characteristics (Figure 1). Perhaps the definition of inheritance of acquired characteristics should be expanded to include all cases of acquisition of foreign nucleic acid, regardless of whether the acquisition is a frequent experimentally controlled event or a rare natural occurrence inferred from molecular studies. For example, the acquisition of the mitochondrial precursor prokaryote by a host cell, perhaps a billion years ago (Cavalier-Smith 1987), can be regarded as a case of inheritance of acquired characteristics that had major effects on today's biological scene.

Acquired traits in evolution and evolutionary saltation

Evolutionary thinking before Darwin was dominated by Lamarck's idea that inherited characteristics change adaptively under the influence of changing environments. Of the experimentally demonstrated changes, only some are adaptive. For example, penicillin-induced L forms are more penicillin resistant than the normal bacilli (Landman and Halle 1963). Yeast cured of their mitochondria by azide are no longer sensitive to this agent (Nagai et al. 1961), and bacteria carrying plasmids derived from an infective agent are immune to further infection by the same agent. Streptomycin-mediated loss of chloroplasts

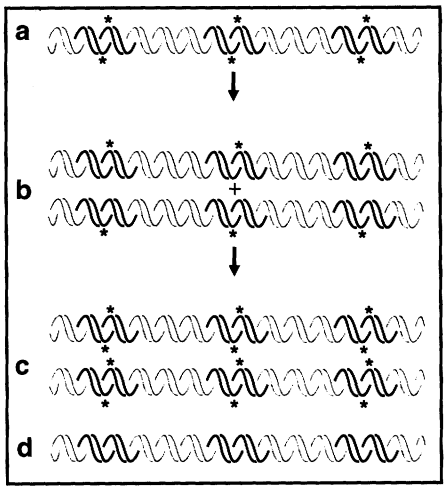
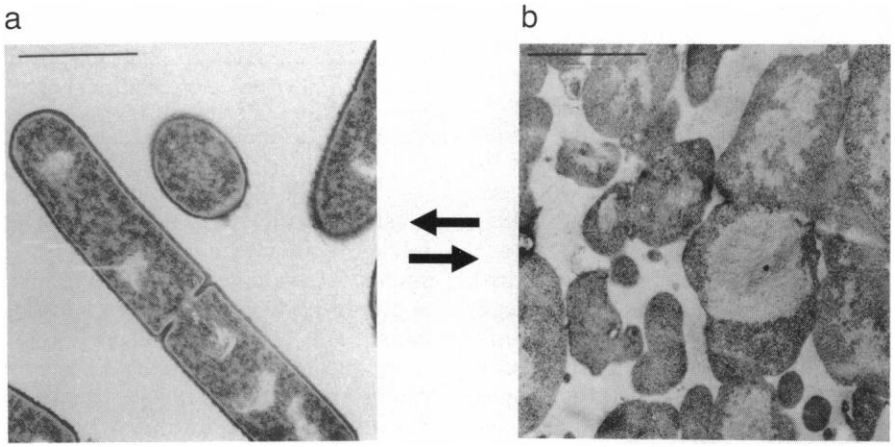


Figure 2. How the perpetuation of CpG methylated sites in DNA is thought to be ensured by a maintenance methylase. (**a**) Resident DNA is methylated (*) at all target sites. (**b**) Replication gives hemimethylated DNA. (**c**) Hemimethylated DNA becomes fully methylated, presumably by action of a maintenance methylase that recognizes hemimethylated sites. (**d**) Demethylation of DNA may activate genes. It may also render DNA susceptible to restriction endonucleases.

Examples of extranucleic acquired inheritance systems

Heritable loss of cell walls in *Bacillus subtilis*

When a suspension of wild-type *Bacillus subtilis* is exposed to the cell-wall-dissolving enzyme lysozyme, each bacterial rod releases one to three protoplasts—naked spherical cells completely devoid of cell wall. Protoplasts in suspension can grow larger, but they cannot divide or replace the missing wall. The situation changes in a surprising way when the protoplasts are transferred to soft agar media. In such media, each protoplast can give rise to a slow-growing L colony consisting of bodies of heterogeneous size, called L bodies, that are lacking cell walls. The soft agar seems to allow the burgeoning naked cells to divide into viable fragments. The fragments, in turn, are capable of indefinite further propagation: upon transfer to soft agar they give rise to new L colonies. By contrast, if L bodies or protoplasts are plated on hard agar or gelatin media, prompt reversion to the walled, rod-shaped state occurs and normal bacterial colonies are produced (see figure below; Landman 1968, Landman and Halle 1963, Landman et al. 1977).



Electromicrographs of the bacillary form (**a**) and the L form (**b**) of *Bacillus subtilis*. Both forms propagate indefinitely on the same soft agar media. Scale bar = 1 µm. Electromicrographs by A. Ryter.

The sharp difference in heritable persistence of L bodies and protoplasts on soft agar on the one hand and hard agar or gelatin on the other is due to a changed equilibrium between ongoing cell wall synthesis and wall dissolution by enzymes (called autolysins). These enzymes loosen the rigid wall of growing cells to allow for cell expansion and for the separation of bacterial chains into shorter rods. Protoplasts are continually producing nascent cell wall and excreting cell-wall-destroying autolysins. In liquid medium or soft agar, wall synthesis is overwhelmed by wall destruction. By contrast, in gelatin or hard agar autolysin activity is inhibited, new cell wall accumulates, and the naked cells promptly revert to the walled state (Landman et al. 1977).

Heritable maintenance of the induced state in *Escherichia coli*

A culture of *Escherischia coli* grown in synthetic medium with a maintenance concentration of thiomethyl-ß-D-galactoside (5×10^{-6} M TMG) does not show any ß-galactosidase activity. But when an inducing concentration of TMG (5×10^{-4} M) is added, ß-galactosidase activity increases until it reaches a maximum. If the cells are then transferred back to medium with the lower concentration of TMG, the ß-galactosidase activity remains high indefinitely (e.g., for 180 cell generations). In contrast, a culture of *E. coli* that is not exposed to the high concentration of TMG shows no induction of ß-galactosidase activity.

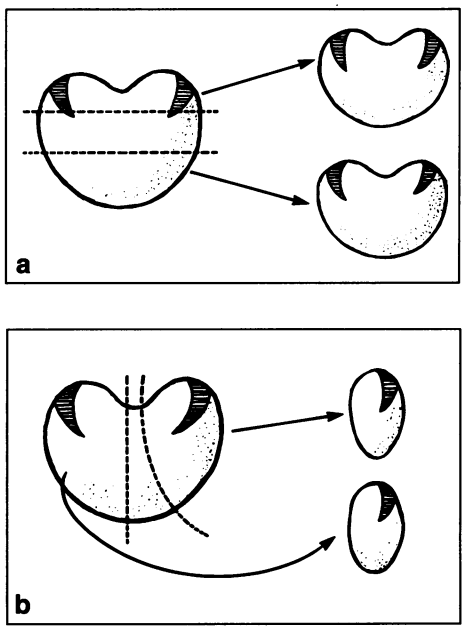
These results may be explained by the observation that during incubation in the high-TMG medium, high levels of ß-galactoside permease as well as ß-galactosidase are induced. During subsequent growth in the low-TMG medium, the cell-envelope-associated permease concentrates the dilute extracellular TMG to a much higher intracellular level (e.g., 100-fold higher), thus maintaining the induction of both enzymes. An uninduced culture lacks permease, so it does not concentrate the dilute extracellular inducer and hence remains uninduced (Novick and Weiner 1957).

Cortical inheritance in ciliates

Cortical inheritance is a special mode of inheritance manifested by structures of the cell cortex of ciliates such as *Paramecium*, *Tetrahymena*, *Stentor*, *Oxytricha*, *Stylonichia*, and *Pleurotricha* (Aufderheide et al. 1980, Sonneborn 1963, 1970). Surgical or accident-caused alterations in cortical morphological features have been observed to propagate clonally. The changes are inherited stably through cell doublings for hundreds of generations, through repeated self-matings, and through matings with morphologically normal partners. Mixed

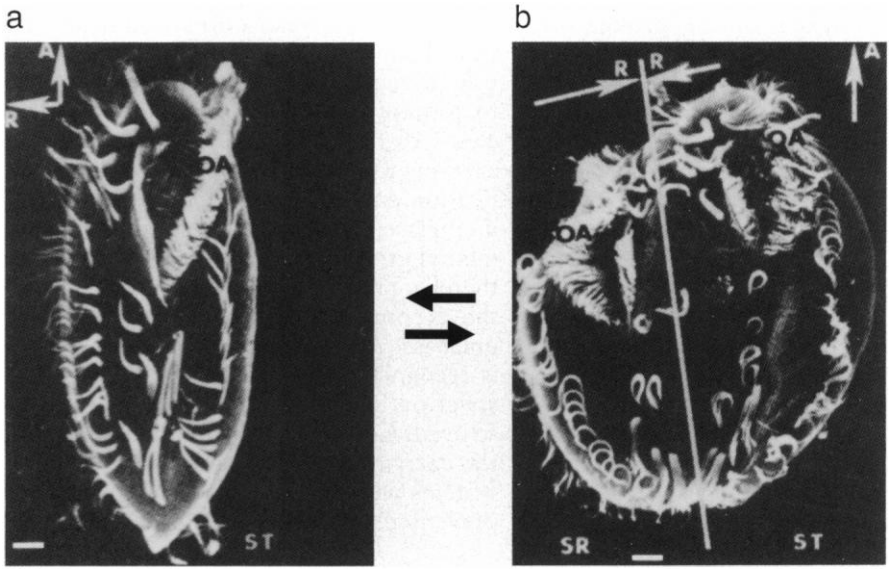
matings can be arranged so that the two former mating partners emerge with identical cytoplasms as well as identical nuclear genetic complements, yet they retain their distinctive cortical differences. The former mating partners then transmit these distinctive features to their progeny indefinitely.

Double monsters, or doublets, have been among the most informative materials in cortical inheritance studies. Doublets are formed when mating pairs of ciliates fail to separate and, instead, fuse. The doublet morphology is inherited clonally through sexual and asexual reproduction as a cortically determined trait. Doublets and singlets of *Oxytricha fallax* both form cysts devoid of ciliature and other cortical features. Therefore, cysts derived from doublets and singlets are indistinguishable, yet doublet-derived cysts always excyst as doublets. This observation permits the important inference that preexisting morphological structures do not play a major role in the heritable continuity of the acquired information. Nevertheless, the space where the morphogenetic information resides can be localized: a single cut separating an excysting doublet into halves gives rise to two clones of singlets if the cut is longitudinal and to two clones of doublets if the cut is transverse (Grimes 1973). There seem to be two oriented regions determining overall pattern in the doublet; when these regions are separated, each gives rise to a singlet animal clone, but when fragments of both are retained in a single piece a doublet regenerates from it (see figure top right).



Cortical inheritance. **a.** Transverse cut of encysting doublet of *Oxytricha fallax* between dotted lines produces two doublets. **b.** Longitudinal cut of encysting doublet between dotted lines produces two singlets. (Diagram modified from Grimes 1973.)

By a combination of heat shock and surgery, clones of doublets were obtained in *Pleurotricha lanceolata* that exhibited mirror-image symmetry: the fused partners both had the same anterior-posterior orientation, but the mirror-image partner showed reversal of left-right orientation of the oral apparatus and the overall ciliature arrangement. Thus, in forming a doublet, the fused cells had acquired bilaterally symmetric morphology (see figure bottom right; Grimes et al. 1980). This apparently occurred without changes in gene sequences previously limited to expression of the asymmetric cell morphology of singlets. In encystment experiments with these doublets, the mirror-image pattern emerged unchanged in excystment and was subsequently propagated clonally. Messages emanating from the grafted-on cell fragments may modify an existing feedback system between the cortex and genes that control morphogenetic patterns to produce completely new structures, such as those having mirror-image morphology. Further, the encystment-excystment experiments show that morphologically distinct preformed structures do not play a direct role in this presumed feedback loop.



Scanning electron micrographs of a singlet (**a**) and a mirror-image doublet (**b**) of *Pleurotricha lanceolata*. A: anterior-posterior axis. R: right-left axis. OA: oral apparatus. ST: standard morphology. (R L) SR: half-doublet with reversed right-left axis (LR). The organisms shown in **a** and **b** reproduce clonally; their gene sequences are thought to be the same. A longitudinal cut of the doublet in **b** produces two singlets that both give rise to normal singlet clones. Bars represent 10 μm. (Photographs reproduced and modified from Grimes et al. 1980 with permission.)

in *Euglena* may be cited as an example of a nonadaptive induced change. Similarly, removal of bacterial cell walls through lysozyme, autolysin, or penicillin results in massive lysis in low-osmotic-strength media. Loss of the cell wall may thus be adaptive or catastrophic, depending on the environment in which it takes place.

Acquired characters thus resemble random mutations: some are adaptive and some are not. Like mutations, acquired characteristics are also subject to natural selection. Nevertheless, as a class, acquired changes differ from the class of ordinary DNA changes such as point mutations, deletions, duplications, or rearrangements: acquisition of such integrated foreign gene banks as plasmids, bacteriophage, retroviruses, or bacteria is much more likely to lead to a major evolutionary advance—a saltation (Goldschmidt 1940) or evolution by a sudden spurt (Eldredge 1989)—than is an ordinary random mutation in a single gene. (After transfer, gene banks are commonly repressed. Full or partial derepression of a gene bank might follow promptly or be delayed for many generations in the recipient genome).

Ancient examples of saltation are the acquisitions of the prokaryotic precursors of mitochondria and chloroplasts by host cells in the Precambrian era (Cavalier-Smith 1987); a contemporaneous example is the acquisition by a wide range of gram positive and gram negative bacteria of a plasmid (R26) that conveys multiple antibiotic resistances and versatile conjugational capabilities (Smith and Thomas 1989). These examples show that a clear-cut line can be drawn between foreign nucleic acid elements horizontally acquired from unrelated species or from the environment and the vertical heredity of traditional genetics—the presence of identical genes in ancestors and descendants—according to Johannsen's (1911) classical definition.

Acquired inheritance systems governed by extranucleic mechanisms also suggest possible examples of saltatory evolutionary events. One such example may be the previously described appearance of bilateral symmetry in doublets of the ciliate *Pleurotricha lanceolata* (see box page 698; Grimes et al. 1980). A partly epinucleic inheritance system, restriction/modification, probably played an important role in bacterial evolution by helping to promote mutual isolation of species. (All of the DNA of a bacterium carrying a gene for a particular modification enzyme—say a modification methylase—is uniquely marked with methyl groups by that enzyme and is thereby protected from cleavage by the accompanying restriction endonuclease. Any incoming foreign DNA is recognized as non-self by this restriction endonuclease and is destroyed. Genetic interaction of bacteria carrying restriction/modification systems with any foreign DNA is thus prevented.)

An epinucleic system based on inherited modification of DNA

Inherited modifications of DNA have been demonstrated in organisms ranging from bacteria to mammals and can sometimes be altered experimentally. The cytidine analog 5-azacytidine, a powerful inhibitor of DNA transmethylase, can replace cytosine in DNA synthesis (Jones and Taylor 1981). Tissue culture cells treated with 5-azacytidine have a lower level of 5-methyl cytosine in their DNA. This loss is accompanied by activation of specific genes (Holliday 1987).

A demonstration of heritably stable gene activation after transient treatment of cells with 5-azacyidine was presented by Konieczny and Emerson (1984) using the mouse embryonic cell line C3H 10T1/2. This cell line is permanent and nonneoplastic, and it has a fibroblastic morphology. C3H 10T1/2 cells were inoculated to a density of 50 cells per plate and treated with 3 μM 5-azacytidine for 20–24 hours. The agent was then washed off and clonal growth continued for 2–4 weeks. Cells in 1500 clones were then examined for altered morphology: 25% of the colonies contained typical myocytes (muscle cells), 7% contained adipocytes (fat storage cells), and 1% contained chondrocytes (matrix-producing cells). The remaining 67% of the clones did not contain differentiated cells. Control clonal and mass cultures of 10T1/2 cells not treated with 5-azacytine did not contain any cells of these mesodermal phenotypes.

Because none of the clones treated with 5-azacytidine consisted entirely of differentiated cells, the key demethylation events seemed to produce stem-cell lineages that were developmentally determined but morphologically undifferentiated. Depending on the particular demethylated control site, the stem cell lines then later gave rise to differentiated myocytes, adipocytes, or chondrocytes. As expected, progeny of myocyte-producing cells subsequently produced no adipocytes or chondrycytes, only myocytes.

A history of ideas concerning inheritance of acquired traits

With a clear-cut definition of the inheritance of acquired characteristics (Figure 1) and an understanding of the diverse molecular mechanisms that may give rise to such inheritance (see boxes page 698 and this page), it is evident that inheritance of acquired traits and Mendelian heredity can coexist comfortably in a common universe of molecular biology. This perception (Landman 1991, 1993) has emerged from a long history of myth, misconception, and controversy. In a brief and episodic sketch of this long history, I summarize a few highlights, largely omitting details, inconsistencies, and complications.

The idea that acquired traits may be passed on to one's descendants is found as early as the folk tales of antiquity. For example: Why do Athenians have lean buttocks? Theseus, their mythical ancestor, trespassed into the underworld, intent on robbery. As punishment, he became rooted to a rock on which he sat to rest. Eventually, Hercules cut him loose, but a piece of his gluteus maximus was left behind. Theseus passed on this defect to his progeny, the Athenians, leaving them all sadly diminished.

An early formulation of the concept of inheritance of acquired characteristics is found in the writings of a physician of the school of Hippocrates

(fifth century B.C.). The text describes the longstanding custom of the legendary macrocephalics to bind the heads of babies to produce longheadedness. After a long time, the early physician believed, the longheadedness became hereditary, just like baldness or blue eyes, and binding was no longer constantly required. However, when the macrocephalics abandoned the custom of skull deformation, the longheadedness trait faded, according to the Hippocratic physician. How were the acquired traits—any traits—thought to be inherited? All parts of the body, both the healthy parts and such acquired "sick" parts as longheadedness, contributed to the makeup of the semen, and the semen, in turn, determined the new individual (Lesky 1950).

This theory of heredity of the Hippocratic school was thoroughly demolished by Aristotle (fourth century B.C.). For example, Aristotle noted that traits such as skin color are not always expressed in the immediate progeny but can be submerged in the children's generation and reappear in grandchildren. He also pointed out that traits not present when the father begets a son, such as white hair, may appear in father and then son long afterward. Aristotle comes close to enunciating the twentieth-century insight that sperm carries an information blueprint that determines the new individual (rather than an assemblage of minisamples of organs, traits, and acquired characteristics, as envisioned by the Hippocratic school): "...why not admit straight away that the semen is such that out of it blood and flesh can be formed, instead of maintaining that the semen itself is both blood and flesh?" (Aristotle 347–335 B.C.).

Despite Aristotle's critique, belief in the inheritance of acquired characteristics persisted through two millennia and was apparently widely accepted by eighteenth and early nineteenth century biologists. Among the best-remembered is Jean-Baptiste de Lamarck, the French evolutionist. Lamarck believed that complex organisms evolved progressively from simple ones, over long time periods, by continual adaptations to changing environments. An animal's use or disuse of an organ affected that organ's development in the animal's offspring (thus, according to Lamarck, successive generations of giraffes developed and then transmitted longer and longer necks that enabled them to reach leaves at ever higher branches).

Lamarck explained organisms' ability to adapt by their need (*besoin*) to respond to the challenges of their environment. Lamarck developed the idea that acquired characteristics are generally adaptive and that adaptive acquired changes are a principal factor in the advance of evolution. He did not explain, however, how the physiological adaptations became heritably established (Burkhardt 1977, Jordanova 1984).

Charles Darwin's explanation of how adaption occurs in evolution differs sharply from Lamarck's, and it is well summarized by the phrase "survival of the fittest." However, Darwin, like Lamarck, believed in the inheritance of acquired characteristics, and he thought that acquired characteristics were partly responsible for the observed variability of species. According to pangenesis, the tentative theory of heredity espoused by Darwin, minute, invisible copies of each body component and organ (gemmules) exist in all cells. The gemmules can change in type and number in response to environmental conditions in the tissues. The gemmules, including those with acquired changes, are transported by the bloodstream to the sex organs and there assemble into the gametes. After fertilization, during development, the maternal and paternal gemmules fan out to constitute their respective tissues and organs (Carlson 1966, Darwin 1868, Strickberger 1985).

During most of the nineteenth century, the idea of acquired inheritance continued to be widely accepted. Mendel's experiments in the 1860s did not affect this acceptance because they remained unknown until their rediscovery in 1900. In the 1880s, however, serious questions concerning the inheritance of acquired characteristics began to be raised by August Weismann (1889).

Weismann made clear the distinction between somatoplasm and germ plasm. He recognized that the continuity of heredity is maintained in the germ line and by the gametes in isolation from the soma. It seemed impossible that modifications of characteristics acquired in the tissues could influence the hereditary message carried forward in the germ line. (The argument is much less compelling in plants, where asexual, vegetative propagation is a common occurrence).

To demonstrate the fallacy of the idea of inheritance of acquired characteristics, Weismann performed an influential experiment: he cut off the tails of male and female mice in five successive generations, thereby creating and reinforcing the acquired trait of taillessness. But even after five generations, all of 901 offspring sported complete and normal tails (Weismann 1889).

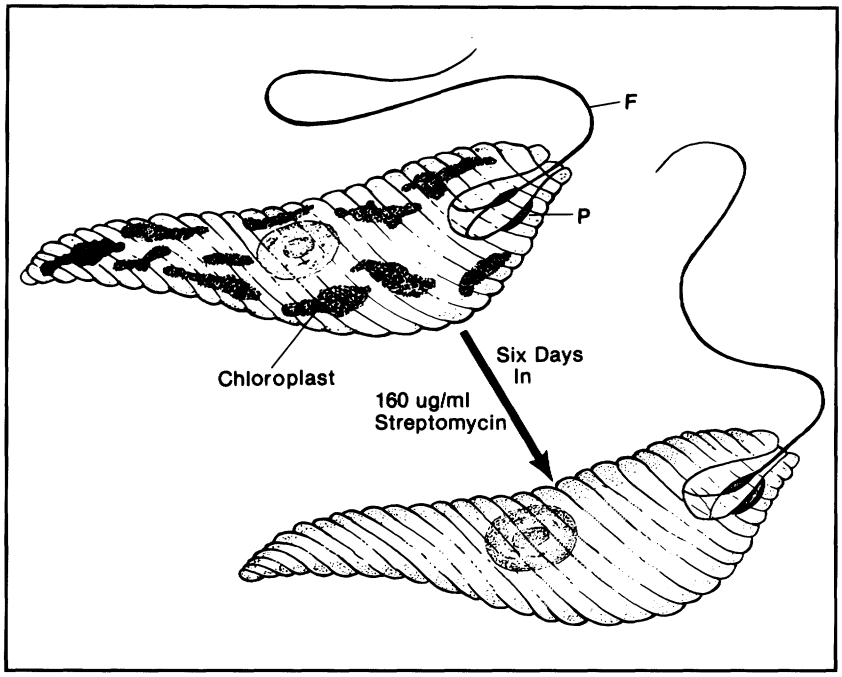
After Weismann, understanding of the mechanisms of inheritance progressed steadily. Important milestones were the rediscovery of Mendel's experiments (1900), the development of the concept of mutation by Hugo De Vries and others (ca. 1902), the connection between the hereditary units of Mendel and cytologically observed chromosomes (by W. Sutton and others ca. 1902), and the adoption of *Drosophila* as a chief vehicle of genetic experimentation by T. H. Morgan (1910). The concept of inheritance of acquired characteristics could not be accommodated in *Drosophila* genetics, the dominant line of genetic research before 1940. To quote Morgan (1932), "It is somewhat depressing to give so much time to destructive criticism of a doctrine that makes wide popular appeal. It sometimes seems as if everybody wanted to believe in the inheritance of acquired characteristics....It is part of the role of science to destroy pernicious superstitions."

In the distinct, rapidly developing field of microbial genetics (much of it single-cell genetics), the issue of acquired characteristics had not been resolved: clearly, in microbial systems, any acquired changes in heredity are not relegated to a germ line but can be transmitted by the same cells that were exposed to the environmental stress. A culture of bacteria exposed to attack by bacteriophage rapidly becomes phage resistant. Did this happen because the phage induced a mass heritable change or because rare resistant mutants multiplied to overgrow the culture? This question was posed by Luria and Delbrück (1943), and it was answered in an apparently

Systems based on induced loss or acquisition of nucleic acid elements

Curing *Euglena gracilis* of its chloroplasts

The antibiotic streptomycin can produce cells lacking chloroplasts. Consider a suspension of *Euglena gracilis* that, when test-plated on solid media, gave rise to 100% green photosynthesizing colonies. Each green cell contained approximately ten chloroplasts. After being incubated for six days in the presence of streptomycin (160 µg/ml), the suspension gave rise to 100% white colonies, whose cells lacked chloroplasts (see figure to right). The streptomycin did not affect the viability of *Euglena*. Although these colorless cells were serially transferred for at least ten generations in streptomycin-free media in the presence of light, no green cells appeared among the progeny (Provasoli et al. 1951).



Euglena cured of its chloroplasts. F: flagellum. P: photoreceptor.

Heat-induced elimination of heritably transmitted infection by virus sigma in *Drosophila*

Approximately one third of the fruit-fly strains of France are sensitive to carbon dioxide: they are killed by 30 seconds of contact, whereas normal flies survive several hours of carbon-dioxide exposure. The trait of carbon-dioxide sensitivity is due to infection by a rhabdovirus called sigma (a single-stranded RNA of approximately 4×10^6 dalton molecular weight). Sigma infection is not contagious, but resistant strains can be rendered sensitive by the injection of virus preparations into adult flies. Crosses between sensitive females and resistant males produce so-called stabilized carbon-dioxide-sensitive strains.

The sensitivity trait shows a predominantly maternal inheritance pattern. Females of stabilized strains transmit sensitivity to virtually all their progeny. It is estimated that oogonia of stabilized females each contain 10–40 virus particles and that each mature oocyte contains approximately 10^6 particles. Males of stabilized strains transmit sensitivity only sporadically, presumably because sperm contain less virus than eggs. Sigma is therefore carried through the germ line of *Drosophila* by abundant infection of the oocyte cytoplasm and not by association with the chromosomes.

Transmission of carbon-dioxide sensitivity in stabilized strains can be cut off completely by keeping egg-laying females for approximately six days at 30°C or warmer. All the progeny of such females are carbon-dioxide resistant. Similarly, when spermatogenesis takes place in males held at approximately 30°C, their spermatozoa no longer transmit carbon-dioxide sensitivity (Brun and Plus 1980, L'Héritier 1951, Preer and Preer 1977).

Acquisition of nucleic acid elements

If an *Escherichia coli* bacterium carrying a plasmid called fertility factor F is added to an *E. coli* suspension lacking the factor, F spreads rapidly through the entire culture by cell-to-cell conjugation. Factor F is subsequently inherited by all the bacterial progeny (Lederberg et al. 1952).

Similarly, most cells in an *E. coli* culture infected with the virus lambda survive the infection. These surviving bacteria have integrated the infecting virus into their chromosomes. They henceforth carry it and pass it along to all of their offspring (Lwoff 1953).

Acquisition of foreign nucleic acid elements by eukaryotes is a well-documented phenomenon. Examples are the acquisition and subsequent chromosomal integration of retroviruses in mammals (Jenkins et al. 1981) and the transfer of DNA from an *Agrobacterium tumefaciens* plasmid to the chromosomes of various plant species, in the context of crown gall formation (Zambryski et al. 1989).

unambiguous way: the culture was overgrown by phage-resistant mutants that were present before any phage were added.

The rise to power of the agronomist T. D. Lysenko in the Soviet Union in the 1930s revived the discussion of the inheritance of acquired characteristics. Lysenko gained Stalin's backing by promising to improve crop yields much more quickly than could traditional plant geneticists. He claimed that by cold treatment (vernalization) he could transform winter wheat into spring wheat. Vernalization proved to be a costly failure. After 1936, when 7 million hectares were planted with vernalized seed, vernalization was quietly and abruptly abandoned (Rolls-Hansen 1985; also see note added in proof, page 705).

Lysenko also ingratiated himself with Stalin by arguing that the principles of dialectical materialism applied to genetics: that all matter was in a state of continuing change and, consequently, there could not be stable hereditary traits independent of the environment. He asserted that genes did not exist and that, in the Soviet Union, "nurture must take charge of nature."

The foremost Mendelian geneticist of the USSR, N. I. Vavilov, labeled Lysenko's "new genetics" an "outbreak of medieval obscurantism" (Ashby 1947). Vavilov and many other Soviet geneticists paid with their lives for opposing Lysenko. Lysenko's ascendancy was based entirely on political intrigue, denunciation and intimidation of scientific opponents as "class enemies," and denigration of "bourgeois capitalist" genetics and geneticists. In the 34 years he wielded power, he and his followers never offered believable experimental evidence to support their claims of environmentally induced heritable changes (Medvedev 1969, Zirke 1949).

The complete politicization of genetics in the USSR evoked a shocked but also political response in the West (Huxley 1949). This response is reflected in the following statement, issued by the Governing Board of the American Institute of Biological Sciences (Butler et al. 1949): "The conclusions of Lysenko and his group regarding the inheritance of adaptive responses in higher organisms have no support in scientific fact. Genetic researches definitely support the reality of the gene and the validity of Mendel's laws. They do not support the official Communist claim that mendelian heredity is an illusion."

Acquired inheritance rejected and resurrected

The Lysenko affair and, before that, the delineation of the germ line concept by Weismann, the experimental demonstration of gene mutation, the comprehensive studies of *Drosophila* genetics of the Morgan laboratory, and the Luria-Delbrück experiment all contributed to the near-universal rejection of the concept of inheritance of acquired characteristics by Western geneticists by 1950. Looking back on this premature demise of the idea of inheritance of acquired characteristics, one may note that the Mendelian geneticists were more judicious than the protagonists of acquired inheritance in the choice of their experimental materials. Had Weismann chosen to cut *Oxytricha* doublets in two rather than to amputate the tails of successive generations of mice, surgery in a single generation would have been sufficient to produce an inherited change. Had Lysenko heated *Euglena* or *Paramecia* or carbon-dioxide-sensitive *Drosophila*, he could have demonstrated the environmentally induced heritable changes that eluded him in all his vernalization experiments. Had Luria and Delbrück used phage lambda for their fluctuation test rather than the virulent phage T1, they would have found that a large proportion of the infected *E. coli* had acquired phage resistance as a result of prior exposure to lambda (actually immunity due to lysogenization). (Recently, experiments by Cairns et al. [1988] and by Hall [1991] have raised yet a different challenge to Luria and Delbrück's conclusions: these authors describe experiments suggesting that media containing particular substrates somehow induce a greatly increased number of mutations adaptive to these substrates. An adaptive-mutation-enhancing mechanism has not been pinpointed so far [Lenski and Mittler 1993].)

The exploration of many acquired inheritance systems, starting with the discovery of heat-induced curing of *Euglena* chloroplasts by Ternitz (1912), took place when the underlying mechanisms were not known: they were premature discoveries that were not understood and consequently ignored by most of the scientific establishment (Stent 1972). The great experimental and conceptual advances in molecular genetics that were needed to understand acquired inheritance systems were made after 1945. Thus, understanding of the mechanisms of gene transcription and transcription controls (developed during the 1960s) is crucial to understanding the mechanisms of extranucleic acquired inheritance systems. Similarly, the identification of mitochondria, chloroplasts, plasmids, and temperate viruses as self-replicating nucleic-acid-containing genetic elements (starting in the mid-1950s) was needed to understand the mechanisms of nucleic aquired inheritance systems. Exploration of DNA methylation and glucosylation—required to understand epinucleic inheritance—came only in the late 1960s.

The study of single-cell systems has shown that extranucleic and epinucleic traits can be induced and stably inherited in single cells. But so far as I know now, only changes in nucleic systems can be transmitted through the germ line.

It is evident that the phenomenon that I have called inheritance of acquired characteristics greatly resembles the concepts of environmentally induced inherited changes that were held by Hippocrates, Lamarck, and Lysenko. If anything, some of the persistent changes in traits induced by brief treatments are more sudden, pervasive, and permanent than those envisaged by the historical proponents of the idea of inheritance of acquired characteristics. The mechanisms that we now know to be responsible for acquired changes in inheritance were, of course, remote from the conceptual universes of these men. A further and decisive distinction lies in current recognition that acquired inheritance is not a universal mechanism of inheritance as implied by Hippocrates, Lamarck, and Lysenko but is instead only one of the many facets of molecular genetics.

Despite this more limited scope, reassessment of the subject of inheritance of acquired characteristics has provided fresh perspectives on impor-

tant topics in genetics and evolution. It underscores the realization that the relationship between heritable transmission of traits and DNA or RNA genes may be quite indirect. (For example, the heritable wall-lessness of *B. subtilis* L forms is due to the destruction of cell wall, a product of cell metabolism, by autolysin, a posttranslational gene product.) The acquisition of foreign nucleic acid elements—horizontal inheritance—is not only a major mechanism for acquisition of new traits but also an important source of organized, integrated genetic information for organisms ranging from bacteria to mammals. There is convincing evidence that acquisition of foreign gene banks has been responsible for great leaps in evolution.

Acquired inheritance changes and differentiation

The subjects of inheritance of acquired characteristics and differentiation are linked conceptually. The sudden appearance of new traits during tissue differentiation and the subsequent clonal transmission of these traits during organ growth is analogous to the induced mass appearance and subsequent inheritance of new traits, especially the extranucleic and epinucleic systems (see boxes pages 698 and 700).

The distinction between differentiation and the acquired inheritance systems is, of course, that the controls triggering developmental changes (for example, gene activation) are highly specific, gene controlled, exerted in the internal milieu of the organism, and terminated by its death. By contrast, the environmental events that induce changes in acquired inheritance systems are not controlled endogenously, and the changed characteristics are propagated to succeeding generations. Despite these distinctions, investigations of both inheritance of acquired characteristics and differentiation continue to shed light on the questions how heritable changes in biological systems may be triggered and how heritable stabilization may be achieved.

Future studies of inheritance of acquired characteristics

In this article, I have reviewed the heterogeneous array of mechanisms on which known acquired inheritance systems are based. It is likely that additional examples of acquired inheritance systems will be found in the future as new nucleic-acid-containing elements are discovered; as new modes of horizontal gene transfer are described (Rennie 1993); as different, potentially heritable structural modifications of DNA are studied (e.g., histone-DNA interaction, supercoiling, DNA condensation, and nucleosome spacing; Weintraub 1985), and as new exotic heritable systems are explored. (For example, the PrP^{sc} prion, an infectious protein and causative agent of fatal neurodegenerative diseases, converts a normal gene product of the host, PrP^{c} prion, to the infectious PrP^{sc} isoform by an unknown posttranslational process [Prusiner 1991]. A second example is that, after cell or protoplast fusion in *B. subtilis*, expression of one of the two component genomes is heritably but not irreversibly repressed, perhaps due to the condensation of one of the two constituent nucleoids [Guillen et al. 1982, Landman and Pepin 1982].)

The best-known acquired inheritance systems are drawn primarily from studies of the major genetic organisms—mouse, *Drosophila*, *E. coli*, yeast, and *Paramecium*. Many additional examples of such systems may be expected to be found in future studies of the rest of the biological world.

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Note added in proof. Long before Lysenko, it had been claimed that vernalization could induce early flowering. The validation or disproof of this claim was obscured by the uncontrolled use and abrupt abandonment of vernalization at Lysenko's instigation. Recent experiments (reported in August at the 17th International Congress of Genetics in Birmingham, United Kingdom, by W. J. Peacock and his colleagues of the CSIRO Division of Plant Industry in Canberra, Australia) have shown that vernalization can be successful and that it is an epinucleic process—that the effect of cold treatment (or 5-azacytidine treatment) of seeds or root fragments is transmitted by mitotic cell-line inheritance throughout the growth and differentiation of plants grown from treated seeds or root fragments. However, the effect is not passed on through the germ line. Vernalization is mediated by partial DNA demethylation, which is triggered by both the cold and the 5-azacytidine treatments.