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Inheritance of acquired traits in plants
Reinstatement of Lamarck

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Since Lamarck proposed the idea of inheritance of acquired traits 200 years ago, much has been said for and against it, but the theory was finally declined after the 1930s. Despite of the negative opinions of the majority of geneticists, botanists and plant breeders have long recognized that altered properties during the growth were occasionally transmitted to the offspring. This was also the case with artificially altered properties such as dwarfism, flowering timing and plant stature, which were induced by a non-mutagenic chemical, 5-azacytidine and its derivatives. As these drugs are powerful inhibitors of DNA methylation in vivo, a close correlation between methylation and phenotypic expression was suggested. Subsequent studies showed that rice plants acquired disease resistance upon demethylation of the corresponding resistant gene, and that both resistant trait and hypomethylated status were inherited by the progeny up to nine generations. Whether or not the methylation pattern changes under natural condition was then questioned, and recent studies have indicated that it indeed naturally changes in response to environmental stresses. Whether or not the altered methylation pattern during the vegetative growth is heritable was also questioned, and studies on toadflax and rice affirmed the question, showing stable maintenance of hypermethylation in the former and hypomethylation in the latter for 250 and 10 years, respectively. The observation strongly suggested that acquired traits can be heritable as far as the acquired methylation pattern is stably transmitted. This concept is consistent with the Lamarck's theory of the inheritance of acquired traits, which therefore should be carefully reevaluated to reestablish his impaired reputation.

In 1809, the French naturalist, Jean Baptiste de Lamarck (1744–1829) proposed two laws of evolution—the law of use/disuse and the law of inheritance of acquired traits. The theory was declined almost completely after the 1930s. In plants, however, phenomena showing apparent inheritance of acquired traits have long been observed. This article briefly summarizes the current view of the "Lamarckian inheritance" in higher plants. Many excellent review articles related to this topic have been published, and readers are strongly suggested to refer to them for further information on molecular aspects.[1-3]

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Inheritance of Traits Acquired during Vegetative Growth

An intriguing example was the experiments with flax performed in the early 1960s.[4] When wild-type flax (*Linum usitatissimum*) was grown under nutrient-rich condition with ammonia, phosphate and potassium, the mature plants exhibited a three-fold heavier weight in comparison with those grown under non-nutrient rich condition. This trait was stably transmitted to the progeny over six generations, irrespective of the culture condition employed thereafter. It was concluded that the induction of heritable change is dependent upon the environmental conditions and the genetic constitution of the plant. The idea of conditioning was further supported by experiments showing heritable differences in flowering time and stature of *Nicotiana rustica* grown under potassium-rich nutritional conditions.[5]

During the 1990s, several cases were reported, indicating that a single treatment with a non-mutagenic chemical agent, 5-azacytidine, induced heritable changes in phenotypes: dwarfism in rice,[6] different stature and ripening timing in triticale (stable hybrid between wheat and rye)[7] and early flowering in flax[8] (**Table 1**). Since then, many works have suggested the inheritance of acquired traits, induced either spontaneously or artificially, in multiple plant species, including *Arabidopsis thaliana*, *Oryza sativa* (rice), *Zea mays* (maize) and others.[9] However, many observed traits displayed unusual properties such as developmental abnormality, growth retardation, sterility and transgene inactivation, apparently not occurring naturally in plants. In this context, the findings were valuable for analytical purposes at the molecular level, but did not necessarily reflect the natural procedure of plant development and evolution. Nevertheless, it appears to have been established that some traits changed during vegetative growth can be transmitted to the progeny.[9]

DNA Methylation

The molecular basis for the inheritance of acquired traits is not fully understood, but it is believed that phenotypic changes are not due to the mutation, but due to the flexible response of plants to their growth environment. In other words, change in expression of genes, which are responsible for phenotype formation, is not induced by the nucleotide sequence alteration, but by some other factors that reversibly respond to external stimuli.

Table 1. Some early observations on transgeneration of acquired traits

Plant	Trait	Causative factor	Transmission	Year	Ref.
Linum usitatissimum (flax)	Blanching/weight	Nutrient	Over 6 generations	1962	4
Nicotiana rustica (tobacco)	Weight/flowering	Nutrient	At least 2 generations	1965	5
Antirrhinum majus (Snapdragon)	Variegation	Crossing	At least 2 generations	1987	25
Oryza sativa (rice)	Dwarfism	5-azacytidine	At least 3 generations	1990	6
Triticale	Height/ripeness	5-azacytidine	At least 2 generations	1990	7
Linum usitatissimum (flax)	Early flowering	5-azacytidine	At least 3 generations	1994	8

Recent data obtained from model plants such as Arabidopsis are not listed due to abnormal properties derived from mutants and transgenics. Such information will be available through refs. 2, 9, 23 and 24.

Table 2. Change of methylation patterns upon environmental stresses

Plant	Causative factor	Induced status	Year	Ref.
Bryonia dioica (white bryony)	Mechanical stress	Hypomethylation[a]	1993	12
Pisum sativum (pea)	Water deficit	Hypermethylation[a]	2002	16
Zea mays (maize)	Low temperature	Hypomethyaltion[b]	2002	13
Trifolium repsens (clover)	Heavy metal	Hypomrthylation[a]	2004	14
Nicotiana tabacum (tobacco)	Pathogen infection	Hypomethylation[b]	2004	17
Arabidopsis thaliana	Pathogen infection	Hypomethylation[a]	2006	18
Nicotiana tabacum (tobacco)	Abiotic stresses	Hypomethylation[b]	2007	15
Nicotiana tabacum (tobacco)	Pathogen infection	Hypermethylation[a]	2007	26

Observations with intact plants are listed. Methylation status was estimated at global DNA (a) or individual gene (b) levels.

Methylation of DNA has been proposed to be one of such factors. In higher plants, it almost exclusively occurs at cytosine residues, conferring 5-methylcytosine.[10] Intensive studies have so far revealed a reverse relationship between cytosine methylation and gene expression.[10] Since cytosine methylation is a post-replication event, its status is always reset after each cell cycle, and eventually in each generation.[10] Such a generation-specific feature was thought to be favorable for reversible control of gene expression during the growth, and several experiments using a methylation inhibitor, 5-azacytidine, pointed to the change in cytosine methylation indeed to correlate with heritable phenotypic changes (**Table 1**). However, most observations were circumstantial, leaving a direct relationship between the phenotypic change and altered methylation of the responsible gene(s) to be determined.

The cause-effect correlation between the two was strongly suggested through a series of experiments using rice plants.[11] When seedlings were treated with 5-azadeoxycytidine, mature plants acquired resistance against the bacterial blight disease, to which most cultivated rice varieties are susceptible. Subsequent screening by the methylation-sensitive amplified polymorphism method identified an *Xa21*-like gene, that confers resistance to the host plant against blight disease bacterium, *Xanthomonas oryzae*, under the gene-for-gene manner. The promoter region was heavily methylated and silent in the wild-type, whereas it was unmethylated and active in the drug-treated plants. Both resistance and hypomethylation were inherited by the progeny over nine generations. Thus it was concluded that acquired traits can be heritable as far as the acquired methylation pattern is stably transmitted.[11]

Methylation Change under Natural Condition

Two questions then arise to generalize the concept that DNA methylation is responsible for the acquired-trait inheritance in nature. First, does the methylation pattern change under naturally growing condition? Second, if so, how is the changed methylation pattern transmitted to the progeny?

The methylation pattern of genomic DNA has occasionally been reported to dynamically change in several plant species upon biotic and abiotic stresses.[3] For example, global hypomethylation was induced by a simple mechanical touching in white bryony,[12] by low temperature in maize[13] and by heavy metals in clover[14] and tobacco plants[15] (**Table 2**). In contrast, global hypermethylation was induced by drought in pea[16] (**Table 2**). Hypomethylation was also induced upon pathogen infection in tobacco[17] and Arabidopsis[18] (**Table 2**). In some cases, decrease of methylation was observed to take place within several hours after the onset of the stress.[13,15] Some hypomethylated genes involved in stress responses were found to be transcriptionally activated.[15,17] Hypomethylation or demethylation is catalyzed by DNA glycosylases through the base excision-repair pathway.[19] The timing when DNA is methylated/demethylated appears to be strictly regulated during development and environmental responses, suggesting a specific machinery and its regulators to be involved.[19] Whatever the mechanism is, these observations suggest that environmental stresses induce alteration of methylation status and eventually regulation of relevant gene expression.

Data showing the inheritance of methylation patterns by the progeny under natural condition have curiously been limited. An outstanding example is the study on *Linaria vulgaris* (toadflax).[20] The wild-type plant forms asymmetric bilateral flowers, whereas a mutant, originally observed 250 years ago by Linnaeus, forms symmetric radial flowers. The responsible gene for flower development (*Lcyc*) was found to be heavily methylated and transcriptionally silent in the mutant. Occasionally observed revertants showed normal flower structure, and demethylation and expression of *Lcyc*. Hence hypermethylation at the *Lcyc* locus has been maintained for at least 250 years. Another example is the study on rice.[11] Azadeoxycytidine-treated rice acquired disease resistance trait, and the responsible gene, *Xa21G*, was totally demethylated at the promoter region. Direct methylation sequencing showed almost all cytosines in the region to have been demethylated in the offspring. This indicates that hypermethylation in the wild-type and hypomethylation in the mutant were faithfully maintained for at least 10 years.

How is methylation patterns acquired or lost during vegetative growth inherited by the progeny? In mammals, the pattern of DNA methylation is totally erased during gametogenesis, and reprogrammed in the next generation.[21] In plants, methylation patterns are not completely reset.[22] Since plant reproduction is conducted through different systems from mammals including double fertilization,[2] methylation patterns of the parent can be transmitted to the offspring. Although its precise molecular mechanism must be determined, recent studies have revealed that methylation/demethylation is finely regulated through highly coordinated functions between methyltransferases and demethylases.[19] This facilitates plants to cope with diverse environmental stresses, by memorizing the best counteraction and flexibly regulating gene expression in both individual and following generations.[23]

Concluding Remarks

The year 2009 marks the 200 years anniversary since the publication of *Philosophie Zoologique* (1809) by the French naturalist, Jean Baptiste de Lamarck (1744–1829). In his book, Lamarck proposed the law of use and disuse of organs, and the law of inheritance of acquired traits. Although the theory was discredited by most geneticists after the 1930s, botanists have long been aware of phenomena implying inheritance of acquired traits: branching and body weight by nutrient condition, spontaneous variegation in ornamental plants, new traits after grafting and others. Since appropriate explanation on molecular basis was not available, these observations have not drawn much attention until the 1990s.

Apart from botanists' classical findings, the theory of epigenetics has become the highlight of developmental biology and genetics since the 1980s.[1] Epigenetics is defined as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence".[24] Among specific mechanisms proposed, DNA methylation was suggested to best meet the above condition, reversibly controlling gene expression and being heritable in its patterns.[1] Supporting evidence has considerably accumulated today, providing detailed knowledge on its molecular mechanisms.[1,2,21,24]

In his later years, Lamarck was much criticized by the scientists of his time. It is said that his daughter, Rosalie, comforted him by assuring that some future generation will reconsider his theory and prove it to be correct. Now the time has come for the Lamarckian inheritance to be reevaluated in the domain of epigenetics.

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