Cent. Eur. For. J. 63 (2017) 173–179 DOI: 10.1515/forj-2017-0024

CENTRAL EUROPEAN Forestry Journal
http://www.nlcsk.sk/fj/

REVIEW PAPER

Epigenetic memory effects in forest trees: a victory of "Michurinian biology"?

Dušan Gömöry[1]*, Matúš Hrivnák[1], Diana Krajmerová[1], Roman Longauer[2, 3]

[1]*Technical University in Zvolen, Faculty of Forestry, T. G. Masaryka 24, SK – 960 53 Zvolen, Slovak Republic*
[2]*National Forestry Centre, Forest Research Institute Zvolen, T. G. Masaryka 2175/22, SK – 960 92 Zvolen, Slovak Republic*
[3]*Mendel University in Brno, Faculty of Forestry and Wood Technology, Zemědělská 2, CZ – 613 00 Brno, Czech Republic*

Abstract
The study reviews trait inheritance, which is in contradiction with the rules of Mendelian genetics, and which was object of controversies among biologists (sometimes with grave political consequences) in the USSR and Soviet-controlled countries in the 1930s–1960s. "Carryover" or "memory" effects of the climate, to which maternal trees are exposed during seed development, on phenological behavior and other adaptively relevant traits of their offspring in conifers are mentioned; similar effects are associated with the germination and early growth environment. Molecular mechanisms underlying these effects include covalent modifications of DNA or DNA-associated proteins (cytosine methylation, various types of histone modifications), micro-RNAs and small interfering RNAs. Tools for the identification of these modifications are reviewed with a focus on cytosine methylation, along with an overview of the hitherto knowledge on the occurrence of DNA modifications in forest trees. The practical implications of epigenetic inheritance in forest trees are discussed with the focus on the adaptation to climate change and legislation on forest reproductive materials.

Key words: epigenetics; carryover effects; cytosine methylation; MSAP; climate change; Lysenko

Editor: Martin Lukac

1. A bit of history

Although naturalists often suffer from the illusion that natural science is independent from external ideological and political influences, more than often this is just an illusion. The almost fifty-year period of so-called Michurinian biology in the Soviet Union and its eastern-European satellites (including Czechoslovakia) is a bitter reminder of detrimental, even fatal effects of political influence in science. This "science", abusing the name of famous Russian and Soviet plant breeder I.V. Michurin, is associated with the name of T.D. Lysenko, a Stalin favourite and long-time director (1938–1962 with a short interruption) of VASKhNIL, the Lenin All-Union Academy of Agricultural Sciences. It was not only the abuse of political power for intimidation, professional and sometimes even physical disposal of scientific opponents, but also absurdity of theories and weakness of experimental fundaments on which their dogmas were built, which characterized Lysenko's era. Even though agricultural plants and animals were the primary focus of Soviet "agrobiologists", the shadow of this pseudoscience did also encompass forestry, including Czechoslovak forestry in the 1950s and early 1960s. In many cases, the theories of Michurinian biology can only be described as charlatan, such as those on the creation of living cells from mixtures of high-molecular organic substances, transformation of viruses into bacteria and back, or saltational changes of species identity under the influence of the environment (Soyfer 2011). Based on practical experience in agriculture and horticulture, Lysenko and his followers hypothesized "soft heredity" mediated by a plethora of organic substances in a cell (not nucleic acids), resulting in strong effects of environment on phenotype and transmission of such environment-induced phenotypic changes into the offspring generation (Flegr 2002).

Even though Lysenko's influence started to decline already in late 1950s, he retained his position even during the Khrushchev era; it required an intervention of prominent Soviet scientists (mostly non-biologists) such as Sakharov and Kapitsa to have his theories proclaimed as pseudoscience, and he lost his position only after Khrushchev's fall. Nevertheless, his effect on biology in the USSR was devastating and remained visible long after his dismissal.

Corresponding author. Dušan Gömöry, e-mail: dusan.gomory@tuzvo.sk

Now, the question is: was all the "Michurinian biology" a pure nonsense? And what does it have to do with forestry?

2. Uncommon trait inheritance in trees

In Scandinavia, conifer seed orchards or clonal collections used for seed production by mass controlled pollination were routinely moved to southern regions in order to obtain higher yields of seed material in seed orchards, as the amount of seeds produced by the trees decreases and the intervals between mast years become longer towards the North due to the harshness of climate. However, the offspring of translocated seed orchards exhibited lower frost resistance compared to the original northern populations (Johnsen 1989; Dormling & Johnsen 1992). A poorer performance of such offspring in terms of frost hardiness was initially attributed to pollen contamination from the local (southern) pollen sources, which is typically high in Scandinavia. However, later investigations showed that progenies originated from controlled crosses behaved in the same manner. It appeared that the seeds produced in southern seed orchards 'remember' in some way the climate at the site of production, despite containing genes inherited from parents originating from the North (hence the designation 'memory' or 'carryover' effects). Similar observations were made on North-American conifers (Greenwood and Hutchison 1996; Stoehr et al. 1998; Webber et al. 2005).

Another line of evidence for heredity not obeying the rules of genetics comes from provenance transfer. In Scandinavia again, quite much Norway spruce originating from Central Europe (Germany, Austria, the Carpathians) was planted, and some plantations already reproduce. Offsprings from such plantations resemble the local indigenous plantations rather than the material translocated from identical Central-European source populations in terms of timing of budburst, budset, growth cessation and other adaptive traits, which means that in spite of unchanged genetic structures, the populations of Central-European origin changed their phenological behavior within a single generation (Skrøppa et al. 2010).

In later controlled-pollination experiments performed in climatic chambers, greenhouses and also under field conditions, it was found that the temperature during seed development of Norway spruce affects important phenological characteristics. Cold environment advanced autumn bud set, cold acclimation, spring dehardening and flushing. On the other hand, the conditions during the pre-zygotic stage and fertilization did not affect phenology (Johnsen et al. 2005a). The effect of temperature also interacts with the daylength effects, plants subjected to high temperature and long days and those subjected to low temperature and short days expressed characteristics similar to cold-subjected individuals from the previously mentioned study: autumn bud set and spring flushing were earlier and they were more frost-hardy in the autumn (Johnsen et al. 2005b).

Environmental conditions during germination and early growth can affect phenology in a similar way. In a nursery experiment with Norway spruce and European larch, Gömöry et al. (2015) found that provenances sown in a warm nursery delayed budburst consistently compared to those grown in a cold nursery, wherever they were later transplanted.

In a certain way, such observations confirm Lysenko's ideas of soft inheritance of traits, meaning that traits induced by environment during the ontogeny of an individual are transmitted to its offsprings. As this type of heredity is not associated with a change of the genetic information *sensu stricto*, it is called epigenetic. This term is used to describe heritable (not necessarily sexually, but at least during the cell division, mitosis) changes to gene expression not caused by changes of the DNA sequence itself.

3. Molecular mechanisms of epigenetic inheritance

Carryover effects and other epigenetic phenomena have important implications for biology, medicine, agriculture or forestry. Nevertheless, they do not contradict to the paradigms of genetics, and can be explained by mechanisms involved in the molecular basis of heredity. Phenotype of a tree does not depend exclusively on genotype (in the meaning of the composition of alleles constituting a genotype). It results from physiological processes depending on the activity of genes. Phenotypic traits from the subcellular level to the level of organism depend on the timing and location of gene expression. The basis of epigenetic inheritance needs to be looked for in molecular mechanisms regulating transcription and translation of genes, which are on one hand inducible by environmental signals, and on the other hand reproducible and transmittable across generations.

Mechanisms underlying epigenetic effects include histone modifications. Histones are proteins that pack the chromosomal DNA in eukaryotic cells into nucleosomes, molecular coils serving for the organization of huge DNA molecules and allowing the cell to manipulate with them during cell division. Each nucleosome consists of eight histone cores, around which approximately 147 bp (basepairs) of DNA are wound. In the nucleosome, each histone can be potentially modified by a number of covalent modifications, including acetylation, methylation, phosphorylation and ubiquitination, whereas the modification status decides whether the chromatin around nucleosomes and the associated genes will be transcriptionally active or inactive (Turner 2000).

Small interfering RNA (siRNA) and micro RNA (miRNA) molecules also play an important role in epi-

genetics. siRNAs are double-stranded RNA molecules with a length of 20 – 25 basepairs, while miRNAs are single-stranded with a length of about 22 nucleotides. Both types regulate gene expression by interfering with the translation of the information contained in the DNA to proteins, usually by cleaving the messenger RNA (mRNA), which is the carrier of information on protein primary structure, or otherwise speeding the degradation of mRNA.

Finally, gene expression is affected by methylation of DNA cytosines. Other DNA base modifications may occur as well (Vanyushin 2005). Adenine methylation was thought not to occur in eukaryotes, but has recently been reported in mouse embryonic stem cells (Wu et al. 2016). Methylated cytosines mostly occur in the context where cytosine is located next to guanine (CG). This configuration allows for symmetric methylation on both strands of DNA, as cytosine is complementary to guanine. In plants methylated cytosines often occur in other contexts as well (CHG and CHH sites; H = A, C or T), having their own maintenance mechanisms and function (Chan et al. 2005). Sometimes only one strand of DNA is methylated, which is called hemimethylation. The methylated CG sites often occur concentrated on the so-called methylated CG islands (Neumann & Barlow 1996). The function of DNA methylation also differs with its different position within the genes. While methylation towards the beginning of a gene (promoter or first exon) inhibits production of proteins, regions more downstream can behave differently (Suzuki & Bird 2008; Brenet et al. 2011). In the downstream regions of gene loci, DNA methylation has been shown to induce alternative splicing, i.e. the removal of introns from the primary transcript, creating different proteins from the same DNA template (Maunakea et al. 2013). There are several mechanisms how DNA cytosine methylation inhibits gene expression. First, DNA methylation seems to be linked with histone methylation and the formation of heterochromatin, a transcriptionally inactive (not protein-producing) state of chromatin (Soppe et al. 2002; Hashimoto et al. 2010). In a more direct manner, methylation interferes with binding of transcription factors, proteins that facilitate transcription of DNA into mRNA or, alternatively, may attract repressors of transcription (Bird 2002).

Epigenetic mechanisms play a role in a plenty of processes such as cellular differentiation (Hsieh & Gage 2005), inactivation of specific genes, transposons (DNA sequences that can change their position or multiply across the genome; Miura et al. 2001) and viral DNA/RNA (Raja et al. 2008).

4. Technical means for the study of DNA methylation

The technically easiest procedure for the identification and quantification of cytosine methylation in the genome is the methylation-sensitive amplification polymorphism (MSAP) technique, which is a modification of the widely used amplified fragment length polymorphism (AFLP) method. AFLP is based on DNA cleaving with a pair of restriction endonucleases (enzymes searching DNA for a particular sequence motif and cleaving it where the motif is found), ligation of short oligonucleotides (adaptors) at the ends of the formed fragments and amplification of fragments by polymerase chain reaction (PCR). MSAP replaces frequent-cutter enzyme from AFLP with a pair of restriction enzymes with different sensitivity to cytosine methylation, HpaII and MspI both targeting identical recognition sequence (CCGG). Both of these enzymes can cut a non-methylated site, while only MspI is able to cut the DNA if the inner cytosine is methylated ($C^{m}CGG$), either on both sides or one side of the double-stranded DNA whereas HpaII is able to cleave sites where the outer cytosine on only one strand is methylated ($^{m}CCGG$). Difference in the presence or absence of a particular fragment in samples treated by either endonuclease allows thus assessing the methylation status of the CCGG sequence on its end.

MSAP can be used on any species without prior knowledge of its genome, it covers the whole genome as the fragment positions are randomly distributed across the genome, and is relatively cost-effective. On the other hand, it screens anonymous loci, which cannot be associated with known genes or identify new genes, except when sequencing of fragments is performed *post hoc*. Moreover, some MSAP patterns are either questionable or cannot distinguish methylation change from a genetic mutation (Fulneček & Kovařík 2014). Also, MSAP is quite sensitive to technical imperfections and it is sometimes difficult to standardize the results.

There are several alternatives to detect DNA methylation. Bisulfite sequencing uses a series of chemical reactions to convert unmethylated cytosines to uracil, which is then replaced by thymine during the PCR reactions. Samples treated and untreated with bisulfite ions are then sequenced and compared to obtain the exact methylation information. This method requires DNA sequencing making it more complicated and expensive than MSAP; in spite of this, it has currently become a standard for epigenetic studies, especially in model organisms. Alternatively, the standard restriction enzymes used in MSAP can be replaced by MseI + Acc65I/KpnI (recognition sequence GGTACC) that has easier interpretation in regards to methylation than the HpaII/MspI pair used in MSAP (Chwedorzewska & Bednarek 2011). The methylation-sensitive restriction enzyme polymerase chain reaction (MSRE-PCR) and the methylation-dependent restriction enzyme PCR (MDRE-PCR) use restriction enzymes sensitive to or dependent on methylation (just like those used in MSAP) to cleave the DNA and subsequently attempt to amplify particular loci (for example candidate genes) with PCR, failing if the DNA between the primers has been cut, which in turn depends

on the methylation of the restriction site. MSRE-PCR can use a variety of available restriction enzymes, including HpaII, Hin6I, NotI or HhaI (Melnikov et al. 2005; Oakes et al. 2006). MDRE-PCR uses enzyme McrBC that cleaves $R^mC(N_{40\text{-}3000})R^mC$ sites (R = G or A). The recognition site of McrBC is a bit more tricky, it requires two R^mC (i.e. G^mC or A^mC) half-sites separated by 40 to 3000 nucleotides (55 to 103 nucleotides are optimal). McrBC cuts the DNA near one of the half-sites, but it is not defined which one (Stewart et al. 2000), therefore it is important to have both half-sites located within the region that is to be amplified. MSRE-PCR and MDRE-PCR are also able to take advantage of the real-time PCR that further simplifies and speeds up the process of analysis (Oakes et al. 2006).

Direct sequencing of base modifications in single-molecule next-generation sequencers is expected to become common in the future. Currently only PacBio R instruments from Pacific Biosciences and nanopore instruments from Oxford Nanopore Technologies are useable in this way (Flusberg et al. 2010; Murray et al. 2012; Schreiber et al. 2013). However, such analysis will be hard to apply to trees, generally having large genomes.

5. Hitherto knowledge of epigenetic variation in trees

Epigenetics was suggested to be one of the mechanisms underlying phenotypic plasticity, i.e., the capability of a single genotype to be expressed in multiple phenotypes depending from the environment (Bossdorf et al. 2008; Jablonka and Raz 2009; Kramer et al. 2017). In forest trees as long-lived organisms with complex life cycles exposed to environmental fluctuations over their long lifetimes, plasticity is of utter importance for their survival and adaptation to rapidly changing climate conditions (Rehfeldt et al. 1999; Rohde and Junttila 2008).

The relation between epigenetics and phenotype in trees is an under-explored area, although first studies in this field exist. Bräutigam et al. (2013) provided an excellent review of various aspects of epigenetic in forest trees. Here we mention only those relevant in terms of responses of trees to environmental signals.

In the case of carryover effects associated with the climate and photoperiod during embryogenesis, a number of possible epigenetic mechanisms, including DNA methylation, were theorized to be responsible. Johnsen et al. (2005a) mentioned unpublished data suggesting that plants from warmer environment had higher levels of the overall DNA cytosine methylation. Yakovlev et al. (2010) found and sequenced 16 micro-RNAs that showed different transcription levels between cold-environment and warm-environment Norway spruce individuals. They also confirmed that these miRNAs indeed affect transcription levels of their predicted target genes.

In angiosperms, most evidence for epigenetic effects on phenotypic traits is available in poplars and eucalypts. In poplar cuttings of the same genotype obtained from different geographic locations, subsequently grown under common environmental conditions, and exposed to drought stress, Raj et al. (2011) observed differences in genome-wide DNA methylation levels and transcriptome composition related to climate conditions, in which parental trees were growing. Genotypic variation for both DNA methylation and yield-related traits and a relationship between them was observed in Euramerican black poplar hybrids (Gourcilleau et al. 2010). DNA methylation may be a mechanism of gene expression regulation in poplar in response to drought stress (Hamanishi & Campbell 2011). A functional link between an epigenetic mark and variation in cellulose content was discovered in eucalypts (Thumma et al. 2009). As for the other genera, Gugger et al. (2016) found an association between specific methylated sites and climatic variables in *Quercus lobata*.

There is also abundant evidence for the participation of DNA methylation or covalent modifications of histones in developmental processes and ontogeny (Fraga et al. 2002; Santamaria et al. 2009; Valledor et al. 2007); however, in this case the durability of epigenetic marking and its transferability across generations is questionable.

6. Implications for forestry practice?

The example of the translocation of conifer seed orchards in Norway clearly demonstrates that the current forestry practices and legislation largely ignore the issue of epigenetics. The current paradigm from which all practical measures are derived is that of classical quantitative genetics: tree phenotype results from the interaction between genotypic and environmental effects, where genotype and environment are independent. Genes remain the same wherever a tree naturally grows or is planted, and conversely, climate, soil, surrounding biota etc. are not affected by tree's genes. Consequently, the location of seed sources or nurseries does not matter, wherever basic materials are situated or wherever forest reproductive materials (FRM) are produced, their genetic structures remain unaffected. The problem of this view is that even though it has been traditionally and successfully applied in breeding, it is quite mechanistic. Indeed, environment cannot change nucleotide sequence in a gene (except for environment-induced mutations, but these are commonly rare in a typical forest environment, and are random, thus may be detrimental, neutral or beneficial). Therefore, neither the European legislation (Directive 1999/105/EC on the marketing of forest reproductive material) nor national legislations set any restrictions on the location of basic materials or growing plants. In fact, the EC directive prohibits setting such restrictions. Any forest company is allowed to estab-

lish a seed orchard or clonal collection at any place of their choice, any nursery is allowed to grow reproductive materials of any origin, and no state is authorized to set legal restrictions on marketing of such materials, provided it comes from approved sources. The underlying logic is that it is only the genes contained in the reproductive materials, which decide about its future behavior. The thing is, the way from the genetic information contained in a gene to the gene-controlled phenotypic trait is long and rarely simple. To become effective, the gene needs to be translated into a polypeptide, which needs then to fold and sometimes to be chemically modified to become a functional protein. For phenotypic expression, not only the quality of a gene product is important (although even here must be reminded that due to alternative splicing and posttranslational modifications a single gene may result in multiple functionally differing proteins). The when and where in the plant's body the protein is produced, and how much of it is produced, is also important. Environment can exert essential effects on timing, location and intensity of gene expression, and these effects can be persistent, even heritable. This is the basis of the epigenetic memory described above, and implies risks, which are not at all considered in the legislation on FRM. On a positive note, epigenetic phenomena may allow for a rapid adjustment of forest tree populations to environmental changes. We deliberately avoid using the term 'adaptation' in this context, as this term is commonly used for changes of allelic structures through natural selection in response to environmental pressures. The capacity of a population to adjust phenology (and potentially other adaptive traits) to local climate and photoperiod as described by Skrøppa et al. (2010) is relevant in the context of climate change: even when the adult trees will be damaged by climatic stress, the offspring generation may already be able to cope with the new climate. Close-to-nature forestry, leaving the broadest space for natural processes, may thus be a viable complementary strategy to assisted migration in mitigation of climate-change effects.

So, returning to the question in the title: does the discovery of epigenetics mean a victory for what was called "Michurinian biology"? Not at all. Even though the mainstream science is known to get the things wrong sometimes, the attitude of Lysenko and his followers was equivalent to that of the present-day climate-change skeptics: highlighting weak points in established theory is fully legitimate but ignoring scientific evidence and even denying everyday experience is not. Mendelian genetics and molecular biology proved their validity. On the other hand, Michurinian biology pointed out to several interesting phenomena (inheritance of environmentally induced traits is just one of them, cf. Flegr 2002), which are fully compatible with genetics and molecular biology but remained largely ignored by biologists and have been (still are) considered irrelevant in agricultural and forestry sciences. The problems resulting from seed orchard translocation clearly demonstrate that dogmatic approaches to biological problems are detrimental whatever authority they rely on.

Acknowledgements

The review was supported by a grant of the Slovak Research and Development Agency no. APVV-0135-12.

References

Bird, A., 2002: DNA methylation patterns and epigenetic memory. Genes & Development, 16:6–21.

Bossdorf, O., Richards, C. L., Pigliucci, M., 2008: Epigenetics for ecologists. Ecology Letters, 11:106–115.

Bräutigam, K., Vining, K. J., Lafon-Placette, C., Fossdal, C.G., Mirouze, M., Marcos, J. G. et al., 2013: Epigenetic regulation of adaptive responses of forest tree species to the environment. Ecology and Evolution, 3:399–415.

Brenet, F., Moh, M., Funk, P., Feierstein, E., Viale, A. J., Socci, N.D. et al., 2011: DNA methylation of the first exon is tightly linked to transcriptional silencing. PLoS ONE: e14524

Chan, S. W., Henderson, I. R., Jacobsen, S. E., 2005: Gardening the genome: DNA methylation in *Arabidopsis thaliana*. Nature Reviews Genetics, 6:351–360.

Chwedorzewska, K. J., Bednarek, P. T., 2011: Genetic and epigenetic studies on populations of *Deschampsia antarctica* Desv. from contrasting environments on King George Island. Polish Polar Research, 32:15–26.

Dormling, I., Johnsen, Ø., 1992: Effects of the parental environment on full-sib families of *Pinus sylvestris*. Canadian Journal of Forest Research, 22:88–100.

Flegr, J., 2002: Was Lysenko (partly) right? Michurinist biology in the view of modern plant physiology and genetics. Rivista di Biologia, 95:258–72.

Flusberg, B. A., Webster, D., Lee, J., Travers, K., Olivares, E., Clark, T. A. et al., 2010: Direct detection of DNA methylation during single-molecule real-time sequencing. Nature Methods, 7:461–465.

Fraga, M. F., Rodriguez, R., Canal, M. J., 2002: Genomic DNA methylation-demethylation during aging and reinvigoration of *Pinus radiata*. Tree Physiology, 22:813–816.

Fulneček, J., Kovařík, A., 2014: How to interpret Methylation Sensitive Amplified Polymorphism (MSAP) profiles? BMC Genetics, 15:2.

Gömöry, D., Foffová, E., Longauer, R., Krajmerová, D., 2015: Memory effects associated with the early-growth environment in Norway spruce and European larch. European Journal of Forest Research, 134:89–97.

Gourcilleau, D., Bogeat-Triboulot, M. B., Le Thiec, D., Lafon-Placette, C., Delaunay, A., El-Soud, W.A. et al., 2010: DNA methylation and histone acetylation: genotypic variations in hybrid poplars, impact of water deficit and relationships with productivity. Annals of Forest Sciences, 67:208.

Greenwood, M. S., Hutchison, K. W., 1996: Genetic after effects of increased temperature in *Larix*. In: Hom, J., Birdsey, R., O'Brian, K. (eds.): Proceedings of the 1995 Meeting of the Northern Global Change Program, USDA Forest Service report, Radnor, p. 56–62.

Gugger, P. F., Fitz-Gibbon, S., Pellegrini, M., Sork, V. L., 2016: Species-wide patterns of DNA methylation variation in *Quercus lobata* and their association with climate gradients. Molecular Ecology, 25:1665–1680.

Hamanishi, E. T., Campbell, M. M., 2011: Genome-wide responses to drought in forest trees. Forestry, 84:273–283.

Hashimoto, H., Vertino, P. M., Cheng, X., 2010: Molecular coupling of DNA methylation and histone methylation. Epigenomics, 2:657–669.

Hsieh, J., Gage, F. H., 2005: Chromatin remodelling in neural development and plasticity. Current Opinion in Cell Biology, 17:664–671.

Jablonka, E., Raz, G., 2009: Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. The Quarterly Review of Biology, 84:131–176.

Johnsen, Ø., 1989: Phenotypic changes in progenies of northern clones of *Picea abies* (L.) Karst. grown in a southern seed orchard. I. Frost hardiness in a phytotron experiment. Scandinavian Journal of Forest Research, 4:317–330.

Johnsen, Ø., Dæhlen, O. G., Østreng, G., Skrøppa, T., 2005b: Daylength and temperature during seed production interactively affect adaptive performance of *Picea abies* progenies. New Phytologist, 168:589–596.

Johnsen, Ø., Fossdal, C. G., Nagy, N., Mølmann, J., Dæhlen, O. G., Skrøppa, T., 2005a: Climatic adaptation in *Picea abies* progenies is affected by the temperature during zygotic embryogenesis and seed maturation. Plant, Cell and Environment, 28:1090–1102.

Kramer, K., Ducousso, A., Gömöry, D., Hansen, J.K., Ionita, L., Liesebach, M. et al., 2017: Chilling and forcing requirements for foliage bud burst of European beech (*Fagus sylvatica* L.) differ between provenances and are phenotypically plastic. Agricultural and Forest Meteorology, 234:172–181.

Maunakea, A. K., Chepelev, I., Cui, K., Zhao, K., 2013: Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. Cell Research, 23:1256–1269.

Melnikov, A. A., Gartenhaus, R. B., Levenson, A. S., Motchoulskaia, N. A., Levenson, V. V., 2005: MSRE-PCR for analysis of gene-specific DNA methylation. Nucleic Acid Research, 33: e93

Miura, A., Yonebayashi, S., Watanabe, K., Toyama, T., Shimada, H., Kakutani, T., 2001: Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*. Nature, 411:212–214.

Murray, I. A., Clark, T. A., Morgan, R. D., Boitano, M., Anton, B. P., Luong, K. et al., 2012. The methylomes of six bacteria. Nucleic Acids Research, 2012:1–13.

Neumann, B., Barlow, D. P., 1996: Multiple roles for DNA methylation in gametic imprinting. Current Opinion in Genetics & Development, 6:159–163.

Oakes, C. C., La Salle, S., Robaire, B., Trasler, J. M., 2006: Evaluation of a quantitative DNA methylation analysis technique using methylation-sensitive/dependent restriction enzymes and real-time PCR. Epigenetics, 3:146–152 .

Raj, S., Bräutigam, K., Hamanishi, E. T., Wilkins, O., Schroeder, W., Mansfield, S. D. et al., 2011: Clone history shapes *Populus* drought responses. Proceedings of the National Academy of Sciences of the United States of America, 108:12521–12526.

Raja, P., Sanville, B. C., Buchmann, C., Bisaro, D. M., 2008: Viral genome methylation as an epigenetic defense against geminiviruses. Journal of Virology, 82:8997–9007.

Rehfeldt, G. E., Tchebakova, N. M. , Parfenova, Y. I. , Wykoff, W. R. , Kuzmina, N. A., Milyutin, L. I., 2002: Intraspecific responses to climate in *Pinus sylvestris*. Global Change Biology, 8:912–929.

Rohde, A., Junttila, O., 2008: Remembrances of an embryo: long-term effects on phenology traits in spruce. New Phytologist, 177:2–5.

Santamaria, M. E., Hasbun, R., Valera, M. J., 2009: Acetylated H4 histone and genomic DNA methylation patterns during bud set and bud burst in *Castanea sativa*. Journal of Plant Physiology, 166:1360–1369.

Schreiber, J., Wescoe, Z. L., Abu-Shumays, R., Vivian, J. T., Baatar, B., Karplus, K. et al., 2013: Error rates for nanopore discrimination among cytosine, methylcytosine, and hydroxymethylcytosine along individual DNA strands. Proceedings of the National Academy of Sciences of the United States of America, 47:18910–18915.

Skrøppa, T., Tollefsrud, M., Sperisen, C., Johnsen, Ø., 2010: Rapid change in adaptive performance from one generation to the next in *Picea abies* – Central European trees in a Nordic environment. Tree Genetics and Genomes, 6:93–99.

Soppe, W. J. J., Jasencakova, Z., Houben, A., Kakutani, T., Meister, A., Huang, M.S. et al., 2002: DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in *Arabidopsis*. The EMBO Journal, 23:6549–6559.

Soyfer, V. N., 2011: Stalin and fighters against cellular theory. Studies in the History of Biology, 3:82–96.

Stoehr, M. U., L'Hirondelle, S. J. , Binder, W. D., Webber, J. E., 1998: Parental environment after effects on germination, growth, and adaptive traits in selected spruce families. Canadian Journal of Forest Research, 28:418–426.

Suzuki, M. M., Bird, A., 2008: DNA methylation landscapes: provocative insights from epigenomics. Nature Reviews Genetics, 9:465–476.

Thumma, B. R., Matheson, B. A., Zhang, D., Meeske, C., Meder, R., Downes, G. M. et al., 2009: Identification of a cis-acting regulatory polymorphism in a Eucalypt COBRAlike gene affecting cellulose content. Genetics, 183:1153–1164.

Turner, B. M., 2000: Histone acetylation and an epigenetic code. Bioessays, 22:836–845.

Valledor, L., Hasbún, R., Meijón, M., 2007: Involvement of DNA methylation in tree development and micropropagation. Plant Cell Tissue and Organ Culture, 91:75–86.

Vanyushin, B. F., 2005: Adenine methylation in eukaryotic DNA. Molecular Biology, 39:473–481.

Webber, J., Ott, P., Owens, J., Binder, W., 2005: Elevated temperature during reproductive development affects cone traits and progeny performance in *Picea glauca – engelmannii* complex. Tree Physiology, 25:1219–1227.

Wu, T. P., Wang, T., Seetin, M. G., Lai, Y. Q., Zhu, S. J., Lin, K. X. et al., 2016: DNA methylation on N6-adenine in mammalian embryonic stem cells. Nature, 532:329–333.

Yakovlev, I. A., Fossdal, C. G., Johnsen, Ø., 2010: MicroRNAs, the epigenetic memory and climatic adaptation in Norway spruce. New Phytologist, 187:1154–1169.