Epigenetics in Forest Trees: State of the Art and Potential Implications for Breeding and Management in a Context of Climate Change

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Abstract

Forest trees are long-lived organisms subject to repeated environmental constraints throughout their long lifetimes. They have developed various mechanisms enabling them to cope with fluctuating environmental conditions during their life span, and to survive to current climate change. Epigenetics has recently emerged as a powerful set of mechanisms regulating various developmental processes, plant growth and responses to environmental variations. Such epigenetic mechanisms, which may remain stable along tree life or across generations, constitute a source of rapid phenotypic variations potentially improving adaptation of the plants in situations in which naturally occurring mutations are very rare.In this review, we summarize recent advances in forest tree epigenomics. We first draw the particularities of trees and the available (epi) genomics resources and strategies. Then, we discuss the potential contributions of epigenetics to cope with global climate change and regulate various developmental processes, such as developmental transitions during the annual cycle, phenotypic plasticity in response to environmental variations and stress memory, as well as local adaptation. Finally, we propose some challenges for forest management and highlighted the need to take epigenetics into account in forest tree breeding strategies.

1. INTRODUCTION: FEATURES OF FOREST TREES BREEDING

Forest tree breeding programs started in Europe and worldwide only a few decades ago, in the 1950s, boosted by ambitious plantation programs. These programs had two main aims: to guarantee the genetic origin of the forest reproductive material (FRM) used in reforestation and to improve tree characteristics. Biomass production was the main selection criterion for many years, together with overall adaptability to different sites. Further criteria have recently been added, including wood quality traits, stem architecture, and more specific criteria linked to adapta-

TABLE 1 Published Forest Tree Genomes With Basic Statistics

Species	Botanical Family	Genome Size (gb/2c)	Assembly Availability (Bioproject Id)	Sequencing Technology	# Scaffolds	Scaffold Cumulat Size (mb
Gymnosperms						
Picea abies	*Pinacea*	39.2	http://congenie.org (ERP002565)	Illumina	10,253,694	4,300
Picea glauca	*Pinacea*	40	NA (PRJNA83435)	Illumina/454FLX	4,300,000	22,500
Pinus taeda	*Pinacea*	44	NA (PRJNA174450)	Illumina	NA	23,180
Pinus lambertiana	*Pinacea*	31	http://www.pinegenome.org/pinerefseq (PRJEB174450)	Illumina	>500bp: 1,089,592	24,700
Ginko biloba	*Ginkgoaceae*	10	NA (PRJNA307642)	Illumina	6,459,773	10,600
Angiosperms						
Quercus robur	*Fagaceae*	1.5	http://www.oakgenome.fr (PRJEB19898)	Illumina/454FLX/Sanger/Moleculo	1,409	814

TABLE 1 (Continued)

Species	Botanical Family	Genome Size (gb/2c)	Assembly Availability (Bioproject Id)	Sequencing Technology	# Scaffolds	Scaffold Cumulat Size (mb
Quercus lobata	*Fagaceae*	1.4	https://valleyoak.ucla.edu/genomicresources/ (PRJNA308314)	Illumina	94,394	1180
Castanea mollisima	*Fagaceae*	1.6	https://hardwoodgenomics.org NA	454FLX/Illumina	41,260	720
Betula pendula	*Betulaceae*	0.84	https://genomevolution.org/coge/api/v1/genomes/35079/sequence (PRJEB14544)	Illumina, SOLID and PacBio	5,642	430
Betula Nana	*Betulaceae*	0.9	http://www.birchgenome.org/ NA	Illumina	551,923	564

TABLE 1 (Continued)

Species	Botanical Family	Genome Size (gb/2c)	Assembly Availability (Bioproject Id)	Sequencing Technology	# Scaffolds	Scaffold Cumulat Size (mb
Juglans regia	*Juglandaceae*	1.2	http://dendrome.ucdavis.edu/ftp/Genome_Data/genome/Reju/ (PRJNA291087)	Illumina/PacBio	186,636	700
Fraxinus excelsior	*Oleaceae*	1.7	http://www.ashgenome.org/assemblies NA	Illumina/454FLX	89,514	867
Eucalyptus grandis	*Myrtaceae*	1.3	http://www.phytozome.net/eucalyptus.php (AUSX00000000)	Sanger	11+4900 unanchored	692

TABLE 1 (Continued)

Species	Botanical Family	Genome Size (gb/2c)	Assembly Availability (Bioproject Id)	Sequencing Technology	# Scaffolds	Scaffold Cumulat Size (mb
Populus trichocarpa	*Salicaceae*	1	https://genomevolution.org/coge/api/v1/genomes/25127/sequence (AARH00000000)	Sanger	1,446	422
Populus pruinosa	*Salicaceae*	1.2	NA (PRJNA353148)	Illumina	78,960	479
Populus tremula x p. Alba	*Salicaceae*	0.9	https://urgi.versailles.inra.fr/Species/Forest-trees/Populus/Clone-INRA-717-1B/NA	Illumina/Ion Torrent	419,969	900
Salix suchowensis	*Salicaceae*	0.86	NA (AVAC00000000)	Illumina/454FLX	>2,000bp: 7516	304
Hevea brasiliensis	*Euohorbiaceae*	1.5	NA (LVXX01000000)	Illumina/Sanger	7,453	1370

TABLE 1 (Continued)

Species	Botanical Family	Genome Size (gb/2c)	Assembly Availability (Bioproject Id)	Sequencing Technology	# Scaffolds	Scaffold Cumulat Size (mb
Macadamian intergrifolia	*Proteaceae*	NA	https://www.ebi.ac.uk/ena/data/view/GCA_900087525 (PRJEB13765)	Illumina	193,493	518
Handroanth impetiginosus	*Bignoniaceae*	1.1	NA (PRJNA324125)	Illumina	13,206	503

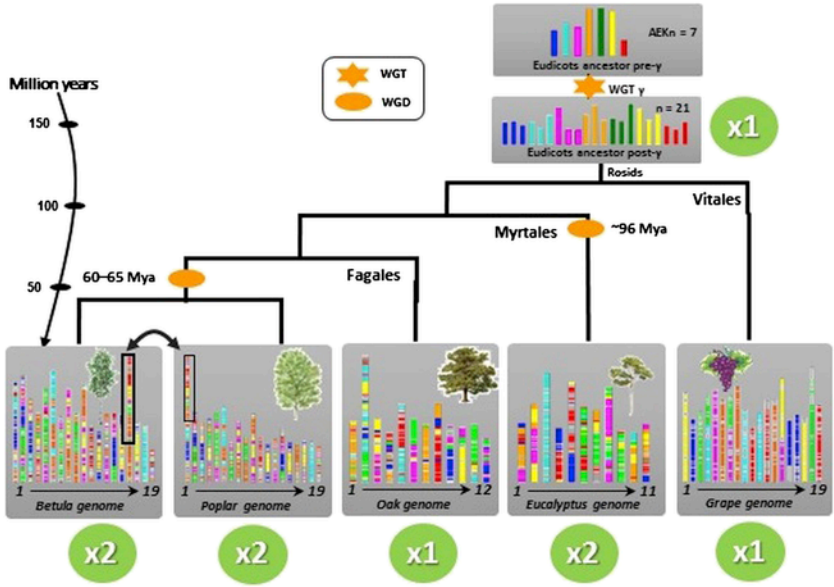

Figure 1. Tree genome evolution from the eudicot ancestor. The present-day tree genomes (birch-poplar-oak-eucalyptus, at the bottom) are represented with color codes to illustrate the evolution of genomic segments from the ancestral eudicot karyotypes (AEKs with 7 and 21 chromosomes, top) over time (time scale on the left in mya). The polyploidization events shaping the structure of the modern tree genomes during their evolution from the AEK are indicated as orange dots (duplication) or stars (triplication). Major angiosperm families are indicated on the tree branches. Green circles show the expected number of modern genes from any ancestral protogene, taking into account the polyploidization events occurring during tree evolution.

tion to abiotic and biotic factors in response to new socioeconomic needs and global changes (Mullin et al. 2011; Pâques, 2013).

Forest tree species are characterized by two key features that render them unique in the domain of plant breeding. Firstly, forest trees were domesticated only recently, so the available gene pools can be considered mostly wide, despite the well-documented introduction of exotic species and/or populations at some sites. Secondly, forest tree species generally cover large areas with contrasting ecological conditions. In Europe, the recolonization routes followed by these species after the most recent period of glaciation have, together with local selection pressures, shaped populations consisting of many different ecotypes. Furthermore, interspecific hybridization is common in forest trees, and several characteristics of the reproductive biology of species (dioecious species, monoecious species with asynchrony between female and male flowers, in-

TABLE 2 Key Characteristics of Most Widely Used Methods for the Genome-wide Analysis of DNA Methylation

Technology	The Technology Behind	Methylated/Unmethylated Fraction	Nucleotide Resolution (Yes/No)	DNA Input in µg	Sequencing Reads per Sample in m	of an Average Genome	Comments
WGBS/ METHYLC-SEQ	Adaptor ligation before or after bisulfite treatment of genomic DNA prior to sequencing	U+M	Yes	0.1–5	500	95%	Comprehensiv coverage of al cytosines in a genome, several variations of library preparation exist, cost-intensive, but will with decreasing sequencing costs become the gold standard for th discovery technologies, no distinction between 5 mC and 5 hmC
RRBS	Restriction digest allows for size selection of small fragments, used for library construction, bisulfite conversion and	U+M	Yes	0.01–1	20	4%	85% of CpG islands are covered, cost-effective sequencing approach, low coverage in CpG poor regions, no distinction between 5 mC and 5 hmC

AGILENT SURESELECT^{XT} METHYLATION CAPTURE	Libraries are prepared form genomic DNA fragments, hybridized to the capture probes, eluted, bisulfite converted, and amplified before being sequenced by a NGS	U+M	Yes	3	50	2.8%	Off the shelf product, captures all known promoters and upstream regions, uses the procedure used for exom sequencing with an additional bisulfite conversion step, relatively expensive, custom design possible

SEQCAPEPI CPGIANT ENRICHMENT	Libraries are prepared form genomic DNA fragments, bisulfite converted, pre-amplified hybridized to the capture probes, eluted, b and amplified before being sequenced by a NGS	U+M	Yes	1	40–50	2.7%	Less input compared to the SureSelec capture as capture is performed on bisulfite treate libraries after pre-amplification, relatively expensive, custom desigr possible

MBD-SEQ/ METHYLCAP-SEQ	Enrichment of methylated DNA using a Methyl-binding domain protein before or after a library construction followed by sequencing	M	No	0.2–1	30–40	95%	Also suitable for FFPE, amenable to high-throughput an analysis of large cohorts, absolute quantification of DNA methylation difficult, specific for 5 mC

MEDIP-SEQ	Library construction, Enrichment of methylated DNA using an antibody against 5 mC followed by sequencing	M	No	0.3–0.5	50–60	95%	Captures and allows to analyze also the transposor of the human genome, specific for 5 mC, absolut quantification of DNA methylation difficult, lowe signal to noise compared to MBD- and MIRA-seq, better coverag in CpG poor regions

MIRA-SEQ	Enrichment of methylated DNA using a Methyl-binding domain protein before or after a library construction followed by sequencing	M	No	0.1–1	40–50	95%	Higher specificity compared to MBD-seq, absolute quantification of DNA methylation difficult, specific for 5 mC
MRE-SEQ	Digestion of genomic DNA in parallel with several methylation-sensitive restriction enzymes, size selection, library construction, sequencing	U	Yes, at restriction site	1.5–2.5	30	4%	Analyses preferentially unmethylated CpGs in high density CpG regions, in combination with MeDIP comprehensiv genome coverage is achieved

HELP	Digestion of genomic DNA in parallel with a methylation-sensitive restriction enzymes and its methylation-insensitive isoschizomer, size selection, library construction, sequencing	U+M	Yes, at restriction site	0.3	30	4%	Normalizatior of the counts signals from the two digestions allows for quantitative analysis at restriction sites, limited coverage due to the use of restriction enzymes, variation allows for the parallel analysis of 5 hmC

MSAP	Digestion of genomic DNA in parallel with a methylation-sensitive restriction enzymes and its methylation-insensitive isoschizomer in parallel in combination with a frequently cutting enzyme, adaptor ligation, size selection, library construction, sequencing	U+M	Yes at restriction site	0.5	15	< 1%	One of the most widely used methods in plants and trees, MSAP has mostly been performed using PAGE electrophoresi as read-out platform using a fluorescently or radioactive labeled amplification primer, rarely by sequencing most diff. methylated regions are located in intergenic regions and repetitive elements

Abbreviations: 5 mC: 5-methylcytosine; 5 hmC; 5-hydroxymethylcytosine; WGBS: Whole Genome Bisulfite Sequencing; RRBS: Reduced Representation Bisulfite Sequencing; MBD: Methyl-Binding Domain; MeDIP: Methylated DNA ImmunoPrecipitation; MIRA: methylated-CpG island recovery assay; MRE: Methylation-sensitive Restriction Enzyme sequencing; HELP: *Hpa*II tiny fragment Enrichment by Ligation-mediated PCR; MSAP: Methylation-Sensitive Amplification Polymorphism.

TABLE 3 Available Epigenomics Data in Forest Tree Species

Species	Family	Approach	Main Conclusion	References
DNA Methylation				
Picea abies	*PINACEAE*	WGBS	The Norway spruce genome is heavily methylated because of high transposon content. Somatic embryogenesis cultures used in the industry show altered DNA methylation patterning.	Ausin et al. 2016
Pinus pinaster	*PINACEAE*	MSAP	DNA methylation varies during aging of embryogenic cultures and in response to 5-azacytidine treatment	Klimaszewska et al. 2009
Pinus radiata	*PINACEAE*	MSAP	DNA methylation variations during needle maturation in relation to the loss of *in vitro* organogenic capability	Valledor, Meijón, Hasbún, Cañal, & Rodríguez, 2010

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Pinus pinea	*PINACEAE*	MSAP	Variable epigenetic markers discriminate individuals and differentiates two well represented populations contrary to genetic variation	Sáez-Laguna et al., 2014
Quercus lobata	*FAGACEAE*	RRBS	While CHG methyl polymorphisms are not playing a significant role and would make poor targets for natural selection, our findings suggest that CpG methyl polymorphisms as a whole are involved in local adaptation, either directly or through linkage to regions under selection.	Platt et al. 2015

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Quercus lobata	*FAGACEAE*	RRBS	Climate and spatial variables explain more overall variance in CG-SMV among individuals than in SNPs, CHG-SMVs or CHH-SMVs. These results from natural oak populations provide initial evidence for a role of CG methylation in locally adaptive evolution or plasticity in plant response	Gugger et al. 2016
Quercus ilex	*FAGACEAE*	MSAP	DNA methylation variations under drought stress	Rico, Ogaya, Barbeta, & Peñuelas, 2014
Eucalyptus globulus	*MYRTACEAE*	MeDIP-seq MRE-seq	Locus-specific methylation could be major regulators of vegetative phase change	Hasbún, Iturra, Bravo, Rebolledo-Jaramillo, & Valledor, 2016

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus trichocarpa	*SALICACEAE*	WGBS	If patterns of methylation are very similar in flowering plants, CHG methylation levels in transposons and repeats were much higher in poplar	Feng et al. 2010
Populus trichocarpa	*SALICACEAE*	MeDIP-SEQ	DNA in recombination hotspots is less methylated	Slavov et al. 2012
Populus trichocarpa	*SALICACEAE*	MeDIP-SEQ	DNA methylation is tissue specific and gene-body DNA methylation has a more repressive effect on transcription than promoter methylation	Vining et al. 2012
Populus trichocarpa	*SALICACEAE*	MeDIP-SEQ	DNA methylation varies in a highly gene- and chromosome-differential manner during *in vitro* differentiation and regeneration. Hypermethylation of gene bodies may serve a protective role against activation of abundant transposable elements	Vining et al. 2013

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus trichocarpa	*SALICACEAE*	WGBS	Differential methylation was associated with site-dependent, different total amounts of miRNA, with few miRNAs sequences directly targeted by differential methylation. This may explain habitat or seasonal memory in perennials and site-dependent growth performance	Schönberger et al. 2016
Populus trichocarpa	*SALICACEAE*	WGBS	DNA methylation in response to stress regulates genes by methylating TEs in promoters and gene body of transcription factors	Liang et al. 2014
Populus tremula x alba	*SALICACEAE*	RNAi strategy	First report for a DNA methylation modified-tree. The phenotypic consequences of reduced *DDM1* activity and DNA methylation appears to increase with cumulative plant propagation and growth	Zhu et al. 2013

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus tremula × *alba*	*SALICACEAE*	Immunofluorescence	CsDML gene induces bud formation needed for the survival of the apical meristem under the harsh conditions of winter	Conde et al. 2017a, 2017b
Populus tremula × *alba*	*SALICACEAE*	WGBS	A chilling-dependent DEMETER-like DNA demethylase mechanisms being involved in the shift from winter dormancy to a condition that precedes shoot apical vegetative growth in poplar	Conde et al. 2017a, 2017b
Populus alba	*SALICACEAE*	MSAP	DNA methylation would adapt gene expression in response to biotic and abiotic stress	Cicatelli et al. 2014

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus alba	*SALICACEAE*	MSAP	The genetic biodiversity of poplars is quite limited but it is counterbalanced by epigenetic inter-population molecular variability. Plant biodiversity should no longer be restricted to genetic aspects, especially in the case of vegetatively propagated plant species	Guarino et al. 2015
Populus tomentosa	*SALICACEAE*	MSAP	DNA methylation sites have the potential to regulate the genes' transcript levels	Song et al. 2012
Poplar latin	*SALICACEAE*	MSAP	DNA methylation is nonlinearly related to the ploidy level	Li et al. 2011
Populus deltoides	*SALICACEAE*	MeDIP-SEQ	The methylation patterns of the parents both partially and dynamically passed onto their hybrids and F1 hybrids has a non-additive methylation level	Gao et al. 2014

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus simonii	*SALICACEAE*	MSAP, Degradome Bisulfite-seq	DNA methylation probably regulates the expression of miRNA genes, thus affecting expression of their target genes, likely through the gene-silencing function of miRNAs, to maintain cell survival under abiotic stress conditions	Ci et al. 2016
P. pseudo-simonii × *P. nigra and P. beijingensis*	*SALICACEAE*	MSAP	Both hybridization and polyploidization contributed to cytosine methylation variation.	Suo, Dong, & Kang, 2015
Populus. simonii	*SALICACEAE*	HPLC MSAP	The SDMR162 region, consisting of Psi-MIR396e and PsiLNCRNA00268512, is regulated by epigenetic pathways and we speculate that PsiLNCRNA00268512 regulates miR396e levels by acting as a target mimic.	Song, Ci, Tian, & Zhang, 2016

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus nigra	*SALICACEAE*	MeDIP-seq	Methylated genes were prevalent in the poplar genome, but that only a few of these participated in diurnal gene expression regulation	Ding, Liang, Diao, Su, & Zhang, 2018
Populus×euramericana (P. deltoides batr.×P. nigra l.)	*SALICACEAE*	MeDIP-chip	Shoot apical meristem responses to changes in water availability involved coordinated variations in DNA methylation, as well as in gene expression, with a specific targeting of genes involved in hormone pathways, a factor that may enable phenotypic plasticity	Lafon-Placette et al. 2018
Jatropha curcas	*EUPHORBIACEAE*	MSAP	DNA methylation polymorphism is observed between individuals and in response to gamma-irradiation	Kanchanaketu, Sangduen, Toojinda, & Hongtrakul, 2012

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Acacia mangium	*FABACEAE*	MSAP	Differences in DNA methylation exist between juvenile and mature plant materials	Baurens, Nicolleau, Legavre, Verdeil, & Monteuuis, 2004
Laguncularia racemosa	*COMBRETACEAE*	MSAP	Higher DNA methylation differentiation than genetic one between adapted populations to distinct environment	Lira-Medeiros et al. 2010
Elaeis guineensis jacq.	*ARECACEAE*	MSAP	DNA methylation polymorphism discriminates between the two phenotypes (normal and mantled) only when they were from the same genetic origin	Jaligot, Beulé, Baurens, Billotte, & Rival, 2004
Histones Modifications				

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Picea asperata	*PINACEAE*	antibody-enrichment, MS	Functional characterization of the acetylated proteins revealed that in the desiccated somatic embryos, LysAc is mainly involved in the response to stress and central metabolism	Xia et al. 2016
Quercus suber l.	*FAGACEAE*	HPCE MS-RAPD Protein Gel Blot	Epigenetic mechanisms such as DNA methylation and histone H3 acetylation have opposite and particular dynamics that can be crucial for the stepwise establishment of cork Oak into high stress, allowing its acclimation and survival	Correia et al. 2013
Eucalyptus grandis	*MYRTACEAE*	ChIP-seq	In developing *E. grandis* xylem, H3K4me3 enrichment is an indicator of active transcription, consistent with its known role in sustaining pre-initiation complex formation in yeast	Hussey, Mizrachi, Groover, Berger, & Myburg, 2015

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus trichocarpa	*SALICACEAE*	HDAC Colorimetric Assay	HDACs were required for de novo organogenesis and normal growth of populus roots.	Ma, Zhang, Zhang, Yang, & Li, 2016
Populus simonii x Populus nigra	*SALICACEAE*	ChIP assay	Histone acetylation positively regulates the tissue dependent expression pattern of the poplar homologs of C4 homologous genes. This regulatory mechanism seems to be conserved among the C3 and C4 species	Li et al. 2017
Small RNAS (miRNA & siRNA)				
Picea abies	*PINACEAE*	sRNA-seq	Norway spruce contains a set of conserved miRNAs as well as a large proportion of novel non-conserved miRNAs. The differentially expression of specific miRNAs indicate their putative participation in the epigenetic regulation	Yakovlev et al. 2010

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Picea abies	*PINACEAE*	sRNA-seq RNA-seq	A significant number of epigenetic regulators were differentially expressed during embryogenesis at different epitype-inducing conditions. This support that methylation of DNA and histones, as well as sRNAs, are pivotal for the establishment of the epigenetic memory	Yakovlev et al. 2016
Picea abies	*PINACEAE*	Rt-qPCR	Epigenetic memory affects the timing of bud burst phenology and the expression of bud burst related genes in genetically identical Norway spruce epitypes in a manner usually associated with ecotypes.	Carneros et al. 2017
Picea abies	*PINACEAE*	sRNA-seq	Fine-tuning of the miRNA production likely participates in both developmental regulation and epigenetic memory formation in Norway spruce	Yakovlev & Fossdal, 2017

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Picea glauca	*PINACEAE*	sRNA-seq	Lacking of 24-nt sRNAs at the late conifer seed developmental phase may result in less constraint in TE activities, thus contributing to the massive expansion of genome size.	Liu & El-Kassaby, 2017
Pinus taeda	*PINACEAE*	miRNA-array	conserved miRNAs are expressed in mature and germinated loblolly pine pollen	Quinn, Iriyama, & Fernando, 2014
Pinus tabuliformis	*PINACEAE*	sRNA-seq PARE-seq	The sRNA pathways have higher activity in female than in male cones, and the miRNA pathways are the main sRNA pathways in *P. tabuliformis*	Niu et al. 2015
Ginkgo biloba l.	*Ginkgoaceae*	sRNA-seq	Identification a large number of miRNAs in mature female and male *G. biloba* leaves	Wang et al. 2015

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus trichocarpa	*SALICACEAE*	sRNA-seq, Degradome sequencing	Five upregulated miRNAs and seven downregulated miRNAs in response to drought stress discovered. This will promote the understanding of miRNA functions during the drought response.	Shuai et al. 2013
Populus balsamifera	*SALICACEAE*	sRNA-seq	A large fraction of miRNAs vary among species. The non-conserved miRNAs may regulate cellular, physiological or developmental processes specific to the taxa that produce them, as appears likely to be the case for those miRNAs that have only been observed in Populus	Barakat, Wall, DiLoreto, dePamphilis, & Carlson, 2007

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus. tomentosa	*SALICACEAE*	sRNA-seq qRT-PCR	Significant changes in the expression of 17 conserved miRNA families and nine novel miRNAs were observed in response to drought stress, and in seven conserved miRNA families and five novel miRNAs in response to flooding stress.	Ren et al. 2012
Populus tomentosa	*SALICACEAE*	Bisulfite sequencing	miRNA 172b might play an important role in the regulation of bisexual flower development-related gene expression in andromonoecious poplar, via modification of methylation. Hyper-methylation in andromonoecious and gynomonoecious poplar might function as an important regulator in bisexual flower development	Song, Tian, Ci, & Zhang, 2015

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Populus euphratica	*SALICACEAE*	sRNA-seq	Expression changes of miRNAs were inversely correlated with the expression profiles of their putative targets and might be involved in some biological process related stress tolerance	Duan et al. 2016
Cunninghamia lanceolata	*CUPRESSACÉES*	sRNA-seq	Identification of a complex population of sRNAs in Chinese fir through high throughput sequencing	Wan et al. 2012
Cryptomeria japonica	*CUPRESSACÉES*	sRNA-seq	Both conserved and species-specific sRNAs contribute to the development of male strobili in *C. japonica*	Ujino-Ihara, Ueno, Uchiyama, & Futamura, 2018
Acer palmatum thunb.	*SAPINDACEAE*	RNA-seq sRNA-seq	RNA-seq and sRNA-seq data show that gene differentiation associates with *Acer palmatum* leaf coloration in different light conditions	Li et al. 2015

TABLE 3 (Continued)

Species	Family	Approach	Main Conclusion	References
Phoenix dactylifera l.	*ARECACEAE*	sRNA-seq	Date palm contains a large population of conserved and non-conserved miRNAs that function at the post-transcriptional level, and are important for adaptation to salinity	Yaish et al. 2015

Abbreviations: WGBS: Whole Genome Bisulfite Sequencing; RRBS: Reduced Representation Bisulfite Sequencing; MeDIP: Methylated DNA ImmunoPrecipitation; MRE-seq: Methylation-sensitive Restriction Enzyme sequencing; MSAP: Methylation-Sensitive Amplification Polymorphism; sRNA-seq: small RNA sequencing; ChIP: Chromatin ImmunoPrecipitation; HDAC: Histone DeACetylase; MS-RAPD: Methylation Sensitive - Random Amplified Polymorphic DNA; HPCE: High-Performance Capillary Electrophoresis; HPLC: High-Performance Liquid Chromatography.

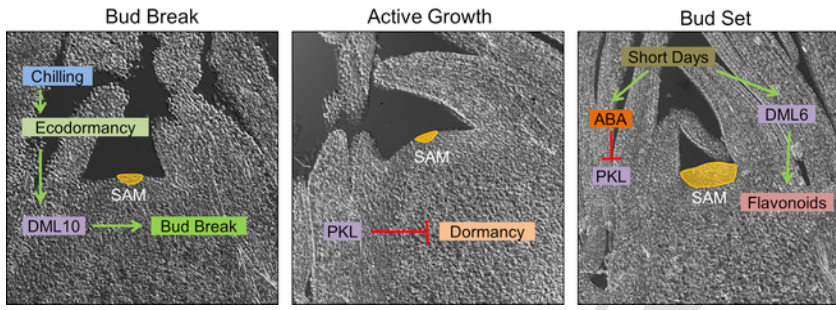

Figure 2. Schematic model of the role of chromatin remodeling in poplar bud dormancy. During bud break, DML10-mediated active demethylation promotes the induction of growth-promoting genes require to re-establish shoot elongation. During active growth, DNA methylation is tightly restricted to the central zone of the SAM (yellow patch). During the growing season the PKL chromatin remodeling factor represses the induction of bud dormancy. During bud set, DNA methylation spreads to the rest of the SAM (yellow patch). Short days induce ABA, which represses PKL, promoting dormancy. Short days and cold temperatures also induce DML6, a DNA demethylase promoting the expression of flavonoid biosynthetic enzymes. Flavonoids accumulate in the SAM and bud scales during apical bud formation.

compatibility genes, etc.) greatly limit consanguinity. All these factors contribute to the considerable genetic diversity observed in many forest trees (Bagnoli et al. 2011).

Over several decades before the advent of tree breeding programs, many studies focused on how diversity in species is geographically structured. Large-scale provenance trial networks and molecular marker analysis were used as complementary approaches to investigating both adaptive and neutral genetic diversity with two major outcomes. Firstly, it provided evidence in support of the delineation of regions of origin and the selection of local seed sources (selected seed stands). These regions, most of which were defined for native species, were designed to protect local genetic resources and, thus, to restrict the circulation and use of FRM. Secondly, it made possible to set up breeding programs for a limited number of species through identification of the best populations adapted to fit for local zones and to establish breeding populations through the mass-selection of individuals in native stands. In parallel, dedicated plantations (first-generation seed orchards) were established with this material, for mass production of FRM. For many species, these plantations remain the main source of seed for reforestation.

This approach has a major drawback. Indeed, core breeding populations are selected on the basis of their performances across contrasting

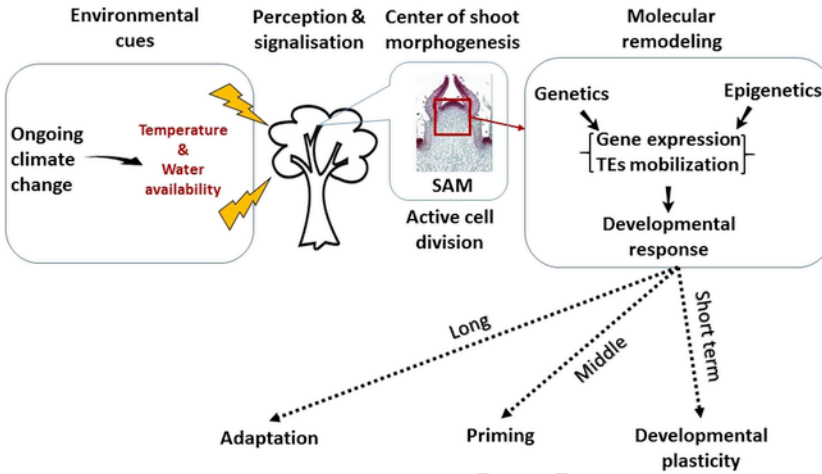

Figure 3. Schematic model of the link between environmental cues and epigenetic regulation in the shoot apical meristem (SAM). SAMs are the center of shoot morphogenesis and undergo constant cell division. During environmental constraints (heat, cold, drought), the signals perceived by the plant induce dynamic changes in the SAM affecting gene expression and TE mobilization. These signals are mostly linked to changes in epigenetic state, but also, to a lesser extent, to genetic changes. At short term, they may affect plant development and promote stress tolerance, leading to developmental plasticity. Once the constraint is released, the plant may develop the ability to remember the stress episode (priming, middle term), allowing it to react more efficiently if subsequently exposed to the same stress, thereby resulting in higher resistance. In cases of acute and long-lasting environmental pressure (long term), these changes may be transmitted to subsequent generations, potentially promoting adaptation to the new environmental conditions.

environments in large common garden networks, but populations are compared on the basis of seed lots collected in a single year, in specific environments. Furthermore, parental contributions to seed lots are probably constrained by a lack of full panmixia and the impact of the environment on seed formation has also been largely ignored although maternal effects on seed weight and subsequent growth are well documented, and evidences for epigenetic memory during embryo formation are also accumulating for forest trees (Carneros, Yakovlev, Viejo, Olsen, & Fossdal, 2017; Skrøppa, Solvin, & Steffenrem, 2016; Yakovlev et al., 2012; Yakovlev & Fossdal, 2017). Consequently, the genetic differences between populations reported in common garden studies are most likely overestimated.

Due to the length and inherent costs of the breeding cycle in trees, breeding *sensu stricto* has been restricted to a few species of consider-

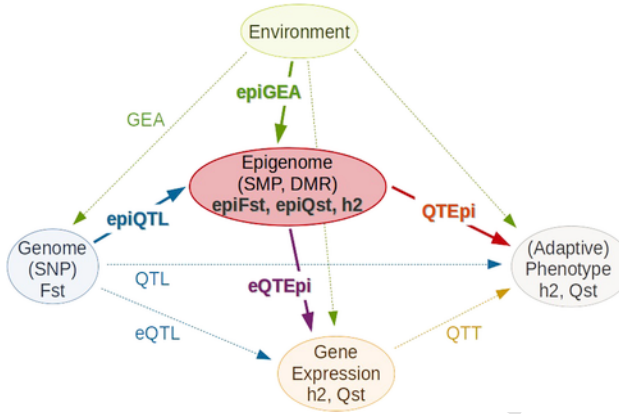

Figure 4. Quantitative and population genomics analyses for the integrative analysis of genetic, epigenetic and environmental effects. GEA: gene–environment association; epiGEA: epigenetics–environment association; QTL: quantitative trait locus; epiQTL: epigenetic QT; eQTL: expression QTL; QTEpi: quantitative trait epilocus; eQTEpi: expression QTEpi; QTT: quantitative trait transcript; h^2: heritability; Fst: genetic differentiation between populations; epiFst: epigenetic differentiation between populations; Qst: quantitative differentiation between populations; epiQst: epigenetic quantitative differentiation between populations; SNP: single-nucleotide polymorphism; SMP: single-methylation polymorphism; DMR: differentially methylated region.

able real or potential economic importance and, generally, to those with a short rotation (mostly conifers and poplars). In most cases, breeding programs target reforestation zones outside the native range of the species. Breeding populations are largely exotic and better adapted to reforestation needs. Except in Scandinavia, characterized by strong environmental gradients, breeding programs have mostly aimed to varieties that are not specialized to specific environmental conditions (low GxE (Genetics by Environment) interactions) or traits.

The breeding objectives for forest trees are directed by various expectations from end-users of FRM, such as the reproductive success of base material required by the seed industry, the biomass production required by forest owners (implying adaptation to abiotic and biotic factors), and the stem architecture and properties of wood required by the wood industry for various end uses (Alia & Majada, 2013; Sanchez et al. 2013). Consequently, forest tree varieties actually represent a compromise between a diversity of target traits. There are many natural trade-offs between characters, and the management of antagonistic traits (e.g. growth and wood density) requires alternative approaches, such as the management of breeding populations in sublines, selection procedures for opti-

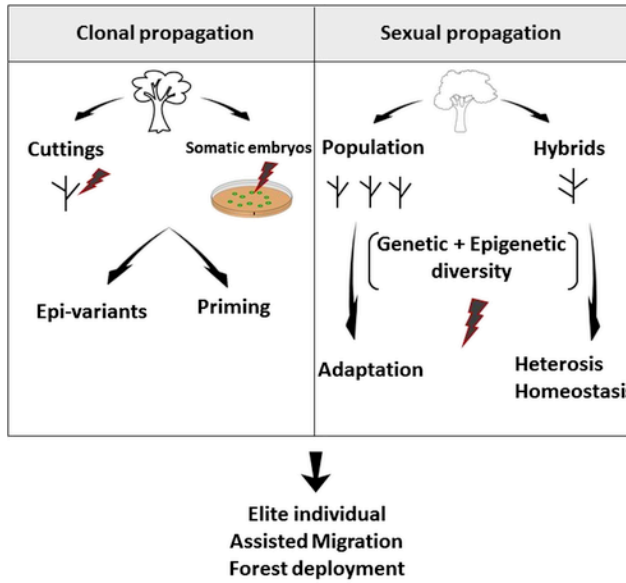

Figure 5. Recommendations for forest management and tree breeding in the face of climate changes. Some trees are vegetatively propagated, which may lead to limited genetic diversity. However, it is now evident that epigenetic diversity plays a key role in adaptation, due to its sensitivity to environmental conditions. During clonal propagation (cuttings or somatic embryos), epigenetic variants (epi-variants) or priming effect may be induced by the application of controlled stresses (heat, drought) early in the life of the plant. The early-induced epigenetic changes may promote developmental plasticity and, subsequently, local adaptation, if these changes are transmitted across generations. Sexually propagated trees tend to display greater genetic and epigenetic diversity, which may be crucial for population adaptation under environmental constraints. Another way of combating forest decline is to produce progenies with higher performances. In this case, the concept of heterosis should not be restricted to genetic aspects, but should also take into account the complex interactions between genetic and epigenetic diversity, because the expression of a trait of interest in forestry may be dependent on its epigenetic state. The trees with the highest performance generated by such strategies could be used in assisted migration and forest deployment programs.

mizing genetic gains, interspecific hybridization to break down unfavorable correlations and the clonal propagation of ideotypes as sources of FRM (Hallingbäck, Sánchez, & Wu, 2014).

With few exceptions (e.g. interspecific hybridization (poplars, larches) and clonal (poplar, wild cherry) strategies), simple recurrent selection is the most common breeding strategy used for forest trees (e.g. spruces, pines, Douglas fir). This approach is based on recurrent cycles of recombination, testing and selection phases aiming to improve the

breeding population over generations and to lead to the release of improved varieties (White, Adams, & Neale, 2007). Simple recurrent selection is based exclusively on additive gene effects, whereas other approaches also exploit non-additive effects (as in reciprocal recurrent selection) or all the available genetic variation (as in clonal strategies). The optimization of mating plans and of selection procedures to increase the frequency of favorable alleles and to discard deleterious alleles whilst maintaining broad genetic variability within the population over breeding cycles is a major challenge that must be met to ensure long-term genetic gains.

Nevertheless, several major morphological and biological constraints slow the whole process, limit breeding possibilities and account for the very small number of forest tree species currently bred beyond a first generation. Space and time are two of the most critical factors. The large size of trees generally limits both the size of breeding populations to a few hundred trees established in clonal banks, and the size and, number of test sites (number of genotypes and replicates tested), decreasing the selection pressure (selection intensity) and the precision of breeding value estimations, thereby restricting genetic gains. Sexual maturity is not reached for a number of years in many tree species, delaying the recombination phase and, thus, the breeding cycle, and the late expression of some traits or their differential expression with age due to ontological effects may postpone the selection phase even further, by several years or even decades. Research efforts to limit these drawbacks and accelerate breeding cycles have focused on flower initiation and stimulation, various techniques to reduce the length of the testing phase (e.g. early selection when there is evidence of strong juvenile–mature correlations for traits, marker-assisted selection) and the timely management of base material establishment for FRM production. Genomic selection, which has only recently been envisaged for some forest trees, shares the same objectives (Muranty et al. 2014; Woolliams, 2013).

The duration of the recombination phase is often limited by estimating the breeding values of genotypes through open-pollinated (or polycross) progenies collected in a single year, as in simple recurrent selection schemes, and, for economic reasons, at a limited number of test sites. Breeding values and other genetic parameters are likely to be biased (overestimated) in cases of strong GxE interactions and/or in the presence of unaccounted for epigenetic effects. As a result, the genetic gains obtained by selection may be smaller than anticipated. Neverthe-

less, the gains achieved in several breeding programs demonstrate the overall success of this approach (Kimberley, Moore, & Dungey, 2015; Toda & Kurinobu, 2002) at least when GxE interactions are properly accounted for.

Various forms of FRM can be produced, depending on the selection scheme used. Synthetic varieties mass-produced by open-pollination in dedicated plantations (seed orchards) including several tens or even hundreds of genotypes selected on the basis of their GCAs (general combining abilities) are the most common. The hypothetical assumption of panmixia is rarely respected, but these varieties are nevertheless preferred because they guarantee a broad genetic base, together with acceptable levels of genetic gain at relatively low cost. The problem of unequal parental contributions can be overcome (together with the consequences of not accounting for environmental effects during seed formation), by mixing seed crops from several years, but this is seldom done in commercial situations. In more advanced schemes, parent-of-family varieties are produced. This involves the use of a limited number of parental clones and artificial crossing (Nanson, 2004).

Finally, various clonal propagation systems (cuttings, somatic embryogenesis) are also available for some species, for the mass production of a few elite genotypes. The gains from elite genotypes are theoretically greater, as they exploit all the genetic variability available and they can be deployed more rapidly, allowing a faster turnover of varieties in some cases, but at the expense of more limited genetic diversity and frequently higher production costs (Lelu-Walter et al. 2013). The success of clonally propagated trees is conditioned by both genetic and non-genetic effects, such as topophysis and cyclophysis (Nanson, 2004), and more broadly by epigenetic marks during zygotic and somatic embryogenesis, with the potential to modify tree behavior in forests (Kvaalen & Johnsen, 2008).

Here, epigenetics is defined as meiotically or mitotically heritable changes that alter phenotype without any changes in the DNA sequence (Russo, Martienssen, & Riggs, 1996; see Chapter 1 of this book). There are different epigenetic modifications that affect the chromatin structure and therefore gene expression or transposable elements mobilization such as DNA methylation, histones modifications or variants and non-coding RNA (see Chapter 2 of this book). Epigenetics is a fundamental mechanism controlling development and response to environmental variations as well as a tool of choice opening perspectives for forest tree

management and breeding notably in the context of actual climate change.

2. FOREST TREE GENOMICS TO SUPPORT EPIGENOMIC RESEARCH

2.1. Sequenced Genomes

All epigenome studies require high-quality genome sequence assemblies. The first forest tree for which a whole-genome sequence was obtained was *Populus trichocarpa* in 2006 (Tuskan et al. 2006), followed, years later by *Eucalyptus grandis* (Myburg et al. 2014). Both species have relatively small genomes (<500 Mb). With advances in NGS technologies, the number of whole-genome sequences available for forest trees has steadily increased (Table 1), although the sequences available remain a drop in the ocean of the 60,000 species of trees in the world. Their quality, in terms of completeness (proportion of the genome covered) and scaffold contiguity (up to chromosome arm-level), varies considerably, but new technologies for the sequencing of large single molecules (Daemer et al., 2016) and new approaches improving scaffolding (Jiao et al. 2017), together with new algorithms capable for assembling separate haplotypes in highly heterozygous species, will clearly help to improve forest tree genome assemblies, including those for conifers, which have particularly large genomes (Mackay et al. 2012). Temperate tree species are quite well represented in the list of newly sequenced tree genomes, with representatives of large botanical families, such as Fagaceae (e.g. oak), Salicaceae (e.g. willow); Betulaceae (e.g. birch), Oleaceae (e.g. ash) and Pinaceae (e.g. pine, spruce). By contrast, progress has been slower for other highly diverse tropical forest tree species (but see Silva-Junior, Grattapaglia, Novaes, & Collevatti, 2018), despite their key role in biodiversity and the multiple ecosystem services they provide to humanity, including the supply of food (Daru, Berger, & Wyk, 2016).

Once reference genomes have been established and made publicly available (Neale, Langley, Salzberg, & Wegrzyn, 2013), they can be used to explore a number of fundamental research questions (reviewed in Holliday et al. 2017; Plomion et al. 2016):

- In functional genomics, they help improving our understanding of the ways in which trees develop and respond to biotic interactions and abiotic constraints (plasticity)

- In population genomics, for studies of population diversification, adaptation to local conditions, speciation and to the capacity of species to survive environmental change. In this context, decoding the epigenetic landscape will provide indispensable insight, improving our understanding of forest tree development and adaptation
- In comparative genomics, in which they provide insight into chromosome evolution in terms of past polyploidy events, tandem duplications and the diversification of biological functions. Here, the functional consequences of the macro-evolutionary history of epigenetic signatures has yet to be studied
- In quantitative genetics, to shed light on the consequences of genetic diversity in terms of phenotypic variability. Here, we need to determine the extent to which epigenetic variation accounts for the phenotypic variation of relevant traits and contributes to the debate concerning the causal factors for "*missing heritability*" (i.e. the fact that single genetic variants cannot account for much of the heritability of quantitative traits).

These genomic resources also offer opportunities for converting whole-genome sequences into practical tools for breeding and conservation programs. For instance, high-quality genome sequences can be used to establish an inventory of genetic (SNPs, CNVs, indels) and epigenetic (DNA methylation, chromatin conformation state) variants. These inventories can be used to determine the links between (epi)genotypes and phenotypes, and to develop genomic prediction strategies (Bartholomé et al. 2016; Grattapaglia, 2017) to increase genetic gain per unit time. Another foreseeable impact of enriching the inventory of variants is that it should provide new knowledge concerning neutral and adaptive polymorphisms to facilitate the conservation of threatened species (McMahon, Teeling, & Höglund, 2014), a major endeavor considering the rate at which forest tree species can become extinct (ter Steege et al. 2015).

2.2. Genome Evolution and Potential Role of Epigenetic Modifications

The access to tree genome sequences in recent decades has made it possible to compare tree evolution with the evolution of other plant genomes. However, of the forest tree genomes available and listed in Table 1, only those well assembled into pseudomolecules, such as those of oak, poplar, eucalyptus and birch from the angiosperm (flowering plants) clade are

suitable for genome-scale comparative genomics and ancestral genome reconstruction (Fig. 1). Angiosperms arose some 120 to 170 million years ago (mya), and diverged 140–150 mya into monocots and eudicots, the two largest and most diverse groups, accounting for 20% and 75% of modern angiosperm species, respectively (Soltis, Bell, Kim, & Soltis, 2008). It has been suggested that the ancestral angiosperm karyotype consisted of a potential repertoire of 22,899 ancestral genes conserved in present-day species and dating back to 190–238 mya, which evolved into the ancestral monocot karyotype (AMK, with 5 protochromosomes and 6707 ordered protogenes) and the ancestral eudicot karyotype (AEK, with 7 protochromosomes and 6284 ordered protogenes), providing an integrative view of the evolution of angiosperm genomes (Murat, Armero, Pont, Klopp, & Salse, 2017). Oak, poplar, eucalyptus and birch evolved independently from the AEK over the last 150 million years. The AEK experienced an ancestral whole-genome triplication (γ) common to all modern eudicots, to reach a post-γ AEK of 21 chromosomes (Salse, 2016a). Grape (Vitales subfamily) is the rosid genome most closely resembling the AEK, with 19 modern chromosomes (Murat et al. 2015). Eucalyptus (Myrtales subfamily) experienced a lineage-specific duplication followed by 69 chromosome fissions and 79 chromosome fusions, which shaped the 11 modern chromosomes (Myburg et al. 2014). Poplar (Tuskan et al. 2006) and birch (Salojärvi et al. 2017) emerged from the AEK through a common lineage-specific duplication with a single large chromosomal arrangement involving chromosomes 1 and 16 that differentiates between poplar and birch, consistent with a translocation event between these two genomic fragments (either from chromosome 1 to 16 in birch or from chromosome 16 to 1 in poplar) rather than independent fusions of ancestral chromosomes (on chromosome 1 in poplar or 16 in birch). Finally, it has been suggested that the oak genome has undergone five fissions and 14 fusions since the AEK to achieve its current genome of 12 chromosomes (Plomion et al. 2018). According to this scenario, oak has experienced no lineage-specific whole-genome duplication in addition to the shared ancestral triplication (γ), whereas birch, poplar and eucalypt have experienced an additional duplication. Thus, for any given AEK gene, a single gene would be expected in oak, corresponding to pairs of genes in birch, poplar and eucalyptus (Fig. 1). Such tree species are thus crucial for addressing the role of duplications in promoting the structural and functional partitioning of duplicated genes following polyploidization events (Salse, 2016b).

DNA methylation has been shown to play an important role in duplicate gene evolution in soybean (El Baidouri et al. 2018), wheat (Shaked, Kashkush, Ozkan, Feldman, & Levy, 2001), spartina (Salmon, Ainouche, & Wendel, 2005), rice (Wang et al. 2017c), *Arabidopsis* (Wang et al. 2017b), Brassicaceae (Chen et al. 2015) and cassava (Wang et al. 2015). Differences in the methylation of duplicate genes are involved in the regulation and maintenance of these genes (Keller & Yi, 2014). Greater functional divergence of gene duplicates has been reported in mammals and plants (*i.e.* organisms that have cytosine methylation) as compared to yeast, nematode and flies (*i.e.* organisms that do not have methylated genomes; Rodin & Riggs, 2003). DNA methylation may play a major role in (1) activating transposable elements, leading to insertional mutagenesis and changes in local patterns of gene expression (see Chapter 5), and (2) silencing one copy or driving organ-specific up- or downregulation of one of the duplicated genes. This may result in unequal expression of the duplicates, facilitating rapid genomic reorganization and the stabilization of the newly formed polyploid, contributing to correct meiotic pairing and isolation of the polyploid from its progenitors, increasing evolutionary potential (Paun, Fay, Soltis, & Chase, 2007). Overall, epigenetic regulation of duplicated genes may be a central phenomenon to understand the impact of polyploidization events in plant survival or adaptation to environmental constraints. Research projects targeting post-polyploidization epigenetic plasticity are needed to be accurately used in breeding programs or conservation practices, especially for perennial plant species such as trees that have to face recurrent biotic and abiotic constraints during their long life span.

3. STATE OF THE ART IN FOREST TREE EPIGENOMICS

3.1. Methods and Recent Advances in Epigenomics

Second/next generation sequencing (NGS) has revolutionized the way of interrogating the epigenomes. NGS-based technologies have largely replaced microarrays as the read-out platform for DNA methylation analysis enabling a count-based absolute quantification of DNA methylation levels and current NGS platforms provide robust and high-quality results. The main approaches for the discrimination of methylation are based on three main principles (Table 2):

1) The use of methylation-sensitive restriction endonucleases, i.e., enzymes that are blocked by methylated cytosines in their recognition sequence (Bird & Southern, 1978) are widely used for the analysis of methylation patterns in combination with their methylation-insensitive isoschizomers found within the recognition sequences of restriction enzymes (Fazzari & Greally, 2004). Information complementary to that obtained by methylation-sensitive restriction digests can be obtained by the methylation-dependent restriction enzymes such as *McrBC*, which cleaves between two non-palindromic G/A^{me}C sites (Stewart, Panne, Bickle, & Raleigh, 2000).

2) The methylated fraction of a genome can be enriched by precipitation with a bead-immobilized antibody specific for 5-methylcytosine (Down et al. 2008; Feber et al. 2011) or by affinity purification of methylated DNA with methyl binding domain (MBD) proteins such as MeCP2 (Brinkman et al. 2010) or MBD2 in combination with MBD3L1 (Rauch & Pfeifer, 2005).

3) The most widely used approach consists of the chemical modification of genomic DNA with sodium bisulfite. This chemical reaction induces hydrolytic deamination of non-methylated cytosines to uracils, while methylated cytosines are resistant to the conversion under the chosen reaction conditions (Frommer et al. 1992; Shapiro, DiFate, & Welcher, 1974). This method translates the methylation signal into a sequence difference. After performing PCR, the methylation status at a given position is manifested in the ratio of C (former methylated cytosine) to T (former non-methylated cytosine) and can be analyzed as a virtual C/T polymorphism spanning the entire allele frequency spectrum from 0%–100% in the bisulfite treated DNA. It should be noted that standard bisulfite conversion protocols cannot discriminate between 5-hydoxymethylcytosine and 5-methylcytosine, which are converted with a similar efficiency requiring specialized protocols such as oxidative bisulfite sequencing (Booth et al. 2012) or TAB-seq (Yu et al. 2012).

Whole-genome bisulfite sequencing (WGBS) or MethylC-seq can be considered as the current gold standard for the genome-wide identification of differentially methylated cytosines (DMCs) and differentially methylated regions (DMRs) at single nucleotide resolution (Lister et al. 2008; Urich, Nery, Lister, Schmitz, & Ecker, 2015). The whole genome read-out after bisulfite conversion overcomes the limitations of cloning and Sanger sequencing, in which the quantitative resolution was limited

by the number of clones analyzed (in most studies < 20), or pyrosequencing limited to a small number of loci of interest. Several protocols have been devised to reduce the loss of material, adding sequencing adaptors through transposition rather than ligation (Adey & Shendure, 2012) or adding these only after bisulfite treatment, which leads to a substantial loss of library complexity using standard protocols (Miura, Enomoto, Dairiki, & Ito, 2012).

These and other technical improvements allow now the comprehensive methylome analysis from limited amounts of cells (Daviaud, Renault, Mauger, Deleuze, & Tost, 2018), or even single cells (Kelsey, Stegle, & Reik, 2017). However, the unprecedented quantitative and spatial resolution that is currently transforming DNA methylation analysis still comes at a high cost requiring substantial sequencing to obtain a proper and even coverage, and requires specialized bioinformatic expertise and resources. Noteworthy, as only part of the analyzed sequences will contain potentially methylated cytosines, a number of strategies have been devised to concentrate the analysis on parts of the genome including Reduced Representation Bisulfite Sequencing (RRBS, Meissner et al. 2005), methylation-sensitive enzymes (MRE-seq; Maunakea et al. 2010) or affinity based approaches using methyl-binding proteins or antibodies (Brinkman et al., 2010; Jung et al., 2015; Taiwo et al., 2012; see also Tost, 2016 for a detailed review). However, these do not necessarily allow the analysis of all regions of interest, due to the absence of restriction-enzyme recognition sites in the size range which is commonly sequenced (RRBS, MRE-seq) or do not provide base-resolution data and only relative quantification of DNA methylation levels (MeDIP/MBD).

Alternative approaches have been developed to focus DNA methylation analysis together with a supposed biological function (i.e. expressed or silenced regions of the genome) making use of the presence of other epigenetic modifications such as histone modifications or open chromatin (Lafon-Placette et al. 2013; Statham et al. 2012). Another now widely used approach captures regions of interest with complementary oligonucleotides similarly to standard exome sequencing analyzing up to 8 M CpGs (Walker et al. 2015; Wendt, Rosenbaum, Richmond, Jeddeloh, & Burgess, 2018). Capture can be made prior or after the bisulfite conversion with the first approach making use of the increased complexity of the genome prior to bisulfite conversion, but prohibiting amplification prior to the capture, while the second approach needs to accommodate a much larger number of probes to capture molecules with

different methylation patterns but permitting DNA amplification prior to capture. If fewer regions of interest need to be interrogated, a number of multiplexed amplification systems have been devised enabling the amplification of up to several hundred regions of interest in parallel (Diep et al. 2012; Paul et al. 2014), which are subsequently analyzed on benchtop NGS sequencers. For locus-specific DNA methylation analysis Sanger sequencing (Frommer et al. 1992), Pyrosequencing (Tost & Gut, 2007), MALDI mass spectrometry (Ehrich et al. 2005), methylation-specific PCR (Herman, Graff, Myohanen, Nelkin, & Baylin, 1996) and its real time variations (Eads et al. 2000; Tost, 2016) have been most-widely used methods, but are also more and more replaced by a NGS-based amplicon sequencing. Benchtop sequencers (MiSeq or PGM) allow generating high levels of coverage (100's-1000's x) that yield precise measurements of the quantitative levels of cytosine methylation. In addition, due to the sequencing of clonal clusters, these methods provide co-methylation patterns on individual molecules within the limits of the length of the reads. With a current output of ~50 M reads for the MiSeq, yielding between 3.8 and 15 GB of sequence depending on the used sequencing kit, several tens to hundreds of target regions can be analyzed simultaneously depending on the desired coverage and number of samples analyzed in parallel. Their short run time, relatively low running costs and wide availability make them a valuable alternative for targeted DNA methylation analysis. A recent large-scale evaluation study including the most widely used methods for locus-specific DNA methylation analysis demonstrated that technologies have reached a level of technical maturity yielding reproducible and accurate results, with pyrosequencing and bisulfite amplicon-sequencing showing slightly improved performance (Blueprint Consortium, 2016; http://www.blueprint-epigenome.eu/).

New read-out technologies enabling a direct-readout of DNA methylation patterns without the need for chemical conversion and/or amplification such as single-molecule real-time (SMRT) and Nanopore sequencing have been devised. SMRT sequencing is based on different kinetics during polymerization by an immobilized polymerase when encountering methylated bases while for nanopore sequencing single DNA molecules are pulled through a protein or synthetic pore and nucleobase dependent changes in the electric potential of the pore are measured (Clarke et al. 2009; Flusberg et al. 2010; Song et al. 2012). Despite recent improvements in the base calling algorithms for nanopore sequencing (Rand et al. 2017; Simpson et al. 2017), these need to be further optimized, the high

error rate associated with the sequencing, which currently requires sequencing with very deep coverage, needs to be reduced before these technologies can be routinely applied to DNA methylation analysis.

In contrast, few methods are available for the analysis of histone modifications now commonly analyzed by chromatin immunoprecipitation (ChIP (Gilmour & Lis, 1984)) followed by qPCR for the detection and quantification of the analyzed modification at a locus of interest using specific primers or NGS (ChIP-seq) for the genome-wide mapping of an histone modification at very high resolution (Landt et al. 2012).

3.2. Epigenomics in Forest Trees: Role in the Response to Environmental Variations

These last years, the available epigenomic data in forest trees are steadily increasing. These studies focused especially on DNA methylation, histones modification and small RNAs. Table 3 summarizes available epigenomic data in forest tree species for DNA methylation, histones modification and small RNAs. For DNA methylation we chose to not include studies only focusing on global DNA methylation level (Plomion et al. 2016). Although miRNAs are mitotically and meiotically hereditable factors that control gene expression without changes in the DNA sequence, they are classically not included in epigenetic mechanisms. However, growing evidences, on cancer studies, show their substantial role in the control of several epigenetic mechanisms. Specifically, miRNAs regulate at the post-transcriptional level many epigenetic-related-genes, a subgroup of miRNAs, epi-miRNAs, can regulate the expression of effectors of the epigenetic mechanisms by directly or indirectly targeting epigenetic-modifying enzymes and molecules or can act in the nucleus by stimulating or repressing genes transcription in a manner strictly correlated to the chromatin state (Ramassone, Pagotto, Veronese, & Visone, 2018; Xia, Guo, & Deng, 2014). According to this, it is possible to consider miRNAs in an enlarged view of mechanisms associated to epigenetic processes. For these reasons, we referred to some miRNAs studies in trees in the Table 3. Few of the epigenomic studies are developed thereafter as examples showing the growing evidences for the role of epigenetics in the response of trees to environmental variations.

3.2.1. Epigenetics and Developmental Transitions During the Annual Cycle

Many studies have reported changes in the developmental stages of cells, tissues or organs from different organisms accompanied by global changes in chromatin epigenetic marks (Kumar, Kumari, Sharma, & Sharma, 2013). These observations suggest that switches in the epigenetic state of chromatin generate a new landscape for gene expression that is required to promote entry into a new developmental stage and has led to research to characterize the role of chromatin remodeling in plant development over the entire life cycle (Liu et al. 2015).

In cold and temperate regions, the annual growth cycles of trees are tightly linked to the succession of environmental conditions during the course of the seasons. The decrease in day length during the fall causes a cessation of growth and induces bud set in several tree species, including poplar. The first stage of this process is called ecodormancy. Greater exposure to short days and low temperatures later in the year induces endodormancy. In several tree species, such as apple, low temperatures alone are sufficient to induce dormancy. Once in endodormancy, the apical meristem and leaf primordia, which are already protected by buds, become insensitive to growth-promoting signals and cell division is stopped by endogenous factors that remain poorly understood. Many species have a chilling requirement to break dormancy, a need for exposure to low, non-lethal temperatures. Once dormancy is released, growth is halted purely by environmental factors, such as low winter temperatures, in particular. An increase in temperature during the spring promotes bud burst and restores growth. Changes in the epigenetic profile of chromatin over the course of the annual cycle have been observed in several tree species. In chestnut, for example, total DNA methylation increases during bud set in the fall and decreases during bud break in the spring (Santamaría et al. 2009). Furthermore, acetylated histone 4 (AcH4) levels in the apical bud are higher during bud break than during bud set (Santamaría et al. 2009). In peach, the dormancy regulators DORMANCY-ASSOCIATED MADS-BOX (DAM), DAM1, DAM4, DAM5 and DAM6, are significantly enriched in H3K27me3 during dormancy release relative to the dormant state (de la Fuente, Conesa, Lloret, Badenes, & Ríos, 2015). H3K27me3 is a chromatin mark associated with stable gene silencing in this situation. In poplar stems, DNA methylation levels are higher and AcK8H4 levels are lower during winter dormancy

than during active growth (Conde, González-Melendi, & Allona, 2013). Moreover, DNA methylation is highly restricted to the central zone of the poplar SAM (shoot apical meristem) during the active growth period, whereas methylation is widespread throughout the SAM and in other bud regions during growth cessation under short days (Conde et al. 2017b). In poplar, the transition from dormancy to the reactivation of cell division in the SAM is accompanied by an initial increase, followed by a gradual decrease in global DNA methylation level (Conde et al. 2017a). In apple (*Malus* x *domestica*), Kumar, Rattan, and Singh (2016) found that DNA methylation levels decreased gradually over four developmental stages, from bud dormancy to fruit set: dormant bud, silver tip, green tip and initial fruit set. This dynamic in apple buds occurs only when apple trees are grown in environmental conditions satisfying the chilling requirement for winter dormancy release, as reported for poplar bud break (Conde et al. 2017a; Kumar et al. 2016), highlighting the importance of environmental conditions for the developmental reprogramming of DNA methylation (see Chapters 10 and 11 of this book).

In addition to these changes in epigenetic profile during annual growth cycle of trees, several transcriptomic analyses have highlighted differential patterns of expression for genes involved in chromatin remodeling over the various stages of tree development during the course of the year: in poplar, FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) is rapidly upregulated following the perception of short days (Ruttink et al. 2007). FIE is part of t POLYCOMB REPRESSION COMPLEX 2 (PRC2), which is involved in the H3K27m3 methylation. Tylewicz et al. (2018) used a transgenic approach to analyze the function in poplar of PICKLE (PKL), a chromatin remodeling antagonist of PRC2 identified in *Arabidopsis*. They showed that PKL was downregulated by abscisic acid (ABA) under short days, and required for plasmodesmata closure and bud dormancy establishment in poplar (Fig. 2). Shim et al. (2014) identified five genes involved in DNA methylation that were differentially expressed at different developmental stages of winter dormancy in poplar stems: two poplar homologs of *Arabidopsis* METHYLTRANSFERASE 1 (MET1), a homolog of CHROMOMETHYLASE 3 (CMT3) and two DEMETER-like (DML) genes. One poplar DML gene was upregulated when growth ceased at the onset of winter dormancy, whereas the other was more strongly expressed during the exit from dormancy after the chilling requirement had been fulfilled.

Based on the changes in DNA methylation and in the expression of genes involved in the establishment or removal of this epigenetic mark over the annual cycle in poplar, Conde et al. (2017a and 2017b) identified and characterized the role of DMLs in trees. One DML induced by short days and low temperatures in poplar and chestnut participates in apical bud formation during autumn, by activating flavonoid biosynthesis enzymes. Flavonoids accumulate in bud scales and the SAM during the onset of winter dormancy, and are required for the survival of the apical meristem under harsh winter conditions (Conde et al. 2017b) (Fig. 2). Poplar transgenic lines with a reduced expression of PtaDML10 showed a delay in bud burst after winter dormancy release, compared to wild type. Genome-wide transcriptome and methylome analysis and data mining have identified gene targets for the active DML-dependent DNA demethylation associated with bud break (Conde et al. 2017a). These data suggest that DEMETER-like mediated DNA demethylation is involved in an environmentally induced developmental stage transition, during the shift from winter dormancy to vegetative growth of the shoot apex in poplar (Fig. 2).

In summary, there is growing evidence to suggest that chromatin remodeling changes are important during the transitions between developmental stages in the annual growth cycle of trees annual developmental stages transitions. The small number of functional characterizations carried out to date has led to the identification of chromatin remodelers involved in both the establishment of bud dormancy in the fall and bud break in the spring. Further studies are required to determine the mechanisms by which the epigenetic state of chromatin senses the environmental conditions under which the tree is growing, throughout its life cycle.

3.2.2. Epigenetics as a Mediator of Phenotypic Plasticity in Response to Environmental Variations

Phenotypic plasticity is defined as the ability of a genotype to express different phenotypes in different environments (Bradshaw, 2006). Significant progress has been made towards understanding the molecular mechanisms underlying phenotypic plasticity (Kelly, Tami, & Andrew, 2012), but the role of epigenetics in this process remains unclear, despite recent findings suggesting that it is of great importance (Bossdorf, Richards, & Pigliucci, 2008; Bräutigam et al. 2013; Herrera & Bazaga, 2013; Lira-Medeiros et al. 2010; Zhang, Fischer, Colot, & Bossdorf, 2013). Using *Arabidopsis thaliana* epigenetic recombinant inbred lines

(i.e. differing only in their level of DNA methylation), Kooke et al. (2015) showed that traits such as leaf area, flowering time, and rosette branching display variable plasticity under neutral and saline conditions, suggesting that the variation of DNA methylation may play a key role in phenotypic plasticity. The capacity of the epigenetic marks (DNA methylation, histone modifications, etc.) to alter genes expression and transposable elements mobility could lead to deep physiological modulation affecting plant fitness.

In forest trees, epigenetic mechanisms may play a crucial role in survival and adaptation under stressful conditions, by mediating phenotypic modifications with beneficial effects in response to the environment. However, it remains difficult to separate the genetic and epigenetic components underlying phenotypic modifications. Many tree species propagate easily through clones, and this feature can be exploited in artificial conditions, such as breeding programs, for dissemination. Clonal propagation opens up possibilities for unravelling the epigenetic basis of phenotypic plasticity. Only a few studies investigating the role of epigenetics in tree phenotypic plasticity have been published, almost certainly due to the lack of fully sequenced genome for trees. In 2010, Gourcilleau et al. investigated a possible link between epigenetics and the plasticity to drought stress response in poplar, and were able to establish correlations between morphological and epigenetic variables. In particular, they found a positive correlation between DNA methylation levels and biomass productivity under well-watered conditions only. Recently, correlations between DNA methylation levels and biomass productivity have been confirmed on a collection of poplar genotypes grown outdoors in distinct water availability conditions (Le Gac et al. 2018). In the same model species, Raj et al. (2011) found differences in transcript abundance levels under drought stress that were probably linked to the geographic origin and correlated with DNA methylation level, suggesting that DNA methylation may modulate gene expression, leading to phenotypic variation under environmental stress. Recently, Lafon-Placette et al. (2018) have shown when comparing the genome wide distribution of differentially methylated regions (DMRs) to the differentially expressed genes (DEGs) that variations in soil water availability induced changes in DNA methylation levels preferentially at genes involved in phytohormones metabolism and signaling, potentially promoting phenotypic plasticity. Sáez-Laguna et al. (2014) showed epigenetic variability in stone pine, which is characterized by very low levels of genetic variation and

high levels of plasticity for many traits. Using epigenetic markers, they were able to discriminate between two populations that were indistinguishable with classical molecular markers. They concluded that the high level of phenotypic plasticity found in stone pine trees, was probably linked to epigenetic variations between individuals or populations.

However, despite the many observations made in these studies, the relationship between epigenetics and phenotypic plasticity in trees remains underexplored. The main challenges for the coming years relate to the role of epigenetics in modulating the phenotype of forest trees under ongoing climate change and the stability of this environmentally-induced plasticity between individuals and across generations.

3.2.3. Epigenetics and Environmental (Stress) Memory

The concept of memory can be defined as the capacity of the organism to benefit from its past experience (Tulving, 1985). In plants, memory, has been linked to epigenetic modifications (Crisp, Ganguly, Eichten, Borevitz, & Pogson, 2016; D'Urso & Brickner, 2016; Latzel, Rendina González, & Rosenthal, 2016; Schönberger, Chen, Mager, & Ludewig, 2016). Indeed, several studies in recent decades have focused on the memory effect in plants and have shown that it is not exclusively dependent on genotype (in terms of the alleles present), but that it results from physiological processes dependent on gene activity (Lauria & Rossi, 2011). In addition to memory, the priming event is followed by a period of stress memory which involves storing information on the priming stress, potentially through an epigenetic phenomenon, and results in a modified response upon a recurring stress or in a sustained response after the priming stress (Lämke & Bäurle, 2017). This memory may last from several days to weeks for somatic stress memory, and in some cases, may even extend to offspring.

In forest trees species, which are characterized by a long life time and complex life cycle, phenological behavior, such as growth, bud phenology and frost hardiness, may be influenced by climatic conditions (Conde et al. 2017a; Johnsen et al. 2005b, 2005a; Johnsen & Skrøppa, 2001; Skrøppa, Tollefsrud, Sperisen, & Johnsen, 2010; Skrøppa & Johnsen, 2000). The best known epigenetic memory in trees is that reported for Norway spruce. In 2005, Johnsen et al. established that differences in day length and temperature during female meiosis affect progeny performance. Cold and short days environment advances bud burst during the spring, whereas high temperature and short days delay bud set

during the summer. Yakovlev et al. (2014) and Yakovlev, Carneros, Lee, Olsen and Fossdal (2016) have identified a temperature-dependent epigenetic memory affecting the timing of bud burst and bud set in trees generated by temperature changes during somatic embryogenesis (Carneros et al. 2017; Yakovlev & Fossdal, 2017). Yakovlev, Fossdal, and Johnsen (2010), Yakovlev et al. (2012 and 2016) subsequently showed that this temperature-dependent "epigenetic memory" may be accounted for in part by the differential expression of specific microRNAs (miRNAs). As already mentioned (see Section 3.2), if miRNAs are not strictly classified in epigenetic mechanisms, growing evidences of their interactions with epigenetic mechanisms propose that it could be relevant to include them in an enlarged view of mechanisms controlling epigenetic phenomenon (Ramassone et al., 2018; Xia et al. 2014) …

Similar epigenetic effects have been found in other species, such as white spruce, Scots pine, longleaf pine and *Larix* spp (Dormling & Johnsen, 1992; Greenwood & Hutchison, 1996; Schmidtling & Hipkins, 2004; Stoehr, L'Hirondelle, Binder, & Webber, 1998; Webber, Ott, Owens, & Binder, 2005). In poplar, Schönberger et al. (2016) showed that, despite their clonal origin, stem cuttings derived from different sites (with different amounts of phosphorus) established differently in a common environment. Cuttings derived from the site with the lowest amount of phosphorus grew in a manner suggesting a memory of the original environment. These differences in behavior were associated with different levels of methylation and amounts of miRNAs as a function of previous habitat, modifying the roots in a habitat-dependent manner under conditions of phosphate starvation, suggesting that these epigenetic mechanisms may account for habitat or seasonal memory in perennials. Le Gac et al. (2018) reported the existence of an environmental epigenetic memory in shoot apical meristems (SAMs) of *Populus* × *euramericana* trees in field conditions. SAMs are the center of shoot morphogenesis, undergoing constant cell division. It therefore seems likely that these meristematic cells are the best candidates for the transmission of environmental variants to the newly formed organs (leafs, stems, etc.), leading to phenotypic plasticity (Fig. 3) (Gourcilleau et al. 2010; Lafon-Placette et al. 2013, 2018). Using trees grown in common gardens under drought or from different sites with a pedoclimatic gradient, Le Gac et al. (2018) showed that winter-dormant SAMs express an epigenetic memory of the drought stress they experienced during vegetative growth. They identified DMRs conserved in different experimental set-ups (greenhouse,

common garden, field conditions) with similar environmental constraints (water availability), and overlapping with genes most strongly related to the stress response, suggesting a potential role in priming.

Despite these important studies, there is still a long way to go before the underlying mechanisms of epigenetic memory in long-lived forest trees species are fully understood. However, progress in NGS technologies and CRISPR-cas9 should rapidly enhance future investigations. The elucidation of epigenetic memory in forest trees species will provide a valuable resources for the adaptation of trees to local environments, because this memory effect can persist for several years under field conditions (Skrøppa, Kohmann, Johnsen, Steffenrem, & Edvardsen, 2007). Indeed, memory could be a good tool for successful dispersion of genetically heterogeneous cohorts in stable conditions, in the sense that imprint could prepare and "correct" new seed for the conditions that prevail at the parents' place, smoothing out different allelic setups. In that sense, memory would prevent natural selection from weeding out all genetic variation, and preserving extra amounts that may become useful in future conditions. It is important to note that the stability of epigenetic modifications, which is still under investigation, is a crucial issue for breeding perspectives (see part 4. of the present chapter; Achour et al. 2017).

Most studies on local adaptation have focused on genetic variation (SNP, CNV, etc.). There is currently no concrete evidence for an involvement of epigenetic mechanisms in this process of local adaptation, but several studies have suggested that epigenetics may explain some of the phenotypic differentiation between populations that cannot otherwise be explained by DNA polymorphisms (Gugger, Fitz-Gibbon, PellEgrini, & Sork, 2016; Platt, Gugger, Pellegrini, & Sork, 2015; Skrøppa et al. 2010; Sork, 2016, 2017; Verhoeven, von Holdt, & Sork, 2016). It has been suggested that epigenetics can modulate plant fitness under environmental constraints (Bewick & Schmitz, 2017; Bräutigam et al. 2013; Kooke et al., 2015; Kawakatsu et al. 2016; Lande, 2009; Meyer, 2015; Seymour & Becker, 2017). This was interesting suggesting that genetics (allelic variation) is not the only source of phenotypic variation between individuals in a population or species. There is also evidence for a link between genetic and epigenetic mechanisms or for genetic control over epigenetic variation (Becker et al. 2011; Dubin et al. 2015). Epigenetic modifications may also generate genetic variants, such as SNPs (deamination of 5 mC to yield T) or CNV (reactivation of transposable elements (TEs) leading to new insertions elsewhere in the genome). Platt et al.

(2015) recently showed, in a comparison of SNPs (single-nucleotide polymorphisms) and SMPs (single-methylation polymorphisms), that population differentiation levels were lower for SNPs in loci without SMPs than for those in loci with methylation polymorphisms. One explanation could be stabilizing selection against locally driven methylations. Plant adaptation is frequently associated with changes in DNA sequence, but it is becoming increasingly evident that changes in epigenetic state (mostly changes in DNA methylation level) under environmental constraints can lead to the reactivation of certain TEs, which may then insert next to coding regions, causing a stable silencing of the genes concerned that is transmissible across generations (Horváth, Merenciano, & González, 2017; Wang et al. 2017a; Zheng et al. 2017). Such epigenetic mechanisms represent a rapid source of adaptation since naturally occurring mutations are very rare (Becker et al. 2011). Indeed, epimutation rate has been estimated at 3.10^{-4} (van der Graaf et al., 2015) which is four times higher than the substitution rate estimated at 7.10^{-9} in *A. thaliana* for example.

Interestingly, Platt et al. (2015) showed, in natural oak tree populations in southern California, that changes in DNA methylation, particularly at CpG sites, are strongly associated with local adaptation, either directly or through linkage to regions under selection. However, no relationship was found between methylation in a CHG context and adaptation. Methylation in the CpG context seems to be more frequent than non-CG methylation in the genes of diverse species, suggesting that CpG methylation may have a higher adaptation potential, due to its tight association with the coding regions subjected to selection. Indeed, Gugger et al. (2016) identified several climate-associated methylation variants that tend to occur in or near genes, highlighting the potential importance of DNA methylation in the CpG context as a determinant process for the plant response to environment leading to local adaptation in natural populations.

Further studies are required to characterize clearly the role of epigenetics in local adaptation. Improvements in our understanding of the role of epigenetics in phenotypic plasticity and memory will be essential for this. Yakovlev et al. (2010, 2012 and 2016) demonstrated the existence of a temperature-dependent "epigenetic memory" during embryo development in Norway spruce that regulates bud set and cold acclimation. This epigenetic memory was found to be of ecological significance, as it

reproduced the differences observed between latitudes in natural conditions (Skrøppa et al. 2010).

4. POTENTIAL USES OF EPIGENETICS IN FOREST TREE BREEDING

4.1. Epigenetic Diversity (Natural or Induced) in Tree Clonally-Propagated or Seed Conservation

Plants have two types of propagation strategy: sexual and asexual. Sexual reproduction is a natural process generating seeds that give rise to plants not genetically identical to the parent plant. By contrast, asexual propagation (division, grafting, air-layering, cuttings, somatic embryogenesis, etc.) is frequently used for forest trees, as it maintains the identity and performance of the parent tree in the context of tree breeding. The clonal propagation of some forest trees may limit their genetic diversity, potentially increasing the importance of epigenetic diversity for adaptation. However, many questions remain unanswered and will be of increasing relevance in the coming years. Is epigenetic diversity fine-tuned by environmental cues? Will it be sufficient to cope with ongoing climate changes?

Forest decline due to drought in conditions of heat stress has already been observed in various regions of the world (Allen et al. 2010). Epigenetics is emerging as a key driver of phenotypic plasticity and adaptation, because epigenetic mechanisms can be induced in a reversible manner by environmental conditions. Several studies have investigated the role of epigenetics in clonally propagated trees (Ausin et al. 2016; Carneros et al. 2017; Guarino, Cicatelli, Brundu, Heinze, & Castiglione, 2015; Klimaszewska et al. 2009; Liang et al. 2014; Schönberger et al. 2016; Teyssier et al. 2014; Vining et al. 2013; Yakovlev, Carneros, Lee, Olsen, & Fossdal, 2016). Epigenetics has been shown to provide an additional layer of regulation for various biological process in clonally propagated tree, which may help clonal trees with limited genetic diversity to modulate their phenotype under changing conditions (Cicatelli et al. 2014; Fresnedo-Ramírez, Chan, Parfitt, Crisosto, & Gradziel, 2017; Guarino et al. 2015; Lafon-Placette et al. 2018; Liang et al. 2014; Schönberger et al. 2016; Shuai, Liang, Zhang, Yin, & Xia, 2013). There is also finding suggesting that embryo development and seed maturation may be the sensi-

tive periods for stable epigenetic modification (Carneros et al. 2017; Yakovlev et al. 2016).

Somatic embryogenesis is an emerging technique for obtaining embryos from somatic or embryonic cells. It is emerging as a powerful tool for studying molecular and physiological mechanisms during embryo development (Lelu-Walter et al. 2013, 2006; Trontin, Klimaszewska, Morel, Hargreaves, & Lelu-Walter, 2016). It is also widely used for the multiplication of elite individuals as part of forest improvement and deployment programs. Plant regeneration via somatic embryogenesis occurs in five steps: initiation, multiplication, maturation, germination and acclimation. Given the suggested sensitivity of embryo development as a period for stable modification, this technique of clonal propagation is a valuable tool for studies of the role of environmental constraints during early plant life. The modification of the annual growth cycle reported in Norway spruce (see Section 3.2.3) due to stable epigenetic changes in the early life of the plant opens up new possibilities for tree deployment. Indeed, somatic embryogenesis could be used to produce trees better able to acclimate to the anticipated global warming. Another advantage of somatic embryogenesis is that the embryogenic masses obtained after the multiplication phase can be cryopreserved in liquid nitrogen (Lelu-Water et al. 2006). This makes it possible to store veritable banks of individuals for years, a major advantage over seed banking.

Seed banking and conservation are used to prevent the total extinction of species due to climate change. Seeds are stored at low temperatures, to maintain them in a dormant state until their use for replanting. However, seed conservation has been reported to lead to yield losses. Little is known about the possible effects of epigenetics on seed storage (Michalak et al. 2013, 2015; Plitta et al. 2014; Plitta-Michalak et al. 2018). For instance, in *Pyrus communis*, Michalak et al. (2013) detected epigenetic changes in severely desiccated seeds, potentially reflecting a specific response of orthodox seeds to drying. Senescence and aging in recalcitrant *Quercus robur* seeds were also correlated with a decrease in global DNA methylation levels, suggesting that epigenetic mechanisms may play a key role in seed viability and should be assessed to improve seed storage practices (Michalak et al. 2015; Plitta-Michalak et al. 2018).

4.2. Epigenetic Component of Heterosis and Hybrid Homeostasis

Hybrid vigor or heterosis is defined as a superior performance of hybrids, for many traits, relative to their parental inbred lines (see Chapter 8 of this book). Hybrids have been widely used in crop and tree breeding since ancient times, although the molecular mechanisms underlying heterosis have yet to be fully elucidated. Various genetic concepts have been put forward to account for this phenomenon: dominance, overdominance and epistasis. Genetic differences between parental inbred lines have long been considered as a determinant of performance when creating hybrids, but it has recently been shown that hybrids may outperform their parents even if the parental genomes are almost identical (Groszmann et al. 2011; Meyer, Törjék, Becher, & Altmann, 2004). This finding raises the interesting question of the origin of this heterosis. In theory, heterosis involves the creation of new genotypic combinations, altering the gene expression profile of the progeny. However, epigenetic mechanisms can alter gene expression without altering the DNA sequence, and there is growing evidence for a role of epigenetic factors in heterosis (Dapp et al. 2015; Groszmann, Greaves, Fujimoto, James Peacock, & Dennis, 2013; Schnable & Springer, 2013). DNA methylation and small RNA-mediated mechanisms are the most epigenetic processes most frequently associated to heterosis (Chodavarapu et al. 2012; Gao et al. 2014; Kawanabe et al. 2016; Lauss et al. 2018; Shen et al. 2017; Zhang et al. 2016).

The importance of these epigenetic mechanisms for heterosis was demonstrated in *Arabidopsis,* in which the inhibition of DNA methylation (5-azacytidine) or the disruption of an RNA-dependent DNA methylation process neutralized heterosis in hybrids (Shen et al. 2012). Lauss et al. (2018) recently suggested that locus-specific epigenetic divergence between the parental lines can directly or indirectly trigger heterosis independently of genetic changes. Very few studies have been published investigating the role of epigenetics in the heterosis of forest trees. In 2014, Gao et al. investigated the role of DNA methylation changes in *Populus deltoides* hybrids from intraspecific parental lines (i.e. with similar genetic backgrounds and different levels of DNA methylation). They found that hybrids outperformed the parental lines for most of the traits studied, and had higher levels of DNA methylation. They suggested that the methylation patterns of the parental inbred lines were passed on, at

least in part, to the progeny, and that these hybrids had non-additive levels of methylation, suggesting a possible key role of DNA methylation in the heterosis of forest trees. Further studies are required to characterize hybrid vigor in trees in more detail. For the moment, it is clear that epigenetic variations, such as decreases in DNA methylation during homologous recombination, can lead to the reactivation of TEs, which can then insert into new regions of the genome, creating epialleles. Environmental stresses may trigger the generation of these epialleles, which create epigenetic diversity, with the potential to improve the performance of phenotypic traits in the progeny. Thus, in the face of ongoing change, heterosis is a tool of choice for the survival of forest trees species in new environmental conditions, at least for those trees for which hybrid breeding programs have been established. The identification of epigenetic markers for trait improvement will help tree breeders to produce plants with higher performances capable of acclimating rapidly to climate change.

4.3. Epigenetic Markers for Trait Improvement and Breeding

Plant breeding programs to date have focused almost exclusively on genetic variation between individuals and populations. Quantitative genetics is generally used merely to explain the genetic basis of variation in complex traits. However, as described here, it is now evident that epigenetics plays a fundamental role in gene expression and transposon silencing leading to phenotypic variation and that epigenetic marks can be inherited by future generations (Law & Jacobsen, 2010; Richards et al. 2017). These epigenetic factors also provide the genome with a certain degree of plasticity, as they can be induced reversibly by environmental conditions. Their extensive involvements in various biological processes and their impact on complex traits have made them essential for population and quantitative genomic studies. In 2014, Cortijo et al. showed that DMRs could account for up to 90% of the heritability of complex traits in *Arabidopsis* epi-mutant lines, suggesting that DMRs could act as quantitative trait loci (epiQTL) potentially used for artificial selection. Indeed, the traditional quantitative genetics methods used to estimate the genetic component of the phenotype could also be used to assess natural epigenetic variations. DNA methylation could therefore be considered as traits, and the estimation of narrow-sense heritability (h^2) or genetic differentiation index ($epiQ_{ST}$), for these marks could help to shed light on the possible relationship between genetic and epigenetic variation and more precisely, on the genetic control of epigenetic variation (Dubin et

al. 2015; Sow et al. 2018). Conversely, epigenetic polymorphisms, such as SMPs (single-methylation polymorphisms) or DMRs could be used as epigenetic markers in genome scan based approaches, to assess the role of epigenetics into population structure and phenotypic variation (epiFST, epiQTL) (Fig. 4). Only few studies based on these epigenetic mapping approaches have been published (Cortijo et al. 2014; Li, Eichten, Hermanson, & Springer, 2014; Long et al. 2011; Zou et al. 2017), and no study has yet reported the use of epigenetic markers for association mapping analyses in forest tree species. However, there is growing interest in the use of these methods in selection. Indeed, marker-assisted selection should no longer focus purely on genetics, but should also consider the interaction between genetics, epigenetics and the environment (Fig. 4). The success of these new integrative approaches will depend principally on the development of powerful statistical models capable of dealing with different variables in multivariate analysis.

4.4. Biotechnology and Epigenome Editing

In recent decades, the development of new tools for genome engineering has opened up new perspectives and helped to decipher the molecular mechanisms underlying different biological process. In *Arabidopsis* and crop plants, the success of genome modification has been well documented and such techniques have been incorporated into breeding programs (Podevin, Davies, Hartung, Nogué, & Casacuberta, 2013). Various tools have recently been proposed for targeting the epigenome. Most of these techniques target genes or mRNAs involved in epigenetic process (readers and erasers of epigenetic marks, chromatin remodelers, etc.). Several studies on *Arabidopsis* have demonstrated the utility of such methods for highlighting the role of epigenetics in important biological process. This is clearly illustrated by the epiRILs (epigenetic recombinant inbred lines) obtained by crossing *A. thaliana ddm1* mutant lines with a wild-type line, leading to the creation of lines differing only in terms of their DNA methylation levels (Johannes, Colot, & Jansen, 2008; see Chapter 4 of this book).

Very few studies on epigenomic engineering in forest tree species have been published. The first hypomethylated trees were generated in 2013. Using an RNAi strategy, Zhu et al. (2013) targeted the homology of the *A. thaliana DDM1* gene in hybrid poplar (*Populus tremula x alba*). This gene encodes a chromatin remodeler that provides DNA methyltransferases with to access to the DNA sequence. It is therefore required

for the maintenance of DNA methylation and heterochromatin assembly. Poplar *ddm1* RNAi lines had lower levels of DNA methylation and a mottled leaf phenotype after a cycle of dormancy. Conde et al. (2017a) used this RNAi strategy in poplar to decrease the expression of *DEMETER-LIKE (DML)* genes. They found that transgenic poplars with *DML8/10* downregulation had significantly higher levels of DNA methylation, which delayed bud break. Conversely, the overexpression of a *DML* from chestnut (*Castanea sativa*) in poplar hybrids resulted in transgenic lines in which bud formation was accelerated (Conde et al. 2017b). No data have yet been published for the knockout of genes implicated in epigenetic processes in forest tree species.

The recent development of new genome editing technologies has opened up new avenues for investigating the role of epigenetics in gene expression, transposon silencing and genome stability. The CRISPR/Cas9 system is emerging as a major technique due to its simplicity, design flexibility and high degree of efficiency and it has been used for the editing of genomes in diverse taxa, from bacteria to eukaryotes (see Chapter 6 of this book). This system has a number of different uses including the suppression or direct insertion at specific loci of a gene, or of a chromosome or gene rearrangement. Few studies involving the use of this technique in forest trees have been published, and none of the existing studies focused on epigenetic marks (Fan et al. 2015; Zhou, Jacobs, Xue, Harding, & Tsai, 2015). Much remains to be done to develop these new advanced technologies for application in forest tree species. The biology of such trees (with their complex life cycle and long generation time) and the strategies used for their propagation (clonal propagation) impose additional constraints, limiting the routine use of these techniques. Although there are still some technical difficulties to be solved, the development of new propagation strategies, such as somatic embryogenesis, should help to overcome some of these limitations, by making it possible to work with embryonic cells, resulting in the correct segregation of transgenes in the final product. The development of these strategies should also make it possible to gain insight into molecular mechanisms for forest tree breeding.

4.5. Integrative Approach, Systems Biology, Statistics, and Modeling

Understanding how tree phenotype is controlled at the molecular level in response to environmental conditions is an important and challenging

task involving studies of the complex interplay between different genetic and epigenetic mechanisms. Modern "-omics" technologies can provide genomic, transcriptomic, and epigenomic data for species growing under different environmental conditions, and opportunities for studying phenotypic plasticity from several perspectives. The integrative statistical analysis of "-omics" data, combining several heterogeneous data sets obtained within a given species, increases study power and provides insight into the interactions between the various mechanisms of molecular regulation determining the phenotype. Studies of the role of epigenetic factors in the context of their relationship to other mechanisms are particularly useful in this respect.

Such analyses are challenging methodologically, because they involve studies of statistical interactions between several high-dimensional "-omics" data sets and the construction of mathematical model for predicting phenotypes from these explanatory data sets taking into account their large numbers of dimensions, different origins and statistical correlation. A recurrent problem in such analyses is that there are large numbers and diverse types of -omics variables for a relatively small number of observations. The following strategies are classically adopted to make it possible to use multivariate statistical models in this context: use of additional knowledge (regularization, Bayesian priors), limitation of the number of variables (variable selection, exploration of candidate models) or modification of data representation through the use of kernel methods or variable compression.

The mathematical tool used depends on the goal of the study and the context. One of the main methods for studying relationships between heterogeneous data sets, such as DNA sequencing, RNA sequencing, and methylation data, for example, is based on mathematical network models. This approach is based on the representation of large biological datasets as networks, with biological entities (e.g. genes) as nodes and edges corresponding to links between these biological entities. If only one type of link is considered (e.g. a transcriptomic link), the resulting network diagram summarizes the system of links of this type between genes (e.g. correlation of expression levels). If several links of different types are considered simultaneously, an integrative network is constructed, representing several layers of cellular information and their interplay in the same network diagram (Fabres, Collins, Cavagnaro, & Rodríguez López, 2017; Wong & Matus, 2017).

Another strategy is required if the goal is to study the link between one or several of the explanatory data sets and the phenotype. For instance, the statistical analysis of epigenetic variation and its relationship to environmental phenotypic plasticity can be performed according to a classical strategy in which the association of each methylation site or region with the considered phenotype is tested separately (Bräutigam et al. 2013; Nicotra et al. 2010). If other explanatory data sets are available, this classical approach may be extended by taking into account influential covariates, such as transcriptomic, genetic, or environmental variables.

Kernels and kernel-based methods are another promising tool for data integration for classification and phenotype prediction. Kernel methods were first proposed as a technique for data integration by Lanckriet, De Bie, Cristianini, Jordan, and Noble (2004). Each dataset is represented by a kernel function, which defines generalized similarity relationships between pairs of entities, such as genes or proteins. The kernel representation is both flexible and efficient, and can be applied to many different types of data. Moreover, several computational algorithms have been developed, making it possible to combine kernel functions derived from different types of data efficiently. Kernel-based methods provide a natural framework for combining variables of different types, as they make it possible to take into account the nature of each type of "-omics" variable and to combine them in multiple kernel methods (Alam, Lin, Calhoun, & Wang, 2017).

5. CONCLUSION

Trees are outstanding organisms in terms of their extreme size, long lifespan, complex life cycles and wood production and forests, play a major role in Earth's ecology, by supplying a range of ecosystem services. In the past decades, widespread forest die-off due to drought and/or heat constraints has been observed around the world for all forest biomes, and is predicted to increase with ongoing climate (Allen et al. 2010). In recent years, epigenetics, that is sensitive to environmental variations, has emerged as a major regulator of various biological processes conferring plasticity to the genome that can be transmitted across generations. However, in forest tree species, there is still a long way to go in to decipher the role of epigenetics in ecological responses particularly in the frame of climate change. Here, we have reviewed the

increasing genomics resources that will support the development of forest tree epigenomics. We have also synthesized the major evidences actually available in forest trees supporting a role of epigenetics in phenotypic plasticity, stress memory and adaptation. Further investments are required to clarify the role and applications of epigenetics in genetic resource conservation, breeding and forest management as for other plants (see other chapters of this book; Achour et al. 2017). Here, we reviewed several potential uses of epigenetics for trees with sexual or clonal propagation (Fig. 5) in the frame of the production of elite individuals, assisted migration and forest deployment programs. Thus, it is now urgent to inform and assist tree breeders and forest managers to include a questioning towards epigenetics in their future program. This is one of the concerns as well as some of the research questions and proposed avenues indicated above that will be addressed within the research project **EPITREE** (**E**volutionary and functional impact of **EPI**genetic variation in forest **TREE**s; ANR-17-CE32–0009–01, 2018–21, https://www6.inra.fr/epitree-project/Le-projet-EPITREE). EPITREE focuses on the evolutionary and functional impact of epigenetic variation in forest trees. Two emblematic trees, poplar and oak, are used in an innovative integrative analysis to assess how genetic and epigenetic variations contribute to phenotypic plasticity and adaptation to local environment.

REFERENCES

Achour, Z., Archipiano, M., Barneche, F., Baurens, C., Beckert, M., Ben, C., et al., 2017. Epigenetics in plant breeding, Article de positionnement du Groupement d'intérêt scientifique Biotechnologies vertes et de l'Alliance nationale de recherche pour l'environnement http://www.gisbiotechnologiesvertes.com/fr/publications/position-paper-epigenetics-in-plant-breeding.

Adey, A., Shendure, J., 2012. Ultra-low-input, tagmentation-based whole-genome bisulfite sequencing. Genome Research 22 (6), 1139–1143.

Alam, M., Lin, H.-Y., Calhoun, V., Wang, Y.-P., 2017. Kernel method for detecting higher order interactions in multi-view data: An application to imaging, genetics, and epigenetics, arXiv preprint arXiv:1707.04368.

Alia, R., Majada, J., 2013. Phenotyping for the future and the future of phenotyping. In: Lee, S., Woolliams, J. (Eds.), Novel tree breeding. PublINIA, Madrid, Spain, pp. 53–62 http://libros.inia.es/libros/download.php?id=709&pdf=products_pdfpreview.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., et al., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259 (4), 660–684. https://doi.org/10.1016/j.foreco.2009.09.001.

Ausin, I., Feng, S., Yu, C., Liu, W., Kuo, H.Y., Jacobsen, E.L., et al., 2016. DNA methylome of the 20-gigabase Norway spruce genome. Proceedings of the National Acad-

emy of Sciences of the United States of America 113 (50), E8106–E8113. https://doi.org/10.1073/pnas.1618019113.

Bagnoli, F., Fady, B., Fineschi, S., Oddou-Muratorio, S., Piotti, A., Sebastiani, F., et al., 2011. Neutral patterns of genetic variation and applications to conservation in conifer species. In: Plomion, C., Bousquet, J., Kole, C. (Eds.), Genetics, genomics and breeding of crop plants science publ. CRC Press, Enfield, NH, USA, Chapter 4.

Barakat, A., Wall, P.K., DiLoreto, S., dePamphilis, C.W., Carlson, J.E., 2007. Conservation and divergence of microRNAs in Populus. BMC Genomics 8 (1), 481. https://doi.org/10.1186/1471-2164-8-481.

Bartholomé, J., Van Heerwaarden, J., Isik, F., Boury, C., Vidal, M., Plomion, C., et al., 2016. Performance of genomic prediction within and across generations in maritime pine. BMC Genomics 17 (1)https://doi.org/10.1186/s12864-016-2879-8.

Baurens, F.-C., Nicolleau, J., Legavre, T., Verdeil, J.-L., Monteuuis, O., 2004. Genomic DNA methylation of juvenile and mature Acacia mangium micropropagated in vitro with reference to leaf morphology as a phase change marker. Tree Physiology 24 (4), 401–407. https://doi.org/10.1093/treephys/24.4.401.

Becker, C., Hagmann, J., Müller, J., Koenig, D., Stegle, O., Borgwardt, K., et al., 2011. Spontaneous epigenetic variation in the Arabidopsis thaliana methylome. Nature 480 (7376), 245–249. https://doi.org/10.1038/nature10555.

Bewick, A.J., Schmitz, R.J., 2017. Gene body DNA methylation in plants. Current Opinion in Plant Biology 36, 103–110. https://doi.org/10.1016/j.pbi.2016.12.007.

Bird, A.P., Southern, E.M., 1978. Use of restriction enzymes to study eukaryotic DNA methylation: I. The methylation pattern in ribosomal DNA from Xenopus laevis. Journal of Molecular Biology 118 (1), 27–47.

Birol, I., Raymond, A., Jackman, S.D., Pleasance, S., Coope, R., Taylor, G.A., et al., 2013. Assembling the 20 Gb white spruce (Picea glauca) genome from whole-genome shotgun sequencing data. Bioinformatics 29 (12), 1492–1497. https://doi.org/10.1093/bioinformatics/btt178.

Blueprint Consortium, 2016. Quantitative comparison of DNA methylation assays for biomarker development and clinical applications. Nature Biotechnology 34 (7), 726–737.

Booth, M.J., Branco, M.R., Ficz, G., Oxley, D., Krueger, F., Reik, W., et al., 2012. Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. Science 336 (6083), 934–937.

Borno, S.T., Fischer, A., Kerick, M., Falth, M., Laible, M., Brase, J.C., et al., 2012. Genome-wide DNA methylation events in TMPRSS2-ERG fusion-negative prostate cancers implicate an EZH2-dependent mechanism with miR-26a hypermethylation. Cancer Discovery 2 (11), 1024–1035.

Bossdorf, O., Richards, C.L., Pigliucci, M., 2008. Epigenetics for ecologists. Ecology Letters 0 (0)https://doi.org/10.1111/j.1461-0248.2007.01130.x, 071117033013002–???.

Bradshaw, A.D., 2006. Unravelling phenotypic plasticity – why should we bother?. New Phytologist 170 (4), 644–648. https://doi.org/10.1111/j.1469-8137.2006.01761.x.

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 (2), 399–415. https://doi.org/10.1002/ece3.461.

Brinkman, A.B., Simmer, F., Ma, K., Kaan, A., Zhu, J., Stunnenberg, H.G., 2010. Whole-genome DNA methylation profiling using MethylCap-seq. Methods 52 (3), 232–236.

Carneros, E., Yakovlev, I., Viejo, M., Olsen, J.E., Fossdal, C.G., 2017. The epigenetic memory of temperature during embryogenesis modifies the expression of bud burst-related genes in Norway spruce epitypes. Planta 246 (3), 553–566. https://doi.org/10.1007/s00425-017-2713-9.

Chen, X., Ge, X., Wang, J., Tan, C., King, G.J., Liu, K., 2015. Genome-wide DNA methylation profiling by modified reduced representation bisulfite sequencing in Brassica rapa suggests that epigenetic modifications play a key role in polyploid genome evolution. Frontiers in Plant Science 6, https://doi.org/10.3389/fpls.2015.00836.

Chodavarapu, R.K., Feng, S., Ding, B., Simon, S.A., Lopez, D., Jia, Y., et al., 2012. Transcriptome and methylome interactions in rice hybrids. Proceedings of the National Academy of Sciences of the United States of America 109 (30), 12040–12045. https://doi.org/10.1073/pnas.1209297109.

Chwialkowska, K., Korotko, U., Kosinska, J., Szarejko, I., Kwasniewski, M., 2017. Methylation sensitive amplification polymorphism sequencing (MSAP-Seq)-A method for high-throughput analysis of differentially methylated CCGG sites in plants with large genomes. Front Plant Science 8, 2056.

Cicatelli, A., Todeschini, V., Lingua, G., Biondi, S., Torrigiani, P., Castiglione, S., 2014. Epigenetic control of heavy metal stress response in mycorrhizal versus non-mycorrhizal poplar plants. Environmental Science and Pollution Research 21 (3), 1723–1737. https://doi.org/10.1007/s11356-013-2072-4.

Ci, D., Song, Y., Du, Q., Tian, M., Han, S., Zhang, D., 2016. Variation in genomic methylation in natural populations of Populus simonii is associated with leaf shape and photosynthetic traits. Journal of Experimental Botany 67 (3), 723–737. https://doi.org/10.1093/jxb/erv485.

Clarke, J., Wu, H.C., Jayasinghe, L., Patel, A., Reid, S., Bayley, H., 2009. Continuous base identification for single-molecule nanopore DNA sequencing. Nature Nanotechnology 4 (4), 265–270.

Conde, D., González-Melendi, P., Allona, I., 2013. Poplar stems show opposite epigenetic patterns during winter dormancy and vegetative growth. Trees 27 (1), 311–320. https://doi.org/10.1007/s00468-012-0800-x.

Conde, D., Le Gac, A.-L., Perales, M., Dervinis, C., Kirst, M., Maury, S., et al., 2017a. Chilling-responsive DEMETER-LIKE DNA demethylase mediates in poplar bud break: Role of active DNA demethylase in trees' bud break. Plant, Cell and Environment 40 (10), 2236–2249. https://doi.org/10.1111/pce.13019.

Conde, D., Moreno-Cortés, A., Dervinis, C., Ramos-Sánchez, J.M., Kirst, M., Perales, M., et al., 2017b. Overexpression of DEMETER, a DNA demethylase, promotes early apical bud maturation in poplar. Plant, Cell and Environment 40 (11), 2806–2819. https://doi.org/10.1111/pce.13056.

Correia, B., Valledor, L., Meijón, M., Rodriguez, J.L., Dias, M.C., Santos, C., et al., 2013. Is the interplay between epigenetic markers related to the acclimation of cork oak plants to high temperatures?. Plos One 8 (1), e53543. https://doi.org/10.1371/journal.pone.0053543.

Cortijo, S., Wardenaar, R., Colome-Tatche, M., Gilly, A., Etcheverry, M., Labadie, K., et al., 2014. Mapping the epigenetic basis of complex traits. Science 343 (6175), 1145–1148. https://doi.org/10.1126/science.1248127.

Crisp, P.A., Ganguly, D., Eichten, S.R., Borevitz, J.O., Pogson, B.J., 2016. Reconsidering plant memory: Intersections between stress recovery, RNA turnover, and epigenetics. Science Advances 2 (2), e1501340. https://doi.org/10.1126/sciadv.1501340.

Dai, X., Hu, Q., Cai, Q., Feng, K., Ye, N., Tuskan, G.A., et al., 2014. The willow genome and divergent evolution from poplar after the common genome duplication. Cell Research 24 (10), 1274.

Dapp, M., Reinders, J., Bédiée, A., Balsera, C., Bucher, E., Theiler, G., et al., 2015. Heterosis and inbreeding depression of epigenetic Arabidopsis hybrids. Nature Plants 1 (7)https://doi.org/10.1038/nplants.2015.92.

Daru, B.H., Berger, D.K., Wyk, A.E., 2016. Opportunities for unlocking the potential of genomics for African trees. New Phytologist 210 (3), 772–778.

Daviaud, C., Renault, V., Mauger, F., Deleuze, J.F., Tost, J., 2018. Whole-genome bisulfite sequencing using the ovation(R) ultralow methyl-seq protocol. Methods in Molecular Biology 1708, 83–104.

de la Fuente, L., Conesa, A., Lloret, A., Badenes, M.L., Ríos, G., 2015. Genome-wide changes in histone H3 lysine 27 trimethylation associated with bud dormancy release in peach. Tree Genetics & Genomes 11 (3)https://doi.org/10.1007/s11295-015-0869-7.

Diep, D., Plongthongkum, N., Gore, A., Fung, H.L., Shoemaker, R., Zhang, K., 2012. Library-free methylation sequencing with bisulfite padlock probes. Nature Methods 9 (3), 270–272.

Ding, C.-J., Liang, L.-X., Diao, S., Su, X.-H., Zhang, B.-Y., 2018. Genome-wide analysis of day/night DNA methylation differences in Populus nigra. Plos One 13 (1), e0190299. https://doi.org/10.1371/journal.pone.0190299.

Dormling, I., Johnsen, , 1992. Effects of the parental environment on full-sib families of Pinus sylvestris. Canadian Journal of Forest Research 22 (1), 88–100. https://doi.org/10.1139/x92-013.

Down, T.A., Rakyan, V.K., Turner, D.J., Flicek, P., Li, H., Kulesha, E., et al., 2008. A Bayesian deconvolution strategy for immunoprecipitation-based DNA methylome analysis. Nature Biotechnology 26 (7), 779–785.

Duan, H., Lu, X., Lian, C., An, Y., Xia, X., Yin, W., 2016. Genome-wide analysis of MicroRNA responses to the phytohormone abscisic acid in populus euphratica. Frontiers in Plant Science 7, https://doi.org/10.3389/fpls.2016.01184.

Dubin, M.J., Zhang, P., Meng, D., Remigereau, M.-S., Osborne, E.J., Casale, F.P., et al., 2015. DNA methylation in Arabidopsis has a genetic basis and shows evidence of local adaptation. Elife 4, e05255.

D'Urso, A., Brickner, J.H., 2016. Epigenetic transcriptional memory. Current Genetics 63 (3), 435–439. https://doi.org/10.1007/s00294-016-0661-8.

Eads, C.A., Danenberg, K.D., Kawakami, K., Saltz, L.B., Blake, C., Shibata, D., et al., 2000. MethyLight: A high-throughput assay to measure DNA methylation. Nucleic Acids Research 28 (8), e32.

Ehrich, M., Nelson, M.R., Stanssens, P., Zabeau, M., Liloglou, T., Xinarianos, G., et al., 2005. Quantitative high-throughput analysis of DNA methylation patterns by base-specific cleavage and mass spectrometry. Proceedings of the National Academy of Sciences of the United States of America 102 (44), 15785–15790.

El Baidouri, M., Kim, K.D., Abernathy, B., Li, Y.-H., Qiu, L.-J., Jackson, S.A., 2018. Genic C-methylation in soybean is associated with gene paralogs relocated to transposable element-rich pericentromeres. Molecular Plant 11 (3), 485–495. https://doi.org/10.1016/j.molp.2018.02.006.

Fabres, P.J., Collins, C., Cavagnaro, T.R., Rodríguez López, C.M., 2017. A concise review on multi-omics data integration for terroir analysis in Vitis vinifera. Frontiers in Plant Science 8, https://doi.org/10.3389/fpls.2017.01065.

Fan, D., Liu, T., Li, C., Jiao, B., Li, S., Hou, Y., et al., 2015. Efficient CRISPR/Cas9-mediated targeted mutagenesis in populus in the first generation. Scientific Reports 5, https://doi.org/10.1038/srep12217.

Fazzari, M.J., Greally, J.M., 2004. Epigenomics: Beyond CpG islands. Nature Reviews. Genetics 5 (6), 446–455.

Feber, A., Wilson, G., Zhang, L., Presneau, N., Idowu, B., Down, T.A., et al., 2011. Comparative methylome analysis of benign and malignant peripheral nerve sheath tumours. Genome Research 21 (4), 515–524. https://doi.org/10.1101/gr.109678.110.

Feng, S., Cokus, S.J., Zhang, X., Chen, P.-Y., Bostick, M., Goll, M.G., et al., 2010. Conservation and divergence of methylation patterning in plants and animals. Proceedings of the National Academy of Sciences of the United States of America 107 (19), 8689–8694. https://doi.org/10.1073/pnas.1002720107.

Flusberg, B.A., Webster, D.R., Lee, J.H., Travers, K.J., Olivares, E.C., Clark, T.A., et al., 2010. Direct detection of DNA methylation during single-molecule, real-time sequencing. Nature Methods 7 (6), 461–465.

Fresnedo-Ramírez, J., Chan, H.M., Parfitt, D.E., Crisosto, C.H., Gradziel, T.M., 2017. Genome-wide DNA-(de)methylation is associated with Noninfectious Bud-failure exhibition in Almond (Prunus dulcis [Mill.] D.A.Webb). Scientific Reports 7, 42686. https://doi.org/10.1038/srep42686.

Frommer, M., McDonald, L.E., Millar, D.S., Collis, C.M., Watt, F., Grigg, G.W., et al., 1992. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proceedings of the National Academy of Sciences of the United States of America 89 (5), 1827–1831.

Fulnecek, J., Kovarik, A., 2014. How to interpret methylation sensitive amplified polymorphism (MSAP) profiles?. BMC Genetics 15, 2.

Gao, M., Huang, Q., Chu, Y., Ding, C., Zhang, B., Su, X., 2014. Analysis of the leaf methylomes of parents and their hybrids provides new insight into hybrid vigor in Populus deltoides. BMC Genetics 15 (Suppl 1), S8. https://doi.org/10.1186/1471-2156-15-S1-S8.

Gilmour, D.S., Lis, J.T., 1984. Detecting protein-DNA interactions in vivo: Distribution of RNA polymerase on specific bacterial genes. Proceedings of the National Academy of Sciences of the United States of America 81 (14), 4275–4279.

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 Science 67 (2), 208.

Grattapaglia, D., 2017. Status and perspectives of genomic selection in forest tree breeding. In: Sorrells, M.E. (Ed.), Genomic selection for crop improvement. Springer, Cham, pp. 199–257.

Greally, J.M., 2018. The HELP-based DNA methylation assays. Methods in Molecular Biology 1708, 191–207.

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, pp. 56–62.

Groszmann, M., Greaves, I.K., Albertyn, Z.I., Scofield, G.N., Peacock, W.J., Dennis, E.S., 2011. Changes in 24-nt siRNA levels in Arabidopsis hybrids suggest an epigenetic contribution to hybrid vigor. Proceedings of the National Academy of Sciences of the United States of America 108 (6), 2617–2622. https://doi.org/10.1073/pnas.1019217108.

Groszmann, M., Greaves, I.K., Fujimoto, R., James Peacock, W., Dennis, E.S., 2013. The role of epigenetics in hybrid vigour. Trends in Genetics 29 (12), 684–690. https://doi.org/10.1016/j.tig.2013.07.004.

Guan, R., Zhao, Y., Zhang, H., Fan, G., Liu, X., Zhou, W., et al., 2016. Draft genome of the living fossil Ginkgo biloba. GigaScience 5 (1)https://doi.org/10.1186/s13742-016-0154-1.

Guarino, F., Cicatelli, A., Brundu, G., Heinze, B., Castiglione, S., 2015. Epigenetic diversity of clonal white poplar (Populus alba L.) populations: Could methylation support the success of vegetative reproduction strategy?. Plos One 10 (7)https://doi.org/10.1371/journal.pone.0131480.

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 (8), 1665–1680. https://doi.org/10.1111/mec.13563.

Gu, H., Smith, Z.D., Bock, C., Boyle, P., Gnirke, A., Meissner, A., 2011. Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. Nature Protocols Other Titles: Protocols 6 (4), 468–481.

Hallingbäck, H.R., Sánchez, L., Wu, H.X., 2014. Single versus subdivided population strategies in breeding against an adverse genetic correlation. Tree Genetics & Genomes 10 (3), 605–617. https://doi.org/10.1007/s11295-014-0707-3.

Hasbún, R., Iturra, C., Bravo, S., Rebolledo-Jaramillo, B., Valledor, L., 2016. Differential methylation of genomic regions associated with heteroblasty detected by M&M algorithm in the nonmodel species Eucalyptus globulus Labill. International Journal of Genomics 2016, https://doi.org/10.1155/2016/4395153.

Herman, J.G., Graff, J.R., Myohanen, S., Nelkin, B.D., Baylin, S.B., 1996. Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands. Proceedings of the National Academy of Sciences of the United States of America 93 (18), 9821–9826.

Herrera, C.M., Bazaga, P., 2013. Epigenetic correlates of plant phenotypic plasticity: DNA methylation differs between prickly and nonprickly leaves in heterophyllous Ilex aquifolium (Aquifoliaceae) trees: Heterophylly and DNA methylation. Botanical Journal of the Linnean Society 171 (3), 441–452. https://doi.org/10.1111/boj.12007.

Holliday, J.A., Aitken, S.N., Cooke, J.E., Fady, B., González-Martínez, S.C., Heuertz, M., et al., 2017. Advances in ecological genomics in forest trees and applications to genetic resources conservation and breeding. Molecular Ecology 26 (3), 706–717.

Horváth, V., Merenciano, M., González, J., 2017. Revisiting the relationship between transposable elements and the eukaryotic stress response. Trends in Genetics 33 (11), 832–841. https://doi.org/10.1016/j.tig.2017.08.007.

Hussey, S.G., Mizrachi, E., Groover, A., Berger, D.K., Myburg, A.A., 2015. Genome-wide mapping of histone H3 lysine 4 trimethylation in Eucalyptus grandis developing xylem. BMC Plant Biology 15 (1)https://doi.org/10.1186/s12870-015-0499-0.

Jaligot, E., Beulé, T., Baurens, F.-C., Billotte, N., Rival, A., 2004. Search for methylation-sensitive amplification polymorphisms associated with the « mantled » variant phenotype in oil palm (Elaeis guineensis Jacq.). Genome 47 (1), 224–228. https://doi.org/10.1139/g03-085.

Jiao, W.-B., Accinelli, G.G., Hartwig, B., Kiefer, C., Baker, D., Severing, E., et al., 2017. Improving and correcting the contiguity of long-read genome assemblies of three plant species using optical mapping and chromosome conformation capture data. Genome Research 27 (5), 778–786. https://doi.org/10.1101/gr.213652.116.

Johannes, F., Colot, V., Jansen, R.C., 2008. Epigenome dynamics: A quantitative genetics perspective. Nature Reviews. Genetics 9 (11), 883–890. https://doi.org/10.1038/nrg2467.

Johnsen, , Dæhlen, O.G., Østreng, G., Skrøppa, T., 2005a. Daylength and temperature during seed production interactively affect adaptive performance of Picea abies progenies. New Phytologist 168 (3), 589–596. https://doi.org/10.1111/j.1469-8137.2005.01538.x.

Johnsen, , Fossdal, C.G., Nagy, N., Mølmann, J., DæHLEN, O.G., Skrøppa, T., 2005b. Climatic adaptation in Picea abies progenies is affected by the temperature during zygotic embryogenesis and seed maturation. Plant, Cell and Environment 28 (9), 1090–1102. https://doi.org/10.1111/j.1365-3040.2005.01356.x.

Johnsen, , Skrøppa, T., 2001. The influence of the reproductive environment during sexual reproduction on adaptations of conifers along latitudinal and altitudinal gradients. In: Huttunen, S., Heikkilä, H., Bucher, J., Sundberg, B., Jarvis, P., Matyssek, R. (Eds.), Trends in European forest tree physiology research, cost action E6 EUROSILVA. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 207–221.

Jung, M., Kadam, S., Xiong, W., Rauch, T.A., Jin, S.G., Pfeifer, G.P., 2015. MIRA-seq for DNA methylation analysis of CpG islands. Epigenomics 7 (5), 695–706.

Kanchanaketu, T., Sangduen, N., Toojinda, T., Hongtrakul, V., 2012. Genetic diversity analysis of Jatropha curcas L. (Euphorbiaceae) based on methylation-sensitive amplification polymorphism. Genetics and Molecular Research 11 (2), 944–955. https://doi.org/10.4238/2012.April.13.2.

Kawakatsu, T., Huang, S.C., Jupe, F., Sasaki, E., Schmitz, R.J., Urich, M.A., et al., 2016. Epigenomic diversity in a global collection of Arabidopsis thaliana accessions. Cell 166 (2), 492–505. https://doi.org/10.1016/j.cell.2016.06.044.

Kawanabe, T., Ishikura, S., Miyaji, N., Sasaki, T., Wu, L.M., Itabashi, E., et al., 2016. Role of DNA methylation in hybrid vigor in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America 113 (43), E6704–E6711. https://doi.org/10.1073/pnas.1613372113.

Keller, T.E., Yi, S.V., 2014. DNA methylation and evolution of duplicate genes. Proceedings of the National Academy of Sciences of the United States of America 111 (16), 5932–5937. https://doi.org/10.1073/pnas.1321420111.

Kelly, S.A., Tami, M.P., Andrew, M.S., 2012. Phenotypic plasticity: Molecular mechanisms and adaptive significance. In: Terjung, R. (Ed.), Comprehensive physiology. John Wiley & Sons, Inc, Hoboken, NJ, USA, https://doi.org/10.1002/cphy.c110008.

Kelsey, G., Stegle, O., Reik, W., 2017. Single-cell epigenomics: Recording the past and predicting the future. Science 358 (6359), 69–75.

Kimberley, M.O., Moore, J.R., Dungey, H.S., 2015. Quantification of realised genetic gain in radiata pine and its incorporation into growth and yield modelling systems. Canadian Journal of Forest Research 45 (12), 1676–1687. https://doi.org/10.1139/cjfr-2015-0191.

Klimaszewska, K., Noceda, C., Pelletier, G., Label, P., Rodriguez, R., Lelu-Walter, M.A., 2009. Biological characterization of young and aged embryogenic cultures of Pinus pinaster (Ait.). In Vitro Cellular & Developmental Biology - Plant 45 (1), 20–33. https://doi.org/10.1007/s11627-008-9158-6.

Kooke, R., Johannes, F., Wardenaar, R., Becker, F., Etcheverry, M., Colot, V., et al., 2015. Epigenetic basis of morphological variation and phenotypic plasticity in Arabidopsis thaliana. The Plant Cell Online 27 (2), 337–348. https://doi.org/10.1105/tpc.114.133025.

Kumar, S., Kumari, R., Sharma, V., Sharma, V., 2013. Roles, and establishment, maintenance and erasing of the epigenetic cytosine methylation marks in plants. Journal of Genetics 92 (3), 629–666.

Kumar, G., Rattan, U.K., Singh, A.K., 2016. Chilling-mediated DNA methylation changes during dormancy and its release reveal the importance of epigenetic regulation during winter dormancy in apple (Malus x domestica Borkh.). Plos One 11 (2), e0149934. https://doi.org/10.1371/journal.pone.0149934.

Kvaalen, H., Johnsen, , 2008. Timing of bud set in Picea abies is regulated by a memory of temperature during zygotic and somatic embryogenesis. New Phytologist 0 (0)https://doi.org/10.1111/j.1469-8137.2007.02222.x, 071009191540003–???.

Lafon-Placette, C., Faivre-Rampant, P., Delaunay, A., Street, N., Brignolas, F., Maury, S., 2013. Methylome of DNase I sensitive chromatin in Populus trichocarpa shoot apical meristematic cells: A simplified approach revealing characteristics of gene-body DNA methylation in open chromatin state. New Phytologist 197 (2), 416–430. https://doi.org/10.1111/nph.12026.

Lafon-Placette, C., Le Gac, A.-L., Chauveau, D., Segura, V., Delaunay, A., Lesage-Descauses, M.-C., et al., 2018. Changes in the epigenome and transcriptome of the poplar shoot apical meristem in response to water availability affect preferentially hormone pathways. Journal of Experimental Botany https://doi.org/10.1093/jxb/erx409.

Lämke, J., Bäurle, I., 2017. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biology 18, 124.

Lanckriet, G.R.G., De Bie, T., Cristianini, N., Jordan, M.I., Noble, W.S., 2004. A statistical framework for genomic data fusion. Bioinformatics 20 (16), 2626–2635. https://doi.org/10.1093/bioinformatics/bth294.

Lande, R., 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. Journal of Evolutionary Biology 22 (7), 1435–1446. https://doi.org/10.1111/j.1420-9101.2009.01754.x.

Landt, S.G., Marinov, G.K., Kundaje, A., Kheradpour, P., Pauli, F., Batzoglou, S., et al., 2012. ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. Genome Research 22 (9), 1813–1831.

Latzel, V., Rendina González, A.P., Rosenthal, J., 2016. Epigenetic memory as a basis for intelligent behavior in clonal plants. Frontiers in Plant Science 7, https://doi.org/10.3389/fpls.2016.01354.

Lauria, M., Rossi, V., 2011. Epigenetic control of gene regulation in plants. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 1809 (8), 369–378. https://doi.org/10.1016/j.bbagrm.2011.03.002.

Lauss, K., Wardenaar, R., Oka, R., van Hulten, M.H.A., Guryev, V., Keurentjes, J.J.B., et al., 2018. Parental DNA methylation states are associated with heterosis in epigenetic hybrids. Plant Physiology 176 (2), 1627–1645. https://doi.org/10.1104/pp.17.01054.

Law, J.A., Jacobsen, S.E., 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Reviews. Genetics 11 (3), 204–220. https://doi.org/10.1038/nrg2719.

Le Gac, A.-L., Lafon-Placette, C., Chauveau, D., Segura, V., Delaunay, A., Fichot, R., et al., 2018. Winter-dormant shoot apical meristem in poplar trees shows environmental

epigenetic memory. Journal of Experimental Botany 69 (20), 4821–4837. https://doi.org/10.1093/jxb/ery271.

Lelu-Walter, M.-A., Bernier-Cardou, M., Klimaszewska, K., 2006. Simplified and improved somatic embryogenesis for clonal propagation of Pinus pinaster (Ait.). Plant Cell Reports 25 (8), 767–776. https://doi.org/10.1007/s00299-006-0115-8.

Lelu-Walter, M.-A., Thompson, D., Harvengt, L., Sanchez, L., Toribio, M., Pâques, L.E., 2013. Somatic embryogenesis in forestry with a focus on Europe: State-of-the-art, benefits, challenges and future direction. Tree Genetics & Genomes 9 (4), 883–899. https://doi.org/10.1007/s11295-013-0620-1.

Liang, D., Zhang, Z., Wu, H., Huang, C., Shuai, P., Ye, C.-Y., et al., 2014. Single-base-resolution methylomes of populus trichocarpa reveal the association between DNA methylation and drought stress. BMC Genetics 15 (Suppl 1), S9. https://doi.org/10.1186/1471-2156-15-S1-S9.

Li, Y., Dong, X.-M., Jin, F., Shen, Z., Chao, Q., Wang, B.-C., 2017. Histone acetylation modifications affect tissue-dependent expression of poplar homologs of C4 photosynthetic enzyme genes. Frontiers in Plant Science 8, https://doi.org/10.3389/fpls.2017.00950.

Li, Q., Eichten, S.R., Hermanson, P.J., Springer, N.M., 2014. Inheritance patterns and stability of DNA methylation variation in maize near-isogenic lines. Genetics 196 (3), 667–676. https://doi.org/10.1534/genetics.113.158980.

Li, A., Hu, B.-Q., Xue, Z.-Y., Chen, L., Wang, W.-X., Song, W.-Q., et al., 2011. DNA methylation in genomes of several annual herbaceous and woody perennial plants of varying ploidy as detected by MSAP. Plant Molecular Biology Reporter 29 (4), 784–793. https://doi.org/10.1007/s11105-010-0280-3.

Lira-Medeiros, C.F., Parisod, C., Fernandes, R.A., Mata, C.S., Cardoso, M.A., Ferreira, P.C.G., 2010. Epigenetic variation in mangrove plants occurring in contrasting natural environment. Plos One 5 (4), e10326. https://doi.org/10.1371/journal.pone.0010326.

Lister, R., O'Malley, R.C., Tonti-Filippini, J., Gregory, B.D., Berry, C.C., Millar, A.H., et al., 2008. Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell 133 (3), 523–536.

Li, Q., Suzuki, M., Wendt, J., Patterson, N., Eichten, S.R., Hermanson, P.J., et al., 2015. Post-conversion targeted capture of modified cytosines in mammalian and plant genomes. Nucleic Acids Research 43 (12), e81.

Liu, Y., El-Kassaby, Y.A., 2017. Landscape of fluid sets of hairpin-derived 21-/24-nt-long small RNAs at seed set uncovers special epigenetic features in Picea glauca. Genome Biology and Evolution https://doi.org/10.1093/gbe/evw283, evw283.

Liu, R., How-Kit, A., Stammitti, L., Teyssier, E., Rolin, D., Mortain-Bertrand, A., et al., 2015. A DEMETER-like DNA demethylase governs tomato fruit ripening. Proceedings of the National Academy of Sciences of the United States of America 112 (34), 10804–10809. https://doi.org/10.1073/pnas.1503362112.

Long, Y., Xia, W., Li, R., Wang, J., Shao, M., Feng, J., et al., 2011. Epigenetic QTL mapping in Brassica napus. Genetics 189 (3), 1093–1102. https://doi.org/10.1534/genetics.111.131615.

Mackay, J., Dean, J.F.D., Plomion, C., Peterson, D.G., Cánovas, F.M., Pavy, N., et al., 2012. Towards decoding the conifer giga-genome. Plant Molecular Biology 80 (6), 555–569. https://doi.org/10.1007/s11103-012-9961-7.

Mader, M., Le Paslier, M.-C., Bounon, R., Bérard, A., Rampant, P.F., Fladung, M., et al., 2016. Whole-genome draft assembly of Populus tremula x P. alba clone INRA 717-1B4. Silvae Genetica 65 (2)https://doi.org/10.1515/sg-2016-0019.

Martínez-García, P.J., Crepeau, M.W., Puiu, D., Gonzalez-Ibeas, D., Whalen, J., Stevens, K.A., et al., 2016. The walnut (Juglans regia) genome sequence reveals diversity in genes coding for the biosynthesis of non-structural polyphenols. The Plant Journal 87 (5), 507–532. https://doi.org/10.1111/tpj.13207.

Maunakea, A.K., Nagarajan, R.P., Bilenky, M., Ballinger, T.J., D'Souza, C., Fouse, S.D., et al., 2010. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature 466 (7303), 253–257.

Ma, X., Zhang, C., Zhang, B., Yang, C., Li, S., 2016. Identification of genes regulated by histone acetylation during root development in Populus trichocarpa. BMC Genomics 17 (1)https://doi.org/10.1186/s12864-016-2407-x.

McMahon, B.J., Teeling, E.C., Höglund, J., 2014. How and why should we implement genomics into conservation?. Evolutionary Applications 7 (9), 999–1007. https://doi.org/10.1111/eva.12193.

Meissner, A., Gnirke, A., Bell, G.W., Ramsahoye, B., Lander, E.S., Jaenisch, R., 2005. Reduced representation bisulfite sequencing for comparative high-resolution DNA methylation analysis. Nucleic Acids Research 33 (18), 5868–5877.

Meyer, P., 2015. Epigenetic variation and environmental change: Fig. 1. Journal of Experimental Botany 66 (12), 3541–3548. https://doi.org/10.1093/jxb/eru502.

Meyer, R.C., Törjék, O., Becher, M., Altmann, T., 2004. Heterosis of biomass production in Arabidopsis. Establishment during early development. Plant Physiology 134 (4), 1813–1823. https://doi.org/10.1104/pp.103.033001.

Michalak, M., Barciszewska, M.Z., Barciszewski, J., Plitta, B.P., Chmielarz, P., 2013. Global changes in DNA methylation in seeds and seedlings of Pyrus communis after seed desiccation and storage. Plos One 8 (8), e70693. https://doi.org/10.1371/journal.pone.0070693.

Michalak, M., Plitta-Michalak, B.P., Naskręt-Barciszewska, M., Barciszewski, J., Bujarska-Borkowska, B., Chmielarz, P., 2015. Global 5-methylcytosine alterations in DNA during ageing of Quercus robur seeds. Annals of Botany 116 (3), 369–376. https://doi.org/10.1093/aob/mcv104.

Miura, F., Enomoto, Y., Dairiki, R., Ito, T., 2012. Amplification-free whole-genome bisulfite sequencing by post-bisulfite adaptor tagging. Nucleic Acids Research 40 (17), e136.

Mullin, T.J., Andersson, B., Bastien, J.C., Beaulieu, J., Burdon, R.D., Dvorak, W.S., et al., 2011. Economic importance, breeding objectives and achievements. In: Plomion, C., Bousquet, J., Kole, C. (Eds.), Genetics, genomics and breeding of crop plants science publ. CRC Press, Enfield, NH, USA, Chapter 2.

Muranty, H., Jorge, V., Bastien, C., Lepoittevin, C., Bouffier, L., Sanchez, L., 2014. Potential for marker-assisted selection for forest tree breeding: Lessons from 20 years of MAS in crops. Tree Genetics & Genomes 10 (6), 1491–1510. https://doi.org/10.1007/s11295-014-0790-5.

Murat, F., Armero, A., Pont, C., Klopp, C., Salse, J., 2017. Reconstructing the genome of the most recent common ancestor of flowering plants. Nature Genetics 49 (4), 490–496.

Murat, F., Zhang, R., Guizard, S., Gavranović, H., Flores, R., Steinbach, D., et al., 2015. Karyotype and gene order evolution from reconstructed extinct ancestors highlight

contrasts in genome plasticity of modern rosid crops. Genome Biology and Evolution 7 (3), 735–749. https://doi.org/10.1093/gbe/evv014.

Myburg, A.A., Grattapaglia, D., Tuskan, G.A., Hellsten, U., Hayes, R.D., Grimwood, J., et al., 2014. The genome of Eucalyptus grandis. Nature 510 (7505), 356–362. https://doi.org/10.1038/nature13308.

Nanson, A., 2004. Génétique et amelioration des arbres forestiers. Presses Agronomiques de Gembloux, Gembloux, 712 p., 103 ill., 25 tab.

Neale, D.B., Langley, C.H., Salzberg, S.L., Wegrzyn, J.L., 2013. Open access to tree genomes: The path to a better forest. Genome Biology 14 (6), 120.

Neale, D.B., Wegrzyn, J.L., Stevens, K.A., Zimin, A.V., Puiu, D., Crepeau, M.W., et al., 2014. Decoding the massive genome of loblolly pine using haploid DNA and novel assembly strategies. Genome Biology 15 (3), R59.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., et al., 2010. Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15 (12), 684–692. https://doi.org/10.1016/j.tplants.2010.09.008.

Niu, S.-H., Liu, C., Yuan, H.-W., Li, P., Li, Y., Li, W., 2015. Identification and expression profiles of sRNAs and their biogenesis and action-related genes in male and female cones of Pinus tabuliformis. BMC Genomics 16 (1)https://doi.org/10.1186/s12864-015-1885-6.

Nock, C.J., Baten, A., Barkla, B.J., Furtado, A., Henry, R.J., King, G.J., 2016. Genome and transcriptome sequencing characterises the gene space of Macadamia integrifolia (Proteaceae). BMC Genomics 17 (1)https://doi.org/10.1186/s12864-016-3272-3.

Nystedt, B., Street, N.R., Wetterbom, A., Zuccolo, A., Lin, Y.-C., Scofield, D.G., et al., 2013. The Norway spruce genome sequence and conifer genome evolution. Nature 497 (7451), 579–584. https://doi.org/10.1038/nature12211.

Pâques, L.E., 2013. In: Pâques, L.E. (Ed.), Forest tree breeding in Europe. Current state-of-the-art and perspectives. Springer, p. 25. https://doi.org/10.1007/978-94-007-6146-9.

Paul, D.S., Guilhamon, P., Karpathakis, A., Butcher, L.M., Thirlwell, C., Feber, A., et al., 2014. Assessment of RainDrop BS-seq as a method for large-scale, targeted bisulfite sequencing. Epigenetics 9 (5), 678–684.

Paun, O., Fay, M.F., Soltis, D.E., Chase, M.W., 2007. Genetic and epigenetic alterations after hybridization and genome doubling. Taxon 56 (3), 649. https://doi.org/10.2307/25065850.

Platt, A., Gugger, P.F., Pellegrini, M., Sork, V.L., 2015. Genome-wide signature of local adaptation linked to variable CpG methylation in oak populations. Molecular Ecology 24 (15), 3823–3830. https://doi.org/10.1111/mec.13230.

Plitta-Michalak, B.P., Naskręt-Barciszewska, M.Z., Kotlarski, S., Tomaszewski, D., Tylkowski, T., Barciszewski, J., et al., 2018. Changes in genomic 5-methylcytosine level mirror the response of orthodox (Acer platanoides L.) and recalcitrant (Acer pseudoplatanus L.) seeds to severe desiccation. Tree Physiology 38 (4), 617–629. https://doi.org/10.1093/treephys/tpx134.

Plitta, B.P., Michalak, M., Bujarska-Borkowska, B., Barciszewska, M.Z., Barciszewski, J., Chmielarz, P., 2014. Effect of desiccation on the dynamics of genome-wide DNA methylation in orthodox seeds of Acer platanoides L.. Plant Physiology and Biochemistry 85, 71–77. https://doi.org/10.1016/j.plaphy.2014.10.014.

Plomion, C., Aury, J.M., Amselem, J., Leroy, T., Murat, F., Duplessis, S., et al., 2018. Oak genome reveals facets of long lifespan. Nature Plants 4, 440–452.

Plomion, C., Bastien, C., Bogeat-Triboulot, M.B., Bouffier, L., Déjardin, A., Duplessis, S., et al., 2016. Forest tree genomics: 10 achievements from the past 10 years and future prospects. Annals of Forest Science 73 (1), 77–103. https://doi.org/10.1007/s13595-015-0488-3.

Podevin, N., Davies, H.V., Hartung, F., Nogué, F., Casacuberta, J.M., 2013. Site-directed nucleases: A paradigm shift in predictable, knowledge-based plant breeding. Trends in Biotechnology 31 (6), 375–383. https://doi.org/10.1016/j.tibtech.2013.03.004.

Quinn, C.R., Iriyama, R., Fernando, D.D., 2014. Expression patterns of conserved microRNAs in the male gametophyte of loblolly pine (Pinus taeda). Plant Reproduction 27 (2), 69–78. https://doi.org/10.1007/s00497-014-0241-3.

Raj, S., Bräutigam, K., Hamanishi, E.T., Wilkins, O., Thomas, B.R., Schroeder, W., et al., 2011. Clone history shapes Populus drought responses. Proceedings of the National Academy of Sciences of the United States of America 108 (30), 12521–12526.

Ramassone, A., Pagotto, S., Veronese, A., Visone, R., 2018. Epigenetics and MicroRNAs in cancer. International Journal of Molecular Sciences 19, 459. https://doi.org/10.3390/ijms19020459.

Rand, A.C., Jain, M., Eizenga, J.M., Musselman-Brown, A., Olsen, H.E., Akeson, M., et al., 2017. Mapping DNA methylation with high-throughput nanopore sequencing. Nature Methods 14 (4), 411–413.

Rauch, T., Pfeifer, G.P., 2005. Methylated-CpG island recovery assay: A new technique for the rapid detection of methylated-CpG islands in cancer. Laboratory Investigation; a Journal of Technical Methods and Pathology 85 (9), 1172–1180.

Ren, Y., Chen, L., Zhang, Y., Kang, X., Zhang, Z., Wang, Y., 2012. Identification of novel and conserved Populus tomentosa microRNA as components of a response to water stress. Functional & Integrative Genomics 12 (2), 327–339. https://doi.org/10.1007/s10142-012-0271-6.

Richards, C.L., Alonso, C., Becker, C., Bossdorf, O., Bucher, E., Colomé-Tatché, M., et al., 2017. Ecological plant epigenetics: Evidence from model and non-model species, and the way forward. Ecology Letters 20 (12), 1576–1590. https://doi.org/10.1111/ele.12858.

Rico, L., Ogaya, R., Barbeta, A., Peñuelas, J., 2014. Changes in DNA methylation fingerprint of Quercus ilex trees in response to experimental field drought simulating projected climate change. Plant Biology 16 (2), 419–427. https://doi.org/10.1111/plb.12049.

Rodin, S.N., Riggs, A.D., 2003. Epigenetic silencing may aid evolution by gene duplication. Journal of Molecular Evolution 56 (6), 718–729. https://doi.org/10.1007/s00239-002-2446-6.

Russo, V.E.A., Martienssen, R.A., Riggs, A.D., 1996. Epigenetic mechanisms of gene regulation Vol. 32, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, i–xii.

Ruttink, T., Arend, M., Morreel, K., Storme, V., Rombauts, S., Fromm, J., et al., 2007. A molecular timetable for apical bud formation and dormancy induction in poplar. The Plant Cell Online 19 (8), 2370–2390. https://doi.org/10.1105/tpc.107.052811.

Sáez-Laguna, E., Guevara, , Díaz, L.-M., Sánchez-Gómez, D., Collada, C., Aranda, I., et al., 2014. Epigenetic variability in the genetically uniform forest tree species Pinus pinea L.. Plos One 9 (8)https://doi.org/10.1371/journal.pone.0103145.

Salmon, A., Ainouche, M.L., Wendel, J.F., 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in spartina (poaceae): Genetic and epigenetic

changes in spartina. Molecular Ecology 14 (4), 1163–1175. https://doi.org/10.1111/j.1365-294X.2005.02488.x.

Salojärvi, J., Smolander, O.-P., Nieminen, K., Rajaraman, S., Safronov, O., Safdari, P., et al., 2017. Genome sequencing and population genomic analyses provide insights into the adaptive landscape of silver birch. Nature Genetics 49 (6), 904–912. https://doi.org/10.1038/ng.3862.

Salse, J., 2016a. Ancestors of modern plant crops. Current Opinion in Plant Biology 30, 134–142. https://doi.org/10.1016/j.pbi.2016.02.005.

Salse, J., 2016b. Deciphering the evolutionary interplay between subgenomes following polyploidy: A paleogenomics approach in grasses. American Journal of Botany 103 (7), 1167–1174. https://doi.org/10.3732/ajb.1500459.

Sanchez, L., Bastien, C., Rozenberg, P., Lee, S., Woolliams, J., Lagercrantz, U., et al., 2013. Novel tree breeding. In: Lee, S.J., Woolliams, J. (Eds.), Monografias INIA, monographies, forestry series.

Santamaría, M., Hasbún, R., Valera, M., Meijón, M., Valledor, L., Rodríguez, J.L., et al., 2009. Acetylated H4 histone and genomic DNA methylation patterns during bud set and bud burst in Castanea sativa. Journal of Plant Physiology 166 (13), 1360–1369. https://doi.org/10.1016/j.jplph.2009.02.014.

Schmidtling, R.C., Hipkins, V., 2004. The after-effects of reproductive environment in shortleaf pine. Forestry 77 (4), 287–295. https://doi.org/10.1093/forestry/77.4.287.

Schnable, P.S., Springer, N.M., 2013. Progress toward understanding heterosis in crop plants. Annual Review of Plant Biology 64 (1), 71–88. https://doi.org/10.1146/annurev-arplant-042110-103827.

Schönberger, B., Chen, X., Mager, S., Ludewig, U., 2016. Site-dependent differences in DNA methylation and their impact on plant establishment and phosphorus nutrition in Populus trichocarpa. Plos One 11 (12), e0168623. https://doi.org/10.1371/journal.pone.0168623.

Seymour, D.K., Becker, C., 2017. The causes and consequences of DNA methylome variation in plants. Current Opinion in Plant Biology 36, 56–63. https://doi.org/10.1016/j.pbi.2017.01.005.

Shaked, H., Kashkush, K., Ozkan, H., Feldman, M., Levy, A.A., 2001. Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. The Plant Cell Online 13 (8), 1749–1759.

Shapiro, R., DiFate, V., Welcher, M., 1974. Deamination of cytosine derivatives by bisulfite. Mechanism of the reaction. Journal of the American Chemical Society 96 (3), 906–912.

Shen, H., He, H., Li, J., Chen, W., Wang, X., Guo, L., et al., 2012. Genome-wide analysis of DNA methylation and gene expression changes in two Arabidopsis ecotypes and their reciprocal hybrids. The Plant Cell Online 24 (3), 875–892. https://doi.org/10.1105/tpc.111.094870.

Shen, Y., Sun, S., Hua, S., Shen, E., Ye, C.-Y., Cai, D., et al., 2017. Analysis of transcriptional and epigenetic changes in hybrid vigor of allopolyploid Brassica napus uncovers key roles for small RNAs. The Plant Journal 91 (5), 874–893. https://doi.org/10.1111/tpj.13605.

Shim, D., Ko, J.-H., Kim, W.-C., Wang, Q., Keathley, D.E., Han, K.-H., 2014. A molecular framework for seasonal growth-dormancy regulation in perennial plants. Horticulture Research 1 (1)https://doi.org/10.1038/hortres.2014.59.

Shuai, P., Liang, D., Zhang, Z., Yin, W., Xia, X., 2013. Identification of drought-responsive and novel Populus trichocarpa microRNAs by high-throughput sequencing and their targets using degradome analysis. BMC Genomics 14 (1), 233. https://doi.org/10.1186/1471-2164-14-233.

Silva-Junior, O.B., Grattapaglia, D., Novaes, E., Collevatti, R.G., 2018. Genome assembly of the Pink Ipê (Handroanthus impetiginosus, Bignoniaceae), a highly valued, ecologically keystone Neotropical timber forest tree. GigaScience 7 (1)https://doi.org/10.1093/gigascience/gix125.

Simpson, J.T., Workman, R.E., Zuzarte, P.C., David, M., Dursi, L.J., Timp, W., 2017. Detecting DNA cytosine methylation using nanopore sequencing. Nature Methods 14 (4), 407–410.

Skrøppa, T., Johnsen, , 2000. Patterns of adaptive genetic variation in forest tree species; the reproductive environment as an evolutionary force in Picea abies. In: Mátyás, C. (Ed.), Forest genetics and sustainability. Kluwer Academic Publications, Dordrecht, the Netherlands, pp. 49–58.

Skrøppa, T., Kohmann, K., Johnsen, , Steffenrem, A., Edvardsen, Ø.M., 2007. Field performance and early test results of offspring from two Norway spruce seed orchards containing clones transferred to warmer climates. Canadian Journal of Forest Research 37 (3), 515–522. https://doi.org/10.1139/X06-253.

Skrøppa, T., Solvin, T., Steffenrem, A., 2016. Epigenetic response to climate conditions during the reproductive phase; an evolutionary factor of northern Norway spruce populations?. In: Final meeting of cost action FP1202 MaP-FGR and conference on "marginal and peripheral tree populations: A key genetic resource for European forests" Arezzo, Italy, 26–29 September 2016.

Skrøppa, T., Tollefsrud, M.M., Sperisen, C., Johnsen, , 2010. Rapid change in adaptive performance from one generation to the next in Picea abies—central European trees in a Nordic environment. Tree Genetics & Genomes 6 (1), 93–99. https://doi.org/10.1007/s11295-009-0231-z.

Slavov, G.T., DiFazio, S.P., Martin, J., Schackwitz, W., Muchero, W., Rodgers-Melnick, E., et al., 2012. Genome resequencing reveals multiscale geographic structure and extensive linkage disequilibrium in the forest tree Populus trichocarpa. New Phytologist 196 (3), 713–725. https://doi.org/10.1111/j.1469-8137.2012.04258.x.

Sollars, E.S.A., Harper, A.L., Kelly, L.J., Sambles, C.M., Ramirez-Gonzalez, R.H., Swarbreck, D., et al., 2017. Genome sequence and genetic diversity of European ash trees. Nature 541 (7636), 212–216. https://doi.org/10.1038/nature20786.

Soltis, D.E., Bell, C.D., Kim, S., Soltis, P.S., 2008. Origin and early evolution of angiosperms. Annals of the New York Academy of Sciences 1133 (1), 3–25. https://doi.org/10.1196/annals.1438.005.

Song, Y., Ci, D., Tian, M., Zhang, D., 2016. Stable methylation of a non-coding RNA gene regulates gene expression in response to abiotic stress in Populus simonii. Journal of Experimental Botany 67 (5), 1477–1492. https://doi.org/10.1093/jxb/erv543.

Song, C.X., Clark, T.A., Lu, X.Y., Kislyuk, A., Dai, Q., Turner, S.W., et al., 2012. Sensitive and specific single-molecule sequencing of 5-hydroxymethylcytosine. Nature Methods 9 (1), 75–77.

Song, Y., Tian, M., Ci, D., Zhang, D., 2015. Methylation of microRNA genes regulates gene expression in bisexual flower development in andromonoecious poplar. Journal of Experimental Botany 66 (7), 1891–1905. https://doi.org/10.1093/jxb/eru531.

Sork, V.L., 2016. Gene flow and natural selection shape spatial patterns of genes in tree populations: Implications for evolutionary processes and applications. Evolutionary Applications 9 (1), 291–310. https://doi.org/10.1111/eva.12316.

Sork, V.L., 2017. Genomic studies of local adaptation in natural plant populations. Journal of Heredity 109 (1), 3–15. https://doi.org/10.1093/jhered/esx091.

Sork, V.L., Fitz-Gibbon, S.T., Puiu, D., Crepeau, M., Gugger, P.F., Sherman, R., et al., 2016. First draft assembly and annotation of the genome of a California endemic oak Quercus lobata nee (Fagaceae). Genes|Genomes|Genetics https://doi.org/10.1534/g3.116.030411.

Sow, M.D., Segura, V., Chamaillard, S., Jorge, V., Delaunay, A., Lafon-Placette, C., et al., 2018. Narrow-sense heritability and PST estimates of DNA methylation in three Populus nigra L. populations under contrasting water availability. Tree Genetics and Genomes https://doi.org/10.1007/s11295-018-1293-6.

Statham, A.L., Robinson, M.D., Song, J.Z., Coolen, M.W., Stirzaker, C., Clark, S.J., 2012. Bisulfite sequencing of chromatin immunoprecipitated DNA (BisChIP-seq) directly informs methylation status of histone-modified DNA. Genome Research 22 (6), 1120–1127.

Stevens, K.A., Wegrzyn, J.L., Zimin, A., Puiu, D., Crepeau, M., Cardeno, C., et al., 2016. Sequence of the sugar pine megagenome. Genetics 204 (4), 1613–1626. https://doi.org/10.1534/genetics.116.193227.

Stewart, F.J., Panne, D., Bickle, T.A., Raleigh, E.A., 2000. Methyl-specific DNA binding by McrBC, a modification-dependent restriction enzyme. Journal of Molecular Biology 298 (4), 611–622.

Stoehr, M.U., L'Hirondelle, S.J., Binder, W.D., Webber, J.E., 1998. Parental environment aftereffects on germination, growth, and adaptive traits in selected white spruce families. Canadian Journal of Forest Research 28 (3), 418–426. https://doi.org/10.1139/x98-012.

Suo, Y., Dong, C., Kang, X., 2015. Inheritance and Variation of Cytosine Methylation in Three Populus Allotriploid Populations with Different Heterozygosity. Plos One 10 (4), e0126491. https://doi.org/10.1371/journal.pone.0126491.

Suzuki, M., Jing, Q., Lia, D., Pascual, M., McLellan, A., Greally, J.M., 2010. Optimized design and data analysis of tag-based cytosine methylation assays. Genome Biology 11, R36. https://doi.org/10.1186/gb-2010-11-4-r36.

Taiwo, O., Wilson, G.A., Morris, T., Seisenberger, S., Reik, W., Pearce, D., et al., 2012. Methylome analysis using MeDIP-seq with low DNA concentrations. Nature Protocols Other Titles: Protocols 7 (4), 617–636.

Tang, C., Yang, M., Fang, Y., Luo, Y., Gao, S., Xiao, X., et al., 2016. The rubber tree genome reveals new insights into rubber production and species adaptation. Nature Plants 2 (6), 16073. https://doi.org/10.1038/nplants.2016.73.

ter Steege, H., Pitman, N.C.A., Killeen, T.J., Laurance, W.F., Peres, C.A., Guevara, J.E., et al., 2015. Estimating the global conservation status of more than 15,000 Amazonian tree species. Science Advances 1 (10), e1500936. https://doi.org/10.1126/sciadv.1500936.

Teyssier, C., Maury, S., Beaufour, M., Grondin, C., Delaunay, A., Le Metté, C., et al., 2014. In search of markers for somatic embryo maturation in hybrid larch (Larix × eurolepis): Global DNA methylation and proteomic analyses. Physiologia Plantarum 150 (2), 271–291. https://doi.org/10.1111/ppl.12081.

Toda, T., Kurinobu, S., 2002. Realized genetic gains observed in progeny tolerance of selected red pine (Pinus densiflora) and black pine (P. thunbergii) to pine wilt disease. Silvae Genetica 51 (1), 42–44.

Tost, J., 2016. Current and emerging technologies for the analysis of the genome-wide and locus-specific DNA methylation patterns. Advances in Experimental Medicine and Biology 945, 343–430.

Tost, J., Gut, I.G., 2007. DNA methylation analysis by pyrosequencing. Nature Protocols Other Titles: Protocols 2 (9), 2265–2275.

Trontin, J.-F., Klimaszewska, K., Morel, A., Hargreaves, C., Lelu-Walter, M.-A., 2016. Molecular aspects of conifer zygotic and somatic embryo development: A review of genome-wide approaches and recent insights. In: In: Germana, M.A., Lambardi, M. (Eds.), In vitro embryogenesis in higher plants Vol. 1359, Springer New York, New York, NY, pp. 167–207. https://doi.org/10.1007/978-1-4939-3061-6_8.

Tulving, E., 1985. Memory and consciousness. Canadian Psychology/Psychologie Canadienne 26 (1), 1–12. https://doi.org/10.1037/h0080017.

Tuskan, G.A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., et al., 2006. The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). Science 313 (5793), 1596–1604. https://doi.org/10.1126/science.1128691.

Tylewicz, S., Petterle, A., Marttila, S., Miskolczi, P., Azeez, A., Singh, R.K., et al., 2018. Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. Science 360 (6385), 212–215. https://doi.org/10.1126/science.aan8576.

Ujino-Ihara, T., Ueno, S., Uchiyama, K., Futamura, N., 2018. Comprehensive analysis of small RNAs expressed in developing male strobili of Cryptomeria japonica. Plos One 13 (3), e0193665. https://doi.org/10.1371/journal.pone.0193665.

Urich, M.A., Nery, J.R., Lister, R., Schmitz, R.J., Ecker, J.R., 2015. MethylC-seq library preparation for base-resolution whole-genome bisulfite sequencing. Nature Protocols Other Titles: Protocols 10 (3), 475–483.

Valledor, L., Meijón, M., Hasbún, R., Cañal, M.J., Rodríguez, R., 2010. Variations in DNA methylation, acetylated histone H4, and methylated histone H3 during Pinus radiata needle maturation in relation to the loss of in vitro organogenic capability. Journal of Plant Physiology 167 (5), 351–357. https://doi.org/10.1016/j.jplph.2009.09.018.

van der Graaf, A., Wardenaar, R., Neumann, D.A., Taudt, A., Shaw, R.G., Jansen, R.C., et al., 2015. Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. Proceedings of the National Academy of Sciences of the United States of America 112 (21), 6676–6681.

Verhoeven, K.J.F., von Holdt, B.M., Sork, V.L., 2016. Epigenetics in ecology and evolution: What we know and what we need to know. Molecular Ecology 25 (8), 1631–1638. https://doi.org/10.1111/mec.13617.

Vining, K., Pomraning, K.R., Wilhelm, L.J., Ma, C., Pellegrini, M., Di, Y., et al., 2013. Methylome reorganization during in vitro dedifferentiation and regeneration of Populus trichocarpa. BMC Plant Biology 13 (1), 92. https://doi.org/10.1186/1471-2229-13-92.

Vining, K.J., Pomraning, K.R., Wilhelm, L.J., Priest, H.D., Pellegrini, M., Mockler, T.C., et al., 2012. Dynamic DNA cytosine methylation in the Populus trichocarpa genome: Tissue-level variation and relationship to gene expression. BMC Genomics 13 (1), 27.

Walker, D.L., Bhagwate, A.V., Baheti, S., Smalley, R.L., Hilker, C.A., Sun, Z., et al., 2015. DNA methylation profiling: Comparison of genome-wide sequencing methods and the infinium human methylation 450 bead chip. Epigenomics 7 (8), 1287–1302.

Wang, J., Blevins, T., Podicheti, R., Haag, J.R., Tan, E.H., Wang, F., et al., 2017b. Mutation of Arabidopsis SMC4 identifies condensin as a corepressor of pericentromeric transposons and conditionally expressed genes. Genes & Development 31 (15), 1601–1614. https://doi.org/10.1101/gad.301499.117.

Wang, D., Qu, Z., Yang, L., Zhang, Q., Liu, Z.-H., Do, T., et al., 2017a. Transposable elements (TEs) contribute to stress-related long intergenic noncoding RNAs in plants. The Plant Journal 90 (1), 133–146. https://doi.org/10.1111/tpj.13481.

Wang, N., Thomson, M., Bodles, W.J.A., Crawford, R.M.M., Hunt, H.V., Featherstone, A.W., et al., 2013. Genome sequence of dwarf birch (Betula nana) and cross-species RAD markers. Molecular Ecology 22 (11), 3098–3111. https://doi.org/10.1111/mec.12131.

Wang, X., Zhang, Z., Fu, T., Hu, L., Xu, C., Gong, L., et al., 2017c. Gene-body CG methylation and divergent expression of duplicate genes in rice. Scientific Reports 7 (1)https://doi.org/10.1038/s41598-017-02860-4.

Wang, L., Zhao, J., Zhang, M., Li, W., Luo, K., Lu, Z., et al., 2015. Identification and characterization of microRNA expression in Ginkgo biloba L. leaves. Tree Genetics & Genomes 11 (4)https://doi.org/10.1007/s11295-015-0897-3.

Wan, L.-C., Wang, F., Guo, X., Lu, S., Qiu, Z., Zhao, Y., et al., 2012. Identification and characterization of small non-coding RNAs from Chinese fir by high throughput sequencing. BMC Plant Biology 12 (1), 146. https://doi.org/10.1186/1471-2229-12-146.

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 (10), 1219–1227. https://doi.org/10.1093/treephys/25.10.1219.

Wendt, J., Rosenbaum, H., Richmond, T.A., Jeddeloh, J.A., Burgess, D.L., 2018. Targeted bisulfite sequencing using the SeqCap epi enrichment system. Methods in Molecular Biology 1708, 383–405.

White, T.L., Adams, W.T., Neale, D.B., 2007. Forest genetics. CABI Publishing, Wallingford and Cambridge, p x + 682.

Wong, D.C.J., Matus, J.T., 2017. Constructing integrated networks for identifying new secondary metabolic pathway regulators in grapevine: Recent applications and future opportunities. Frontiers in Plant Science 8, https://doi.org/10.3389/fpls.2017.00505.

Woolliams, J., 2013. Genome wide selection: A radical re-think or more of the same?. In: Lee, S.J., Woolliams, J. (Eds.), Novel tree breeding, Monografias INIA: serie Forestal no 24.

Xia, J., Guo, X., Deng, K., 2014. Epigenetics, MicroRNAs and human cancer. In: Babashah, S. (Ed.), MicroRNAs: Key regulators of oncogenesis. Springer International Publishing, Cham, pp. 29–57. https://doi.org/10.1007/978-3-319-03725-7_2.

Xia, Y., Jing, D., Kong, L., Zhang, J., OuYang, F., Zhang, H., et al., 2016. Global lysine acetylome analysis of desiccated somatic embryos of picea asperata. Frontiers in Plant Science 7, https://doi.org/10.3389/fpls.2016.01927.

Yaish, M.W., Sunkar, R., Zheng, Y., Ji, B., Al-Yahyai, R., Farooq, S.A., 2015. A genome-wide identification of the miRNAome in response to salinity stress in date palm (Phoenix dactylifera L.). Frontiers in Plant Science 6, https://doi.org/10.3389/fpls.2015.00946.

Yakovlev, I.A., Carneros, E., Lee, Y., Olsen, J.E., Fossdal, C.G., 2016. Transcriptional profiling of epigenetic regulators in somatic embryos during temperature induced formation of an epigenetic memory in Norway spruce. Planta 243 (5), 1237–1249. https://doi.org/10.1007/s00425-016-2484-8.

Yakovlev, I.A., Fossdal, C.G., 2017. In silico analysis of small RNAs suggest roles for novel and conserved miRNAs in the formation of epigenetic memory in somatic embryos of Norway spruce. Frontiers in Physiology 8, https://doi.org/10.3389/fphys.2017.00674.

Yakovlev, I.A., Fossdal, C.G., Johnsen, , 2010. MicroRNAs, the epigenetic memory and climatic adaptation in Norway spruce. New Phytologist 187 (4), 1154–1169. https://doi.org/10.1111/j.1469-8137.2010.03341.x.

Yakovlev, I., Fossdal, C.G., Skrøppa, T., Olsen, J.E., Jahren, A.H., Johnsen, , 2012. An adaptive epigenetic memory in conifers with important implications for seed production. Seed Science Research 22 (02), 63–76. https://doi.org/10.1017/S0960258511000535.

Yakovlev, I.A., Lee, Y., Rotter, B., Olsen, J.E., Skrøppa, T., Johnsen, , et al., 2014. Temperature-dependent differential transcriptomes during formation of an epigenetic memory in Norway spruce embryogenesis. Tree Genetics & Genomes 10 (2), 355–366. https://doi.org/10.1007/s11295-013-0691-z.

Yang, W., Wang, K., Zhang, J., Ma, J., Liu, J., Ma, T., 2017. The draft genome sequence of a desert tree Populus pruinosa. GigaScience 6 (9), 1–7. https://doi.org/10.1093/gigascience/gix075.

Yu, M., Hon, G.C., Szulwach, K.E., Song, C.X., Jin, P., Ren, B., et al., 2012. Tet-assisted bisulfite sequencing of 5-hydroxymethylcytosine. Nature Protocols Other Titles: Protocols 7 (12), 2159–2170.

Zhang, Y.-Y., Fischer, M., Colot, V., Bossdorf, O., 2013. Epigenetic variation creates potential for evolution of plant phenotypic plasticity. New Phytologist 197 (1), 314–322. https://doi.org/10.1111/nph.12010.

Zhang, Q., Li, Y., Xu, T., Srivastava, A.K., Wang, D., Zeng, L., et al., 2016. The chromatin remodeler DDM1 promotes hybrid vigor by regulating salicylic acid metabolism. Cell Discovery 2, 16027. https://doi.org/10.1038/celldisc.2016.27.

Zheng, X., Chen, L., Xia, H., Wei, H., Lou, Q., Li, M., et al., 2017. Transgenerational epimutations induced by multi-generation drought imposition mediate rice plant's adaptation to drought condition. Scientific Reports 7, 39843. https://doi.org/10.1038/srep39843.

Zhou, X., Jacobs, T.B., Xue, L.-J., Harding, S.A., Tsai, C.J., 2015. Exploiting SNPs for biallelic CRISPR mutations in the outcrossing woody perennial Populus reveals 4-coumarate:CoA ligase specificity and redundancy. New Phytologist 208 (2), 298–301. https://doi.org/10.1111/nph.13470.

Zhu, R., Shevchenko, O., Ma, C., Maury, S., Freitag, M., Strauss, S.H., 2013. Poplars with a PtDDM1-RNAi transgene have reduced DNA methylation and show aberrant post-dormancy morphology. Planta 237 (6), 1483–1493. https://doi.org/10.1007/s00425-013-1858-4.

Zou, M., Lu, C., Zhang, S., Chen, Q., Sun, X., Ma, P., et al., 2017. Epigenetic map and genetic map basis of complex traits in cassava population. Scientific Reports 7, 41232. https://doi.org/10.1038/srep41232.

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