Chapter 19
Forest Tree Species Traced with a DNA-Based Proof for Illegal Logging Case in Poland

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Abstract Precise identification of biological samples remains the most important proof in the forensic science. Illegal logging has become the urgent issue in Poland during the last decades, and conventional methods of investigation turn out to be often insufficient. Recently, the DNA-based markers (SSR and cytoplasmic genes) can remarkably help in the forensic botany performed by the Forest Service Guards and the Police investigation in illegal logging of timber. The identification method relies on comparison of the piece of evidence (i.e., stolen wood fragments) with the piece of reference (e.g., tree parts remained in the forest). We present the usefulness of the DNA neutral markers (i.e., microsatellite loci) and cytoplasmic genes in forensic botany based on several case studies of illegal wood identification in Poland, concerning the most economically important coniferous tree species such as *Pinus sylvestris* L., *Picea abies* (L.) Karst., *Abies alba* Mill., and *Larix decidua* (L.). Thanks to the DNA profiles established on the basis of minimum 4 microsatellite nuclear DNA loci, and at least one cytoplasmic organelle (mitochondrial or chloroplast) DNA marker, the determination of the DNA profiles provided fast and reliable comparison between material of evidence (also wood and needles) and material of reference (first of all tree stumps) in the forest. These data strongly supported the decision taken by several District Courts in Poland, as far as the

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identification of wood samples was proved with a high probability (approximately 98–99 %). The aim of the below publication is to present Polish case study on DNA use to fight illegal logging which became very successful among foresters.

19.1 Introduction

Production of quicker and larger ships as well as globalization phenomenon stimulates a rapid increase of worldwide trade. The wood as a raw material was recently recognized as renewable source of energy. The pattern of traditional division among wood constructing and pulp and paper industry has changed because the new customers appeared on the market dealing with biofuels and biorefineries. They strongly compete with wood panel industry using even wood residues left after tree harvesting, e.g., to produce particle boards. At the same time, societies in developed countries prefer to conserve high forests rather than mobilize their exploitation. New values such as tourism and recreation, and picking berries or mushrooms are going to become fully marketable goods, soon. The international agreements concerning sequestration of CO_2 also limit the use of forests. The fast increment of human population which will reach 9 billion in 2050 will only increase demand on wood and its products. The price of wood timber significantly increased during last years and became precious product being willingly harvested illegally and smuggled to Europe. It causes steady diminishing of the world forest area. Forest area covers c.a. 30 % of the world's land surface, providing renewable fuel, wood, timber, and bark (www.fao.org). Moreover, it offers many forest externalities such as a source of medicinal products, carbon sequestration, water protection, and habitat for many terrestrial species. In this sense, the illegal logging is much more than only a value of wood timber. It causes a major problem for many timber-producing developing countries, including the environmental damage; it promotes pathogen and pest transport and generates costs of billions of dollars in government's lost revenue. The international trade of illegally logged wood is a major problem for the countries legally producing timber in many Southeast Asian and South American countries. It is estimated that around 20 % of illegally harvested wood timber is reaching European Union. Without stopping the deforestation phenomenon, it is not possible to meet Europe's 2020 goal on the reduction of greenhouse gases by 20 %.

The European Commission decided to tackle with this topic as its priority and developed proper legislation. Firstly, the action was based on voluntary agreements (FLEGT) and recently thanks to the new EU Timber Regulation. The European Union Regulation No 995/2010 of the European Parliament and of the Council[1]

[1]Regulation (EU) No 995/2010 of the European Parliament and of the Council of 20 October 2010 laying down the obligations of operators who place timber and timber products on the market. Official Journal of the European Union L 295/23-34.

also known as the (illegal) Timber Regulation (EUTR) laid down the obligations of operators who place timber and timber products on the market. The Regulation entered into application on March 3, 2013. It counters the trade in illegally harvested timber and timber products. The wood stolen from the forest is equally treated as those wood timbers from which the VAT is not paid or the wood was harvested from the stand managed in non-sustainable way (e.g., too much cuttings). The EU Timber Regulation covers a wide range of timber products listed in its Annex using EU Custom's code nomenclature. If this initiative has to slow down or stop deforestation process in endanger parts of the world, the scientists should support policy makers with the proper tools. Such a chance is given by the quickly developing genetics and DNA-analyzing technologies (e.g., New-Generation Sequencing). Recent advances in forensic genetics provide unique opportunity for such an investigation, which is the focus of this chapter.

19.2 Scientific Bases for the Genetic Tool to Combat the Illegal Harvesting and Trade

It is estimated that timber theft generates annually the greatest losses (among other forms of crimes) incurred by the Polish State Forests, and thieves stay unpunished for such misconduct (Nowakowska and Pasternak 2014). Since far, the dendrochronological methods are used to trace illegal tree cuttings. The advantage of this technique basically resides in approximate data indication when the illegal cutting occurred (Yaman and Akkemik 2009). But, the insufficient number or lack of clear annual rings due to wood decay severely limits the use of dendrochronological identification of wood samples.

Recently, the forensic botany has become a useful tool, which can help to save forest resources. The method based on the developed set of DNA markers to identify timber genetic profiles could then provide new evidence in a litigation cases faced by the Forest Guard Service or the Police. It is based on the rule that each organism genome contains a large amount of DNA that represents a huge dataset for genetic profiling. For the first time the DNA-based proof was used to determine the identity of a crime perpetrator in United Kingdom (Seton 1988), nearly 20 years after the discovery of the DNA molecules in human cells. Molecular markers can solve the problem of illegal logging as far as DNA molecules are well conserved in wood during harvesting and processing, and the nucleic acid isolation follows standardized methods. The noncoding DNA fragments are a source of variation (polymorphism) of an organism and constitute unique characteristics (pattern) of an individual. Many changes occurring at the DNA molecule level result from a single base-pair mutation, deletions, or substitutions of nucleic acids during replication errors. If those mistakes in gene structure are transmitted to the progeny, the phylogenetic study confirming the relationship of people with animals in the parental line is possible.

Particularly, the microsatellite sequences, known as short sequence repeats (SSR) or short tandem repeats (STR), are prone to the genetic identification at the individual level. The conifer's microsatellite sequences are randomly distributed in the whole genome. The repeated base-pair motifs mostly occur around intergenic regions, in other repeat elements such as satellites or retro-elements. Uneven distribution of repeat motifs may be connected with their functional role in gene expression of some of them, and this kind of distribution has been reported for eukaryotic genomes (Echt and May-Marquardt 1997). For example, motifs $(AC)_n$ and $(AG)_n$ occur in different parts of conifer genomes, and this distribution is conserved in gymnosperms (Smith and Devey 1994; Schmidt et al. 2000). The precise identification of biological samples based on microsatellite loci remains a crucial proof in forensic science, including animal and human probes (Schmidt et al. 2000). In many genomes, the microsatellites in general arise by interaction between DNA motifs or can be carried by the transposons. They mutate by replication slippage and unequal crossing over during the recombination. After the mutation process, the SSRs can reach expansion equilibrium and random mutations, which can lead to break repetitive patterns or the mutation accumulation in some parts of genome (Buschiazzo and Gemmell 2006). Such a phenomenon can be connected with the replacing of the interrupt repetitive motives in particular hot spots (Buschiazzo and Gemmell 2006). So far, a few of STR-rich regions were studied in plants, mostly in nuclear genome (Karhu et al. 2000), but they were also observed in mitochondrial DNA (Jaramillo-Correa et al. 2013).

19.3 Genetics to Provide Evidence for the Courts

Forensic genetics aims to provide evidence in criminal cases basing on the characteristics of the genetic code of the seized material (collected at the crime scene) and the reference one (taken from the alleged perpetrator). The analysis of the genetic profiles for forensic case work includes all kind of organisms ranging from humans and animals, to insects, microorganisms, and plants. The plant material can generate very discriminating DNA profiles as far as their genomes are the biggest among all living organisms. This allows the match of the biological evidence from the scene of crime to an individual with a high degree of confidence.

The forensic botany compares the DNA profiles between the tree tissue (wood, needles as material of evidence) and tree stumps (material of reference) in the forest (Fig. 19.1). The phenomenon of illegal logging has become an urgent issue to be solved in Polish forestry during the last decades, and conventional methods of investigation often turned out to be insufficient. The DNA-based proof becomes of great assistance in many investigations performed by the Polish Forest Service Guards, District Courts, and the Police. The basic identification method relies on the comparison of the piece of evidence (i.e., wood fragments originating from the stolen timber) with the piece of reference (e.g., stumps remained in the forest stand). A small amount of material (100 mg of wood, leaves, or needles) collected in the

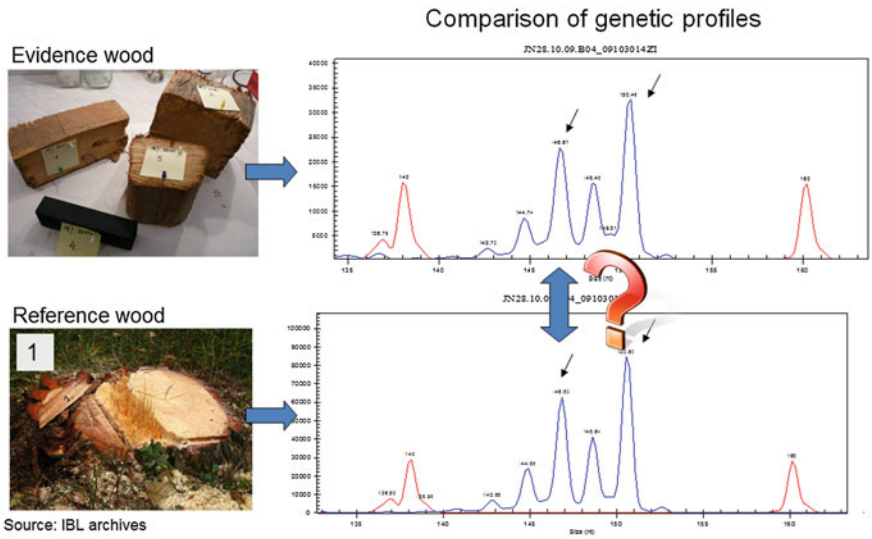

Fig. 19.1 General scheme of genetic profile comparison in wood sample identification procedure

field is required for biochemical analyses. The most frequent cases of illegal conifer wood identification in Poland concern Scots pine, Norway spruce, European silver fir, and European larch. Basic methodology for the DNA study relies on the following stages, i.e., DNA extraction (based on the lysis of cell walls), DNA amplification via polymerase chain reaction (PCR) method with specific primers for nuclear microsatellite or organelle alleles, genotyping of the PCR products in automated sequencer, and finally comparison of the DNA profiles obtained for all samples. This method avoids timely collection of data such as tree age, diameter, height, and thickness, although such a piece of information may be advantageous in wood identification process.

19.4 Difficulties Occurring in DNA-Based Analyses of Wood

The main difficulty in DNA-based analyses remains in proper DNA extraction method from wood tissues, because of the high amount of polysaccharides and polyphenolic compound residuals which inhibit the Taq polymerase during the PCR (Tibbits et al. 2006). The removal of those contaminants guarantees the success of further amplification and accurateness of DNA fragment (allele or gene) detection after the capillary electrophoresis performed in automated sequencer. Many methods of DNA extraction from plant tissue (including wood) have been proposed, e.g., CTAB-based isolation described by Doyle and Doyle (1990),

DNeasy Plant Mini Kit (Qiagen®) used by Dumolin-Lapègue et al. (1999), and MagAttract 96 DNA Plant Core Kit (Qiagen®) (Rachmayanti et al. 2006). Those methods yielded c.a. 2 μg of DNA per 50–100 mg of dried wood sample suitable for nuclear and organelle DNA amplification. The tissue type, i.e., cambium, sapwood, or hardwood, can generate different yield of the DNA molecules, in favor of cambial cells in *Pinus radiata* (Tibbits et al. 2006) and *Quercus robur* (Nowakowska 2011). Good-quality DNA was also extracted by Asif and Cannon (2005) and Tibbits et al. (2006), who supplemented the classical CTAB method with buffer containing NaCl and BSA effectively removing coextracted contaminants. In our case study, the DNeasy Plant Mini Kit (Qiagen®) used for Scots pine, NucleoSpin Plant II (Macherey-Nagel®) applied for Norway spruce, and Phire® Plant Direct PCR kit (Finnzymes®) method used in case of European silver fir and European larch wood resulted in correct amplification patterns of the studied forensic samples.

Generally, the DNA molecules are well preserved in the recently seized wood material. The best DNA isolation is achieved with an actively growing plant tissue (i.e., leaves, needles, buds), while the wood material seized in the field is often subjected to unfavorable climatic conditions during weeks or months. A potential problem occurs with ancient and/or potentially damaged DNA extracted from low amounts of tissues, which leads to the artefacts due to polymerase errors occurring during the amplification process. In many cases, chloroplast or mitochondrial DNA molecules represented by numerous copies per cell even in 100-year-old wooden material provide better templates for the PCRs than the nuclear genome being present in only two copies in each cell and being susceptible to all types of mutations (Dumolin-Lapègue et al. 1999). During archaeological investigations, cytoplasmic markers helped in the study of the geographical origin or taxonomic status of wood used in buildings, furniture, handcrafts, or barrels (Marco et al. 1994; Asif and Cannon 2005).

19.5 Cases of Different Forest Tree Species Recognition by DNA Method

In 2013, **five Scots pine samples** were seized by the Forest Service Guard in the Forest District of G. in Poland (Table 19.1) and sent to the Laboratory of Molecular Biology (LMB) in FRI (IBL, Poland) for relevant genetic analyses. The genomic DNA was extracted with DNeasy Plant 250 Kit (Qiagen®) according to manufacturer's instructions. Six nuclear microsatellite loci were amplified, i.e., SPAG 7.14, SPAC 11.4, SPAC 11.6, and SPAC 12.5 (Soranzo et al. 1998), and SsrPt-ctg4363, Rptest11 (Chagné et al. 2004) according to the LMB procedures (Nowakowska 2011). Eight chloroplast microsatellite DNA markers, i.e., PCP26106, PCP30277, PCP36567, PCP450712, PCP719872, PCP873142, Pt302042, and Pt71936 (Provan et al. 1998, Vendramin et al. 1996), were analyzed in order to generate the DNA profile of the samples.

Table 19.1 Genetic profiles of evidence and reference wood samples of Scots pine with 6 nuclear[1)] and 8 chloroplast[2)] microsatellite DNA markers

No.	Material[a]	SPAG 7.14[1)]		SPAC 12.5[1)]		SPAC 11.4[1)]		SPAC 11.6[1)]		SsrPt-ctg 4363[1)]		Rptest11[1)]	
1	Evidence wood	199	225	131	197	114	146	109	141	96	98	211	214
2	**Evidence wood**	**199**	**223**	**131**	**197**	**114**	**146**	**107**	**141**	**101**	**103**	**211**	**214**
3	Evidence wood	199	223	133	199	116	144	107	141	134	138	202	205
1	**Reference wood**	**199**	**223**	**131**	**197**	**114**	**146**	**107**	**141**	**101**	**103**	**211**	**214**
2	Reference wood	189	191	163	165	112	118	105	109	94	96	193	199

No.	Material[a]	PCP 26106[2)]	PCP 30277[2)]	PCP 36567[2)]	PCP 45071[2)]	PCP 71987[2)]	PCP 87314[2)]	Pt 30204[2)]	Pt 71936[2)]
1	Evidence wood	148	136	111	153	109	115	140	146
2	**Evidence wood**	**148**	**136**	**111**	**153**	**109**	**113**	**140**	**146**
3	Evidence wood	148	135	111	155	109	113	142	148
1	**Reference wood**	**148**	**136**	**111**	**153**	**109**	**113**	**140**	**146**
2	Reference wood	147	135	110	155	109	116	142	146

[a]Sampled material comprises
Evidence wood—pieces of wood seized onsite by the Police or the Forest Guard Service
Reference wood—a stump from the forest
Identical profiles are highlighted in bold
[1)]Nuclear microsatellite markers
[2)]Chloroplast microsatellite markers

Table 19.2 Genetic profiles of evidence and reference wood samples of Norway spruce with 9 nuclear[1)] microsatellite loci and 1 mitochondrial[2)] DNA marker

No.	Material[a]	EATC1 B02[1)]		EATC1 E3[1)]		EATC2 G05[1)]		SpaGG3[1)]		SpaGC1[1)]		EATC2 B02[1)]		EATC1 G2[1)]		SpaGC2[1)]		SpaG2[1)]		*nad*1[2)]
1	**Evidence wood**	**195**	**195**	**132**	**135**	**222**	**228**	**126**	**130**	**94**	**98**	**194**	**197**	**210**	**213**	**83**	**87**	**90**	**92**	**c**
1	**Reference wood**	**195**	**195**	**132**	**135**	**222**	**228**	**126**	**130**	**94**	**98**	**194**	**197**	**210**	**213**	**83**	**87**	**90**	**92**	**c**
2	Reference wood	193	201	129	135	240	246	126	130	90	96	182	182	207	213	105	111	92	92	a
3	Reference wood	213	217	123	123	210	213	146	148	102	104	191	194	180	189	131	133	100	104	a

[a]Sampled material comprises
Evidence wood—pieces of wood seized onsite by the Police or the Forest Guard Service
Reference wood—a stump from the forest
Identical profiles are highlighted in bold
[1)]Nuclear microsatellite markers
[2)]Chloroplast microsatellite markers

In 2014, **four Norway spruce wood samples** (Table 19.2) from the Forest District in N. were analyzed with the help of nine microsatellite loci, i.e., EATC1B02, EATC1E3, EATC2G05, SpAGG3, SpAGC1, EATC2B02, EATC1G2, SpAGC2, and SpAG2 (Pfeiffer et al. 1997) according to the LMB procedures. The mitochondrial *nad1* gene (Sperisen et al. 2001) variation was examined in DNA 1000 chip electrophoresis in Bioanalyser® (USA).

Genetic profiles of **four silver fir wood samples** (Table 19.3) seized in 2012 in the S. Forest District were established on the basis of four nuclear microsatellite loci SF1, SFb4, SF333, and SF239 (Cremer et al. 2006), as well as two chloroplast microsatellite loci Pt30204 and Pt71936 (Vendramin and Ziegenhagen 1997) with Phire® Plant Direct PCR Kit (Thermo Scientific, USA; ABO Ltd., Poland) according to manufacturer's instructions.

In 2013, the Phire® Plant Direct PCR Kit was also used to analyze the genetic profiles of **six European larch wood samples** (Table 19.4) seized in the Forest District of K., taking into account four nuclear microsatellite loci bcLK263, bcLK211, bcLK225, and bcLK228 according to the description of Isoda and Watanabe (2006). For larch samples, additional taxonomic identification was performed, thanks to chloroplast and mitochondrial markers described by Acheré et al. (2004).

For the investigated wood material, the polymerase chain reactions (PCRs) were conducted in Veriti 96 Thermal Cycler (Life Technologies™, USA), and the quality of DNA prior amplification was checked with NanoDrop® ND-1000 spectrophotometer (Wilmington, USA). The PCR products were analyzed with the 3500 Genetic Analyzer (Life Technologies™, USA) using the 3500 Data Collection Software and GeneMapper® version 5 (Life Technologies™, USA). Mean *PIC* (polymorphism information content) values were established for each set of markers in MolKin software version 2.0 (Gutiérez et al. 2005).

19.6 Identity of Compared Wood Samples

In order to avoid the accidental identity of two compared wood samples with the material of reference in the stand, the probability of identity P_{ID} is calculated according to Hedrick (2000):

$$P_{G_{hj}|k_j} = \prod_{i \in \Omega_h}^{\text{samples in } \Omega_h} P_{g_{ij}|k_j}.$$

where $P_{g_{ij}|k_j}$ signifies frequencies in ij alleles in k loci.

The P_{ID} estimator illustrates the probability that two individuals drawn at random from a population will have the same genotype at multiple loci and is generally used to assess the statistical confidence of the marker system for individual

Table 19.3 Genetic profiles of evidence and reference wood samples of European silver fir with 4 nuclear[1)] and 2 chloroplast[2)] microsatellite DNA markers

No.	Material[a]	SF1[1)]		SFb4[1)]		SF333[1)]		SF239[1)]		Pt30204[2)]	Pt71936[2)]
1	Evidence wood	214	218	144	172	166	168	123	123	142	149
2	Evidence wood	218	220	146	174	166	166	108	112	142	149
3	**Evidence wood**	**222**	**224**	**148**	**174**	**164**	**168**	**108**	**110**	**142**	**149**
1	**Reference wood**	**222**	**224**	**148**	**174**	**164**	**168**	**108**	**110**	**142**	**149**

[a]Sampled material comprises

Evidence wood—pieces of wood seized onsite by the Police or the Forest Guard Service

Reference wood—a stump from the forest

Identical profiles are highlighted in bold

[1)]Nuclear microsatellite markers

[2)]Chloroplast microsatellite markers

Table 19.4 Genetic profiles of evidence and reference wood samples of European larch 4 nuclear[1)] microsatellite DNA markers

No.	Material[a]	bcLK228[1)]		bcLK225[1)]		bcLK211[1)]		bcLK263[1)]	
1	Evidence wood	179	195	160	184	207	209	184	240
2	**Evidence wood**	**179**	**199**	**160**	**184**	**211**	**213**	**184**	**240**
3	Evidence wood	179	195	160	184	207	209	184	240
1	Reference wood	199	199	160	184	209	209	184	240
2	Reference wood	177	199	158	158	207	209	182	182
3	**Reference wood**	**179**	**199**	**160**	**184**	**211**	**213**	**184**	**240**

[a]Sampled material comprises
Evidence wood—pieces of wood seized onsite by the Police or the Forest Guard Service
Reference wood—a stump from the forest
Identical profiles are highlighted in bold
[1)]Nuclear microsatellite markers
[2)]Chloroplast microsatellite markers

identification. In practice, the $P_{ID} = 0.001$ for a set of DNA markers means 0.1 % of probability for the identical genotype of tree existing in the forest. Therefore, the genetic profiles of wood from material of evidence and material of reference are identical with 99.9 %.

In all coniferous samples studied for forensic purposes, the P_{ID} estimator ranged from 0.0001 (in Scots pine wood) to 0.011 (in European silver fir wood), proving the very low probability of identical genotypes among 100 randomly examined trees in the stand. These data strongly supported the decision taken by several District Courts in Poland, as far as the identification of wood samples was proved with the high probability (approximately 98.89–99.99 %).

19.7 DNA Profiles as a Strong Proof in the Court Cases

The essence of comparative studies based on DNA analysis is to provide a set of genetic markers with high discrimination power between individuals. Current SSR-based multiplex kit taking into account biallelic single nucleotide polymorphism (SNPs) ensures almost 100 % of likelihood for the match between two human profiles with an error ratio of 1 to billion (Goodwin et al. 2011). Human population is sufficient to analyze c.a. 9 STR loci with the probability close to $1{:}10^6$. Due to the high polymorphism level of the SSR loci in woody species, the minimum of 4 microsatellite DNA loci is sufficient to exclude or confirm the identity of investigated coniferous material at the level of 99.99 %.

Based on 6 nuclear and 8 chloroplast DNA markers of **Scots pine wood** from the G. Forest District, the genetic identity of sample n° 2 (material of evidence) and wood sample n° 1 (material of reference) was concluded (Table 19.1). The final decision sent to the court was supported by the genetic identity between those samples proved with a high probability of 99.99 % ($P_{ID} = 0.0001$).

A wood comparison study performed for **Norway spruce wood** from the N. Forest District revealed the genetic identity between evidence material n° 1 and reference material n° 1 (Table 19.2) with 99.98 % of probability (P_{ID} = 0.002).

The DNA profile comparison between **European silver fir wood** samples from the S. Forest District proved the genetic identity of evidence between the material n° 3 and the reference material n° 1 (Table 19.3) with 98.90 % of probability (P_{ID} = 0.011).

Based on 4 nuclear DNA markers of **European larch wood** from the K. Forest District, the genetic identity between the sample n° 2 (material of evidence) and the wood sample n° 3 (material of reference) was determined (Table 19.4), with the high probability of 98.89 % (P_{ID} = 0.0102).

All selected nuclear DNA markers were characterized by the high level of polymorphism content, with the mean *PIC* = 85.6 % for microsatellite nuclear DNA loci and *PIC* = 95.4 % for cytoplasmic DNA (*PIC* = 40.0 % for mitochondrial and *PIC* = 65.7 % for chloroplast DNA loci).

19.8 Selection Pressure and Environment-Driven Forces

The genomes of many species undergo long evolutionary processes resulting in Hardy–Weinberg equilibrium (HWE) observed in numerous forest tree populations. That means the genotype frequencies can be predicted from the frequencies of the SSR alleles. The effects of genetic drift (more pronounced in small, isolated populations) and self-pollination phenomenon are less frequent in wind-pollinated pine, spruce, fir, and larch stands. Moreover, the DNA loci are less prone to selection pressure and environment-driven forces.

Coniferous forests cover mostly areas in the Northern Hemisphere where they have a huge environmental and economic value, especially in wood industry. However, despite the high importance of this group of woody plants, the knowledge of their genomes is still limited. The genomes are not well characterized, although there are some reports pointing that gene families in conifers are much bigger than those in their angiosperm equivalents, which additionally contain a number of pseudogenes and are rich in high repetitive elements (Ahuja and Neadle 2005; Buschiazzo et al. 2012).

19.9 Genome Complexity and SSR Markers

Due to the big size of the conifer genome (ca. 20–30 Gb), its molecular analysis is a major challenge. However, the genome size is not the only obstacle in the molecular analysis, but also the large effective population size, the high heterozygosity, and the low substitution rate connected to their long life span. One of the hypotheses of an evolution of conifer genome suggests that the basal number of 12 chromosomes

($2n = 24$) slowly expanded according to the activity of transposable elements (LTR—long terminal repeats), which are shared in many conifer genomes. This expansion probably started early and in opposite to angiosperms, and the LTR copies remained in the genomes of conifers, because of less effective mechanism of removal of transposable elements in conifer than in other organisms (Nystedt et al. 2013). Moreover, huge chromosomes can be formed as a result of chromosome replication with genes separated with long fragments of transposable elements, pseudogenes, and highly polymorphic noncoding regions with low recombination frequencies. Conservative structure of the genome, marginal DNA rearrangements, and lack of the whole-genome duplication can explain a high degree of conservation and low phenotypic variation. However, the high degree of the adaptability of different conifers in varied ecosystems may be closely related to the high genetic variability of their huge genome sizes.

Nowadays, the SSR markers are widely used for the analysis of the population diversity, gene flow, parentship, construction of linkage maps, etc. Therefore, the knowledge of their importance, the evolution, and the rate of mutation is very valuable in many fields of research. Thanks to the DNA profiles established on the basis of minimum 4 microsatellite nuclear DNA loci, and at least one cytoplasmic (mitochondrial or chloroplast) DNA marker, the determination of the DNA profiles provided fast and reliable comparison between material of evidence (wood, needles) and material of reference (tree stumps) in the forest. Confirmed by the present and previous data obtained during the illegal logging investigation in Poland, the low probability of occurrence (P_{ID} value) of the identical genotype among randomly chosen trees in a forest stand proved the appropriateness of all nuclear and cytoplasmic DNA markers designed in the Forest Research Institute (FRI) for Scots pine, Norway spruce, silver fir, and European larch wood material investigation (Nowakowska 2011). Similar markers assigned for European larch identification may be successfully used for Japanese larch (*L. kaempferi* Sorg.) wood genetic identification (unpublished data). Still, the most crucial in the forest practice against illegal logging remains the proper manner of wood collection, avoiding material deterioration or contamination. Detailed instructions for seizing wood samples for DNA analyses were presented in a video training film entitled "DNA analysis of wood in combating timber illegal trade" (www.ibles.pl).

19.10 Perspectives of DNA Profiling Methods to Investigate Illegal Logging in Forests

The investigation focusing on DNA markers of wood resulted in designing the efficient method to be used for forensic purposes. The presented methodology helps to identify any single tree species forming major forest stands in Poland, i.e., Scots pine (*P. sylvestris*), Norway spruce (*P. abies*), European silver fir (*A. alba*), and European larch (*L. decidua*), with the high probability c.a. of 99.99 %. The

comparison based on detailed DNA patterns can be used for diagnostics of individual wood samples, taking into account the random probability of identical trees existing in the stand.

Forensic wood material (shafts, logs, sawmill assortment, and stumps) has been proved to be appropriate for the DNA-based identification studies. In order to prohibit the placing on the EU market illegally harvested timber and products derived from such timber, the genetic tool based on DNA (SSR and cytoplasmic markers) helps to identify and match the timber logs in question with the stumps found in the forest with very high probability, practically close to 100 %. It is also possible to match logs with needles (leaves), branches, or roots left on the spot of illegal harvesting. Thanks to this tool the EU traders who place their timber products on the EU market for the first time can check if wood was legally harvested.

The methods based on DNA profiling applied to investigate illegal logging in European forests may contribute to the international actions like Forest Law Enforcement, Governance and Trade (FLEGT) focusing on wood trade between overseas countries and Europe, as well as promoting wood from certified and sustainable managed forests. It is also consistent with the assumptions of the European Parliament Directive on Timber Regulation (EUTR), which came into effect in 2013 to stop the circulation of illegally logged wood in the European Union.

In future, developed genetic techniques can help to identify the wood imported from the disqualified forest regions in the world (e.g., from the forest not meeting the sustainable forest management). Also, they may help to facilitate the traceability of timber products by economic operators in this part of the supply chain referred to as traders in the EU Timber Regulation.

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