SHORT REVIEW

MICROSATELLITE SEQUENCES: A NEW GENERATION OF MOLECULAR MARKERS FOR FOREST GENETICS

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ABSTRACT

Microsatellites are tandem DNA repeats characterised by short sequence motifs, and have been identified and characterised in the nuclear as well as in the chloroplast and mitochondrial genomes of many forest tree species. They are very abundant, randomly dispersed in the genome, highly polymorphic and they show a co-dominant inheritance and a selectively neutral behaviour. The availability and usefulness of nuclear and chloroplast microsatellite markers in genome mapping and population genetics of temperate broad-leaved and tropical species and in conifers is presented and demonstrated.

Keywords: microsatellites, nuclear genome, chloroplast genome, forest species, genome mapping, population genetics

INTRODUCTION

Microsatellites are tandem repeats characterised by short motifs (1 to 6 bp), a low degree of repetition (5 up to 100 repeat units) and a randomly dispersed distribution of about 10^4 to 10^5 per genome (TAUTZ 1993). They are also called simple sequence repeats (SSR), short tandem repeats (STR), or simple sequence length polymorphisms (SSLP) but microsatellites and simple sequences repeats (SSR) are now the most widely accepted denomination. Though some can be found in transcription units (PERRY & BOUSQUET 1998), they have no known function and appear to be solely maintained by their ability to replicate and expand in the genome within the limits established by the negative selection pressure of the fitness loss that they may cause (CHARLESWORTH *et al.* 1994). They display a selectively neutral behaviour, a co-dominant inheritance that allows discrimination of homo- and heterozygotic states in diploid organisms, have a frequent occurrence and an even distribution throughout the nuclear genome, and can also be found in the chloroplastic (VENDRAMIN *et al.* 1996) and mitochondrial genomes (SORANZO *et al.* 1998a). Their high length polymorphism, due to a different number of repeats within the microsatellite regions, can be easily and reproducibly detected via the polymerase chain reaction (PCR). Their main applications are in genome mapping and in population analysis, but microsatellites are also useful for taxonomy, parentage analysis, identification of individuals in forensic studies and human cancer diagnostics.

NUCLEAR MICROSATELLITES (nSSR)

Temperate broad-leaved species

Few species have been screened for microsatellite loci in temperate broad-leaved species until now, and sequences and primers are available for *Quercus* (DOW *et al.* 1995; ISAGI & SUHANDONO 1997; STEINKELLNER *et al.* 1997a) and *Fraxinus* (LEFORT *et al.* 1997 and 1998a). Primers sets are also available for *Populus* (STRAUSS *et al.* 1998; Poplar Molecular Genetics Cooperative SSR Database http://poplar2.cfr.washington.edu/pmgc/ssr/pmgcssr.html) and *Citrus* (KIJAS *et al.* 1995) species. nSSR markers, published for different *Quercus* species, are informative

throughout the natural geographic range of the genus (*Q. macrocarpa*, North America, *Q. myrsinifolia*, Asia, and *Q. petraea*, Europe). The abundance of microsatellite loci in *Quercus* genome is comparable to other tree and plant species (STEINKELLNER *et al.* 1997a). The observed polymorphism was extremely high with 15 to 32 alleles per locus in a forest stand of *Q. petraea* and *Q. robur* (STREIFF *et al.* 1998), and 13 to 20 alleles per locus in a natural population of *Q. macrocarpa* (DOW & ASHLEY 1996). Two SSR markers developed in *Q. macrocarpa* showed amplification and equivalent polymorphism level in *Q. petraea* and *Q. robur* (STREIFF *et al.* 1998). Fourteen primers pairs developed for *Q. petraea* amplified in the closely related *Q. robur* species and in some species from other genera (STEINKELLNER *et al.* 1997b). In the same way, primers developed for *Q. myrsinifolia* were conserved in other Asian oak species (ISAGI & SUHANDONO 1997), and ten primers pairs from *Fraxinus excelsior* amplified in 14 other *Fraxinus* species and in 11 other *Oleaceae* (LEFORT *et al.* 1998). Microsatellites developed on temperate angiosperm forest tree species have been used for gene flow analysis and mapping purposes. Hence, DOW and ASHLEY (1996) characterised gene flow by pollen and by seeds with a parentage analysis on naturally established seedlings in a natural stand of *Q. macrocarpa*. STREIFF *et al.* (1999a), in a same way, used microsatellite loci to characterise gene flow by pollen in a managed oak stand of two species, *Q. petraea* and *Q. robur*. Six highly polymorphic loci allowed the assignment of one male parent in the population without ambiguity for more than 95% of the analysed offspring. This paternity analysis allowed precise characterisation of the mating system parameters, the pollen dispersal function and the gene flow originating from outside the population (STREIFF *et al.* 1999b).

Microsatellites are powerful mapping tools due to their polymorphism, codominance and cross priming in different species and are also good candidates as anchor points within and across genetic maps. Eighteen microsatellite loci were mapped in *Q. robur* (BARRENECHE *et al.* 1998) and showed a homogeneous coverage of the genome.

Tropical species

Microsatellites sequences are already available for several tropical species : *Acacia* sp. (DECROOCQ *et al.* 1997), *Eucalyptus nitens* (BYRNE *et al.* 1996), *Gliricidia sepium* (*Robinieae*) (DAWSON *et al.* 1997), *Piper reticulatum* (*Piperaceae*), *Malmea* sp. nov. (*Annonaceae*), *Virola sebifera* (*Myristicaceae*), *Trophis racemosa* (*Moraceae*), *Poulsenia armata* (*Moraceae*) (CONDIT & HUBELL 1991), *Dryobalanops lanceolata* (*Dipterocarpaceae*) (TERAUCHI 1994), a large canopy tree *Pithecellobium elegans* (CHASE *et al.* 1996a), *Rhododendron metternichi* (NAITO *et al.* 1998), *Swietenia humilis* (*Meliaceae*) (WHITE & POWELL 1997a) and *Symphonea globulifera* (ALDRICH *et al.* 1998). They should be soon available for tek (*Tectona grandis*) and palm oil tree (*Elaeis guineensis* Jacq.) (D. Kusamawati & H. Sitti Nuroniah, Technological Institute, Bandung, Indonesia, personal communication). The identified loci (from 1 to 11 per species) are mainly of the dinucleotide types $[ac/gt]_n$ and $[ag/ct]_n$ and polymorphism varies from 4 to 24 alleles per locus. Some have shown a potential for cross-specific amplification among the same family (WHITE & POWELL 1997b). One SSR locus (6 alleles) of *Gliricidia sepium* was used to genotype 398 progenies from 23 individual trees in order to assess directly pollen-mediated gene dispersal in a natural stand. *G. sepium* is a insect-pollinated tree and microsatellite data showed that 1.8% of gene transfer events were recorded over a distance of 75 m. If only the rarest alleles were considered, 6.1% of transfers could be ascribed to a distance of greater than 75 m. These data, showing that gene transfer events mainly occurred within a diameter of 75 m, are consistent with the behaviour of the pollinators of *G. sepium* which display patterns of near-neighbour foraging with occasional long intervening flight distances. In *Pithecallobium elegans* (CHASE *et al.* 1996), nine SSR loci were characterised and allelic diversity ranged from 5 to 24 alleles per locus. Gene-flow and mating patterns have been studied in a natural rain forest, recently converted to pasture, among 28 adult trees and 167 seeds from progeny arrays of six trees representing 72 independent mating events. The number of alleles and their high allelic diversity allowed an unambiguous identification of all the pollen donors for 97.2 % of the mating events and 28.6 % of the fathers were not of the marked population. Gene flow from known fathers averaged 142 m with a maximum distance of 350 m which is consistent with the long-distance forageing behaviour of the pollinator (hawkmoth). Most surprising was that most mating events were with the most distant trees (more than 10 trees away). These two examples illustrate well the usefulness of microsatellite markers: gene flow information, genetic structure, sociobiological studies correlated to the ecology data.

Conifer species

SSR sequences are abundant in conifers (SMITH & DEVEY 1995; ECHT & MAY-MARQUARDT 1997; PFEIFFER *et al.* 1997), as they are in plants in general (MORGANTE & OLIVIERI 1993, WANG *et al.* 1994). Given the economic

importance of coniferous tree species, there isconsiderable interest in obtaining informative SSR markers. A number of laboratories have begun marker development efforts and conifer SSR markers are now available from *Larix occidentalis* (KHASA *et al.* 1998), *Pinus contorta* (HICKS *et al.* 1998), *Picea abies* (PFEIFFER *et al.* 1997; PAGLIA *et al.* 1998a), *Picea sitchensis* (VAN DE VEN & MCNICOL 1996), *Pinus radiata* (SMITH & DEVEY 1994; FISHER *et al.* 1996), *Pinus strobus* (ECHT *et al.* 1996) and *Pinus sylvestris* (KOSTIA *et al.* 1995; SORANZO *et al.* 1998b). In addition, there are on-going SSR marker development efforts in various laboratories for *Pinus taeda* (C. S. Echt, unpublished results), *Pinus halepensis* (G. G.Vendramin, unpublished results), *Pinus monticola* and *Thuja plicata* (C. Newton, British Columbia Research Institute, USA, personal communication), *Pseudotsuga menziesii* and *Tsuga heterophylla* (J. Carlson, Pennsylvania State Univ. USA, personal communication). For most of these species there are still only a handful of useful SSR primer pairs available, but for a few species there are many. The most SSR primer pairs currently available for a conifer species is 122 from *Picea abies* (M. Morgante, University of Udine, Italy, personal communication), followed by 51 from *Pseudotsuga menziesii* (J. Carlson, personal communication) and 37 from *Pinus radiata* (G. Moran, CSIRO Forestry and Forest Products, Canberra, Australia, personal communication). For *P. taeda*, 110 single-locus SSR markers have been identified (ECHT *et al.*, in preparation), and within the next year we expect to have available hundreds of more primer pairs for *Pinus radiata* and *P. halepensis*. Some of the pine SSR primers are also commercially available.

ECHT *et al.* (1999), using SSR primer pairs from *Pinus strobus* and *Pinus radiata*, found that while primers for monomorphic loci could amplify loci from a wide range of species, the primers for informative dinucleotide repeat loci could only amplify loci from species within a subgenus. Conifer SSR markers have been used for monitoring genetic diversity in managed stands (ECHT 1999; THOMAS *et al.* 1998), genome mapping (DEVEY *et al.* 1996; ECHT & NELSON 1997; PAGLIA *et al.* 1998b) and DNA fingerprinting and parentage analysis (PAGLIA & MORGANTE 1998).

CHLOROPLAST MICROSATELLITES (cpSSR)

CpSSRs have been identified in the chloroplast genome of both conifers (POWELL *et al.* 1995a; VENDRAMIN *et al.* 1996) and angiosperms (POWELL *et al.* 1995b). The availability of the entire sequence of the chloroplast genome of *Pinus thunbergii* (WAKASUGI *et al.* 1994) allowed the identification of twenty - microsatellites, constituted of single nucleo-tide repetitions (19$[a/t]_n$ and 1 $[g/c]_n$) (VENDRAMIN *et al.* 1996). In conifers, the chloroplast genome is paternally inherited (CATO & RICHARDSON 1996; VENDRAMIN & ZIEGENHAGEN 1997) whereas in angiosperm it is maternally inherited (DUMOLIN *et al.* 1995). As the chloroplast genome does not recombine, cpSSRs variants accumulate in a chloroplastic lineage, and thus provide a clonal record of haplotypes which can be used to study the history of populations. The "universality" of most of the cpSSR markers developed from *Pinus thunbergii* chloroplast microsatellite sequences has been established in many *Pinaceae* species (POWELL *et al.* 1995a 1995b; VENDRAMIN *et al.* 1996) and even angiosperms (CATO & RICHARDSON 1996). Universal primers for the amplification of cpSSRs in different species advantageously lowers development costs for individual species. Chloroplast microsatellite variation within and among populations has been analysed in *Abies alba* (VENDRAMIN & ZIEGENHAGEN 1997), *Pinus halepensis* (MORGANTE *et al.* 1997), *Pinus leucodermis* (POWELL *et al.* 1995a), *Pinus pinaster* (VENDRAMIN *et al.* 1998), *Pinus resinosa* (ECHT *et al.* 1998a) and analysis are in progress for *Picea abies* and *Pinus lambertiana*. The distribution of chloroplast haplotypes and of the haplotypic diversity is geographically structured in *Abies alba, Picea abies, Pinus halepensis* and *Pinus pinaster* and appears to be associated with the migration processes from glacial refugia occurring in the most recent post-glacial period. A large proportion of "population specific" haplotypes was detected in *Pinus halepensis* and *Pinus brutia* (89%, BUCCI *et al.* 1998), *Pinus pinaster* (73%, VENDRAMIN *et al.* 1998) and *Pinus resinosa* (61%, ECHT *et al.* 1998) confirming that cpSSR markers can be a useful tool for certification of seed-lots as well as for provenance identification. In *Pinus halepensis*, almost all of the actual haplotypic diversity is concentrated in Greece, which is thought to be the centre of origin of the species (MORGANTE *et al.* 1997). Strong evidence of introgression of *Pinus halepensis* haplotypes into *Pinus brutia* and of unidirectional gene flow in a natural sympatric population was also detected using cpSSR markers (BUCCI *et al.* 1988). ZIEGENHAGEN *et al.* (1998) demonstrated the potential usefulness of the cpSSR approach towards paternity analysis in *Abies alba*. In summary, the chloroplast microsatellite approach shows a relevant usefulness for studying population history, for monitoring gene flow, for identifying areas harbouring high levels of variability, and for certification of seed-lots and provenances. CpSSR analysis thus has an important role in population and conservation genetics of plants.

CONCLUSION

In the past 4 years, nuclear and chloroplast microsatellite sequences have been characterised as suitable molecular markers in a wide range of tree species, broadleaves or conifers, temperate or tropical, of prime economic or ecological importance and more microsatellites markers will be available in a near future. The use of these markers in fundamental research (genetic structure, gene flow studies, genome mapping and molecular phylogenetics) and applied research (marker-aided breeding, provenance testing, commercial certification) is now under way. Given the time and costs needed to identify such markers, potential cross-amplification of primers designed for a species between species and genera has to be extensively examined, although successful amplification tends to decrease with an increasing evolutionary distance. International collaboration of all the scientific teams involved in this field is much needed to build databases providing with microsatellites and amplifying primers sequences as well as alleles sizes data as already suggested by THOMAS *et al.* (1995) for *Vitis vinifera*. Such a dissemination of knowledge will help to reduce research and development costs. Until then, the USDA Forest Service offers information about SSR markers in some tree species through the Dendrome web site (http://dendrome.ucdavis .edu /Data/ primer.html).

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