African Journal of Biotechnology Vol. 9(29), pp. 4510-4515, 19 July, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Review

Cytoplasmic and nuclear DNA markers as powerful tools in populations' studies and in setting conservation strategies

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Accepted 18 June, 2010

Plants are distinguished among eukaryotes in possessing two DNA-containing organelles, the mitochondrion and the plastid, whereas, most eucaryotes contain only the mitochondrial genome. Recently, both organelles are used efficiently in population studies as plant geneticists developed molecular techniques that facilitated the study of plant diversity and evolution. In this paper, some comparisons among organelle and nuclear DNA, their mode of inheritance, examples of their use in genetic investigation of natural plant populations and the different sampling strategies for both markers were provided. The availability of completely sequenced genomes facilitated the development of markers (for example, consensus cp DNA markers). The use of the organelle markers as a tool in intra-specific studies of plant populations, can aid in clarifying their complex behavior by studying their respective distribution area and population dynamics such as in several phylogeography studies. Such studies can help in suggesting conservation management strategies in future for the populations under study.

Key word: Chloroplast DNA, Mitochondria DNA, Nuclear DNA, DNA Markers, Population Studies

INTRODUCTION

Populations are normally defined as geographic entities within a species, categorized either ecologically or genetically (Ehrlich and Daily, 1993). The genetically based definition define population as a group of individuals evolving independently from other groups due to limited gene flow which makes them genetically distinguishable from each other (Hughes et al., 1997). Genetic studies in natural vegetations have become widespread. These ecosystems are known to be dynamic. There is a need for their protection from fragmentation, loss of species, excessive hybridization that can occur between closely related species, colonization by invasive species among others. Several researchers found that it is possible to make conclusions about the history and status of forest trees and especially those endangered, by measuring genetic variation and interpreting these data in a population genetics context. The theory of population genetics furnishes a powerful approach to interpret the measured amounts of genetic variation which is informative in future management and conservation strategies.

Reliable estimates of population differentiation within and between the populations as well as the dynamics of this diversity are crucial in conservation biology as it is often necessary to understand the mechanism of evolution and to understand whether populations are genetically isolated from each other and to what extent.

Population geneticists always recommend avoiding inbreeding and maintaining as possible high genetic variation (Hedrick and Miller, 1992). However, the recommendations did not consider the real genetic variation. There are several forces shaping the genetic variation. A comparison across loci permits inferences about migration, mutation and drift.

In the last decade, the applications of new molecular

Abbreviations: LSC, Large single copy regions; **SSC,** small single copy regions; **IR,** inverted repeat; **PCR,** polymerase chain reaction; **RFLP,** restriction fragment length polymorphism.

Table 1. Comparison of the evolutionary processes between nuclear genomes and cytoplasmic organelles.

Evolutionary unit	Nuclear	Cytoplasmic
Cell (within and among cells)	Mutation	Mutation
		Selection
		Drift
		Recombination (rare)
Individual (among individuals)	Selection	Selection
	Drift	Drift
	Migration	Migration
	Recombination	Recombination

Source: Korpelainen (2004).

techniques have boosted and refined the level of genetic analysis. Molecular genetic markers have played a great role in giving clues in description and quantification of population divergence (Avise, 1994) and population structure (Hamza, 2005). Their use is increasing and detailed maps of the distribution of several species are now available and require a wide range collection of populations (Petit et al., 2002).

Here, an overview of the three DNA markers, with emphasis on the organelles and their difference from the nucleus are provided. Up to date achievements as well as accessible and available recent important databases, especially those that avail large number of organelle primers for use in population studies were also provided, giving some examples of how these types of genetic variation can help in setting guidelines for the conservation. Discussion on considerations and strategies for sampling when using organelle markers, as well as proving chloroplast markers as a good tool in revealing the population structure in plants were carried out.

COMPARISONS OF ORGANELLE AND NUCLEAR GENOMES

Plants are characterized by three types of genomes in the cellular compartment: the nuclear, mitochondrial and the chloroplast. These genomes present different characteristics according to the organisms in which they evolve. The significant differences in evolutionary processes between the nuclear and cytoplasmic genomes (Table 1) are that in multiple-copied cytoplasmic genomes, selection and drift exist at both the individual and cell level; also intermolecular recombination is of restricted importance (Korpelainen, 2004). Sequence comparisons identified proteobacteria and cyanobacteria as ancestors of mitochondria and chloroplasts, respectively (Gray and Doolittle, 1982). It was proposed that some genes had been relocated from the ancestral organelles to the nucleus during evolution (Weeden, 1981; Korpelainen, 2004; Timmis et al., 2004) and vice versa (Timmis et al., 2004). The multiple genomes of chloroplasts and mitochondria are inherited in a non-Mendelian way in many organisms (Korpelainen, 2004). Both chloroplast and mitochondria generally contain multiple circular haploid genomes that are present as monomers and multimers (Timmis et al., 2004). The first higher plant cpDNA genome to be completely sequenced was *Nicotiana tabacum* (tobacco) (Shinozaki et al., 1986). The cpDNA genome is estimated to be 10 times longer than mammal mtDNA genome and consists of 42% non-coding sequences (splited into 30% of intergenic spacers and 12% introns) (Petit and Vendramin, 2007). After ten years, the first complete sequence of seed plant mitochondrial genome (*Arabidopsis thaliana*) was achieved (Unseld et al., 1997). In photosynthetic plants, the size of the chloroplast genome range from 120 to 217 kb, with most angiosperm species having genomes of 135 to 160kbp (Downie and Palmer, 1992).

The chloroplast genome is a single circular chromosome, which consists of two single copy regions (Large: LSC and Small: SSC), separated by two inverted repeat (IR) regions of 10-76 kbp, averaging 20-30 kbp in most species. The IR regions encode the 18S and 23S ribosomal RNA genes (Palmer 1985, Petit and Vendramin, 2007). The coding capacity of organelle genomes varies markedly across eukaryotic lineages. Sequenced plastid genomes contain from 20 to 200 coding genes (Martin and Herrmann, 1998; Simpson and Stern, 2002) and mitochondrial genomes encode from 3 to 67 coding genes (Gray et al., 1999; Lang et al., 1999).

The major differences between chloroplast and nuclear genomes are that the former shows a uniparental mode of inheritance in most plant species (Reboud and Zeyl, 1994; Birky, 1995), a clonal mode of evolution and slow rate of evolutionary change (Wolfe et al., 1987).

The plant mitochondrial genome displays a low rate of nucleotide substitution compared to the plant nuclear and chloroplast genomes; it changes three times slower than cpDNA (Wolfe et al., 1987). It is likely to evolve mainly in structure by intragenomic recombination via small repeated sequences dispersed within the genome (Lonsdale et al.,

Table 2. Some examples of the completed sequence of chloroplast genomes.

Latin name	Common name	Classification	Size (kbp)	Accession no.
**Arabidopsis thaliana*	Arabidopsis	Angiosperm-dicot	154	AP000423
**Nicotiana tabacum*	Tobacco	Angiosperm-dicot	156	Z00044
**Oryza sativa*	Rice	Angiosperm-monocot	134	X15901
**Zea mays*	Maize	Angiosperm-monocot	140	ZMA86563
**Pinus thunbergii*	Pinus	Gymnosperm	120	PINCPTRPG
**Marchantia polymorpha*	Marchantia	Bryophyte	121	X04465
Populus trichocarpa	Poplar	Angiosperm-dicot	157	http://genome.jgi-psf.org/ Poptr1/Poptr1.home.html

*Source: http://megasun.bch.umontreal.ca/ogmp/projects/other/cp_list.html

Table 3. Some examples of the completed sequence of mitochondrial genomes.

Latin name	Common name	Classification	Size (kbp)	Accession no.
Arabidopsis thaliana	Arabidopsis	Angiosperm- Dicot	367	MIATGENA
Nicotiana tabacum	Tobacco	Angiosperm- Dicot	431	BA000042
Marchantia polymorpha	Marchanita	Bryophyte	187	MPOMTCG

Source: http://megasun.bch.umontreal.ca/ogmp/projects/other/mt_list.html

Table 4. Some examples (of Angiosperms) of organelle DNA transmission in plants.

Family	Species	cpDNA	mtDNA	References
Angiosperms	**Dicotyledons**			
Asteraceae	*Helianthus annus*	M	M	Rieseberg et al. 1994
Brassicaceae	*Arabidopsis thaliana*	M	M	Martinez-Zapater et al. 1992
Fabaceae	*Lens culinaris*	MP	MP	Rajora & Mohon 1994, 1995
Fagaceae	*Quercus robur*	M	M	Dumolin et al. 1995; Dumolin-Lapegue et al. 1998
Salicaceae	*Populus spp.*	M	M	Mejnartowicz 1991; Rajora and Dancik 1992; Radetzky 1990, Rajora et al. 1992
Solanaceae	*Nicotiana tabacum*	M(P)	M	Medgysey et al. 1986
Angiosperms	**Monocotyledons**			
Musaceae	*Musa acuminata*	M	P	Faure´ et al. 1994

Source : Petit and Vendramin (2007). Where, M = Maternal inheritance; P = paternal inheritance.

1988; Palmer 1992). The complete sequencing of several genomes is accomplished as can be seen in an outstanding up-to-date list of sequenced organelle genomes available at "*All complete organelle genome sequences* website: (http://megasun.bch.umontreal.ca/ogmp/projects/other/all_list.html). Till now, a total of 1898 complete mitochondrial genome sequences are achieved, of which a total of 1283 complete sequences of different organisms are available online. On the other hand, 122 chloroplast genome sequences have being completed, of which 114 are available online. Some examples of these are presented for chloroplast genome (Table 2) and for mitochondria genomes (Table 3).

INHERITANCE OF THE ORGANELLE GENOMES

The mode of inheritance of the chloroplast and the mitochondrial genomes range from strictly maternal to strictly paternal (Reboud and Zeyl, 1994; Petit and Vendramin, 2007). Many exceptions to this common inheritance pattern of genes in mitochondria and chloroplast are present, of which some are shown in Table 4.

In an investigation, bi-parental transmission was recorded in 27% out of 88 families investigated, 21% of the 233 genera and 27% of the 398 species (Petit and Vendramin, 2007). In banana (*Magnolia acuminata*), chloroplasts are maternally inherited and mitochondria

are paternally inherited (Table 4). Such cases of discordant uniparental inheritance are interesting to study for their effects on differential levels of gene flow via seeds and pollen on levels of geographical structure (Burban and Petit, 2003). The nuclear and organelle genomes are transmitted differently. Thus, it is expected to be in linkage equilibrium, except if there is a recent admixture of differentiated populations or species (Asmussen and Arnold, 1991), or when there is strong epistasis (nucleo-cytoplasmic interaction). On the contrary, full disequilibrium is expected when both organelle genomes are normally transmitted by same parent (Petit and Vendramin, 2007). Some examples of organelle DNA transmission patterns in plants are illustrated in Table 4.

ORGANELLE DNA AS MOLECULAR MARKERS FOR POPULATION STUDIES

Population geneticists commonly use cytoplasmic markers, for estimating the relative seed and pollen dispersal within and among populations (Cruzan, 1998).

Determining the mode of transmission (inheritance) of organelle genomes is of outmost importance before investigating any efforts in population surveys (Cruzan et al., 1993). The mode of organelle inheritance has a major effect on the distribution of organelle DNA diversity and is probably an important factor determining the level of geographic structure in plants (Petit and Vendramin, 2007).

Gene flow in plants occurs mainly via two different components, seed migration and pollen dispersal (Forcioli et al., 1998). In general, both seeds and pollen have different dispersal rates and/or patterns; therefore, it is necessary when studying the gene flow in plant populations to differentiate which component affected it (Forcioli et al 1998). In both angiosperms and gymnosperms, pollen is a major contributor in connecting extant populations with gene flow. Meanwhile, seeds (also other parts) are significant in establishing new populations of plants (Petit and Vendramin, 2007).

Organelle genomes such as cpDNA and mtDNA are found to be a significant source of molecular markers. For instance, cytoplasmic markers (cpDNA and mtDNA markers) have been widely used in the past few years to understand the recovering of post-glacial migration routes for a range of organisms and trees (Taberlet et al., 1998; Hewitt, 2000; Petit et al., 2003). However, as plant mtDNA is known to exhibit a lower rate of nucleotide substitution and liable to extensive intramolecular recombination (Newton, 1988; Palmer, 1992), the chloroplast DNA has therefore been used preferentially in plants, mostly for maternally inherited species (Schaal et al., 1998; Desplanque et al., 2000). The geographical distribution attained by organelle molecular markers provides a better scenario of past migration history than nuclear markers due to their uniparental mode of inheritance and the small effective population size induced by haploid markers (Vettori et al., 2004). Therefore, the haploid markers provide a stronger clue of seed migration (Petit et al., 2003). Additionally, cytoplasmic markers (mainly cpDNA) were also used in many phylogenetic studies (Samuel et al., 2005).

However, the joint use of both markers (cpDNA and mtDNA) in population studies is uncommon in plants where both DNA organelles are inherited from the same parent; (with few exceptions, for example the studies of Dumolin-Lapegue et al., (1998) and Desplanque et al., (2000)). Thanks to the availability of the complete cpDNA sequences which facilitated the design of the consensus primers and "*boosted"* the studies carried on phylogeography and population surveys (Petit and Vendramin, 2007).

CONSENSUS PRIMERS

The accessible DNA sequence added to the conserved order of the genes in cpDNA genomes has facilitated the design of numerous "consensus" or "universal" primers (Taberlet et al. 1991; Demesure et al., 1995; Dumolin-Lapegue, 1997; Weising and Gardner, 1999; Grivet et al., 2001) which greatly promoted population and phylogenetic studies.

The consensus primers match to the most conserved coding regions of the chloroplast genome, and flank the more variable non-coding regions (Dumolin-Lapeque et al., 1997). The primers were used for detecting genetic diversity, mode of inheritance, phylogenetic surveys (Grivet et al., 2001), phylogeography and post glaciation colonisation routes of species (Hamza, 2005), in identification of hybrids when combined with nuclear DNA markers (Heinze, 1998) and for studying closely related species (Hamza et al., 2009). A useful database of polymerase chain reaction (PCR) primers for the study of the cpDNA genome in plants is availed by Heinze (2007).

DIFFERENT SAMPLING STRATEGIES FOR BOTH MARKERS

For the determination of the haplotype frequencies, it is recommended to sample more populations with lower sample sizes in each (Pons and Petit, 1995), as 2-3 individuals per population are sufficient to determine the partitioning of diversity (Tremblay and Schoen, 1999). The distribution of haplotypes will be better reconstructed when more points are sampled (Petit and Grivet, 2002) with homogeneous sampling of populations (Cruzan and Templeton, 2000). In Hamza (2005), a first investigation on seven naturally growing *Salix* populations along the River Nile, the range per population was between 22- 35 individuals.

The methods used to study the variation should be

effective in detecting within-individual variation. In several population surveys of organelle DNA variation, PCR-based techniques was the choice, which often reveal only the most frequent haplotype compared to restriction fragment length polymorphism (RFLP) (Petit and Vendramin, 2007). Indels (insertion/deletion) are useful for comparing restriction site mutation because incomplete digests will not lead to false interpretations, both for southern-blot based analysis and for PCR-RFLP- based approaches.

COMPARISON BETWEEN CHLOROPLAST AND NUCLEAR DNA MARKERS IN POPULATION STUDIES

In the conservation and management of genetic resources, studying or tracing the structure of populations on a wider geographic scale will help clarifying the native populations that requires conservation and management actions. That can be through understanding their geographic structure based on the real genetic diversity as well as phylogenetic relationships. Maternally inherited organelles are mainly dispersed via seeds, while paternally inherited organelles migrate by both seeds and pollen. Therefore, knowing the mode of inheritance (maternal or paternal) of organellar markers and nuclear loci permits the withdrawal of inferences on the migration rates of seeds versus pollen. As haploid organelles have smaller effective population size than diploid nuclear loci, they give clearer picture of the geographic structure. In most angiosperms, organelles are maternally inherited and always show higher F_{ST} values than do the nuclear markers (Latta, 2004).

In Hamza (2005), two *Salix* species (*Salix subserrata* and *Salix murielii*) populations have been studied, in order to resolve the genetic relationship with respect to spatial genetic arrangement, among all individuals as well as to infer maternal and paternal contribution. Nuclear loci indicated presence of hybrids and clones. In a multivariate spatial autocorrelation analysis, nDNA revealed no spatial structure; the same result was given by the cpDNA. However, cpDNA was more informative, which might be attributed to the small effective population size of the organelle markers. Also, cpDNA is dispersed via seeds, which presumably has lower dispersal capability than does pollen. Our findings in the *Salix* study, confirm the predictions of population genetic theory. According to which differentiation should be higher for maternally inherited markers, and reduced for paternally and biparentally inherited markers.

The combined analysis of the two marker types should allow us to reconstruct past population processes in great detail, and to understand their spatial structure and the dynamics of genetic diversity in *Salix* forest stands. Geneticists thus cannot escape the need to incorporate different types of molecular markers. Using different approaches, one can determine in confidence the actual genetic diversity of the species under investigation.

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