

Application of microsatellite molecular markers in studies of genetic diversity and conservation of plant species of Cerrado

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Abstract: Advances in molecular biology in recent decades and the growing interest of the scientific community in assessing the genetic consequences of habitat fragmentation on species have allowed the creation of new perspectives for research on population genetics, where biodiversity is investigated by means of molecular diversity. The initiatives for conservation genomics have been invaluable to delineate effective strategies for genetic conservation in the short and long term. In this paper we discuss the use of microsatellite molecular markers in studies of genetic diversity focused on species of Cerrado. Markers are highly informative and occur profusely in the genome of plants. Despite the high costs involved in developing microsatellites, their use has grown exponentially in reproductive ecology, conservation and population management. This tool allows the identification of the reproductive system of species and possible hybridizations, as well as the estimation of genetic risks of extinction, detection of vulnerable populations and determination of the minimum viable area of conservation, among others.

Keywords: Conservation Genetics, Genetic Diversity, Genetic Landscape, SSR

1. Introduction

The genetic consequences of habitat fragmentation on species have led scientists to question the genetic fragility of populations and the strategies involved in the conservation of endangered species. Consequently, it is necessary to obtain more detailed information on the structure and genetic diversity of natural populations.

Genomics has become an important tool in conservation programs of the biota, as it allows biodiversity to be studied looking at the molecular diversity existent in natural populations of several species, either under direct or indirect anthropogenic impact [1]. Strongly criticized by conservationists in the late 1980s for being restricted to the estimates of heterozygosity in populations subjected to population bottlenecks, the initiatives related to the conservation genomics are currently being leveraged to the level of paramount importance in international conservation programs [2].

These studies allow us to describe the genetic variation of populations and their mechanisms of variation maintenance [3], providing a powerful tool for the detection of relationships and boundaries between species and lineages

[4], recognition of reproductive dynamics and genetic structure of populations [5-6], identification of dynamics of migration and dispersal of species and populations [4], besides uncovering discontinuities in population genes [7].

As a result, Landscape Genetics has recently emerged [5], integrating landscape ecology, spatial statistics and population genetics. Genetic analysis of landscapes has allowed the identification of specific anthropogenic barriers that reduce genetic flow and diversity, therefore enabling the establishment of potential biological corridors for conservation [8]. These analyses have been combined with Conservation Genetics, whose goal is to generate input for the understanding of population and evolutionary processes relevant to the conservation of endangered species [9]. Knowledge of the genetic variation of species and populations has been of great value to design effective strategies for genetic conservation in the short and long term.

2. Microsatellite Molecular Markers

Genetic diversity is continuously introduced into populations by mutation, recombination and gene flow and can be lost by genetic drift, inbreeding and most types of

natural selection [3]. The main tool to assess how genetic variability is distributed within and between populations is the molecular marker [10-11]. A molecular marker is any molecular phenotype derived from an expressed gene or from a specific DNA segment corresponding to regions (expressed or not) of the genome [12].

Microsatellite (or SSR - Simple Sequence Repeats) markers are based on short repeats (1-6 pairs) of bases [13] repeated in tandem (side by side). They represent unstable regions of the genome, randomly distributed, forming polymorphic genetic loci that are under mutational changes at higher rates than those observed in other sequences.

Microsatellites are good DNA markers because they are informative in plants and can be analyzed by PCR (Polymerase Chain Reaction) based on the amplification of individual regions containing simple repeated sequences using a specific pair of primers (20-30 base pairs), complementary to unique sequences flanking the microsatellite. Each amplified segment has a different size and corresponds to a different allele from the same locus [12]. Moreover, they are abundant and uniformly distributed in the genome [14]. They also represent the more polymorphic class of molecular markers currently known and used [12].

The microsatellite islands are unstable regions of the genome under mutational changes, usually addition or deletion of repeats at higher rates than those observed in non-repetitive DNA sequences. This rate can reach up to 1 per 1000 pairs of bases [15]. This instability generates a large number of polymorphic loci, which is useful for the study of populations and gene mapping, thus favoring a more complete coverage of the genome.

Rafalski et al. [16] consider that the SSR are the markers closest to the ideal marker for genetic studies of populations. This is because they have a number of alleles and heterozygosity much higher than other markers, such as those generated by isoenzymes. SSR markers started to be used in ecology to determine the degree of parentage among individuals [17-18]. They were also employed to clarify the genetic structure and variation among individuals, populations and species [19-21]. The main disadvantages of this technique are the high costs involved in the process of marker development. However, the use of markers has grown exponentially in recent years, with examples including applications to the study of plant population genetics, including species of Cerrado [12].

3. Application of Microsatellites in Studies of the Brazilian Cerrado

The Cerrado is the second largest biome of Brazil (Fig 1.), with an estimated biodiversity of more than 13.000 plant species [22]. It has the richest flora among the world's savannas, and its endemism reaches 44% [23]. Nevertheless, it is under constant human action and this, among other factors, has led to habitat fragmentation and loss of genetic diversity [22].

Recent studies showed that the Brazilian Cerrado has been reduced to 50% of its original area, thus evidencing a great need for comprehensive conservation programs. Nevertheless, the few genetic studies for species of the cerrado that have been carried out have focused on species of economical interest, without taking into account ecological factors [24].

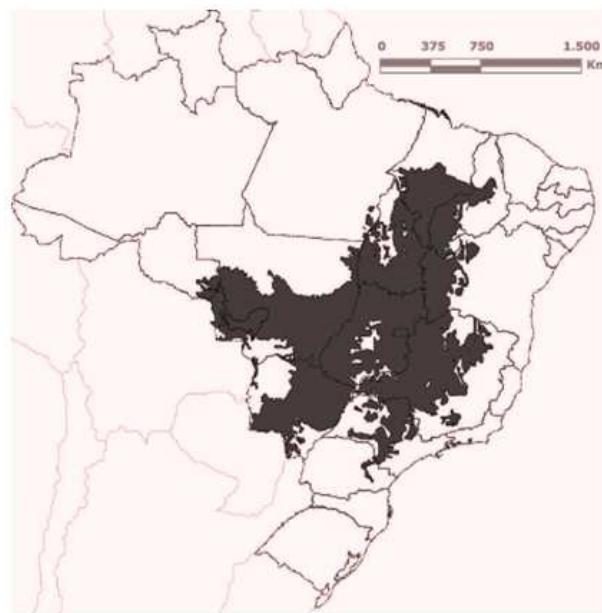


Figure 1. Occurrence area of the Cerrado biome in Brazil. Source: WWF Brazil.

Deforestation has led to loss of genetic variability of many species of Cerrado [25]. Anthropogenic changes reduce this variability for reasons such as: the founder effect or bottleneck, genetic drift, restricted gene flow and increasing inbreeding, isolation and genetic divergence among populations. Furthermore, inbreeding can lead to fixation of deleterious alleles, leading to extinction some populations of this habitat [15, 26-28].

Research on the Brazilian Cerrado has gained importance only recently. There is little bio-ecological knowledge on Cerrado species, since local research has traditionally focused on the tropical forest biomes [28]. The results obtained so far have pointed out some important directions to be taken in order to minimize environmental impact in these ecosystems [29].

Microsatellites have been increasingly developed for the analysis of typical species of the Brazilian Cerrado, such as *Caryocar brasiliense* Camb [30], *Copaifera langsdorffii* Desf [31], *Tabebuia aurea* (Silva Manso) Benth. & Hook. f ex S. Moore [32], *Lychnophora pinaster* Mart [33], *Anacardium humile* Saint Hill [34], *Tibouchina papyrus* (Pohl) Toledo [35] and *Qualea grandiflora* Mart [36].

Some of these studies focused on the reproductive ecology of species of great economic interest, such as *Caryocar brasiliense* [21, 27, 30, 37]. Others aimed at characterizing and collecting accesses for use in programs of genetic pre-breeding, such as for *Eugenia dysenterica* DC [15].

Furthermore, significant results were obtained on the conservation of populations of typical species of Cerrado, such as *Dipteryx alata* Vogel [38-39], *Copaifera langsdorffii* Desf [40-41], *Hymenaea stygonocarpa* Mart. ex. Hayne [42], *Tabebuia ochracea* (Cham.) Standl. [43] and *Tabebuia aurea* (Silva Manso) Benth. & Hook. f ex S. Moore [44].

To study genetic variability employing microsatellite markers we must consider two different aspects of diversity according to Frankel and Söule [45]: allelic richness, measured by the total number of alleles in the population or sample (A), and evenness in the frequency of different alleles in the population, measured by the coefficient of gene diversity (He). Other indices are: (i) observed heterozygosity (Ho), which, according to Weir [46], is an important indicator of genetic diversity, since each heterozygote carries different alleles and best represents the variation in autogamous and allogamous populations; (ii) the percentage of polymorphic loci (P) that is employed together with the number of alleles per locus, in order to characterize and compare the levels of genetic variation in populations [47]; (iii) and Wright's fixation index (f), which measures the excess or deficiency of heterozygote's compared to expected in the Hardy-Weinberg equilibrium model. According to Futuyma [48], the discrepancies existent between a Hardy-Weinberg "ideal" population and actual populations are the ingredients of evolution.

In addition to these indices, it is usually taken into account the polymorphic information content (PIC), which indicates the quality of the marker used. The PIC value ranges from 0 (monomorphic profile) to 1 (highly polymorphic profile). Markers with PIC values greater than 0.5 are considered very informative, whereas values between 0.25 and 0.50 indicate a moderate informative level. Values under 0.25 are considered little informative [49].

The results obtained with this type of study allow: (i) the identification and understanding of the sexual and reproductive system, (ii) the assessment of possible occurrences of hybridization or introgression in populations, (iii) the estimation of genetic risks of extinction, (iv) the definition of strategies of translocation/reintroduction of species in recovery programs, (v) the identification of vulnerable or conservation priority populations, (vi) the estimation of the effective population size, (vii) the determination of the minimum viable area of reserves and (viii) the identification of Evolutionary Significant Units and Management Units [50,51].

Moreno et al. [42] argues that recent studies have adopted broad approaches to understand how genetic diversity is generated within populations and to more precisely quantify the possible ranges of gene flow via pollen and seeds, as well as to study historical and contemporary gene flow. The existence of asexual propagation within populations and their interference in the calculation of genetic indices diversity have also been investigated in order to jointly apply this knowledge to the planning of long term effective strategies for genetic conservation [25,52-53].

4. Concluding Remarks

Genetic variation of species is essential for population survival over generations, and the understanding of the distribution patterns of this variability allows us to comprehend the effects of habitat fragmentation that put species at risk of extinction. Investigations using microsatellite markers have increased in recent years despite being severely limited by the high costs involved in the construction of genomic libraries. Nevertheless, this technique has been proved beneficial by allowing the adoption of broad approaches to the understanding of genetic variation of species. In summary, the use of microsatellite markers has provided subsidies for conservation and management programs, thus allowing a sustainable use of resources.

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