Chromosome diversity in population: Defining conservation units and their micro-identification through genomic *in situ* painting*

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The basic unit of conservation in vogue is the morpho-species, where the role of genetics is concerned with the assessment of diversity for single loci or random DNA sequences. However, to underpin the evolutionary potential at population level it is of utmost importance that chromosomal divergence is duly taken into account in a conservation programme. Cryptic/cytotypic/ploidy variations are common in vegetative populations that influence genetic architecture and reproductive potential, but may not be immediately recognizable in a phenotype and also escape screening through random DNA markers. Further issues arise in planning management action for conservation when there is intraspecific cytological variation and the populations of cytotypes are spatially separated. It is in this context that the chromosomes have a definite role in elucidating the biodiversity and defining population groups for conservation. Combined with cytological markers, a simple approach of comparative chromosome painting resolved by in situ hybridization with genomic DNA of a reference species could unequivocally facilitate micro-identification of numerical and/or cryptic chromosome diversity, and help complement conservation plans.

OVER six decades ago, Vavilov predicted the value of wild relatives as sources of genes for improving agriculture. This motivated the establishment of germplasm banks – living seed collections that serve as repositories of genetic variation. Recent upsurge in consideration of ethical basis of conservation of biological resources has aroused political consciousness leading to 'Convention on Biodiversity' that implicits (i) conservation of biological diversity, (ii) its sustainable use, and (iii) the equitable sharing of benefits derived from the use of components of biological diversity. Three levels of biological diversity are recognized, ecosystem and habitats, species and communities, and genomes and genes. Curiously, evolutionary process is not included in the convention, although evolution cannot proceed without genetic diversity. The process of evolution thus underlies studies of biodiversity[1]. Traditionally, the collection and preservation of germplasm were mainly centred around clearly defined characters, recognizable in a phenotype, but there is now a paradigm shift in this direction looking for genes based on molecular markers. Such data help to provide an empirical basis for decisions about allocation of resources towards maintenance and utilization of genetic diversity. Genetic linkage maps have further made it possible to study the chromosomal location of genes, to facilitate crop improvement and unleash the genetic potential of wild and cultivated germplasm resources for the benefit of the society[2]. However, a central question still remains as to what should be conserved at species level to represent the evolutionary dynamics in population: whether scoring just for gene diversity is sufficient, or even the structural and cytotypic variations that influence reproductive potential and gene expression are equally important?

Intra-populational chromosome variation and conservation

Unlike in animals, flowering plants harbour tremendous amount of polyploid variation at interspecific level. Nearly three-fourths of angiosperms are polyploids[3,4] and almost all taxa in grasses encompass a polyploid series. The stonecrop, *Sedum suaveolens*, with highest number of chromosomes for any angiosperm ($2n = 640$) is estimated to be about 80-ploid[5]. Of course, in most cases such changes are on account of allopolyploidy consummated through evolutionary fixation from interspecific hybridization among two or more genomes, e.g. wheat, cotton, mustard, and artificial allopolyploidization, e.g. noble canes, etc. During speciation, chromosomes are

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constantly changing in size and morphology by differential amplification of DNA sequences, and also by structural alterations. A classical example of involvement of species-specific translocation has been documented in the evolution of *Triticum aestivum*[6]. Although evolution of polyploid forms almost always arises by functioning of unreduced gametes leading to multiplication of basic number, the basic number may itself either increase or decrease (aneuploid changes) with gain or loss of centromeres and telomeres. Even in artificial wide hybrids (e.g. the synthesis of noble canes) and their progenies, such structural and numerical changes are the natural consequences.

Evidences have recently been provided that allopolyploid formation accompanies large-scale genomic adjustments involving elimination of certain repetitive genome and/or chromosome specific-sequences[7,8]. Patterns of genetic diversity for neutral markers may provide useful information on co-ancestry, gene flow and ecology over species and populations, but the very issue of genetic identity gets confounded when the variation in the population is on account of intraspecific cytological variation. Further, if the populations of cytotypes are spatially separated, then there are important implications for the conservation of existing populations. Such intraspecific cytological variation is especially frequent in the species that reproduce obligatorily, vegetatively. To cite as examples: somatic chromosome number in *Rutidosis leptorrhynchoides* ranges from 22 to 44 with $x = 11$, 12 or 13 (ref. 9), extensive karyotype polymorphism has been recorded in *Stylidium crossocephalum* showing 42 different haploid genomic combinations[10], the morpho-species of *Scilla autumnalis* constitute huge cytotypic differentiation comprising dysploid variation from $n = 5$, 6 to 7 (ref. 11); somatic chromosome number in *Nymphaea rubra* varies from 42 to 112, and in *Sprekelia formosissima* the number ranges between 27 and 180 (ref. 12). *Allium stracheii*, *Aloe elgonica*, *Nardostachys grandiflorum*, *Costus speciosus*, *Mentha* spp. all evince environment specific ploidy level variations. A three-fold variation in copy number for long terminal repeats among individuals and populations of *Hordeum spontaneum* is encountered on a local ecological scale[13]. Also, the genetic resources of several cultivated plants, especially pulses and cereals, are endowed with cryptic structural variations[12], karyotype and nucleolar diversity[14] that have value in breeding programmes.

Cytotype as the unit of conservation for population groups

Although no definitive effort has been made to ascertain the frequency of occurrence of intraspecific chromosome variation in plant populations in general, there are numerous reports that show wide occurrence and clear geographic separation of chromosome races and variants within species, especially those reproducing vegetatively. Extensive discontinuous and disjunct species-specific variation over geographical regions has been encountered in the genera *Rutidosis*[9], *Stylidium*[10], *Scilla*[11,15], *Briza*[16], *Xanthisma*[17], *Aloe*[18], *Prospero*[19], etc. The total gene pool of a species may comprise different cytotypes, many of which may occupy different ecological niches thus meriting their separate conservation. Further, while planning conservation strategies, it is desirable that the individual cytotype units are maintained in isolation, lest they lead to any hybrid dysgenesis. Therefore, it would be useful to understand the patterns of genetic and cytotypic variation within a species, and value such information in defining population groups for conservation. The approaches mentioned below in combination with conventional chromosome analysis may be useful in fine-scale identification of genetic variation among the cytotypes.

Chromosome markers and large-scale molecular architecture

Plant and animal genomes consist largely of repetitive DNA; there are ~30 sequence motifs ranging in size from dinucleotides to more than 10,000 bp. Copy number of individual repetitive DNA motifs can vary from several hundred to hundreds of thousands, and single motifs may represent 10 or even 50% of a genome. The repetitive DNA motifs are characteristically dispersed along the length of the chromosomes and could therefore facilitate linear differentiation of chromosomes[20]. Although plant chromosomes evince characteristic linear distribution of dispersed repetitive DNA motifs along the chromosomes, their distribution is homogenous with respect to genomic complement *per se* unlike animal systems, where each chromosome shows characteristic GC:AT ratio. Such situation limits the development of chromosome-specific probes in plants. Therefore, one has to depend on characteristic linear distribution of dispersed repetitive sequences in the genome as a whole *vis-à-vis* chromosome-specific distribution[21,22]. Nevertheless, there are certain conserved sites that facilitate structural and functional differentiation of eukaryotic chromosomes into heterochromatin, centromeres, telomeres, and nucleolus-organizing regions. High-copy, tandem repeat elements are preferentially located in the constitutive heterochromatin. Whereas ribosomal RNA (rRNA) genes are organized in high-copy number tandem repeats and show heterochromatic structure, the telomeres and centromeres have a prominent tandem-repeat. Telomere has a consensus repeat TTTAGGG conserved in most plants with minor variations, but two different repetitive DNA elements have been identified in centromeres among cereals[23,24]. Localization of centromeric/telomeric sequences, coupled with banding patterns can facilitate

chromosome identification to a good extent. Further refinement in chromosome diagnostics would be feasible if the distribution of repetitive DNA motifs and their copy number could be visualized through a sort of chromosome printing. The latter situation has in fact been realized in *Allstromeria*, where the otherwise similar-looking karyotype in its two species, *A. aurea* and *A. inodora*, could be differentiated by species-specific physical localization of repetitive segments[25].

Repetitive DNA-based chromosome painting to facilitate core collections

Notwithstanding recent upsurge in genome-mapping initiatives, sequencing of entire chromosomes and genomes, and the identification of gene synteny, technical advances in cytogenetic applications to genome mapping are occurring at a rapid pace[6]. The molecular cytogenetic technique of fluorescence *in situ* hybridization is one of the most appropriate methods for physical mapping of DNA sequences on chromosomes, facilitating identification of individual chromosomes with different DNA markers[26–28]. Microscopic visualization of chromosomes offers distinct advantages over molecular characterization on the gel and filters in certain specific situations; typical examples are on account of cryptic structural variations and/or numerical chromosome variations in autopolyploids/aneuploids and dysploids. Such a situation with respect to chromosome diversity is sufficiently common in vegetatively-propagating populations. Therefore, in order to facilitate core collections it is of utmost importance that representative chromosome variants are taken into account, and duly characterized. Categorization of such collections is possible only if the chromosome diversity is characterized through necessary means that generate a sort of differentiating unique chromosome-printing. A simple approach of repetitive DNA bar-coding for chromosome identification as outlined below could hold wide applicability.

As already discussed, the various repetitive DNA sequence motifs occur across the plant genomes, and a few of them are species-specific. Nevertheless, their linear

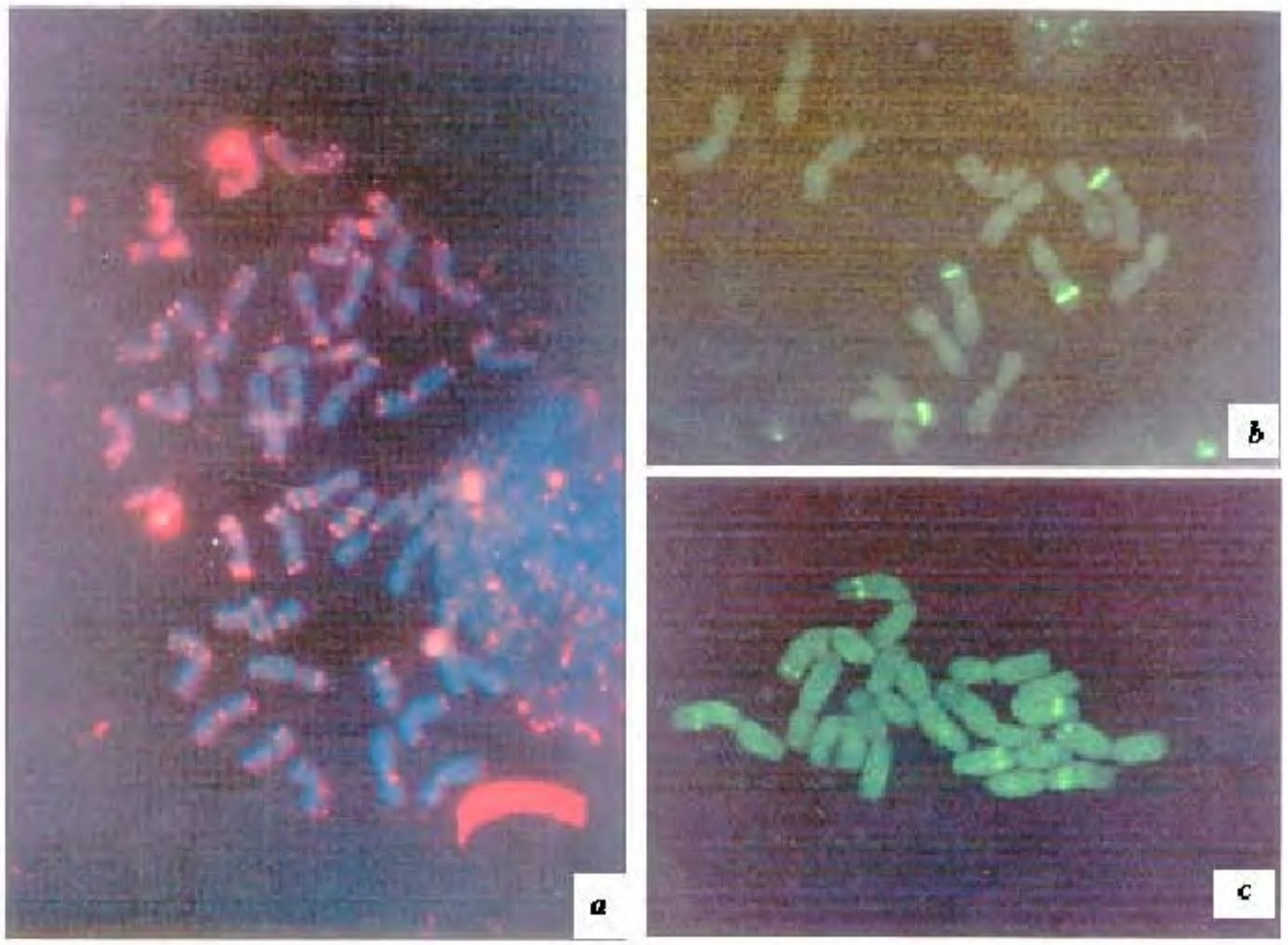

Figure 1. Fluorescence *in situ* hybridization painting on somatic chromosomes. ***a***, Metaphase chromosomes of wheat showing fluorescence *in situ* hybridization with total genomic DNA from rice; ***b–c***, *In situ* localization of rRNA multigene families on somatic chromosomes of barley to facilitate chromosome identification; ***b***, *In situ* hybridization sites homologous to 45S rRNA (on two pairs), and ***c***, *In situ* hybridization sites homologous to 5S rRNA (on four pairs).

distribution is largely chromosome-specific. Therefore, an effort to look for linear distribution of repetitive DNA can facilitate identification of individual chromosomes in a better way than hitherto made possible. This could be possible just through a simplified version of whole genomic *in situ* hybridization. Total genomic DNA from a reference species with low DNA content (e.g. *Oryza sativa* or *Arabidopsis thaliana*) is labelled with fluorophores by nick translation, and hybridized *in situ* following the standard procedure of genomic *in situ* hybridization[26,27]. For the target species, this would generate chromosome-specific fluorescence patterns that are homologous to repetitive DNA of reference species (Figure 1 *a*). Further, as an improvement to this approach, the hybridization mix may be supplemented with excess amount of unlabelled conserved DNA such as rDNA/centromeric DNA as a DNA block, to suppress hybridization to their homologous sites in order to improve the fluorescence resolution at unique hybridization sites. Alternatively, simultaneous detection of such conserved sites through multicolour FISH or reprobing[26–28] could be used as markers for chromosome identification. It may be pertinent to mention here that the region-specific repetitive DNAs, although quite conserved, in certain cases do show polymorphism with respect to their distribution, thus facilitating chromosome identification to a certain extent, e.g. localization of 45S and 5S rRNA could facilitate identification of all the seven linkage groups in barley (Figure 1 *b* and *c*).

This approach would not only help specific chromosome painting for the target species to facilitate chromosome identification, but could also be used over a range of taxa to deduce affinities. This comparative genomic *in situ* hybridization approach has earlier been outlined by the present author using total genomic DNA, taking rice as a reference species[26]. Further, a similar approach has now been extended for comparative cytogenetic banding analysis of plant chromosomes[29]. Here, *Arabidopsis* genomic DNA has been used to deduce fluorescence banding pattern in several complex genomes from monocots to dicots, suggesting that the banding patterns generated are species-specific and can be utilized for the purpose of chromosome characterization as well as in deducing species affinities.

Conclusion

In order to supplement the conservation programmes, it is important to know the structure and behaviour of chromosomes and genomes to elucidate evolutionary potential of population, and also to underpin the structural rearrangements, B-chromosome segments so as to unravel the elements of chromosome variation – all of which influence genetic architecture, but may not be detectable just through DNA marker analysis. Availability of a repertoire of chromosome techniques, including chromosome banding and fluorescence *in situ* hybridization can aid microscopic visualization of genes, chromosomes and chromosome segments, and genomes to deduce chromosome diversity to facilitate conservation programmes. A simple approach of repetitive DNA barcoding of chromosomes resolved by *in situ* hybridization with genomic DNA of a reference species combined with numerical analysis could further facilitate unequivocal identification of chromosomal and cytotypic variations.

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