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Traditional karyomorphological studies: can they still provide a solid basis in plant systematics?*

Abstract

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Chromosomes are helpful tools for systematic purposes, but their use has not always been rigorous. In this contribution, we review and discuss the use of karyomorphological data from the beginning of cytotaxonomy to present day. We highlight the use of incorrect karyomorphological parameters and the erroneous merging of different parameters in some case studies. In addition, we suggest complementing the karyological data with information from other independent sources to infer systematic reconstructions.

Key words: chromosome number, karyotype asymmetry, multivariate analysis, phylogeny.

Background

Since early works (e.g. Strasburger 1882, 1910), chromosomes have played an important role in plant systematics, because their changes in number and structure has always been linked to plants evolution and speciation (Greilhuber & Speta 1978; Greilhuber 1982; Cerbah & al. 1998; Weiss-Schneeweiss & Schneeweiss 2003; Guerra 2012).

Traditional techniques, such as Feulgen staining, for evaluating phenotypic aspects of chromosome complement revealed to be some of the most used techniques for the purpose of studying relationships among taxa. In addition, they are quite cheap and easy to apply. For these reasons, a big amount of data has been collected during times (Stace 2000; Bedini & al. 2010 onwards, 2012; Garbari & al. 2012; Rice & al. 2014; Peruzzi & al. 2014).

Phenotypic features of chromosome complements, i.e. karyotype, mainly deals with number, size and position of centromere in chromosomes of a given complement. These karyomorphological features are not simply taxonomic characters, such as the number of petals or the leaf shape, used for taxonomic issues, but they can be crucial for depicting evolutionary scenarios involving systematic relationships (e.g. Levin 2002, Doyle & al. 2004; Leitch & Leitch 2008; Mandáková & Lysak 2008; Siljak-Yakovlev & al. 2008; Weiss-Schneeweiss & al. 2009; Siljak-Yakovlev & Peruzzi 2012).

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One of the most important concepts in karyomorphology is the karyotype asymmetry: a concept introduced by Levitsky (1931) and later developed by Stebbins (1971), who for first proposed a classification of chromosome complements based on both inter-chromosomal and intra-chromosomal asymmetry. According to these authors, inter-chromosomal asymmetry is a measure of the heterogeneity of chromosome lengths within a single complement. Hence, a symmetrical complement is characterized by having chromosomes more or less of the same length. Instead, intra-chromosomal asymmetry depends on the position of centromere along single chromosomes. A metacentric chromosome is considered symmetric while a telocentric chromosome is considered asymmetrical. Therefore, an asymmetrical karyotype is mostly constituted by telocentric chromosomes, whereas a symmetrical complement is mostly constituted by metacentric chromosomes.

The problem of describing karyotype features

During times, many indices have been proposed for measuring karyotype asymmetry, starting from the quali-quantitative indices proposed by Stebbins (1971). In place of these quali-quantitative indices, many quantitative ones have been proposed by later authors, some of them combining both inter- and intra-chromosomal aspects. Much more indices of intrachromosomal asymmetry have been proposed with respect to interchromosomal ones. Indeed, as stated by Paszko (2006), the Coefficient of Variation of Chromosome Length (CV_{CL}) has been commonly considered the best choice for describing interchromosomal asymmetry, while less accordance exists concerning intrachromosomal asymmetry. The contribution of Paszko (2006) represents the first critical review dealing with conceptual and statistical reliability of these indices and she proposed to use, along with CV_{CL}, the Coefficient of Variation of Centromeric Index (CV_{CI}), for measuring intrachromosomal asymmetry. In addition, she introduced the Asymmetry Index (AI), an index combining both the aforementioned indices.

Five years later, Zuo & Yuan (2011) criticized the use of CV_{CI}, because it does not measure the intrachromosomal asymmetry sensu Levitsky (1931), but it only evaluates the variation of centromeric position in a complement, instead of evaluating the frequency of telocentric chromosomes (asymmetrical chromosomes). Based on these considerations, Peruzzi & Eroğlu (2013) confirmed the use of CV_{CL} and rejected the use of CV_{CI}, replaced by M_{CA}. The latter parameter corresponds to the A index proposed by Watanabe & al. (1999), but expressed in percentage. M_{CA} index is consistent with the intrachromosomal asymmetry concept and has a solid statistic basis. In addition, Peruzzi & Eroğlu (2013) discouraged the use of combined indices and supported the use of scatter plots of the two asymmetry indices plotted one against the other, for a better comparison of taxa. Indeed, merging such different concepts may cause an important loss of information leading to an underestimation of the differences among taxa.

A year later, further suggestions came from Peruzzi & Altinordu (2014), who proposed the use of six parameters, of general applicability and uncorrelated each other, in multivariate analysis based on karyological data. These parameters are the following: the chromosome number (2*n*), the basic chromosome number (*x*), the total haploid length (THL), the interchromsosomal CV_{CL}, MCA and CVCI. This last index could be useful as additional parameter, measuring the heterogeneity of centromere position. However, if CV_{CI} is correlated to M_{CA} it should be discarded from the analysis. More recently, a tool for measuring karyotypes and automatically calculating these parameters has also been released (Altinordu & al. 2016).

A critical survey of the karyomorphological literature

Despite the criticisms moved against the use of biased karyotype asymmetry indices, they are still used today (Fig. 1). The indices proposed by Romero Zarco (1986) and the TF% (Huziwara 1962) are the most used, but we can see that in the last years their use is decreasing, probably due to the critical reviews of Paszko (2006) and Peruzzi & Eroğlu (2013). The use of an index instead of another appears often related to the geographical location of manuscripts' authors (Fig. 2), or to their affiliation's working group (not shown). For instance, in India and Iran the TF% (Huziwara 1962) seems the first choice, while in South America the indices by Romero Zarco (1986) are definitely the most frequently used, and in Italy in the last 10 years the most used are those proposed by Paszko (2006).

One of the reasons why old (incorrect) parameters are highly represented is because in the last 10 years, these indices were used altogether in many studies, often in a multivariate

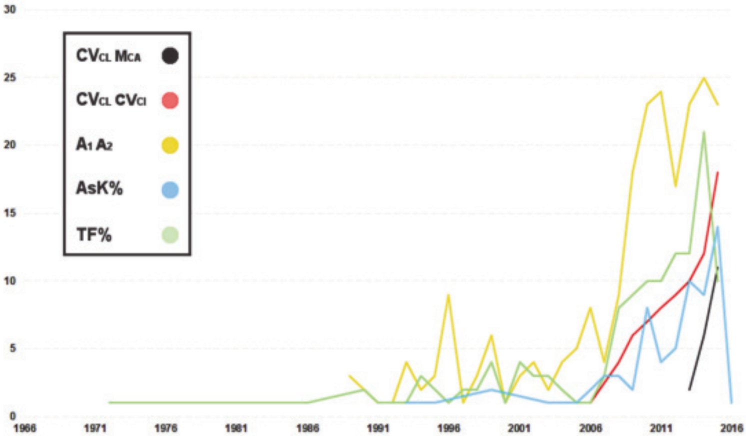

Fig. 1. Plot showing how frequently (number of papers found on ISI Web of Science) karyotype asymmetry indices were used during times, at May 31st 2016. Only the five most popular methods are shown, i.e. CV_{CL} & M_{CA} (Peruzzi & Eroglu 2013); CV_{CL} & CV_{CI} (Paszko 2006); A_1 & A_2 (Romero Zarco 1986); AsK% (Arano 1963); TF% (Huziwara 1962).

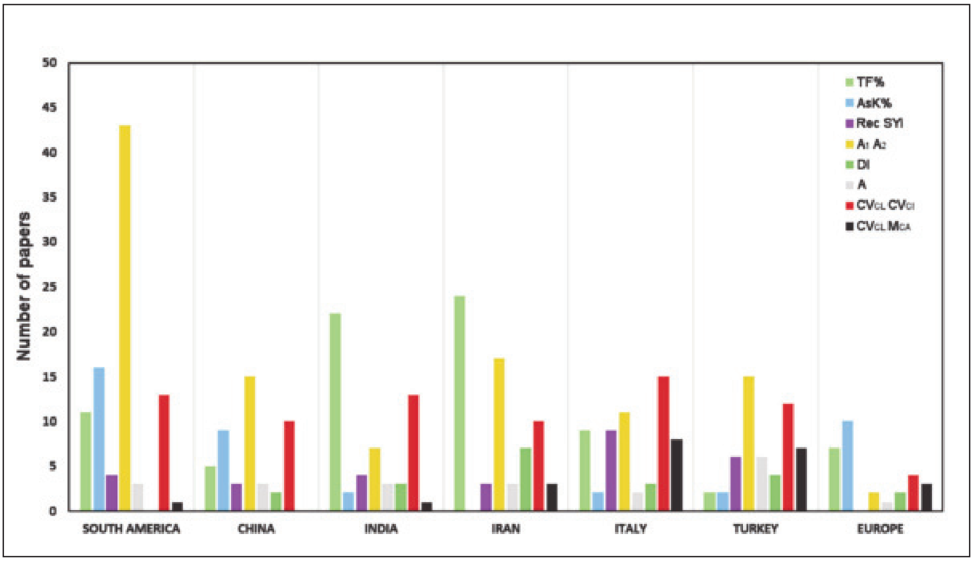

Fig. 2. The frequency (number of papers found on ISI Web of Science) of karyotype asymmetry indices and the geographical affiliation of the authors who included these indices in their papers, at May 31st 2016. For references on indices, see the caption of Fig. 1. For the remaining indices: Rec & SYi (Greilhuber & Speta 1976); DI (Dispersion Index; Lavania & Srivastava 1992); A (Watanabe & al. 1999).

approach. Being these indices correlated to each other (see Paszko 2006; Peruzzi & al. 2009; Peruzzi & Eroğlu 2013), such an approach may be affected by redundancy and may thus provide unreliable results. Indeed, as summarized in the case regarding tribes within *Liliaceae*, when performing a PCoA with six uncorrelated parameters (see Fig. 2 in Peruzzi & Altinordu 2014), a better separation of groups than using 11 mostly correlated parameters (Fig. 3) can be obtained. In addition, using these six parameters in *Crocus* L. series *Verni* B.Mathew (Peruzzi & Altinordu 2014), we can see that resolution of groups is better with respect to the simple scatter plot with the two asymmetry indices (Harpke & al. 2015). For instance, if we look at *C. tommasinianus* Herb. and *C. heuffelianus* Herb., they mostly overlap in the scatter plot, whereas they are well-separated in the PCoA with six parameters. A further problem regarding many studies with multivariate analyses is that often parameters not of general applicability are included, such as the absolute lengths of chromosome arms, satellite numbers and positions, etc. It is obvious that these can be useful only when comparing closely related taxa.

Another kind of misleading result can be found when phylogenetic relationships are inferred exclusively using karyological data. For example, in the study of Caputo & al. (2013), a dendrogram from a cluster analysis was produced, using karyological data of species within *Vicia* L. subgen. *Vicia*. In addition, the authors of this study also used molecular data to clarify the relationships among taxa. Conflicting results between the two different analyses were found, but despite these incongruences, they tried to draw some conclusions merging the two kinds of data in a single matrix, from which a phylogenetic tree was obtained. One of the authors' conclusions is that a trend of increasing asymmetry can be found in this group of plants, with plesiomorphic condition represented by more symmetrical karyotypes, typical of *Vicia* sect. *Atossa* (Alef.) Stank. However, the two species belonging to this section (i.e. *V. sepium* L. and *V. oroboides* Wulfen) cluster with two different sections (i.e. *V.* sect. *Narbonensis* (Radzhi) Maxted and *V.* sect. *Vicia*, respectively) having different overall karyotype asymmetry. Moreover, the authors hypothesized that *V.* sect. *Narbonensis* retains more plesiomorphic characters, because a part of it (four species) diverges earlier in the cluster analysis. Nevertheless, a cluster analysis is not properly a phylogenetic tree, and cannot be used for depicting evolutionary trends. In fact, in the ITS tree *V.* sect. *Narbonensis* is monophyletic and its position among other sections is unresolved. Moreover, it is worth to stress again the possible biases introduced by redundancy: in this analysis on subgenus *Vicia*, 37 karyological parameters were used, most of them being redundant. This redundancy might have led to a loss of signal of karyotype asymmetry information and to an increase of weight of the character "*chromosome number*". An example of this, could be found considering the cluster in the dendrogram corresponding to species with $2n = 10$, which instead are scattered throughout the ITS tree.

On the contrary, in the study on *Liliaceae* by Peruzzi & al. (2009), karyological data were superimposed upon a reliable phylogenetic framework, in order to evaluate direction of changes involving karyotype features in an evolutionary perspective. In this case, we can only visually appreciate karyotype changes, for instance those concerning the basic chromosome number *x*, without having a statistical support of what we can perceive or hypothesize by eye. A more advanced way to infer trends in the evolution of karyological traits is to perform phylogenetic regression analyses. For instance, in the study on the subgenera within *Allium* L. by Peruzzi & al. (2017), plesiomorphic character-states concern-

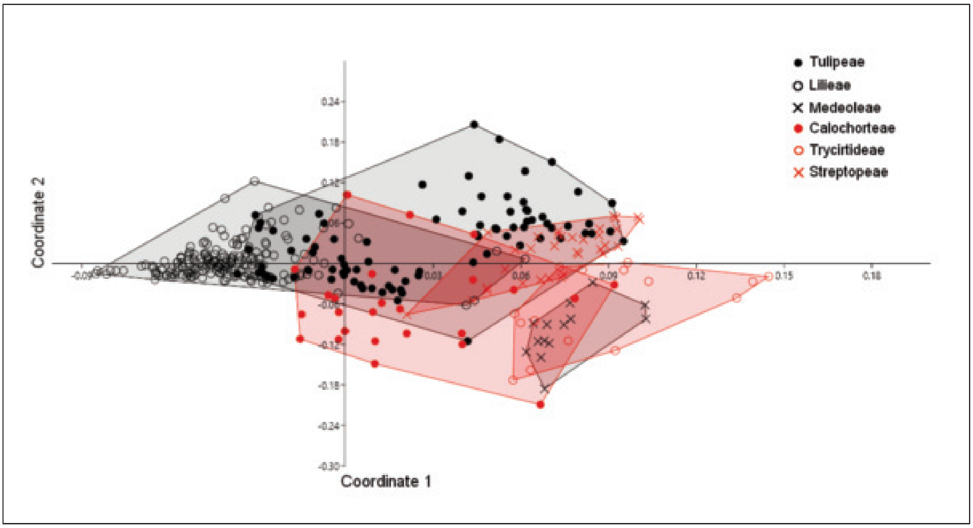

Fig 3. PCoA with Gower's distance performed on the tribes within *Liliaceae*, using 11 karyological parameteres. Original karyomorphological data derived from Peruzzi & al. (2009).

ing CV_{CL} and M_{CA} were inferred by a statistical model. Indeed, in the phylogenetic tree, M_{CA} resulted changing with a trend going from asymmetrical to more symmetrical karyotypes, confirming that the common assumption that symmetrical karyotypes are plesiomorphic (Stebbins 1971) is often wrong.

Concerning modern cytotaxonomy, molecular cytogenetics has reached great advances, for example in tracing karyotype evolution in *Brassicaceae* through chromosome painting techniques using BAC (Lysak & Lexer 2006; Lysak & al. 2007; Schranz & al. 2007; Mandáková & Lysak 2008; Mandáková & al. 2010; Mandáková & al. 2012; Lysak 2014). In addition, in the near future Next Generations Sequencing technique could implement the construction of BAC libraries (Dolezel & al. 2014) for chromosome painting comparison, and could facilitate the development of probes for comparative FISH analysis (Soltis & al. 2013). However, this kind of analysis cannot completely replace traditional karyomorphology. Indeed, molecular cytogenetics is often limited to comparisons of very closely related taxa (e.g., comparisons of 45S and 5S positions using FISH, investigations of recent hybridization events using GISH, etc.; see also Siljak-Yakovlev & Peruzzi 2012). Moreover, molecular cytogenetic data are still poor in terms of number, lacking a wide coverage of plant diversity, with respect to the vast amount of basic karyological data (Peruzzi & Bedini 2014). For example, up to now comparative chromosome painting has been widely applied almost exclusively within *Brassicaceae* family.

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