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KARYOMORPHOMETRICAL ANALYSIS OF SELECTIVE MEMBERS OF HELIANTHEAE (ASTERACEAE)

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ABSTRACT

A Study of chromosome number and chromosome morphology was carried out in fourteen taxa of the family Asteraceae belonging to the tribe Heliantheae. The chromosome number varies from 2n = 18 to 2n = 72. Two cytotypes (2n = 24 and 2n = 22) were observed in *Cosmos bipinnatus* and *Eclipta prostrata* (2n = 18 and 2n = 22). The chromosome complements in the various members differ in minute karyotypical details. With regard to the gross morphology, nearly submedian to nearly median chromosomes were observed. The size of chromosomes ranges from 2.23 to 0.41 μm in length The chromosome with secondary constriction was 2 to 8 in number. Variations found in the karyotype parameters suggest that Heliantheae members are characterized by symmetrical to slightly asymmetrical karyotypes. The study revealed that polyploidy, aneuploidy and structural changes in the chromosomes have played a significant role in speciation within the tribe.

Key Words: Asteraceae, chromosome number, chromosome morphology, Heliantheae, karyotype

INTRODUCTION

The tribe Heliantheae is one of the largest and most diverse group in the family Asteraceae, consisting of 1500 species and 160 genera. The Heliantheae are well known for the cytological studies (Robinson *et al.* 1981). However, there are

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some genera in which chromosomal information is still scarce or absent. The cytological data already available for the tribe show many different basic chromosome numbers that ranges from x= 8 to x = 17, having multibasic genera with large aneuploid series such as *Bidens, Coreopsis,Cosmos, Dahlia and Thelesperma* (Stuessy, 1977).

The cytological studies undertaken so far in Asteraceae were concentrated mainly on the determination of chromosome numbers and have provided very few data on chromosome morphology and structure in detail. Moreover most of the karyomorphological studies conducted on this family were on the basis of conventional methods. There are limitations for the conventional measuring and characterization of chromosome complement by visual evaluation, especially for very small chromosomes. In some groups karyotypic differences between species are highly quantitative and have been difficult to assess by conventional quantitative methods. Karyomorphological studies by computer based image analysis provide a better knowledge on the cytogenetic constitution of various species over conventional method. Karyomorphometric studies based on image analysis system are available in other tribes of Asteraceae, like Eupatorieae, Astereae and Inuleae and selected members such as *Tagetes, Spilanthes and Emilia* (Rajalakshmi and Joseph, 2002, 2004, 2006, 2009, 2011, 2014). The present study provides additional information on the chromosome spectrum of selective members of the Heliantheae by using semiautomatic image analysis system.

MATERIALS AND METHODS

Fourteen taxa were investigated for the present study. For mitotic analysis, root tips were pretreated with mixtures of cytostatic chemicals such as saturated aqueous solution of Para-dichlorobenzene and Aesculine. The pretreated root tip cells, after washing thoroughly in distilled water were fixed in 1: 3 acetic acid - ethyl alcohol mixture overnight, followed by aceto-orecin squash techniques. Squash preparations were made in 1% acetocarmine (Sharma and Sharma, 1980).

Detailed Karyomorphological studies by image analysis system

a. Photographs and image processing

Well spread metaphase plates were photographed using 125 ASA 35 mm Orwo film in a Leitz photographic attachment and suitably enlarged. A 200 dpi image scans were obtained for each original photograph. The software Adobe Photoshop was used for digitizing and reproduction. The contrast of each image has been increased by raising the resolution upto a satisfactory level. The generated images were checked by the visual inspection comparing with the original photomicrographs. After storage of original digital images of the metaphase

spreads in discs, these images were analyzed by using computer devices. The image was recovered from the disc and the original digital image for the analysis was then generated automatically. A binary image that was essential for the object identification by the computer was generated by interactive setting of the lower and upper thresholds of the gray levels. These thresholds were defined properly so that the gray values of the pixels that consisted of chromosome images were included. Binarization was automatically carried out by changing the pixel values stood between the two thresholds to white and all other to black. The large background dust particles and spots whose gray values also fell between the two thresholds were eliminated by adjusting the gray values of the pixels outside the chromosomal region by interactive setting of lower and upper thresholds of the gray levels. This will result in a binary chromosomal image with a clean background.

b. Quantitative karyotyping of the chromosomes

Measurements of each chromosome from enlarged image were made by AutoCAD (Version 2000) loaded on a personal computer. This image was automatically coloured differently by the computer generated colouration based on the actual gray values of the pixels. Pseudo-colouration considerably improved the density distribution of the objects and the recognition by humans. This help to detect the primary and secondary constriction of the chromosomes. These constrictions were marked by the overlay straight lines on the pseudo-coloured images. After this midrib lines were drawn interactively on each chromosome. Extraction of the midrib lines, breakage at the position of the constriction and identification by different colours were subsequently carried out automatically. The outer margin of each chromosome image was also marked by drawing surrounding line. Numerical data such as the arm length, area, perimeter and an apparent visual three dimensional volume of each chromosome were obtained in pixel units. In all the karyotypes, ratio of the short arm to the total length of the chromosome was changed into percentage. Forma percentage or centromeric index (F %) is determined (Rajalakshmi and Joseph, 2009). On the basis of F% the nature of primary constriction in the chromosomes are classified as follows:

F%	**Nature of primary constriction**
50	Median
49.9 - 37.6	Nearly median
37.5 - 25.1	Nearly sub median
25	Sub median
24.9 - 18.76	Nearly sub median
18.75 - 12.6	Nearly sub terminal

12.5	Sub terminal
12.4 - 6.26	Nearly sub terminal
6.25 - 1	Extremely sub terminal
1	Terminal

The values obtained for the chromosome morphology were:

Total arm length of each chromosome (long arm length + short arm length) and relationship of the arm (RB).

RB is calculated by the following formula:

RB = long arm length / short arm length

Arm ratio (AR) has been widely utilized for the classification of chromosome types has been considered empirically to be a more stable parameter of the chromosomal morphology. The AR was defined as the ratio of the length of the short arm to that of the long arm (S/L) for each chromosome.

The following indices were also calculated for each chromosome.

$$\text{Relative long arm length (RLL)} = \frac{2ai}{\sum_{j=1}^{2n} (aj+bj)}$$

$$\text{Relative short arm length (RSL)} = \frac{2bi}{\sum_{j=1}^{2n} (aj+bj)}$$

Relative chromosome length (RL) = RLL +RSL

Arm difference ratio (AD) = (ai - bi)/ (ai + bi) where ai = long arm length of chromosome i, bi = short arm length of chromosome i, $\sum_{j=1}^{2n} (aj+ bj)$ total diploid chromosome length

The disparity index (DI) of chromosome in a karyotype was calculated by the formula:

$$DI = \frac{\text{longest chromosome - shortest chromosome}}{\text{longest chromosome + shortest chromosome.}} \times 100$$

The variation coefficient among chromosome complements (VC) was determined as follows:

$$VC = \frac{\text{Standard deviation}}{\text{Mean lengths of chromosome}} \times 100$$

The total forma percentage or the mean centromeric index value (TF%) was calculated in each taxa.

$$TF\% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$$

The uniformity coefficient (perimeter/area) was also calculated. All the numerical data were prepared after comparing at least five well spread metaphase plates. The various calculations were done by the computer package Microsoft Excel.

c. Quantitative idiograms of chromosomes

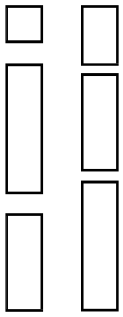
Based on the data relating to the length, the idiograms were prepared combined with the results of quantitative image analysis of chromosomes. The chromosomes were arranged semiautomatically according to the length, arm ratio, uniformity coefficient, three-dimensional volume and idiograms generated with the aid of computer software Adobe Photoshop/Microsoft Excel.

The general description of the common chromosome types is given below followed by the karyotype description of each of the members investigated.

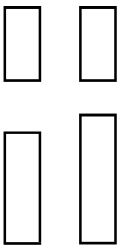
Type A: Comparatively long chromosomes with two constrictions, one median to nearly median and the other nearly sub median.

Type B: Relatively long chromosomes (>0.8) with nearly median to nearly sub median primary constriction.

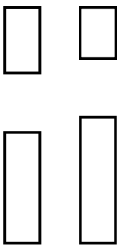
Type C: Small chromosomes (<0.8) with nearly median to nearly sub median primary constriction.



Type A



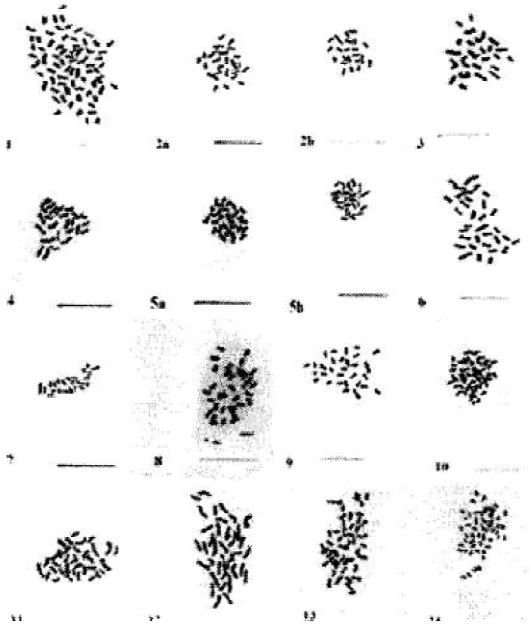
Type B



Type C

RESULTS

The chromosome number varied from 2*n* = 18 to 2*n* = 72. With majority of species concentrated in the number 2*n* = 24. Chromosome numbers observed in the present study were 2n = 72 in *Bidens pilosa;* 2*n* = 24 in *Cosmos bipinnatus* and *C. caudatus;* 2*n* = 22 in *Eclipta prostrata*; 2n = 32 in *Galinsoga parviflora;* 2*n* = 24 in *Melampodium paludosm*; 2*n* = 36 in *Parthenium hysterophorus;* 2*n* = 30 in *Sigesbeckia orientalis;* 2n = 40 in *Synedrella nodiflora;* 2*n* = 34 in *Tithonia diversifolia;* 2*n* = 36 in *Tridax procumbens;* 2*n* = 50 in *Wedelia chinensis* and in *W. trilobata.* Somatic variants were seen in *Cosmos bipinnatus* (2*n* = 22) and *Eclipta prostrata* (2*n*=18).



1. *Bidens pilosa* (2n = 72); 2a. *Cosmos bipinnatus* 'Orange' (2n = 24); 2b. Somatic variant (2n = 22); 3.*Cosmos bipinnatus* 'Yellow' (2n = 24); 4. *C. caudatus* (2n = 24); 5a. *Eclipta prostrata* (2n = 22); 5b. Somatic variant (2n = 18); 6. *Galinsoga parviflora* (2n = 32); 7.*Melampodium paludosm* (2n = 24); 8. *Parthenium hysterophorus* (2n = 36); 9. *Sigesbeckia orientalis* (2n = 30); 10. *Synedrella nodiflora* (2n = 40); 11. *Tithonia diversifolia* (2n = 34); 12. *Tridax procumbens* (2n = 36); 13. *Wedelia chinensis* (2n = 50); 14. *Wedelia trilobata* (2n = 50).

Bar represents 5µm

Figure 1. Photomicrographs of mitotic metaphase of fourteen taxa of Heliantheae

DISCUSSION

Mariano and Morales (1999) reported a numerical chromosome variation for the *Bidens pilosa* from 2n = 24 to 2n = 96 and also reported that about 70% of the population showed 2n = 72. The present study confirmed the chromosome number 2n = 24 in both species of *Cosmos* (C. *bipinnatus* and *C. caudatus),* similar to the previous reports (Morton 1962). However, two cytotypes (2n = 24 and 2n = 22) were observed in *Cosmos bipinnatus* 'Orange'. *Eclipta prostrata* also showed two cytotypes, one with 2n = 18 and other with 2n = 22. Mohan *et al.* (1962) also reported 18 and 22 somatic chromosome numbers for this medicinal plant. The presence of identical numbers in unrelated genera is a very noteworthy feature in Heliantheae. The presence of widely different series of chromosome numbers in different species of same genus and different accessions of same species indicated that the different chromosome numbers can be derived one from the other.

Stuessy (1977) reported that basic chromosome number vary considerably in the tribe Heliantheae, and it ranges from x = 4 to x = 19. However, x = 10 is rare, while x = 8 and 9 are common and higher basic numbers of x= 17, 18 and 19 are also prevalent. The basic chromosome numbers reported in the investigated members of this tribe include x =9, 11, 12, 13, 17 and 20 (Fedorov, 1969).

Fedorov (1969) reported secondary basic number x = 12 for *Bidens pilosa*. Therefore the investigated accession of *Bidens pilosa* was hexaploid. Mariano and Morales (1999) reported that polyploidy play an important role in the evolutionary process of *B. pilosa*.

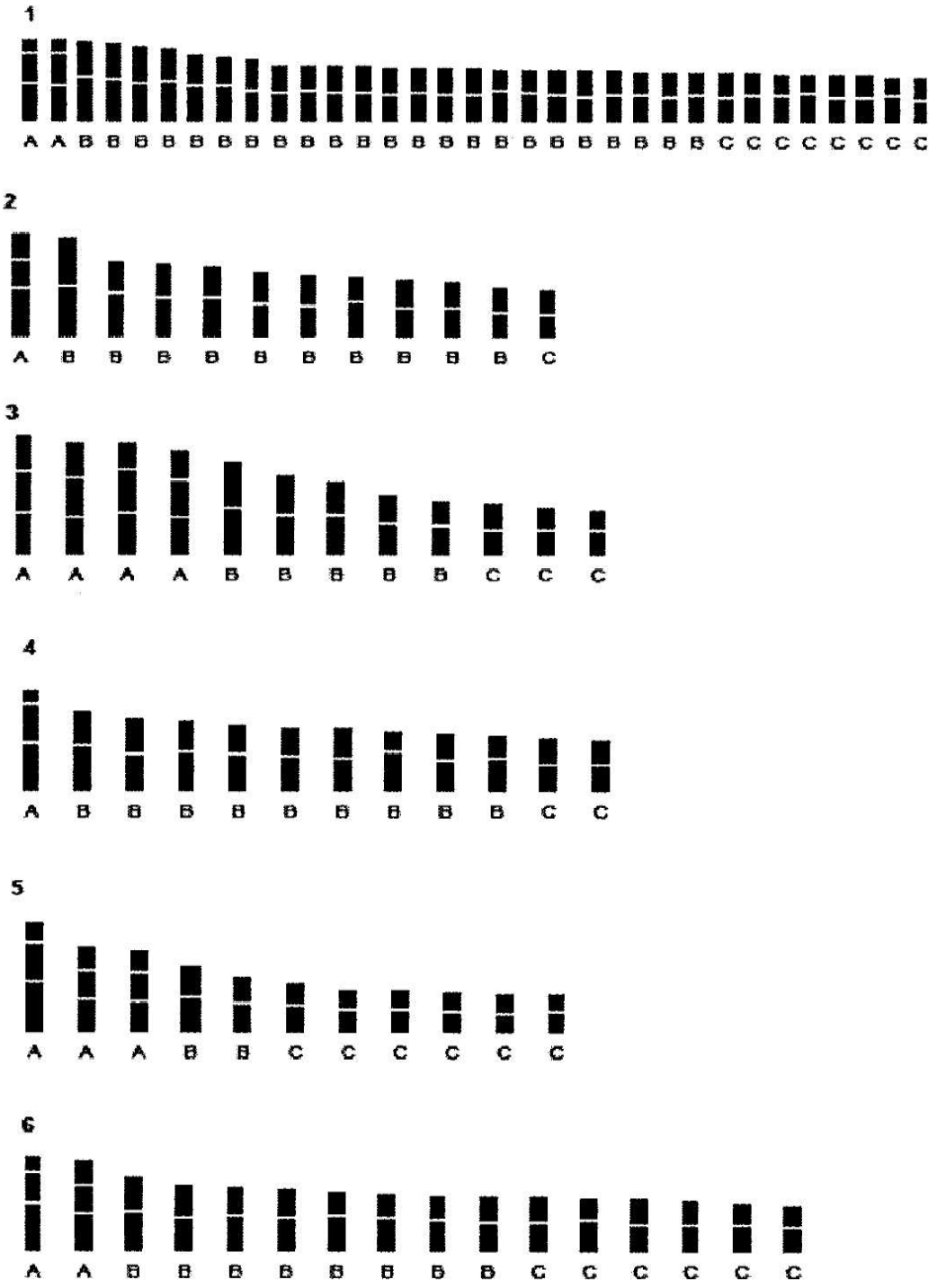
In the present study *Cosmos bipinnatus and Eclipta prostrata* were characterized by different cytotypes. Two different chromosome numbers (n = 12 and 11) have been reported for new developmental changes leading to a later shift towards the asexual mode of reproduction (Stebbins ,1980). Thus polyploidy was found to play an important role in the evolution of *Wedelia*.

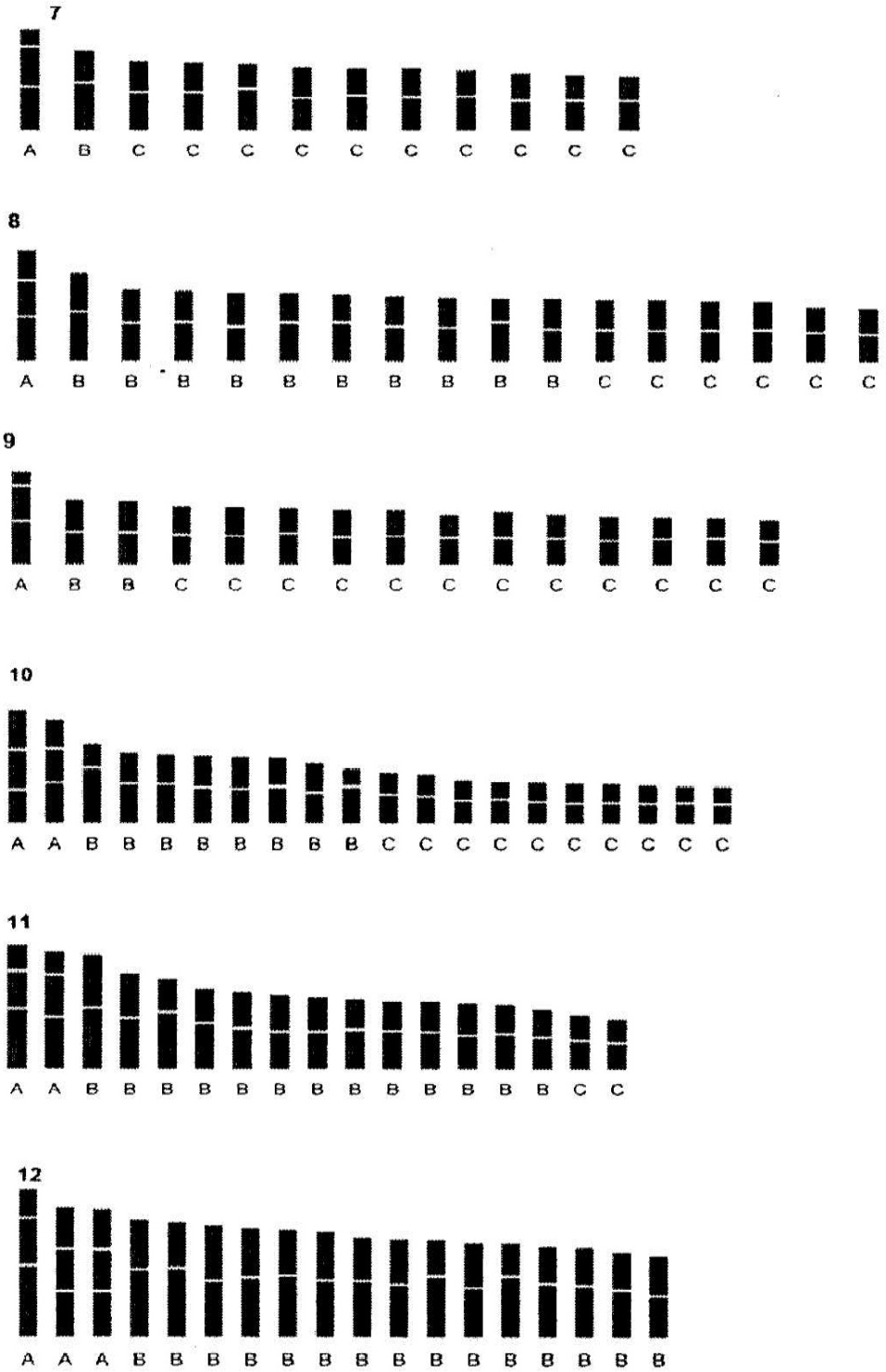
Cosmos bipinnatus by Morton (1962), indicate that this species exist at least in two cytotypes. Previous workers confirmed the existence of x = 11 in *Eclipta* (Fedorov, 1969). Hence chance for the occurrence of amphipolyploidy from the primary base numbers x = 5 and x = 6 as well as ascending or descending dysploidy from the respective secondary base numbers are equal during the process of evolution.

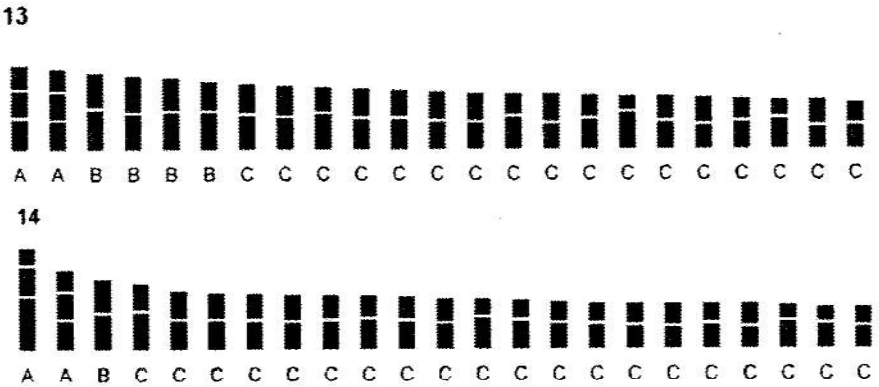
Galinsoga parviflora in this present investigation showed n = 16. In the absence of authentic basic chromosome number reports on this species, the question regarding the basic number remains a matter of speculation. Mathew (1978) reported base number x = 12 in *Melampodium paludosum* which is secondary in nature. Basic chromosome numbers advocated in the present study are x = 9 *(Parthenium* and *Tridax),* x = 15 *(Sigesbeckia),* x = 17 (*Tithonia)* and x = 20 *(Synedrella)* (Fedorov, 1969).

Based on the occurrence of n = I 5 and 25 in Indian species of *Wedelia,* Mehra and Remanandan (1974) suggested that x = 5 as the possible basic number. Two species of *Wedelia* examined in the present study suggested the secondary base number x = 10 is the basic number of this genus. If x = 10 is taken as the basic number of the genus, the present two species should be pentaploid. High chromosome numbers have been considered to be the result of polyploid increase (King and Robinson ,1987). Moreover, the vegetative mean of reproduction, which is prevalent in *Wedelia* seem to bear a correlation with the high degree of polyploidy. Polyploidization might have led to the establishment of new gene combinations that have triggered off.

Figure II: Comparative idiogram of fourteen taxa investigated




7
A B C C C C C C C C C C
8
A B B B B B B B B B B B C C C C C C
9
A B B C C C C C C C C C C C C C
10
A A B B B B B B B B C C C C C C C C C C
11
A A B B B B B B B B B B B B B C C
12
A A A B B B B B B B B B B B B B B B



Scale 1 μm

Fig. 1 to 14- Comparative idiogram chart of various taxa studied

1 .	*Bidens pilosa*	(2*n* = 72)
2 .	*Cosmos bipinnatus* 'Orange'	(2*n* = 24)
3.	*Cosmos bipinnatus* 'Yellow'	(2*n* = 24)
4.	*Cosmos caudatus*	(2*n* = 24)
5.	*Eclipta prostrata*	(2*n* = 22)
6.	*Galinsoga parviflora*	(2*n* = 32)
7,	*Melampodium paludosum*	(2*n* = 24)
8.	*Parthenium hysterophorus*	(2*n* = 36)
9.	*Sigesbeckia orientalis*	(2*n* = 30)
10.	*Synedrella nodiflora*	(2*n* = 40)
11.	*Tithonia diversifolia*	(2*n* = 34)
12.	*Tridax procumbens*	(2*n* 36)
13.	*Wedelia chinensis*	(2*n* = 50)
14.	*Wedelia trilobata*	(2*n* = 50)

Robinson *et al.* (1981) proposed that polyploidy played an important role in the early history of the Heliantheae, based on the distribution of chromosome number. Generally the polyploidized organism has a greater genetic plasticity, due to a greater genetic variability present in the genome. In *Cosmos* the chromosome number 2*n* = 24 was confirmed in both species *(C. bipinnatus and C caudatus)* suggest that it belongs to a diploid level with basic number x = 12. *Cosmos bipinnatus* 'Orange' (2n = 24) exhibit aneuploid somatic variant (2*n* =22), which is common in this genus. It is caused by the gain or loss of one or more chromosomes from the haploid set. *Eclipta prostrata*, 2*n* = 22 based on x = 11. This agrees with the previous report (Fedorov, 1969). Somatic variant (2*n* = 18) was also found in this species which are multiples of the lowest one.

The suggested ploidy level of other members investigated in this tribe are *Galinsoga parviflora* (2n = 2x = 32), *Melampodium paludosum* (2n = 2x = 24), *Siegesbeckia orientalis* (2*n* = 2x = 30), *Synedrella nodiflora* (2*n* = 2x = 40) and *Tithonia diversifolia* (2*n* = 2x = 34). High chromosome numbers such as n = 15, 16, 17 and 20 have been considered to be the result of polyploid increase followed by dysploid loss (King and Robinson, 1987). The high basic number considered as a secondary one. Such a high basic numbers might have originated either through polyploidy followed by aneuploidy or dibasic polyploidy involving the primary base number x = 7, 8 and 9.

Two taxa investigated in this tribe, *Parthenium hysterophorus* and *Tridax procumbens* revealed the existence of tetraploidy from the primary base number x = 9. This agrees with the previous report (Fedorov, 1969). An increase in the number of chromosomes through autopolyploidy provides increased possibilities for new gene combinations, which are of considerable importance in evolution. Both ascending and descending dysploidy, autopolyploidy and amphipolyploidy were found to be responsible for the evolution of various taxa coming under this tribe (King and Robinson, 1987).

The general feature noted in the present karyological study was the presence of very small chromosomes in most of the species. The chromosome complements in the various investigated members differ in minute karyotypical details. With regard to the gross morphology, chromosomes were nearly sub median to nearly median in nature. The size varied from 2.23 to 0.41 μm in length. The chromosomes with secondary constriction ranged from 2 to 8 in number. The average chromosome length (ACL) varied from 0.60 to 1.62 μm. The total chromosome length (TCL) showed a very wide variation with 17.94 being the minimum and 63.12 being the maximum value. The disparity index values ranged between 28.16 - 47.43, the coefficient of variation ranged from 15.38 to 35.91 and total chromatin index (TF%) ranged from 40.30 - 46.90.

Karyomorphological data indicate that, all the taxa have more or less symmetrical karyotype, which is considered to be primitive. On a close examination of karyotype of these members revealed that karyotype asymmetry is progressively greater among the higher polyploids. Similarly karyotypic size difference of chromosome which is brought about by differential deletion of segments of individual chromosomes as well as through occurrence and establishment of unequal transloction between non-homologous chromosomes is also seemed to be greater among the polyploids. Species of *Cosmos* with x = 12, the size ranged from 0.7 - 1.49 μm. However, species of *Wedelia* with x = 10, the size ranged from 0.41 - 1.15 μm. *Wedelia* species are polyploids compared to the

diploid species of *Cosmos*. Thus the significant chromosome difference between genera is also confirmed. In *Wedelia* the lesser values for all the parameters of karyomorphology revealed its evolved nature. Such type of variation is important from the evolutionary view point. Long chromosomes with symmetrical karyotype are further evidence of primitiveness (Sharma, 1984). In *Tridax procumbens* eventhough the karyotype showed some trend towards asymmetry, the higher values of average chromosome length, chromatin length of basic complement revealed its trend towards primitiveness. The chromosome pairs with secondary constriction varied from one to four in various taxa. The karyomorphological differences found among these taxa fully justify that speciation and evolution has been principally affected by minute structural alterations.

These variations found in the karyotypic parameters suggest that Heliantheae members are characterized by symmetrical to slightly asymmetric karyotypes. An increase in the range of chromosome length as well as the increase of sub-metacentrics at the expense of metacentrics is accompanied by an increase in the coefficient of variation leading to symmetry (Stebbins, 1958). Various details of the karyotype such as differences in absolute chromosome size, position of centromere, total chromosome length, karyotype formula and in the number as well as position of the satellites vary from species to species. These variations found in the karyotype parameters suggest that Heliantheae members are characterized by symmetrical to slightly asymmetrical karyotypes.

Individuals with same chromosome number but with differences in karyomorphological details reflect the ongoing evolutionary processes at micro-level. The presence of a wide range of chromosome numbers, numerical variations and structural changes of chromosomes found in many genera mark the significant role in the evolutionary process within the tribe. Besides that both aneuploidy and polyploidy have played an important role in the evolution of various taxa of the tribe at the generic and species level.

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