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CHROMOSOME NUMBERS AND POLYPLOIDY IN THE LEGUMES OF PAKISTAN

SURAYYA KHATOON AND SYED IRTIFAQ ALI

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Original counts of 15 species of leguminous plants of Pakistan are reported here including 3 counts new to science. This brings the total number of chromosomally known leguminous species in the flora of Pakistan to 208 belonging to 68 genera i.e., 40.15% of the total leguminous species in our flora. The incidence of polyploidy is discussed in relation to taxonomic groups, their phylogenetic position, habit classes and phytogeographic regions. The more archaic subfamilies i.e. Caesalpinioideae and Mimosoideae show the retention of higher basic numbers (x=12-14) with secondary cycle of polyploidy non-existent in the former (in our sample) while exhibited by some species like *Acacia* spp., and *Prosopis juliflora* in the latter. In Papilionoideae, the comparatively archaic tribes like Sophoreae and Millettieae exhibit higher basic numbers, but more advanced herbaceous tribes have lower basic numbers (x=6-8). In habit classes, woody species show the retention of high basic numbers with little intrageneric polyploidy (1.92%), while the herbaceous and weedy species show lower basic numbers but higher intrageneric polyploidy. The highest percentage of intragenetic polyploidy among various habit types in the sample is met with in annual herbs (3.8%), which are mostly weedy species belonging to the tribes Indigofereae and Trifolieae. The modal basic number in the sample is found to be 8 followed by 11, at the specific as well as at generic level. Basic numbers higher than 11 are most frequent in the Tropico-subtropical phytogeographic elements followed by Sino-Japanese and Indian elements. The intrageneric polyploids of Papilionoideae do not show any obvious correlation with any phytogeographic region. The overall percentage of intrageneric polyploidy in the sample is found to be 10.096% (0% in Caesalpinoideae, 26.6% in Mimosoideae, 10.1% in Papilionoideae). It is somewhat lower than the world average for the family Leguminosae.

Introduction

Leguminosae is more or less equivalent to Gramineae in economic importance due to its known and potential food plants and a multitude of other uses. With about 727 genera and 19325 species, legumes constitute the third largest family of flowering plants after Compositae and Orchidaceae (Lewis *et al*., 2005). Due to their economic importance, legumes have attracted the attention of cytologists and more than 50% of their total genera are cytologically known (Goldblatt, 1981).

In the Flora of Pakistan, legumes are represented by 107 genera and 518 species (Ali, 1973a, b; 1977). Up to mid-sixties, no cytological work was done on the legumes of Pakistan and up to 1989, only 26 species were chromosomally known (Perveen & Khatoon, 1989). Later, some large scale studies were taken up (Khatoon & Ali, 1991, 1993 and Jahan *et al*., 1994). By compiling all chromosome counts based on the material collected from Pakistan and those reported from the neighbouring areas of Pakistan, the total number of chromosomally known leguminous species comes to be 208 (i.e., 40.15% of the total species) representing 68 genera.

The total expanse of Pakistan has very contrasting geographic and climatological features. Altitudinally it varies from sea-level in the South to 8611 m (K2, the second highest peak after Mt. Everest) in the North, and the mean annual rainfall varies from 50 mm in the deserts to 2033 mm in the moist hilly, northern areas (Ali, 1978). Due to these contrasting features, four phytogeographic regions are recognized in Pakistan (Ali & Qaiser, 1986). The present paper deals with two aspects; firstly it provides voucher details and photographs for the original counts by the authors which were preliminarily published in the Chromosome Atlas of the Angiosperms of Pakistan (Khatoon & Ali, 1993), and secondly the incidence of polyploidy is analysed from various aspects like taxonomic group, habit, phytogeographic regions etc.

Material and Methods

For original counts, the material was collected from various parts of the country. Exact localities are shown in Fig. 1. Young floral buds were fixed in Carnoy's Solution (3:1 ethanol-glacial acetic acid) in small vials and stored at 5°C. Slides were prepared by squashing the anthers in 1% propionic carmine. Photo micrographs were taken from the temporary mounts in most of the cases with a Zeiss photomicroscope; the slides were later made permanent by passing through ethanol and mounting in Euparal. The voucher specimens are deposited in the Karachi University Herbarium (KUH).

For the analysis of polyploidy, chromosomal counts pertaining to Pakistan and adjacent parts of India, Afghanistan, Kashmir and Iran were taken from the literature in addition to the original counts.

Results

Information about the original chromosomal counts, along with reference to the photographs is given in the Table 1, while the details about other counts which have been taken into account for the analysis of polyploidy are available in Khatoon & Ali (1993).

The original counts include one count in Mimosoideae and 14 counts in Papilionoideae. Counts for three species i.e. *Lotus garcinii*, *Ononis antiquorum* and *Crotalaria persica* are new to science; while the counts for another 5 species are new for the flora of Pakistan. Count for one species (i.e., *Indigofera linnaei*) differs from the previous reports. This species had been reported as diploid from India, but our material is found to be tetraploid. The assignment of genera to tribes and the arrangement of the tribes in the Table 1 and 5, is in accordance with Lewis *et al*., (2005).

By pooling the present counts with other counts published earlier by us (Khatoon & Ali, 1982, 1991) and other workers from Pakistan (Baquar *et al*., 1965, 1966; Baquar & Husain, 1967; Baquar & Warsi, 1968; Baquar & Askari, 1970; Quraish & Faruqi, 1970; Faruqi, 1977; Jahan *et al*., 1994); plus those counts available for our legumes based on areas adjacent to Pakistan, the total number of chromosomally known species comes to be 208. The break up according to subfamilies and native and introduced species is given in Table 2.

The distribution and analysis of polyploidy are given in Tables 3-5 and Figs. 3-6. The highest intrageneric polyploidy (26.6%) in the sample is exhibited by Mimosoideae, followed by Papilionoideae (10.1%). Caesalpinioideae has not shown any intrageneric polyploidy, but 100% of its species in the sample have basic numbers higher than 11. Basic numbers higher than 11 are also common in Mimosoideae (93.33%), the only exception is the genus *Calliandra* (x=8). Higher basic numbers are least common in Papilionoideae (4.16%).

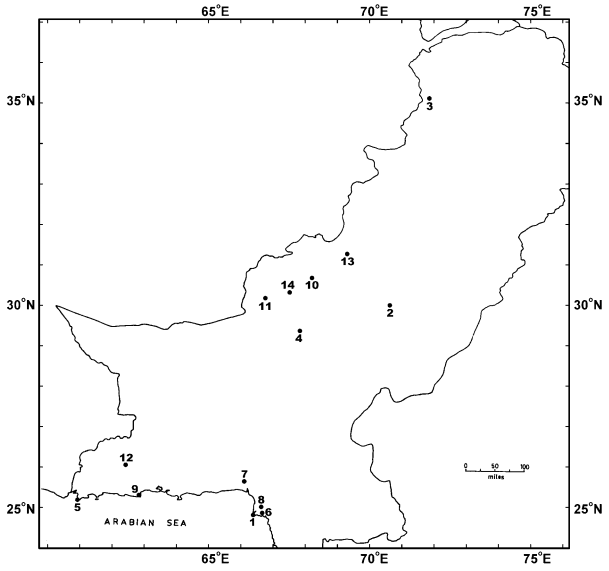

Fig. 1. Map of Pakistan showing the collection localities. (1. Cape Monze, 2: D. G. Khan, 3: Dir, 4: Gishkor, 5: Jiwani, 6:Karachi, 7:Liari, 8:Monghopir, 9:Pasni, 10:Qila Saifullah, 11:Quetta, 12:Turbat, 13: Zhob, 14: Ziarat).

Table 2. Subfamily-wise breakup of chromosomally known species versus total species known from Pakistan (Number of introduced species is given in parenthesis).

Subfamily	Total number of species in flora of Pakistan	No. of chromosomally known species from Pakistan
Caesalpinioideae	18 + (34) = 52	9 + (16) = 25 (48.07%)
Mimosoideae	19 + (29) = 48	7 + (8) = 15 (31.25%)
Papilionoideae	391 + (27) = 418	140 + (28) = 168 (40.19%)
Total	429 + (90) = 518	156 + (52) = 208 (40.15%)

Table 3. Distribution of diploid and polyploid species in the subfamilies

Subfamily	Number of species				
	Diploid	Intrageneric polyploid	Cytotypes	x=>11	n=>11
Caesalpinioideae (25)	25 (100%)	-	-	25 (100%)	25 (100%)
Mimosoideae (15)	11 (73.33%)	3 (20.00%)	1 (6.6%)	14 (93.33%)	14 (93.33%)
Papilionoideae (168)	151 (89.88%)	10 (5.95%)	7 (4.166%)	7 (4.166%)	24 (14.28%)

Table 4. Species with cytotypes*.

Species	Chromosome no.
Indigofera hochstetteri Baker	n = 8; 2n = 16, 32
Indigofera linnaei Ali	n =16; 2n = 16
Tephrosia purpurea (L.) Pers.	n =11, 22
Lotus corniculatus L.	n = 6, 12; 2n = 12
Medicago sativa L.	n = 8, 16
Trifolium repens L.	n = 16, 32
Prosopis juliflora (Swartz) DC.	n = 13; 2n = 52

*Information extracted from Khatoon & Ali (1993)

Table 5. Distribution of polyploidy in the tribes of Papilionoideae (Name of tribe followed by number of species in the sample in parenthesis).

Tribe	Intrageneric polyploids	Species with x=>11	Species with n=>11
Sophoreae (5)	2 (40%)	1 (20%)	3 (60%)
Crotalarieae (11)	1 (9.09%)	0	1 (9.09%)
Genisteae (3)	0	3 (100%)	3 (100%)
Amorpheae (1)	1 (100%)	0	1 (100%)
Dalbergieae (3)	1 (33.33%)	0	1 (33.33%)
Indigofereae (17)	2 (11.76%)	0	2 (11.76%)
Millettieae (11)	2 (18.18%)	0	2 (18.18%)
Phaseoleae (16)	1 (6.25%)	1 (6.25%)	1 (6.25%)
Desmodieae (18)	0	1 (5.55%)	1 (5.55%
Psoraleae (3)	0	0	0
Loteae (2)	1 (50%)	0	1 (50%)
Robineae (4)	0	0	0
Galegeae (36)	1 (2.7%)	0	1 (2.77%)
Hedysareae (4)	0	0	0
Trifolieae (18)	5 (27.77%)	1 (5.55%)	6 (33.33%)
Cicereae (1)	0	0	0
Fabeae (16)	0	0	0

Discussion

Polyploidy has been a pivotal factor in plant evolution; an increase in ploidal level with or without hybridization often has been associated with speciation and the origin of novel adaptations (Levin, 2002). All the three subfamilies of Leguminosae are believed to have a polyploid origin (Goldblatt, 1981). The subfamilies Caesalpinioideae and Mimosoideae are characterized by having basic numbers higher than 11, therefore Goldblatt (1981) has suggested an early tetraploid origin with secondary basic number x=14 for entire Leguminosae, on the basis of x=7 in the original diploid genus *Cercis*. Hence all genera in all subfamilies (except *Cercis*) are ancient polyploids.

The basic numbers lower than 14 in the family are believed to have evolved through descending aneuploidy (Goldblatt, 1981). The comparatively archaic and more tropical subfamilies, i.e., Caesalpinioideae and Mimosoideae still retain the high basic numbers (x=14-12) in most of their genera, with the highly specialized genus *Calliandra* (x=8) being an exception. In the advanced subfamily Papilionoideae with a more temperate distribution, basic numbers as low as x=6 have evolved, therefore superficially they do not appear like archaic polyploids, but the comparatively primitive tribes within the subfamily like Sophoreae, Millettieae etc. bear higher basic numbers (x=14-11).

The secondary cycle of polyploidy in Leguminosae is not very frequent. Goldblatt (1981) in his comprehensive review of legume cytology records intrageneric polyploidy as 6.4% in Caesalpinoideae, 13.4% in Mimosoideae and 14.3% in Papilionoideae. In our sample from flora of Pakistan, Caesalpinoideae has not shown any intrageneric polyploidy, but 100% of its species in the sample have basic numbers higher than 11 (Table 3 and Fig. 4). Basic numbers higher than 11 almost surely indicate archaic polyploidy in all groups of higher plants according to Stebbins (1971) and Goldblatt (1980). We have also accepted it as a standard.

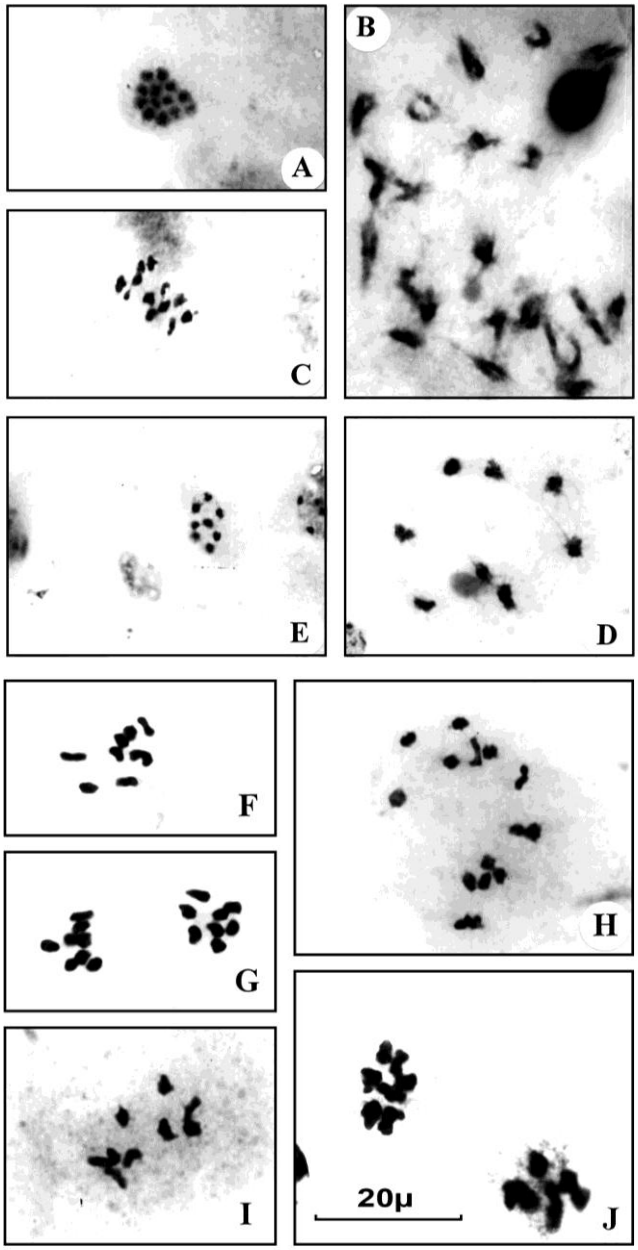

Fig. 2. Meiosis in pollen mother cells. **A.** *Albizia saman*, metaphase-I, n=13 (*Kh.* s. n.); **B**. *Tephrosia apollinea*, late diplotene, n=22 (*Q. 81*62); **C**. *Desmodium triflorum*, metaphase-I, n=11 (*Kh*. 564); **D**. *Alhagi maurorum*, diakinesis, n=8 (*Kh.* 532); **E**. *Taverniera glabra*, early metaphase-II, n=8 (*Gh.* 3613); **F**. *Medicago lupulina*, metaphase-I, n=8 (*Kh.* 548); **G**. *Melilotus officinalis*, anaphase-I, n=8 (*Kh.* 503); **H**. *Ononis antiquorum*, early metaphase-I, n=15 (*Kh.* 545); **I.** *Trifolium resupinatum*, metaphase-I, n=8 (*Kh.* 550); **J.** *Lens orientalis*, metaphase-II, n=7 (*Gh*. 3281).

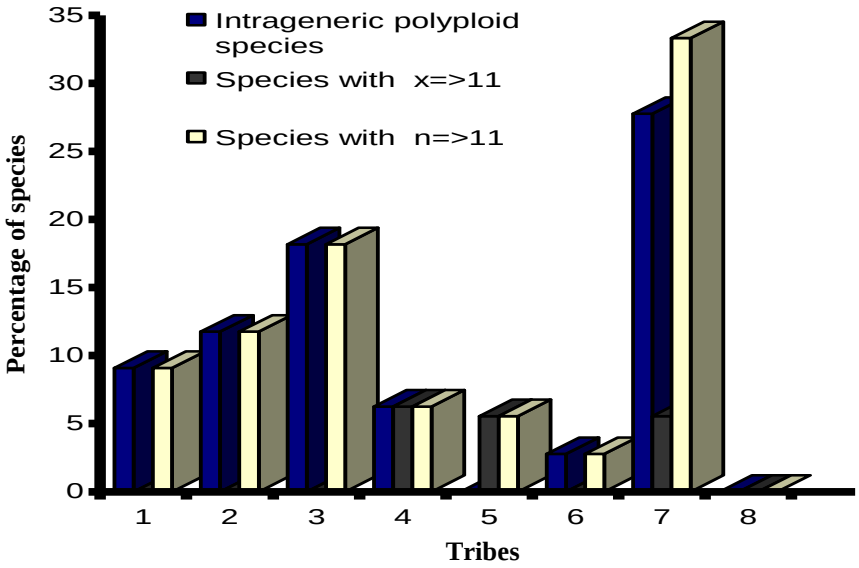

Fig. 3. Distribution of polyploidy in the tribes of Papilionoideae. Only those tribes are included which are represented by more than ten species in the sample (1: Crotalarieae, 2: Indigofereae, 3: Millettieae, 4: Phaseoleae, 5: Desmodieae, 6: Galegeae, 7: Trifolieae, 8: Fabeae).

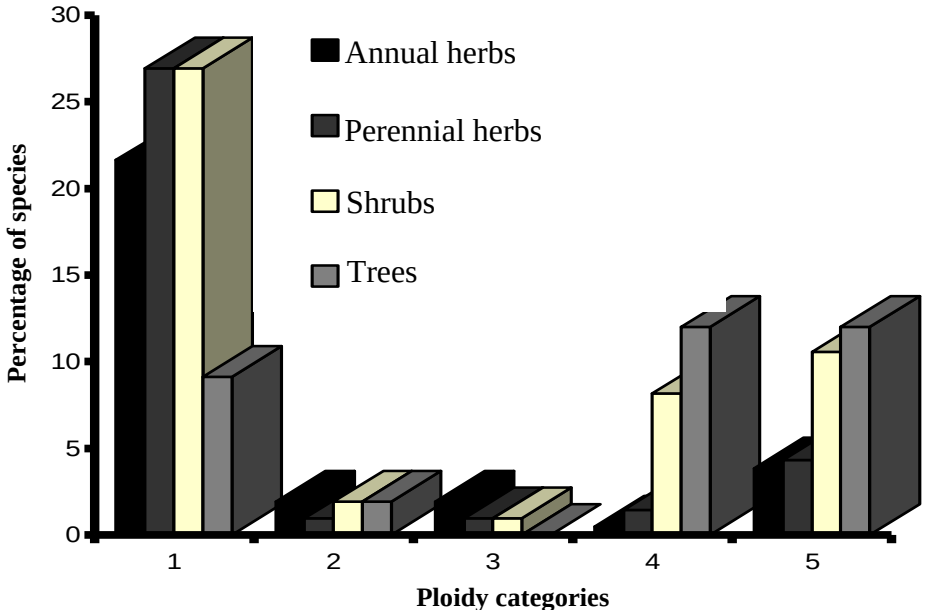

Fig. 4. Distribution of polyploidy in various habit categories. 1: Diploids, 2: Intrageneric polyploids, 3: Species with diploid and polyploid cytotypes, 4: Species with x=>11, 5: Species with n=>11.

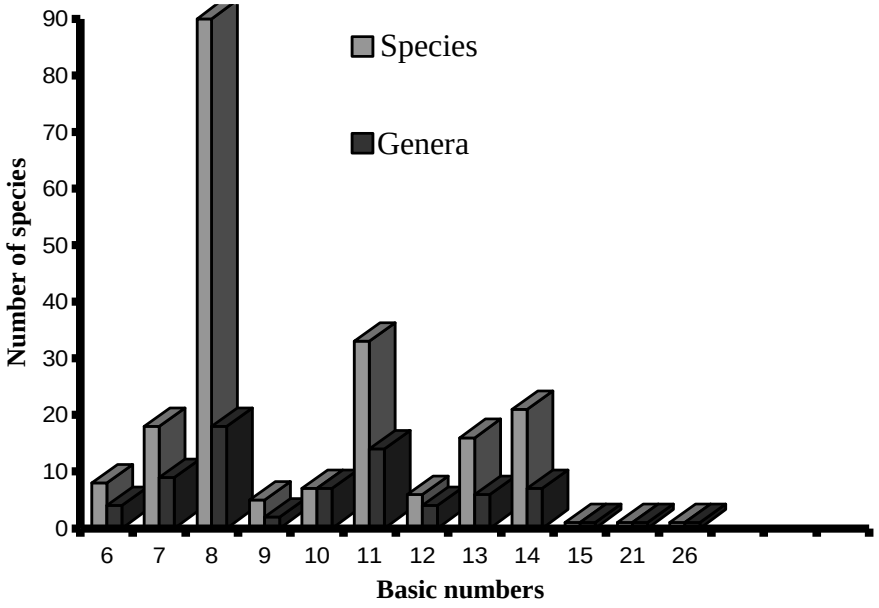

Fig. 5. Frequency of different basic numbers in species and genera of Leguminosae.

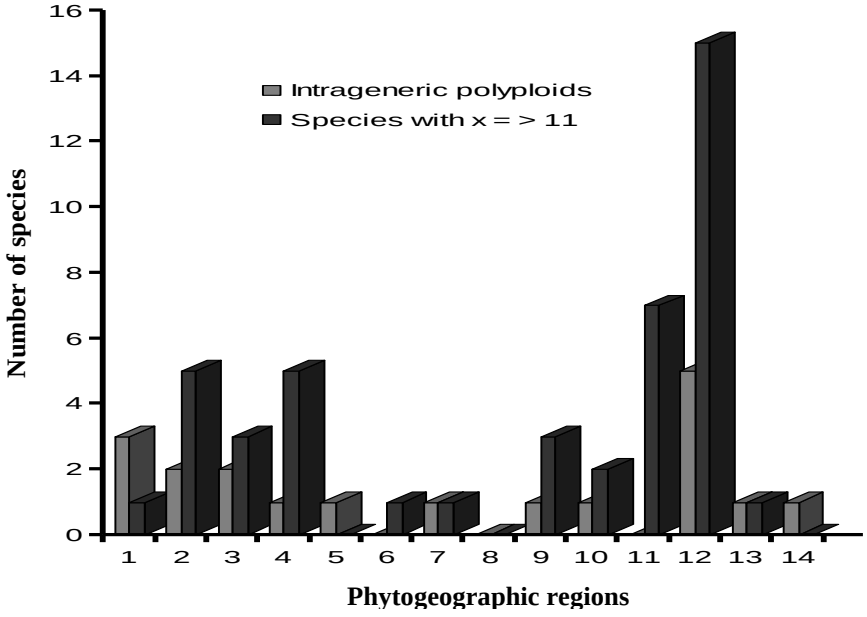

Fig. 6. Distribution of polyploid species in different phytogeographic regions. (1: Irano-Turanian region, 2: Sino-Japanese region, 3: Saharo-Sindian region, 4: Indian region, 5: Euro-Siberian region, 6: Mediterranean region, 7: Irano-Turanian-Mediterranean, 8: Irano-Turanian-Euro-Siberian, 9: Indo-Malayan region, 10: Saharo-Sindian-Indian, 11: Sino-Japanese-Indo-Malayan, 12: Tropical, 13: Sub-cosmopolitan, 14: Boreal.

The sample of Mimosoideae has shown higher basic numbers in 93.33% species and intrageneric polyploidy in 26.6% species. This percentage of intrageneric polyploidy is much higher than the world average of 13.4% (Goldblatt, 1981). However, this could be due to the smaller size of our sample for this subfamily.

In the sample of Papilionoideae, Sophoreae shows 40% intrageneric polyploidy, followed by Trifolieae with 27.7% intrageneric polyploidy. Higher basic numbers are met with in the tribes Sophoreae, Millettieae, Desmodieae, Phaseoleae, Trifolieae (in *Ononis* only) and Genisteae. All the three species of Genisteae studied have higher basic numbers (Table 5 & Fig. 3). The over all intrageneric polyploidy in Papilionoideae i.e. 10.1% is lower than the world average of 14.3%. According to Goldblatt (1981), the secondary cycle of polyploidy in the subfamily is more evident in North temperate species with Eurasia as the secondary centre of radiation, while the primary cycle of polyploidy occurred in the tropics.

The correlation of polyploidy with habit categories, detected as early as in 1938 by Stebbins and supported by Levin & Wilson (1976), Levin (2002), holds true for this sample. The minimum frequency of intrageneric polyploidy is shown by trees (1.92%) with complete absence of any species with cytotypes. On the other hand, the frequency of higher basic numbers is maximum in trees i.e., 12.02%, followed by 8.17% shrubs and subshrubs (Fig. 4). However, the frequency of intrageneric polyploids and species with cytotypes in the annual herbs is found to be greater than perennial herbs (3.85% vs. 1.92%) which is not in conformity with Stebbins' generalization, according to which perennial herbs show highest incidence of polyploidy. This discrepancy could be due to the fact that most of the intrageneric polyploid annual species in this sample are weedy species (mostly belonging to tribes Indigofereae and Trifolieae). According to Stebbins (1971), weediness is correlated with polyploidy. In all habit classes, generally those species show intrageneric polyploidy which occupy rather unstable or disturbed habitats like *Prosopis juliflora*, *Acacia nilotica* and *A. farnesiana* in Mimosoideae and mostly herbaceous, weedy species in Papilionoideae. This conforms to Stebbins' (1984; 1985) Secondary Contact Hypothesis, according to which the taxa with a 'patchy' distribution tend to have more incidence of polyploidy than taxa with more continuous distribution; because the former get a better chance of hybridization between differently adapted races. As polyploidy stabilizes the gene pool acquired through hybridization therefore such taxa have a better ability to colonize newly opened ecological niches in the newly opened or unstable and disturbed habitats.

The basic numbers in the sample vary from x=6 to x=26; of which x=8 is found to be the modal number both in species and genera, followed by x=11 (Fig. 5). On world level, Goldblatt (1981) also found x=8 as modal for species, but he found x=11 as modal for the genera. In our sample, x=8 had been modal at generic level due to the fact that the genera of Papilionoideae with lower basic numbers form the main stock of legumes in our flora, like Galegeae, Indigofereae, Trifolieae, Crotalarieae etc., and the comparatively tropical genera with higher basic numbers have a lesser proportion in the flora. The basic numbers higher than x=11 are most frequent in the tropico-subtropical phytogeographic elements followed by Sino-Japanese and Indian elements (Fig. 6), suggesting that these elements have not changed much since the primary cycle of polyploidy in the family. The frequency of intrageneric polyploids of Mimosoideae is greater in the Tropico-subtropical elements; whereas the intrageneric polyploids of Papilionoideae do not show any obvious correlation with any phytogeographic region.

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