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Cytomorphological Characterization in 28 Species of Papilionaceae from Indian Cold Deserts

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Abstract: ***Present cytomorphological investigation in Papilionaceous members from cold desert region of Kinnaur (India) record the first ever chromosome count in Astragalus malacophyllus (n=8), A. strobiliferus (n=8), Campylotropis eriocarpa (n=11), Colutea nepalensis (n=8), Indigofera himalayensis (n=8), Trigonella pubescens (n=8), Vicia rigidula (n=12), V. tenera (n=7) and in variety nutans of Desmodium elegans (n=11) at world level and in Caragana gerardiana (n=8), Desmodium racemosum (n=11), Medicago falcata (n=8), and Piptanthus nepalensis (n=9) at India level. New or variable chromosome counts have been recorded for four species named Indigofera heterantha, Oxytropis mollis, Trigonella emodi, and Vicia pallida. Based on x=8, in Indigofera heterantha existence of intraspecific diploid (n=8) and hexaploid (n=24) cytotypes have been reported. In this species, the diploid and hexaploid individuals can be distingiushed from each other on the basis of vegetative and floral characters. Further meiotic course in majority of species was observed perfectly regular which resulting into high pollen fertility. However, 11 species depicted the phenomenon of cytomixis of inter PMCs chromatin transfer which consequences various irregular meiotic products yielded unstained/sterile and fertile pollen grains of variable sizes.***

Keywords: chromosome counts, intraspecific polyploidy, cytomixis, pollen grains

1. Introduction

The Papilionaceae is the third largest and economically important family of angiosperms [7] with approximately 476 genera and over 13860 species worldwide, which are easily recognized by their legume fruit and their compound and stipulated leaves. The family is well represented throughout temperate and tropical regions of the world [10] ranging in habit from large trees to annual herbaceous plants. The present communication covers the chromosomal counts, detailed male meiotic course and pollen fertility %age in some members belonging to family Papilionaceae from Kinnaur district of Himachal Pradesh in India (Map 1). Kinnaur is tribal and hilly district with altitude ranging from 1600-6800m, represents a number of lush green valleys, mountain ranges, snow-clad peaks and cold desert conditions. In spite of harsh climatic conditions, the area is rich in plant diversity which includes plenty of flowering plants of dry alpine and temperate zones. Besides some of the species are confined to specific habitats like rock crevices, dry sandy slopes, among stones, moist open and shaddy slopes and along stream side, river and water channels. Owing to rich plant diversity, Kinnaur district has been well explored for its medicinal and aromatic plants wealth [3] [9]. Presently an attempt has been made to explore the cytomorphological diversity in papilionaceous members from this high altitudinal and cold desert region of northwest Himalayas.

2. Material and Methods

2.1 Collection and Submission of Samples

For exploration of cytomorphological diversity, study materials were collected by surveying the different localities of Kinnaur district during the months of April to September for five years (2007-2011).Voucher specimens of the cytomorphologically worked out individuals were deposited in the Herbarium, Department of Botany, Punjabi University Patiala (PUN).

2.2 Cytological Analysis

Young panicles/floral buds were fixed in freshly prepared Carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid v: v: v) for 24h and preserved in 70% alcohol at 4°C. Anthers were squashed in 1% acetocarmine and in each case 400-500 sporads were analyzed. Pollen viability was estimated through stainability tests for which anthers from mature flowers were squashed in glycerol-acetocarmine (1:1) mixture and 1% aniline blue dye. Well filled pollen grains with fully stained nuclei and cytoplasm were scored as fertile while shrivelled with partially or unstained cytoplasm were counted as sterile.

2.3 Morphometric Analysis

The morphological analysis is evaluated by field observations to mark out the different morphovariants by focusing on vegetative (plant height, number of branches per plant, number of leaflets per leaf, size and colour of leaves) and reproductive (number of flowers per inflorescnce and colour of flowers) parameters. Stomatal studies were made from the abaxial epidermal peels of the leaves through KOH treatment. Following expression was used to calculate the stomatal index: $SI = S/E+S \times 100$
Where, SI = stomatal index, S = number of stomata per field, E = number of epidermal cells per field.

2.4 Photomicrographs

Photomicrographs of chromosome counts, meiotic abnormalities, sporads, pollen grains, and stomata were made from the freshly prepared slides using Leica Qwin Digital Imaging System (X290, X900 and X2180) and Nikon Eclipse 80*i* microscope (X330, X1340 and X3400).

Results and Discussions

Present work includes detailed cytomorphological studies on 28 species (41 accessions) under 14 genera of family Papilionaceae from different localities of Kinnaur district between altitudes of 1680-3660m. Data on species, localities with altitudes, GPS coordinates, accession number (PUN), meiotic chromosome number, ploidy level, PMCs involved in cytomixis (%age), pollen fertility (%age) and previous chromosome counts are provided in Table 1. Some of the interesting findings include-

3.1 First ever chromosome counts

All the species of the family have been worked out cytologically for the first time from geographical isolated and cold desert region of Kinnaur. *Astragalus malacophyllus* (n=8; Fig. 1), *A. strobiliferus* (n=8; Fig. 2), *Campylotropis eriocarpa* (n=11; Fig. 3), *Colutea nepalensis* (n=8; Fig. 4), *Indigofera himalayensis* (n=8; Fig. 5), *Trigonella pubescens* (n=8; Fig. 6), *Vicia rigidula* (n=12; Fig. 7), *V. tenera* (n=7; Fig. 8) are counted chromosomally for the first time at world level. The variety *nutans* of *Desmodium elegans* (n=11; Fig. 9) is also recorded chromosomally for the first time. The study also includes the first ever chromosomal count from India for *Caragana gerardiana* (n=8; Fig. 10), *Desmodium racemosum* (n=11; Fig. 11), *Medicago falcata* (n=8; Fig. 12), and *Piptanthus nepalensis* (n=9; Fig. 13).

3.2 Additional/variable cytotypes

On the basis of present chromosome study, new or variable counts have been recorded for four species named *Indigofera heterantha*, *Oxytropis mollis*, *Trigonella emodi*, and *Vicia pallida*. The diploid (2n=16; Fig. 17) cytotype in *Indigofera heterantha* is recorded for the first time from India but already counted from Pakistan [2]. *Oxytropis mollis* with 2n=16 (Fig. 14) adds a new intraspecific diploid cytotype against already existing tetraploid

Table 1: Information on locality with altitude and GPS Coordinates, accession number/s (PUN*), meiotic chromosome number, ploidy level, PMCs involved in cytomixis (%age), pollen fertility (%age), and previous chromosome reports on the cytologically investigated species of Family Papilionaceae.

S. No.	*Taxon*	*Locality with altitude (m)*	*Localities with GPS Coordinates*	*Accession number/s (PUN*)*	*Meiotic chromosome number (n)*	*Ploidy level*	*PMCs involved in cytomixis (%age)*	*Pollen fertility (%age)*	*Previous chromosome reports***
1.	*Astragalus candolleanus* Royle	Rakchham, 3115	31°22'49"N 78°22'5"E	53678	8	2x	-	100	2n=16
2.	*A. chlorostachys* Lindl.	Sangla, 2680	31°25'36"N 78°15'51"E	50909	8	2x	-	100	2n=16
3.	*A. grahamianus* Royle ex Benth.	Rakchham, 3115	31°22'49"N 78°22'5"E	53673	8	2x	17.27	92	2n=16
4.	*A. graveolens* Buch.-Ham. ex Benth.	Ropa, 3000 Ropa, 3000 Sangla, 2680 Rakchham, 3115 Karchham,1900	31°47'48"N 78°25'22"E ,, 31°25'36"N 78°15'51"E 31°22'49"N 78°22'5"E 31°29'46"N 78°10'47"E	55758 55759 50900 53672 50910	8 8 8 8 8	2x 2x 2x 2x 2x	17.02-20.55	100 100 97 96 100	2n=16
5.	*A. malacophyllus* Benth. ex Bunge	Rakchham, 3115	31°22'49"N 78°22'5"E	53676	8	2x	-	100	#
6.	*A. rhizanthus* Royle ex Benth.	Chittkul, 3450	31°21'7"N 78°26'7"E	53677	8	2x	-	100	2n=16
7.	*A. strobiliferus* Royle	Ropa, 3000	31°47'48"N 78°25'22"E	53680	8	2x	-	98	#
8.	*A. zanskarensis* Benth. ex Bunge	Chittkul, 3450	31°21'7"N 78°26'7"E	53682	8	2x	-	100	2n=16
9.	*Campylotropis eriocarpa* Schindler	Bhabanagar, 1900	31°33'50"N 77°56'17"E	50913	11	2x	-	100	#
10.	*Caragana gerardiana* Royle	Nako, 3660	31°52'50"N 78°37'37"E	53683	8	2x	-	98	2n=16, 18
11.	*Colutea*	Pooh, 2840	31°45'47"N 78°35'20"E	53686	8	2x	-	100	#

	nepalensis Sims	Khab, 2800 Moorang, 2590 Thangi, 2700	31°48'0"N 78°38'38"E 31°35'18"N 78°26'53"E 31°33'6"N 78°28'57"E	53684 53685 53687	8 8 8	2x 2x 2x		95 99 98	
12.	*Desmodium elegans* DC. (= *D. tiliaefolium* (D. Don) G. Don, = *Hedysarum tiliaefolium* D. Don)	Kalpa, 2760 Kuppa, 2650 Sangla, 2680	31°31'55"N 78°15'5"E 31°25'44"N 78°14'40"E 31°25'36"N 78°15'51"E	54277 54278 54279	11 11 11	2x 2x 2x	-	98 99 100	2n=22
	D. elegans DC. var. *nutans* (Hook.) H. Ohashi	Rakchham, 3115 Tapri, 1680 Chaura, 1890 Bhabanagar, 1900 Karchham, 1900 Karchham, 1900 Palingi, 1900	31°22'49"N 78°22'5"E 31°31'4"N 78°6'0"E 31°33'50"N 77°56'17"E 31°33'50"N 77°56'17"E 31°29'46"N 78°10'47"E 31°29'46"N 78°10'47"E 31°33'23"N 77°52'58"E	53670 53671 53667 53665 53668 53666 53669	11 11 11 11 11 11 11	2x 2x 2x 2x 2x 2x 2x	-	100 100 100 100 100 100 100	#
13.	*D. racemosum* (Thunb.) DC.	Kalpa, 2760	31°31'55"N 78°15'5"E	53664	11	2x		100	2n=22
14.	*Indigofera heterantha* Wall. ex Brandis **Cytotypes (x=8)** (i) The diploid (ii) The hexaploid	Bhabanagar, 1900 Ponda, 1980 Kuppa, 2600 Sangla, 2680 Sangla, 2680 Rakchham, 3115 Rakchham, 3115	 31°33'50"N 77°56'17"E 31°33'50"N 77°56'17"E 31°25'44"N 78°14'40"E 31°25'36"N 78°15'51"E ,, 31°22'49"N 78°22'5"E ,,	 53718 53719 53722 53720 50911 50912 53723	 8 8 24 24 24 24 24	 2x 2x 6x 6x 6x 6x 6x	 21.11-26.00	 100 100 100 100 100 98 99	2n=16, 48
15.	*I. himalayensis* Ali	Kangoos, 1980	31°33'9"N 77°55'47"E	53717	8	2x	-	100	#
16.	*Lotus corniculatus* L.	Chittkul, 3450 Moorang, 2590 Thangi, 2700	31°21'7"N 78°26'7"E 31°35'18"N 78°26'53"E 31°33'6"N 78°28'57"E	53690 53691 53692	6 6 6	2x 2x 2x	18.34	88 98 96	2n=12, 24, 28, 32, 36
17.	*Medicago falcata* L.	Pooh, 2840 Pooh, 2840 Moorang, 2590 Moorang, 2590 Thangi, 2700	31°45'47"N 78°35'20"E 31°45'47"N 78°35'20"E 31°35'18"N 78°26'53"E ,, 31°33'6"N 78°28'57"E	53694 53697 53693 53696 53695	8 8 8 8 8	2x 2x 2x 2x 2x	15.43-52.11	90 91 92 92 89	2n= 16, 32
18.	*Melilotus alba* Lamk.	Spello, 2800 Pooh, 2840 Reckong Peo, 2670 Thangi, 2700	31°39'30"N 78°26'28"E 31°45'47"N 78°35'20"E 31°33'6"N 78°16'22"E 31°33'6"N 78°28'57"E	53700 50908 50907 53699	8 8 8 8	2x 2x 2x 2x	8.67	100 100 95 96	2n=16, 24, 32, 36
19.	*Oxytropis mollis* Royle ex Benth.	Rakchham, 3115 Chittkul, 3450	31°22'49"N 78°22'5"E 31°21'7"N 78°26'7"E	53702 53701	8 8	2x 2x	-	100 100	2n=32
20.	*Piptanthus nepalensis* D. Don	Sangla, 2680	31°25'36"N 78°15'51"E	53703	9	2x	-	100	2n=18
21.	*Trifolium pratense* L.	Sangla, 2680	31°25'36"N 78°15'51"E	50917	7	2x	-	98	2n=14,16, 26, 27, 28, 29, 32, 48
22.	*T. repens* L.	Sangla, 2680 Nichar, 2150	31°25'36"N 78°15'51"E 31°33'20"N 77°57'21"E	50916 53711	16 16	4x 4x	5.34-6.02	90 91	2n=16, 22, 28, 30, 32, 48, 64
23.	*Trigonella emodi* Benth.	Rakchham, 3115 Chittkul, 3450	31°22'49"N 78°22'5"E 31°21'7"N 78°26'7"E	53714 53713	8 8	2x 2x	15.88-17.24	94 93	2n=16, 24
24.	*T. pubescens* Edgew. ex	Rakchham, 3115	31°22'49"N 78°22'5"E 31°21'7"N 78°26'7"E	53716 53715	8 8	2x 2x	47.05-62.88	38 54	#

	Baker	Chittkul, 3450							
25.	*Vicia pallida* Turcz. (= *V. sylvatica* Benth.)	Chaura, 1890 Bhabanagar, 1900 Palingi, 1900	31°33'50"N 77°56'17"E 31°33'50"N 77°56'17"E 31°33'23"N 77°52'58"E	53707 53706 53708	12 12 12	4x 4x 4x	1.52-2.20	99 73 79	2n=14
26.	*V. rigidula* Royle	Kalpa, 2760 Kalpa, 2760	31°31'55"N 78°15'5"E 31°31'55"N 78°15'5"E	50919 51044	12 12	4x 4x	6.52	80 81	#
27.	*V. sativa* L.	Reckong Peo, 2670 Kuppa, 2600	31°33'6"N 78°16'22"E 31°25'44"N 78°14'40"E	50918 53709	6 6	2x 2x	-	100 100	2n=12, 14, 24
28.	*V. tenera* Grah.	Kuppa, 2600 Sangla, 2680 Sangla, 2680 Sangla, 2680 Sangla, 2680	31°25'44"N 78°14'40"E 31°25'36"N 78°15'51"E ,, ,, ,,	53710 50920 51950 51951 51952	7 7 7 7 7	2x 2x 2x 2x 2x	-	100 94 93 94 93	#

***** Code of Herbarium maintained by the Department of Botany, Punjabi University, Patiala, India as per "Index Herbariorum" by Holmgren and Holmgren (1998).

**Darlington and Wylie (1955), Fedorov (1969), Moore (1967-1971, 1968, 1969, 1970, 1972 1973-1974), Löve and Löve (1982a,b, 1986), Goldblatt (1975-1978, 1979-1981, 1982-1983, 1984-1985), Goldblatt and Johnson (1986-1987, 1988-1989, 1990-1991, 1992-1993, 1994-1995, 1996-1997, 1998-2000, 2001-2003), Kumar and Subramanian (1986), Khatoon and Ali (1993) and IAPT/IOPB Chromosome Number Reports published in Taxon and Index to Plant Chromosome Numbers (wide internet site: http://mobot.mobot.org/W3T/Search/ipcn.html and www.tropicos.org/Project/IPCN) and other journals.

[#] First ever chromosome count in the species.

cytotype of 2n=32 as recorded by Ashraf and Gohil, 1986 [1] from Kashmir Himalayas. For *Trigonella emodi* (2n=16, 2x) (Fig. 15), Sareen and Singh (1976) [11] earlier recorded the triploid cytotype with 2n=24 from Indian North West Himalayas. In *Vicia pallida*, a new tetraploid cytotype with a chromosome count of 2*n*=24 (Fig. 16) is added against the earlier diploid chromosome reports of 2*n*=14 (based on x=6) recorded from Kumaon Hills in Western Himalayas [6].

3.3 Intraspecific polyploid cytotypes

Presently, in *Indigofera heterantha* existence of intraspecific diploid (n=8; Fig. 17) and hexaploid (n=24; Fig. 18) cytotypes (based on x=8) are reported. The values for morphological characters, stomatal size and index were found to be more in the hexaploid individuals compared to the diploid individuals (see: Table 2).

Table 2: Comparison of macro- and microscopic morphological characters of the diploid and hexaploid cytotypes of *Indigofera heterantha* Wall. ex Brandis

↓	*Cytotypes*	
Characters	Diploid (n=8)	Hexaploid (n=24)
Plant height (cm)	30.12-40.68	58.88-99.24
No. of branches per plant	Less branched	More branched
No. of leaflets per leaf	7-13	19-23
Size of leaf (cm)	2.50-5.50	6.00-10.50
Size of leaflet (cm)	0.40-1.00 × 0.10-0.60	0.50-1.60 × 0.20-1.00
Colour of leaves	Light green	Dark green
No. of flowers per inflorescence	6-12	12-21
Colour of flowers	Dark purple	Purple
Stomatal size (μm)	22.62-24.50 × 21.86-23.37	26.39-27.14 × 25.63-26.39
Stomatal index	16.67	20.92

3.4 Meiosis, microsporogenesis, and pollen fertility

Meiotic course in majority of species which include chromosomal pairing, bivalents formation, segregation of chromosomes during A-I/T-I, A-II/T-II was observed to be perfectly regular resulting into normal sporads and high pollen fertility. However, 11 species viz., *Astragalus grahamianus*, *A. graveolens*, *Indigofera heterantha*, *Lotus corniculatus*, *Medicago falcata*, *Melilotus alba*, *Trifolium repens*, *Trigonella emodi*, *T. pubescens*, *Vicia pallida*, and *V. rigidula* depict the phenomenon of cytomixis involving chromatin transfer among neighboring PMCs (Figs.19-20). Consequent to chromatin transfer, the PMCs involved in cytomixis depict various meiotic irregularities which includes hypo-hyperploid PMCs (Fig. 21), enucleated PMCs (Fig. 22), chromatin stickiness (Fig. 23), interbivalents connections, out of plate bivalents and laggards (Fig. 24) due to spindle irregularities, sporads with micronuclei (Fig. 25) and polyads (Fig. 26). The products of such sporads yielded unstained/sterile and fertile pollen grains of variable sizes (Figs. 27-28). Such an impact of cytomixis in inducing meiotic irregularities and pollen malformation has also been detected earlier in *Vicia faba* [4], *Clematis graveolens* [12], *Dianthus angulatus* [5], and *Lindelofia longiflora* var. *falconeri* [8].

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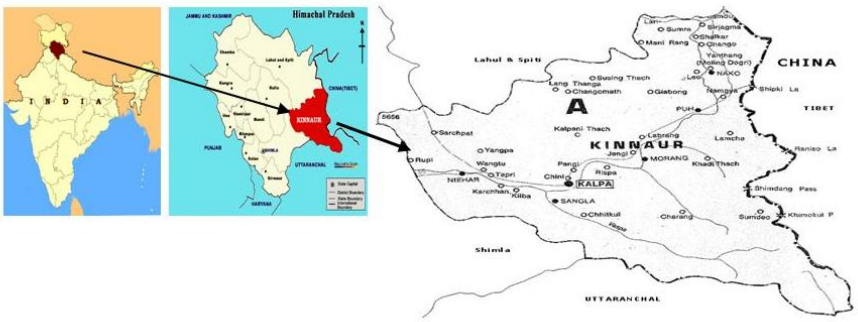
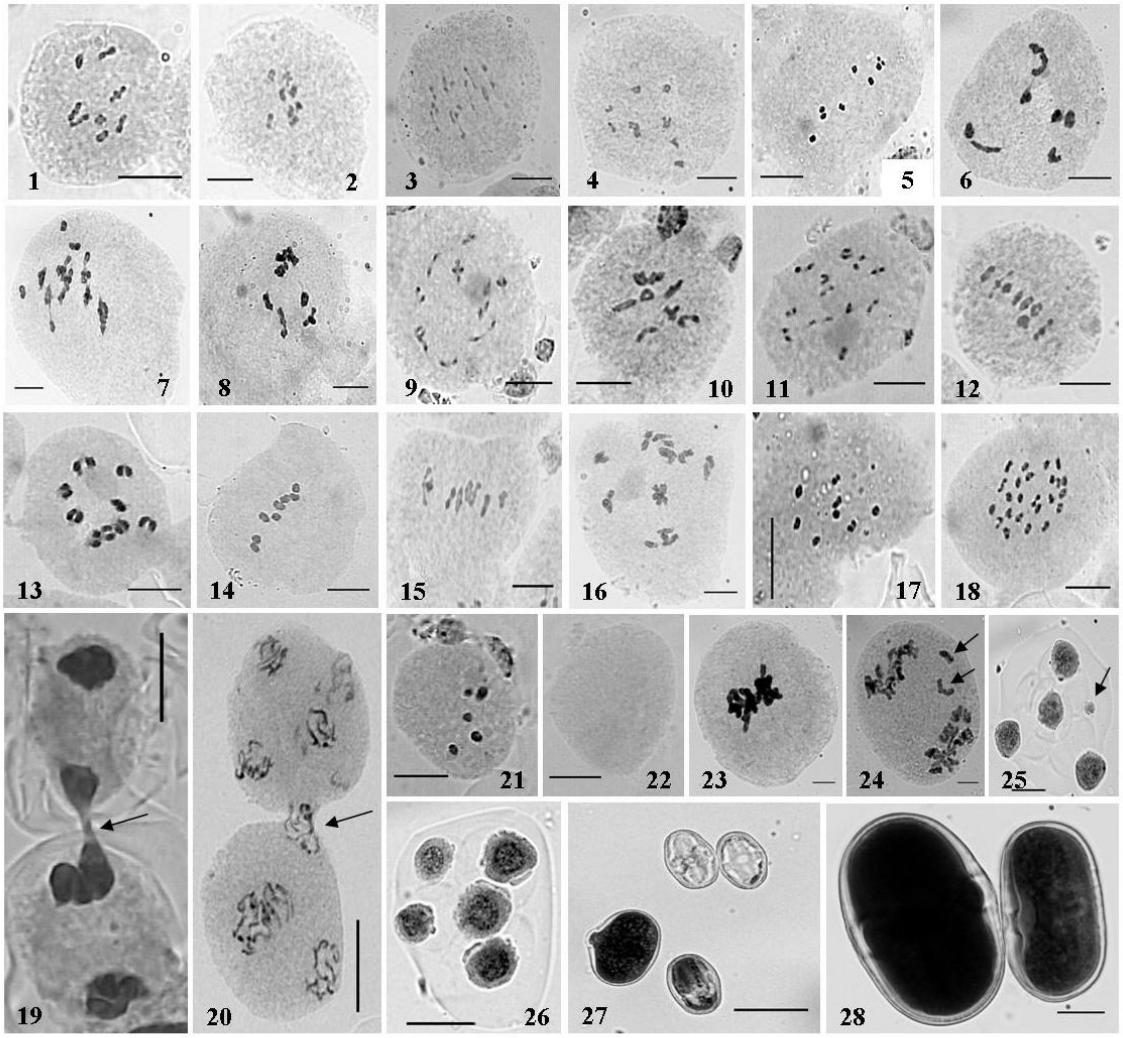


Map 1 : The map showing various localities of Kinnaur district of Himachal Pradesh in India.



Legends of figures

Fig. 1) *Astragalus malacophyllus*, A PMC with 8_{II} at M-I. 2) *A. strobiliferus*, A PMC with 8_{II} at M-I. 3) *Campylotropis eriocarpa*, A PMC with 11_{II} at M-I. 4) *Colutea nepalensis*, A PMC with 8_{II} at M-I. 5) *Indigofera himalayensis*, A PMC with 8_{II} at M-I. 6) *Trigonella pubescens*, A PMC with 8_{II} at diakinesis. 7) *Vicia rigidula*, A PMC with 12_{II} at M-I. 8) *V. tenera*, A PMC with 7_{II} at M-I. 9) *Desmodium elegans var. nutans*, A PMC with 11_{II} at diakinesis. 10) *Caragana gerardiana*, A PMC with 8_{II} at M-I. 11) *Desmodium racemosum*, A PMC with 11_{II} at diakinesis. 12) *Medicago falcata*, A PMC with 8_{II} at M-I. 13) *Piptanthus nepalensis*, A PMC with 9_{II} at diakinesis. 14) *Oxytropis mollis*, A PMC with 8_{II} at M-I. 15) *Trigonella emodi*, A PMC with 8_{II} at M-I. 16) *Vicia pallida*, A PMC with 12_{II} at diakinesis. 17) *Indigofera heterantha*, A PMC with 8_{II} at M-I. 18) *Indigofera heterantha*, A PMC with 24_{II} at M-I. 19) Two PMCs involved in chromatin transfer (arrowed) at A-I. 20) Two PMCs involved in chromatin transfer (arrowed) at A-II. 21) A hypoploid PMC. 22) A enucleated PMC. 23) A PMC at M-I showing chromatin stickiness. 24) A PMC at A-I showing laggards (arrowed). 25) A tetrad with one micronucleus (arrowed). 26) A polyad. 27) Sterile pollen grains. 28) Apparently fertile heterogeneous sized pollen grains.