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Cytological Studies in the Genus *Trigonella* Linn.

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Genus *Trigonella* L. belongs to tribe Trifolieae of family Fabaceae. It is represented by about 100 species (Willis 1966), which are mainly distributed in eastern part of Mediterranean region, spreading from north to central Europe and in North Africa and temperate Asia. Though this is an economically important genus, not much cytological studies have been undertaken so far. Only 29% of the species have been reported either for chromosome counts or for karyotype analysis (Chekhov 1932, Fryer 1930, Senn 1938, Singh and Roy 1970, Fernandes and Santos 1971, Singh and Singh 1976). The genus was also subjected for inducing mutations (Raghuvanshi and Singh 1974a, 1974b) and for making interspecific crosses (Singh 1973). In view of this an attempt has been made by us to study meiosis and mitosis, specially with a view to study intraspecific variability. The present paper deals with the results of meiosis and root tip mitosis (including karyotypes) involving seven species of *Trigonella* L.

Material and methods

Material was obtained in the form of seeds from various sources (Table 1). Plants were raised in pots at Meerut University Experimental Farm, Meerut. Dormancy of seeds was broken by rubbing them with glass fibre paper. Voucher specimens were prepared and deposited in the Department of Agricultural Botany, Meerut University, Meerut.

For meiosis, flower buds were collected in the forenoon, fixed for at least 24 hrs in Carnoy's fluid (absolute alcohol: chloroform: acetic acid, 6: 3: 1) and stored in 70% ethyl alcohol. The anthers were squashed in 2.0% acetocarmine.

For mitosis, young and healthy root tips were pretreated with saturated solution of α-bromonaphthalene for about one to two hours, fixed in acetic alcohol (1:3) for 24 hours and squashed in 2.0% acetocarmine.

Photomicrographs were taken from temporary preparations and measurements were made with the help of Olympus micrometer eye piece. TC1% and TF% were calculated as earlier done in *Crotalaria* (Gupta and Gupta 1978).

Results

1. *Meiosis*

Meiosis was studied in six species (Figs. 1–3). Chromosome associatoins and

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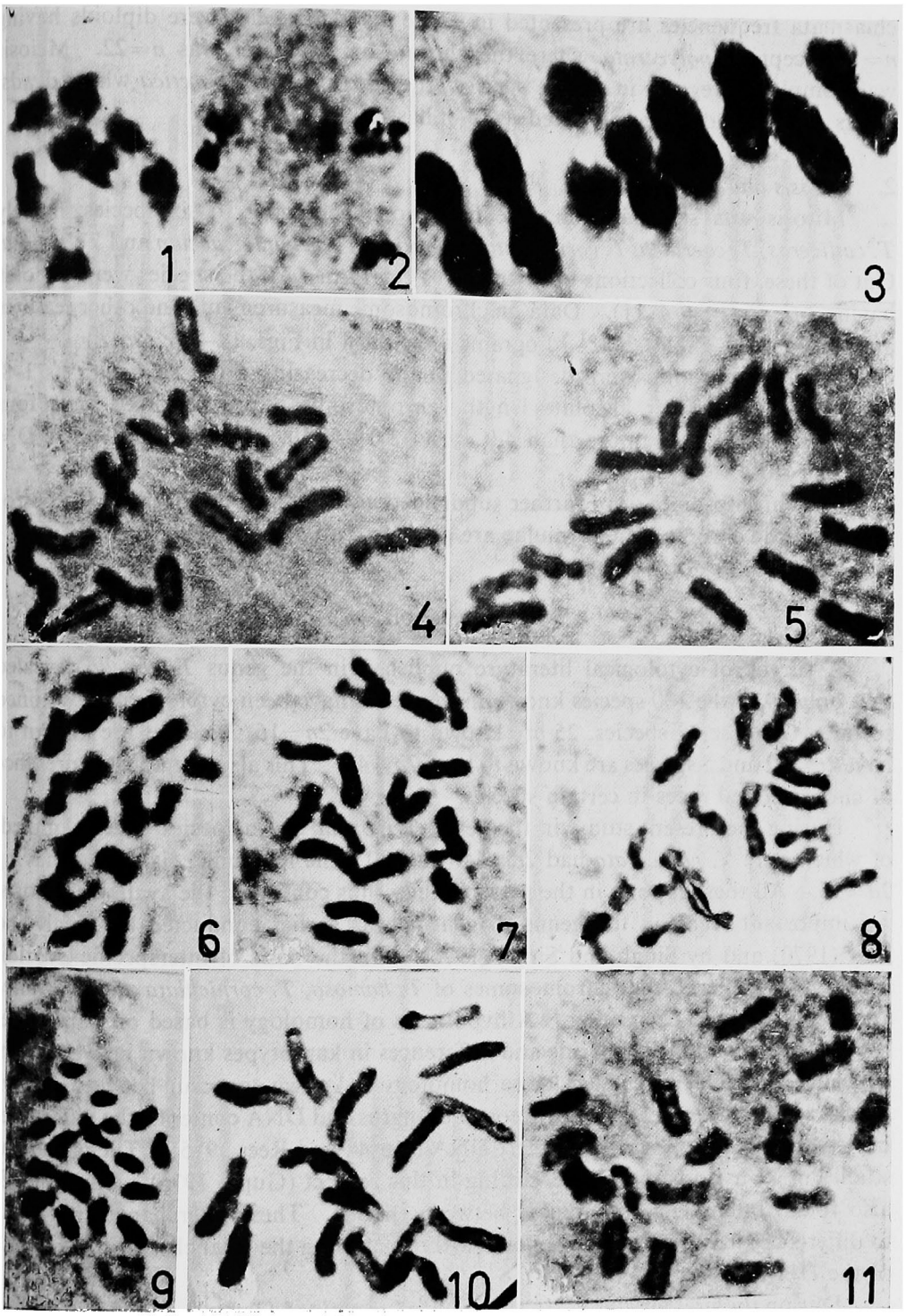
Table 1. Sources of material, gametic chromosome numbers and chiasmata frequencies in the genus *Trigonella*

Species	Coll. number	Gametic number (*n*)	Chiasmata		Sources	Accession number given by source
			Xta/PMC	Xta/bivalent		
T. calliceras Fisch	1760	8	15.10	1.90	Ottawa Research Station, Ontario	AL 263
T. coerulea L.	1769	8	15.67	1.96	RBG, Kew, England	91/06
T. coerulea L.	1762		—	—	Botanischer Garten Der, Leipzig	
T. coerulea L.	1763		—	—	Ottawa Research Station, Ontario	ML 3927
T. coerulea L.	1764		—	—	CSIRO, Canberra, Australia	CPI 135774
T. corniculata L.	1754	8	15.80	1.98	Local market, Meerut, India	
T. cretica L.	1818	8	15.50	1.93	RBG, Kew, England	121/05
T. foenum-graecum L	1753	8	14.19	1.77	RBG, Kew, England	
T. hamosa L.	1819		—	—	Ottawa Research Station, Ontario	1893348
T. polycerata L.	1820	22	—	—	Meerut University Campus, Meerut	



Figs. 1–11. 1–3. Meiosis in different *Trigonella* species. 1, *T. coerulea* (1761), metaphase I, 8^{II}. 2, *T. cretica*, telophase II. 3, *T. foenum-graecum*, metaphase I, 8^{II}. 4–11. Mitotic metaphase plates in different *Trigonella* species. 4, *T. calliceras*. 5, *T. coerulea* (1769). 6, *T. coerulea* (1762). 7, *T. coerulea* (1763). 8, *T. corniculata*, 9. *T. cretica*, 10. *T. foenum-graecum*, 11. *T. hamosa*.

chiasmata frequencies are presented in Table 1. All species were diploids having $n=8$, except *T. polycerata* where the chromosome number was $n=22$. Meiosis was completely regular in all the species studied except in *T. cretica* where dyads, triads and polyads were observed at telophase II (Fig. 2).

2. *Mitosis and karyotype*

Mitosis was studied from nine collections belonging to six species, namely *T. calliceras, T. coerulea, T. corniculata, T. cretica, T. foenum-graecum* and *T. hamosa.* Out of these, four collections belonged to *T. coerulea.* All the species were diploids having $2n=16$ (Figs. 4–11). Data on chromosome measurements and other features are presented in Tables 2–4. Idiograms are shown in Figs. 12–20.

The chromosomes were designated in the decreasing order of length as 1–8. Depending upon their absolute length, chromosomes were classified into four categories, namely A=more than 4.0 μ; B=3.0 μ–4.0 μ; C=2.0 μ–3.0 μ and D=less than 2.0 μ.

The chromosomes were further subdivided according to the position of centromere and the chromosome formulae are given in Table 4.

Discussion

A survey of cytological literature published in the genus *Trigonella* revealed that only 29 of the 100 species known in the genus have been cytologically examined so far. Of these 29 species, 25 are known to have $2n=16$, 3 species are known to have $2n=32$ and 5 species are known to have $2n=44$. This also suggests the presence of chromosomal races in certain species.

During the present study, in the genus *Trigonella* seven species were examined, of which only *T. polycerata* had $2n=44$ (Table 1), the remaining six species having $2n=16$. All these reports in the present study thus confirmed the available reports. An important study in the genus *Trigonella* was earlier conducted by Singh and Roy (1970) and by Singh and Singh (1976). On the basis of interspecific hybridization, they believed that chromosomes of *T. hamosa, T. corniculata* and *T. cretica* are homologous to each other. A hypothesis of homology is based on pairing behaviour at meiosis in F_1 hybrids and differences in karyotypes known between them will have no relevance. Such close homology is known to occur between *Lolium* species which differ greatly in chromosome lengths and DNA contents, the differences being attributed to repetitive or inert DNA (Gupta and Rees 1975). The pachytene studies of F_1 hybrids may be rewarding in this respect (Gupta 1976). Singh (1973) also found interchange differences between species. These interchanges may lead to differences in karyotype asymmetry without altering the total chromosome length or the DNA content.

During the present study, six species were examined (Table 2–4) for mitosis and all were found to be diploids having $2n=16$. The chromatin length among the

Figs. 12–20. Idiograms prepared from mitotic metaphase in different *Trigonella* species. 12, *T. calliceras.* 13, *T. coerulea* (1769). 14, *T. coerulea* (1762). 15, *T. coerulea* (1964). 16, *T. coerulea* (1763). 17, *T. corniculata.* 18, *T. cretica.* 19, *T. foenum-graecum.* 20, *T. hamosa.*

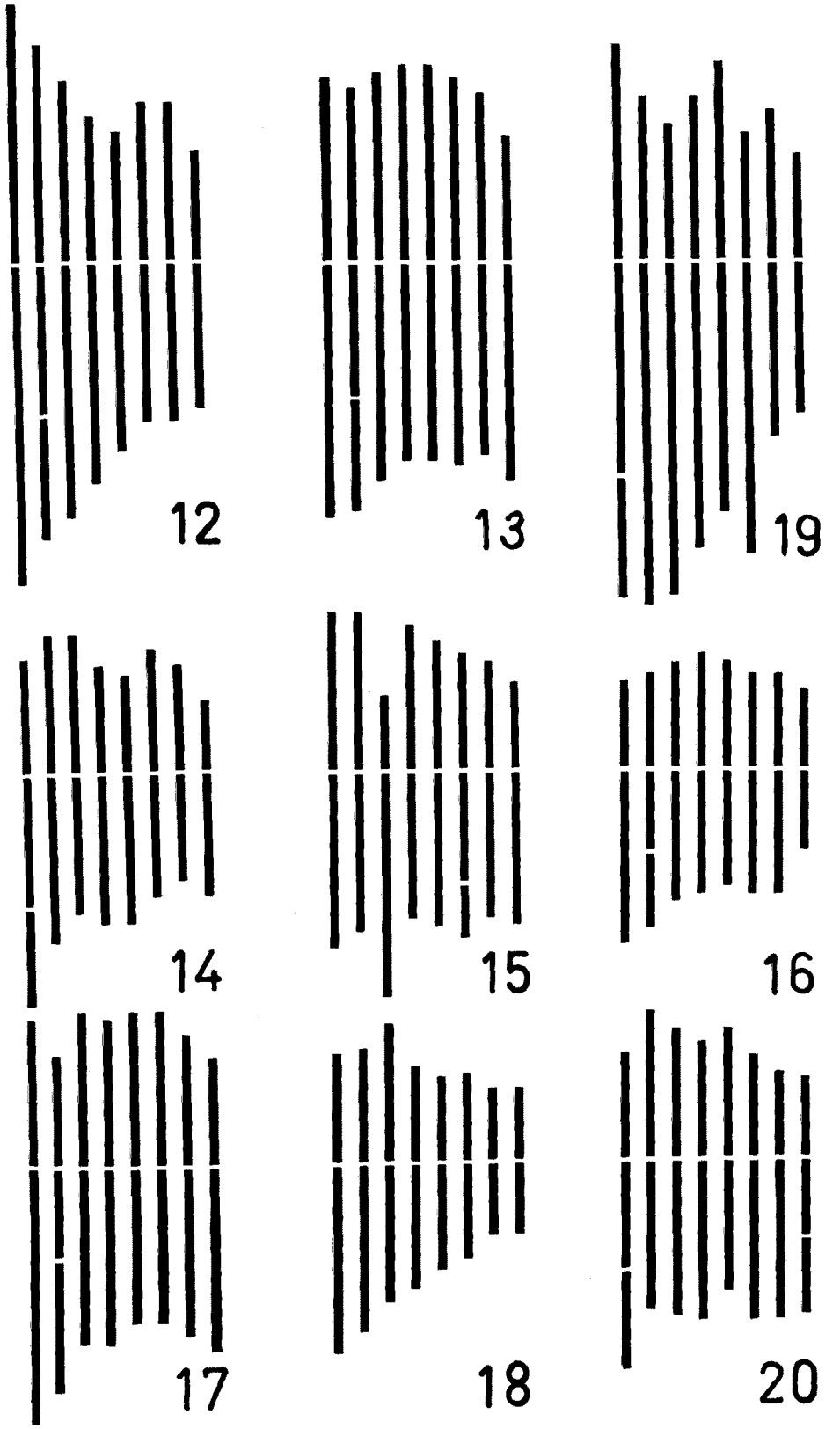

12
13
19
14
15
16
17
18
20

Table 2. Analysis of mitotic chromosomes in different species of *Trigonella* L. (In each species, the first row represents absolute length of chromosome in μ, the second row gives arm ratios; the third row gives relative chromosome length and the fourth row gives TCl%

Species and collections	Chromosome pairs							
	1	2	3	4	5	6	7	8
T. calliceras	5.52	4.62	4.14	3.45	3.04	3.04	3.04	2.42
($2n=16$)	1.22	1.23	1.40	1.50	1.44	1.00	1.00	1.33
	100.00	83.75	75.00	62.50	55.00	55.00	55.00	43.75
	18.87	15.80	14.15	11.79	10.38	10.38	10.38	8.26
T. coerulea	4.19	3.99	3.89	3.76	3.76	3.69	3.44	3.26
Coll. no. 1769	1.39	1.46	1.13	1.00	1.00	1.10	1.12	1.73
($2n=16$)	100.00	95.22	92.84	89.73	89.73	88.06	82.10	77.80
	13.97	13.30	12.97	12.54	12.54	12.30	11.47	10.87
T. coerulea	3.22	2.89	2.64	2.39	2.31	2.30	1.98	1.81
Coll. no. 1762	1.98	1.18	1.00	1.41	1.53	1.00	1.00	1.74
($2n=16$)	100.00	89.75	81.98	74.22	71.73	71.42	61.49	56.21
	16.47	14.79	13.51	12.23	11.82	11.77	10.13	9.26
T. coerulea	3.17	3.04	2.83	2.76	2.69	2.62	2.42	2.28
Coll. no. 1763	1.09	1.00	3.10	1.00	1.67	1.38	1.33	1.75
($2n=16$)	100.00	95.65	89.13	86.96	84.78	82.61	76.09	71.74
	14.56	13.92	12.98	12.66	12.34	12.03	11.08	10.44
T. coerulea	2.48	2.35	2.28	2.28	2.14	2.07	2.07	1.52
Coll. no. 1764	2.00	1.61	1.20	1.06	1.07	1.31	1.31	1.00
	100.00	94.44	91.67	91.67	86.11	83.33	83.33	61.11
	14.46	13.65	13.25	13.25	12.45	12.05	12.05	8.84
T. corniculata	3.80	3.11	3.11	3.04	2.90	2.90	2.83	2.76
($2n=16$)	1.75	2.00	1.14	1.20	1.00	1.00	1.28	1.67
	100.00	81.82	81.82	80.00	76.36	76.36	74.54	72.73
	15.54	12.71	12.71	12.43	11.86	11.86	11.58	11.30
T. cretica	2.83	2.62	2.62	2.07	1.73	1.73	1.38	1.38
($2n=16$)	1.73	1.45	1.00	1.31	1.26	1.08	1.00	1.00
	100.00	92.68	92.68	73.17	61.29	61.09	48.78	48.78
	17.23	15.97	15.97	12.60	9.05	10.50	8.40	8.84
T. foenum-graecum	5.24	4.90	4.55	4.35	4.35	4.07	3.11	2.48
($2n=16$)	1.53	2.09	2.47	1.74	1.25	2.28	1.14	1.40
	100.00	93.42	86.84	82.90	82.90	77.63	59.21	47.37
	15.87	14.82	13.78	13.15	13.15	12.32	9.40	7.52
T. hamosa	2.97	2.76	2.69	2.62	2.48	2.48	2.28	2.14
($2n=16$)	1.82	1.00	1.17	1.38	1.57	1.00	1.75	1.82
	100.00	93.02	90.70	88.37	83.72	83.72	76.74	72.09
	14.53	13.51	13.18	12.84	12.16	12.16	11.15	10.47

six species examined during the present study was found to be highest in *T. foenum-graecum* (33.05 μ) and lowest in *T. cretica* (16.36 μ). *T. foenum-graecum* also had relatively more asymmetric karyotype suggesting that it was an advanced species (Table 5), and that it has evolved due to increase in chromatin content and change in karyotype from symmetric to asymmetric. Such a conclusion receives support

from earlier study conducted by Singh and Singh (1976).

Another important feature of the present study is the intraspecific variability in *T. coerulea* for which four accessions ranged from 17.19 μ to 29.98 μ. It is remarkable that such a variability should be found within the same species. To

Table 3. Chromosome data in 9 collections of *Trigonella* L.

Species and collection number	Total chromatin length (μ)	Mean chromosome length (μ)	Longest/shortest ratio	TF%	Chromatin volume (μ^3)
T. calliceras	29.27	3.66	2.28	44.34	24.52
T. coerulea Coll. no. 1769	29.98	3.75	1.29	45.06	49.41
T. coerulea Coll. no. 1762	19.54	2.44	1.78	43.09	17.61
T. coerulea Coll. no. 1763	21.81	2.73	1.39	42.72	19.52
T. coerulea Coll. no. 1764	17.19	2.15	1.63	43.37	16.41
T. corniculata	24.45	3.06	1.38	42.65	24.77
T. cretica	16.36	2.04	2.05	44.54	10.32
T. foenum-graecum	33.05	4.13	2.11	36.95	56.98
T. hamosa	20.42	2.55	1.39	41.89	19.50

Table 4. Karyotypic formulae of 9 collections belonging to 6 species of *Trigonella* (A, B, C, and D represent length of the chromosome in decreasing order; sc used as subscript represents secondary constriction in long arm; superscripts 'm', 'sm', and 'st' represent respectively the median, submedian and subterminal position of centromeres

S. no.	Species and collection number	Karyotypic formulae
1	*T. calliceras*	$1scA^{sm}+2A^{sm}+2B^{m}+2B^{sm}+1C^{sm}$
2	*T. coerulea* Coll. no. 1769	$1A^{sm}+5B^{m}+1scB^{sm}+1B^{sm}$
3	*T. coerulea* Coll. no. 1762	$1scB^{sm}+3C^{m}+2C^{sm}+1D^{m}+1D^{sm}$
4	*T. coerulea* Coll. no. 1763	$2B^{m}+1C^{m}+1scC^{sm}+3C^{sm}+1C^{st}$
5	*T. coerulea* Coll. no. 1764	$3C^{m}+1sc^{sm}+3C^{sm}+1D^{m}$
6	*T. corniculata*	$2B^{m}+1scB^{sm}+1B^{sm}+2C^{m}+2C^{sm}$
7	*T. cretica*	$1C^{m}+3C^{sm}+3D^{m}+1D^{sm}$
8	*T. foenum-graecum*	$1scA^{sm}+5A^{sm}+1B^{m}+1C^{sm}$
9	*T. hamosa*	$3C^{m}+2scC^{sm}+3C^{sm}$

what extent this variability can be attributed to personal error in measurements will be difficult to evaluate, but in view of the same technique used, degree of chromosome condensation being same and the measurements taken with Olympus micrometer eye piece, the degree of error can not be of a high order. Therefore, the intraspecific variability in chromatin length is real rather than apparent and calls for attention

and explanation. It is not surprising, however, in view of the fact that a species is dynamic, always undergoing a process of change.

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Table 5. Placement of different *Trigonella* species in 12 categories proposed by Stebbins (1971), on the basis of karyotypes

Longest/Shortest	Proportion of chromosomes with arm ratio 2:1			
	0.0	0.01–0.50	0.51–0.99	1.00
<2:1	1A *T. coerulea* Coll. no. 1769 *T. coerulea* Coll. no. 1762 *T. hamosa*	2A *T. coerulea* Coll. no. 1763 *T. coerulea* Coll. no. 1764 *T. corniculata*	3A	4A
2:1–4:1	1B *T. calliceras* *T. cretica*	2B *T. foenum-graecum*	3B	4B
>4:1	1C	2C	3C	4C

Summary

1. In the genus *Trigonella*, male meiosis was studied in six species and mitosis with the help of root tip was studied in nine collections belonging to six species. All the species were diploid having $n=8$ and $2n=16$, except *T. polycerata* where the chromosome number was $n=22$.
2. Meiosis was normal in all the cases. Chiasmata frequency/bivalent varied from 1.77 (*T. foenum-graecum*) to 1.98 (*T. corniculata*).
3. Total chromatin length varied from 16.36 μ (*T. cretica*) to 33.05 μ (*T. foenum-graecum*).
4. The intraspecific variability in chromatin length was observed in four accessions of *T. coerulea*, suggesting that the species was dynamic and always undergoing a process of change.
5. Species were classified according to classification of Stebbins (1971). Only four of the 12 catagories were represented, indicating that the degree of asymmetry was low.

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