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Chromosome Differentiation in Diploid Species of *Lotus* (Leguminosae)

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Summary. Meiotic chromosome behavior was studied in seven diploid species of *Lotus* (*L. alpinus* Schleich., *L. japonicus* (Regel) Larsen, *L. filicaulis* Dur., *L. schoelleri* Schweinf., *L. krylovii* Schischk. and Serg., *L. tenuis* Waldst. et Kit., *L. corniculatus* var. *minor* Baker) and in 51 interspecific hybrids from 16 different crosses. Meiosis in the diploid species was quite regular. In a high proportion of the PMC's of the hybrids there was close chromosome homology with a normal association of 6 II's. However, meiotic irregularities including bridges, lagging chromosomes, univalents, and quadrivalents, occurred in a small percentage of the cells. The late separation of bivalents, the presence of quadrivalents, and inversion bridges with fragments, would indicate for some hybrids that certain chromosomes were structurally differentiated. The large number of rod bivalents observed at diakinesis was also highly suggestive that genetic nonhomology in one chromosome arm could contribute to the frequency of this type of bivalent. Therefore, the maximum number of 6 II's which occurred in a high percentage of cells may be misleading in that cryptic structural differences between chromosome arms, or segments, are not revealed. Pollen fertility in the species and hybrids was not correlated with meiotic irregularities suggesting that pollen fertility is genotypically controlled.

Introduction

Our knowledge of the cytogenetic affinities within the group of diploid species closely related to the cultivated tetraploid species, *Lotus corniculatus* L. ($2n = 4x = 24$), is still very limited. In order to utilize the potentialities of these species and their hybrids in the improvement of this forage legume which is now grown commercially in many countries throughout the world further data on their meiotic behavior and fertility are required. In an attempt to study the importance of interspecific hybridization on the evolutionary development of the genus and to assess the economic importance of these diploid species, a number of interspecific diploid hybrids have been produced (Grant *et al.*, 1962; de Nettancourt and Grant, 1963, 1964a). Limited cytological data on the behavior of the meiotic chromosomes in some of these hybrids indicated close homology as well as a small degree of structural differentiation between the chromosomes of the diploid species. This paper deals with 1) the meiotic chromosome behavior of seven diploid species, *L. alpinus* Schleich, *L. japonicus* (Regel) Larsen, *L. filicaulis* Dur., *L. schoelleri* Schweinf., *L. krylovii* Schischk. and Serg., *L. tenuis* Waldst. et Kit., and *L. corniculatus* var. *minor* Baker, 2) further data on the cytology of some of the hybrids previously investigated in this laboratory, namely, *L. japonicus* × *L. krylovii*, *L. japonicus* × *L. filicaulis*, *L. japonicus* × *L. schoelleri*, *L. japonicus* × *L. alpinus*, *L. alpinus* × *L. japonicus*, *L. krylovii* × *L. schoelleri*, *L. krylovii* × *L. filicaulis* and, 3) the cytology of nine new hybrids, *L. japonicus* × *L. corniculatus* var. *minor*, *L. corniculatus* var. *minor* × *L. alpinus*, *L. corniculatus* var. *minor* × *L. filicaulis*, *L. alpinus* × *L. schoelleri*, *L. alpinus* × *L. filicaulis*, *L. alpinus* × *L. krylovii*, *L. krylovii* × *L. corniculatus* var. *minor*, *L. krylovii* × *L. tenuis*, and *L. filicaulis* × *L. schoelleri*, which were obtained by interspecific hybridization under experimentally controlled conditions. The crossing behavior of the diploid species and a morphological description of the nine hybrids have been reported previously (Somaroo and Grant, 1971).

Material and Methods

A minimum of two plants for each species and two hybrids from each cross were used for meiotic studies, except for the cross *L. krylovii* × *L. tenuis* for which only one plant was available. Altogether 51 hybrids from the 16 different crosses were analyzed. Flower buds collected from plants growing in the field, were fixed in a 6:3:2 ratio of 100% methanol, chloroform, and propionic acid, for 6 to 24 hours. A modified Snow's (1963) methanolic hydrochloric acid-carmine solution was used for staining; the anthers were placed in the stain for a period of 2–4 weeks and then squashed in 45% acetic acid for an examination of the microsporocytes. In certain cases where poor staining was observed, heating the flower buds in the staining solution at 60 °C for 3–4 hours in a paraffin oven quite often made an improvement.

Chromosome behavior was studied in the pollen mother cells (PMC's) from diakinesis through telophase II (TII); in the first meiotic division (DI) only those cells were analyzed in which the pairing relationships of every chromosome was clear.

Estimates of pollen viability were obtained by scoring ten microscopic fields at a magnification of 400 × from each of two preparations per plant for ten plants for each diploid species and for each of the nine new crosses. The pollen was stained with aceto-carmine according to the method outlined by Marks (1954); stained pollen grains possessing a diameter in the size range of normal pollen of the species were scored as normal and tabulated as stainable pollen.

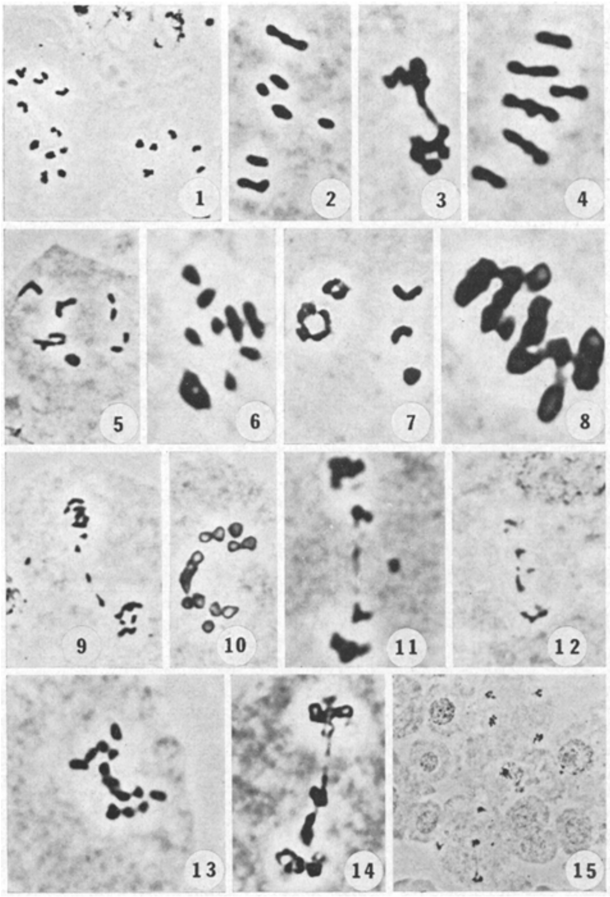
Results

Table 1. *Diakinesis and/or Metaphase I chromosome behavior in Lotus diploid species*

Species	Accession no.	Total no. of cells examined	Means and ranges per cell of			
			I's	%	II's	%
L. alpinus	B77	544	0.19 (0—2)	1.62	5.90 (5—6)	98.38
L. japonicus	B129	392	0.12 (0—2)	0.98	5.94 (5—6)	99.02
L. tenuis	B109	368	0.09 (0—4)	0.77	5.95 (4—6)	99.23
L. filicaulis	B37	530	0.22 (0—4)	1.86	5.89 (4—6)	98.14
L. schoelleri	B166	255	0.09 (0—4)	0.72	5.96 (4—6)	99.28
L. krylovii	B86	320	0.03 (0—2)	0.26	5.98 (5—6)	99.74
L. corniculatus var. *minor*	B303	139	0.20 (0—2)	1.68	5.90 (5—6)	98.32

Chromosome behavior at diakinesis and MI in the PMC's of the diploid species was characterized by a high degree of bivalent pairing (Fig. 1) with over 98% of the chromosomes associated as bivalents (Table 1). At MI the chromosomes were united by a terminal chiasma, or less often by a terminal chiasma at either end, which formed rod or ring shaped bivalents, respectively. Less frequently, cells with 5 II's + 2 I's were observed (Fig. 2). Anaphase I and TI and AII and TII appeared largely regular and only a few lagging chromosomes were observed. Since micronuclei were rarely seen in quartets, it would appear that lagging chromosomes reached the poles and were included in TII nuclei. Of 419 AI cells of *L. japonicus* examined, 10 cells had bridges, and for 214 AI cells of *L. krylovii*, bridges were observed in 3 cells (Fig. 3). No accompanying fragments were seen. In general, meiosis in the diploid species was quite regular (Table 2).

A high degree of bivalent pairing was exhibited in all of the hybrids (Table 3). The bivalents were generally rod shaped and the chiasmata were usually terminal (Fig. 4). Occasional ring-shaped bivalents were also detected. Loose bivalents in which thread-like connections joined some of the chromosomes (Fig. 5) were observed at both diakinesis and MI in all of the hybrids. Relatively few such bivalents were observed in hybrids derived from the crosses *L. alpinus* × *L. schoelleri* and *L. krylovii* × *L. corniculatus* var. *minor*; in plants from the cross *L. alpinus* × *L. krylovii*, loose bivalents were somewhat more frequent and these appeared to constitute between 5 and 8% of the total bivalents.



Figs. 1—15. Meiotic chromosomes of *Lotus* species and hybrids

Fig. 1. Prometaphase in *L. krylovii* showing three cells each with 6 II's. × ca. 760. Fig. 2. MI in *L. alpinus*, 5 II's + 2 I's. × ca. 2000. Fig. 3. *L. krylovii*, AI bridge. × ca. 2500. Figs. 4—5. *L. filicaulis* × *L. schoelleri*. Fig. 4. 6 II's, MI. × ca. 2500. Fig. 5. 4 II's + 2 loose II's, prometaphase. × ca. 1330. Fig. 6. MI in *L. japonicus* × *L. schoelleri*, 3 II's + 6 I's. × ca. 2500. Fig. 7. Prometaphase in *L. alpinus* × *L. schoelleri*, 4 II's + 1 IV. × ca. 1200. Fig. 8. MI in *L. japonicus* × *L. krylovii*, 2 I's + 3 II's + 1 IV. × ca. 3900.

Fig. 9. Early TI in *L. japonicus* × *L. schoelleri* showing lagging chromosomes. × ca. 1330.

Figs. 10—11. *L. japonicus* × *L. krylovii*

Fig. 10. Late separation of one bivalent at AI. × ca. 1750.

Fig. 11. TI inversion bridge and fragment. × ca. 2400

Fig. 12. TI inversion bridge and fragment plus lagging chromosome in *L. japonicus* × *L. alpinus*. × ca. 1330

Figs. 13—14. *L. corniculatus* var. *minor* × *L. alpinus*

Fig. 13. AI bridge, late separation of chromosomes. × ca. 2000. Fig. 14. AI bridge, failure of chiasma terminalization. × ca. 2100

Fig. 15. TII in *L. japonicus* × *L. corniculatus* var. *minor*, lower left, inversion bridge plus acentric fragment, upper right, normal telophase. × ca. 600

Table 2. *Meiotic abnormalities in Division I and Division II and pollen stainability in the diploid Lotus species*

Species	Division I		Division II		Pollen grains	
	Total no. of cells examined	Cells with abnormal division (%)	Total no. of cells examined	Cells with abnormal division (%)	Number examined	Percentage stainable pollen
L. alpinus	593	9.95	117	3.42	3142	67.19
L. japonicus	811	5.18	281	0.36	2663	96.43
L. tenuis	692	3.76	113	2.65	2337	82.41
L. filicaulis	884	9.16	148	2.70	2960	77.30
L. schoelleri	470	2.98	129	1.55	2145	74.41
L. krylovii	534	2.25	—	—	2470	87.04
L. corniculatus var. *minor*	373	5.90	101	4.76	2604	92.93

Table 3. *Diakinesis—Metaphase I chromosome behavior in the interspecific diploid hybrids*

Hybrid	No. of cells examined	Means and ranges per cell of							
		I's	%	II's	%	III's	%	IV's	%
L. japonicus × *krylovii*	177	0.54 (0—4)	4.52	5.50 (4—6)	91.71	—	—	0.11 (0—1)	3.77
L. japonicus × *filicaulis*	324	1.00 (0—4)	8.33	5.50 (4—6)	91.67	—	—	—	—
L. japonicus × *schoelleri*	418	0.74 (0—4)	6.14	5.63 (4—6)	93.86	—	—	—	—
L. japonicus × *corniculatus* var. *minor*	322	0.75 (40—4)	6.21	5.63 (4—6)	93.79	—	—	—	—
L. japonicus × *alpinus*	250	0.30 (0—4)	2.53	5.77 (4—6)	96.13	0.02 (0—1)	0.40	0.03 (0—1)	0.93
L. alpinus × *japonicus*	255	0.83 (0—4)	6.93	5.49 (3—6)	91.50	0.03 (0—1)	0.78	0.02 (0—1)	0.78
L. alpinus × *krylovii*	606	0.77 (0—4)	6.41	5.62 (4—6)	93.59	—	—	—	—
L. alpinus × *filicaulis*	570	0.95 (0—8)	7.92	5.51 (2—6)	91.81	0.01 (0—1)	0.26	—	—
L. alpinus × *schoelleri*	379	0.73 (0—6)	6.05	5.37 (2—6)	89.49	0.06 (0—1)	1.39	0.09 (0—1)	3.08
L. krylovii × *schoelleri*	221	0.94 (0—4)	7.84	5.53 (4—6)	92.16	—	—	—	—
L. krylovii × *filicaulis*	259	0.87 (0—4)	7.27	5.42 (3—6)	90.35	— (0—1)	—	0.07	2.45
L. krylovii × *corniculatus* var. *minor*	163	0.74 (0—6)	6.13	5.63 (3—6)	93.87	—	—	—	—
L. krylovii × *tenuis*	320	0.79 (0—6)	6.61	5.60 (3—6)	93.39	—	—	—	—
L. filicaulis × *schoelleri*	558	0.75 (0—4)	6.57	5.32 (4—6)	93.43	—	—	—	—
L. corniculatus var. *minor* × *alpinus*	486	0.73 (0—4)	6.10	5.53 (3—6)	92.18	—	—	0.05 (0—1)	1.71
L. corniculatus var. *minor* × *filicaulis*	444	0.89 (0—6)	7.43	5.55 (3—6)	92.57	—	—	—	—

In each hybrid, chromosome behavior in some cells was abnormal. As a rule, irregularities were caused by the smaller chromosomes which behaved as univalents at diakinesis and MI. Some appeared to be the result of precocious separation of bivalents (desynapsis), in which they lay opposite each other on either side of the equatorial plate. From 0—4 I's were recorded for many of the hybrids but as many as 6 or 8 I's occurred in some hybrids (Fig. 6). The frequency of univalents varied between the different crosses and from hybrid to hybrid.

Trivalents and quadrivalents were also observed in a number of hybrids (Table 3). Quadrivalents in the form of closed rings, or a chain of four chromosomes, were frequently seen at diakinesis and prometaphase (Fig. 7). The rare quadrivalents seen at MI consisted mainly of chains of four chromosomes or as N-shaped configurations (Fig. 8). Trivalents occurred

Table 4. *Meiotic chromosome behavior at AI—TI and AII—TII in the interspecific diploid hybrids and pollen grain stainability*

Cross	Anaphase I—Telophase I				Anaphase II—Telophase II				Pollen grains	
	No. of cells	Normal (%)	Bridges (%)	Laggards (%)	No. of cells	Normal (%)	Bridges (%)	Laggards (%)	No. examined	Stainable pollen (%)
L. japonicus × *L. krylovii*	308	88.64	3.25[a]	8.11	130	82.31	6.92[a]	10.77	—	—*
L. japonicus × *L. filicaulis*	358	87.15	—	12.85	153	88.24	—	11.76	—	—*
L. japonicus × *L. schoelleri*	629	91.73	0.64	7.63	424	88.44	—	11.56	—	—*
L. japonicus × *L. corniculatus* var. *minor*	553	87.89	3.25[b]	8.86	338	89.05	1.48[c]	9.47	2272	55.59
L. japonicus × *L. alpinus*	212	90.57	2.83[b]	6.60	249	91.40	3.58[d]	5.02	—	—*
L. alpinus × *L. japonicus*	346	91.04	0.29[c]	8.67	96	82.29	1.04[c]	16.67	2381	21.00
L. alpinus × *L. krylovii*	436	94.04	—	5.96	390	89.74	—	10.26	2103	86.11
L. alpinus × *L. filicaulis*	732	91.39	—	8.61	106	91.51	—	8.49	1612	74.25
L. alpinus × *L. schoelleri*	543	93.55	—	6.45	233	96.57	—	3.43	2046	22.83
L. krylovii × *L. schoelleri*	224	92.41	—	7.59	258	92.64	—	7.36	—	—**
L. krylovii × *L. filicaulis*	374	88.77	2.41	8.82	277	90.25	2.89	6.81	—	—*
L. krylovii × *L. corniculatus* var. *minor*	439	93.39	—	6.61	173	94.80	—	5.20	2382	53.02
L. krylovii × *L. tenuis*	267	91.01	0.37[c]	8.61	332	94.88	0.60[a]	4.52	1791	21.72
L. filicaulis × *L. schoelleri*	553	93.13	—	6.87	217	97.24	—	2.76	2549	16.63
L. corniculatus var. *minor* × *L. alpinus*	597	86.93	5.19[c]	7.87	362	88.95	4.70[a]	6.35	2625	50.29
L. corniculatus var. *minor* × *L. filicaulis*	602	90.86	—	9.14	267	86.89	—	13.11	1998	32.28

a — fragments also seen in 2 cells
b — fragments also seen in 4 cells
c — fragments also seen in 1 cell
d — fragments also seen in 3 cells
* — See Grant *et al.* (1962)
** — See de Nettancourt and Grant (1964a)

less frequently than quadrivalents and were generally present as a chain of three chromosomes mainly at MI. The decrease in the frequency of quadrivalents at MI, in comparison with early diakinesis, would indicate that some of the chromosomes had separated to form trivalents or bivalents as terminalization occurred. The percentage of chromosomes associated as multivalents in the hybrids *L. japonicus* × *L. krylovii*, *L. japonicus* × *L. alpinus*, *L. alpinus* × *L. japonicus*, *L. alpinus* × *L. filicaulis*, *L. alpinus* × *L. schoelleri*, *L. krylovii* × *L. filicaulis* and *L. corniculatus* var. *minor* × *L. alpinus* was 3.77, 1.33, 1.56, 0.26, 4.47, 2.45, and 1.71%, respectively. In the cross *L. alpinus* × *L. filicaulis* trivalents but no quadrivalents were observed, whereas the reverse relationship was observed in the cross *L. corniculatus* var. *minor* × *L. alpinus* (Table 3).

Chromosome behavior at post-MI stages appeared to be normal in most of the PMC's. At least 86% of the AI cells and 82% of the AII cells were devoid of bridges and lagging chromosomes (Table 4). Irregularities observed were lagging chromosomes (Fig. 9), asynchronous separation of chromosomes, bridges, and bridges with associated fragments. The number of lagging chromosomes at AI and AII varied from 0 to 3 for many of the hybrids. The laggards appeared to be some of the smaller chromosomes which had separated during late prophase I and which presumably had failed to move to the poles during AI. The different number of univalents observed at AI may be due to the fact that some lagging chromosomes divided whereas others remained undivided. In some meiocytes, medium to large sized chromosomes remained at the equatorial plate at AI (Fig. 10). In some cases, the TI chromosome complement consisted of more, or less, than the normal chromosome number as a result of unequal distribution of the chromosomes at AI.

Bridges, some with associated acentric fragments, were observed in cells at AI—TI and AII—TII in a number of hybrids (Table 4; Figs. 11—15). The frequency of these bridges was somewhat higher at AI; AII bridges were usually broken by the end of this stage. These bridges with fragments would indicate that certain hybrids were heterozygous for inversions. In some bivalents, the chromosomes had difficulty in separating and remained partially attached. As a result they divided late in AI. A configuration in which the chromosomes remained partially attached until late AI was quite different from that of the inversion bridges (Fig. 13). Also, a failure of chiasmata to terminalize contributed to the presence of bridge configurations. Quartets formed in a normal manner following meiosis and micronuclei were observed in less than 2% of the cells.

The percentage viable pollen based on staining reaction was low in many of the diploid species. Only two species, *L. japonicus* and *L. corniculatus* var. *minor* had over 90% stainable pollen (Table 2).

There was a considerable difference between the percentage stainable pollen of any given hybrid and its parental species, but between different hybrids with the same parentage the percentage did not vary markedly. Wide differences in pollen stainability were found between certain crosses which made it possible to group crosses with similar pollen viabilities (Table 4). For example, *L. alpinus* × *L. krylovii* and *L. alpinus* × *L. filicaulis* with 86.11% and 76.25% stainable pollen, respectively, could be grouped into one class, *L. corniculatus* var. *minor* × *L. alpinus* (50.29%), *L. krylovii* × *L. corniculatus* var. *minor* (53.02%) and *L. japonicus* × *L. corniculatus* var. *minor* (55.59%) would form a second group, and the remaining crosses in which stainable pollen ranged from 32.38% (*L. corniculatus* var. *minor* × *L. filicaulis*) to 16.63% (*L. filicaulis* × *L. schoelleri*) may be placed in a third group.

Discussion

Meiosis in the species and hybrids: As might be expected, univalents were rare in the parental species but were considerably more frequent in the interspecific hybrids. Some chromosomes which were lagging at AI were considered to be univalents that had failed to become oriented on the MI plate. Such univalents are believed to have originated from the precocious separation of the bivalents as had been postulated in another *Lotus* hybrid (Grant, 1963). The occurrence of a higher frequency of bivalents than univalents at both diakinesis and MI in the interspecific hybrids would suggest that there is considerable homology between the chromosomes of the species. At the same time, the large number of univalent chromosomes observed as early as diakinesis would suggest that precocious separation of chromosomes alone could not fully account for the occurrence of these univalents and that segmental and genetic differences are more likely to play an important role in their production. The loose bivalents observed in a number of cells at MI, are suggestive of some lack of specificity in pairing of the homologous chromosomes, which had undergone a certain amount of structural differentiation.

The preponderance of bivalents in the PMC's does not prove that the chromosomes are not structurally differentiated. Rather it would suggest that the structural differences may not be very extensive and are cryptic. It is known that cryptic structural differences may be so slight that pairing at meiosis is not affected (Stebbins, 1950). Furthermore, what might appear to be normal bivalent formation at diakinesis and MI could occur with a minimum number of chiasmata per bivalent. In the hybrids, a very high frequency of rod bivalents was apparent; since the presence of rods could imply that a chiasma was formed only in one arm of the chromosome, it is quite possible that genetic nonhomology could contribute to the lack of pairing in one arm, and hence, to the high frequency of this type of bivalent.

Quadrivalents of the closed ring and chain types were observed in a number of hybrids. Also trivalents, predominantly of the chain type, were detected, and many of these are considered to have resulted from the falling apart of one homologue in a quadrivalent association. The presence of quadrivalents is interpreted to mean that these morphologically closely allied species differ by segmental interchanges. One of the two parental species might be homozygous for a reciprocal translocation where quadrivalent formation was found in a hybrid. Multivalent configurations at MI in *Lotus* interspecific hybrids were also observed by Grant *et al.* (1962) and de Nettancourt and Grant (1963, 1964a). These authors also suggested that multivalent formation may result from translocations through which some of the chromosomes have differentiated from their homologous partners.

Anaphase bridges observed in the diploid species *L. japonicus* and *L. krylovii* are considered to be the result of delayed terminalization of chiasmata in these species. In the interspecific hybrids, bridges, with and without acentric fragments, were observed both at AI and TI, and AII and TII. Chromosome stickiness, failure of chiasmata to completely terminalize, and delayed separation of chromosomes, are possible reasons for the presence of bridges without accompanying fragments. The presence of dicentric chromatid bridges with acentric fragments, however, indicated that the particular hybrid was heterozygous for an inversion. Inversion heterozygosity in the hybrids would indicate that the homology within inverted segments of parental chromosomes was strong and that crossingover within these regions had taken place.

The occasional delayed separation of certain bivalents at AI in some hybrids may be due to difficulty in terminalization. Delayed separation of chromosomes in certain *Lotus* hybrids has been reported by Grant *et al.* (1962) and de Nettancourt and Grant (1963, 1964a).

Pollen fertility: Normal pollen production in the diploid species was not as regular as one would expect where normal bivalent formation and chromosome behavior was observed. Various processes such as hybridization among diploid taxa, or parthenogenesis in tetraploid populations, would account for pollen abortion in a species. Larsen (1954) has suggested that natural hybridization occurs between certain *Lotus* species and controlled interspecific hybridization experiments have indicated that hybridization is likely an evolutionary force within the genus (Somaroo and Grant, 1971). Therefore, pollen abortion in some of these diploid taxa may result from the incorporation of foreign germ plasm through a process of restricted hybridization that took place in their natural habitats.

The variation in the incidence of stainable pollen observed between the different crosses reflected the difference in the genetic constitution of the parental species. Meiotic irregularities recorded for the hybrids did not seem to be correlated with the frequency of nonstainable pollen. It is possible, therefore, that the parental species may differ in several genes which influence pollen fertility. Harland (1936) suggested that gene complexes play an important role in affecting pollen fertility and species differentiation in *Gossypium*. In *Lotus* such gene complexes could be the result of inversions. A more likely possibility is that these species differ by small structural changes which are not extensive enough to disturb pairing between partially homologous chromosomes in the hybrids but at the same time give rise to genetic imbalance which lowers the fertility of hybrids heterozygous for these cryptic structural differences. Also, cytoplasmic and environmental factors could be partly responsible for the low percentage of stainable pollen in the *Lotus* hybrids as was suggested by de Nettancourt and Grant (1964a).

Chromosome differentiation and its bearing on speciation in *Lotus*: Studies concerned with pollen fertility and the cytological relationships of chromosome aberrations found in the interspecific hybrids have furnished valuable information on mechanisms underlying species differentiation. In the interspecific hybrids, high pollen sterility was observed in the absence of extensive meiotic irregularities; therefore, one can infer from these observations that sterility is partly genic in nature. It is evident that structural differentiation of chromosomes both cryptic and patent, plays an important role in the speciation of these closely related taxa. However, gross repatterning of chromosomes as evidenced by the low frequencies of translocations and inversions, and by karyotypic studies (Zandstra and Grant, 1968) appears to be minimal. Therefore, the evidence suggests that cryptic structural differentiation has proceeded to a greater extent. This is based on the following observations:

1) Loose bivalents (partial desynapsis) and precocious separation of the chromosomes were detected in all the F_1 hybrids.

2) Reduced chiasmata formation in the hybrids was apparent by the high frequency of rod bivalents indicating that chiasma formation may have occurred only in one chromosome arm. In an interspecific *Gossypium* hybrid, Stephens (1950) suggested that the replacement of a ring bivalent by a rod might indicate that an entire arm had failed to pair because of reduced structural homology.

3) Delayed separation of bivalents occasionally occurred during AI in some hybrids. Such delayed chromosome separation has been considered to be the consequence of a change in homology (Lawrence, 1931).

4) The interspecific hybrids were highly pollen sterile. If the parental species differed by a number of cryptic structural differences then it should be expected that the random distribution of these complexes to the gametes in the hybrids would lead to unbalanced combinations. Thus, in light of the relatively poor pollen stainability in the hybrids, it is considered that cryptic structural differences between many of the species may be fairly extensive.

Also, it should be noted that distorted segregation ratios were consistently observed in diploid F_2 plants and in backcross progenies of *Lotus* by de Nettancourt and Grant (1964b) for such characters as HCN production, stem color, keel tip coloration, bud striping, and seed speckling. Stephens (1949) explained such distorted ratios in *Gossypium* in terms of selective elimination of genes from donor parents. He stated that selective elimination of genes required some form of balanced "polygenic complexes" which may be none other than structurally differentiated chromosomes.

Stebbins (1947) pointed out that structural differences between chromosomes could conceivably coexist with a high degree of regular meiotic pairing. He also indicated that such cryptic differences may be able to effectively prevent the free exchange of genes located within or very close to such regions and provide the means for the differentiation of taxa into new species. In *Lotus*, it seems probable that cryptic differentiation is acting in coordination with other factors to differentiate the species and to make them more distinct from one another. From a consideration of the factors underlying species differentiation in this genus, it is evident that gene substitution, and gross and cryptic structural changes of the chromosomes have played an important role in the evolution of the diploid species.

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