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The Origin and Evolution of Lotus as Revealed Through Ecology, Cytogenetics, Cyanogenesis, Herbivores, Phenols, Rhodanese, and Tannins

A report on a Symposium: **Biology and Microevolution of Lotus (Leguminosae)**. Organized by Krystyna M. Urbanska, Zurich, Switzerland, and held during the Third International Congress of Systematic and Evolutionary Biology, University of Sussex, Brighton, England, July 7, 1985.

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The genus Lotus contains a heterogeneous assemblage of annual and perennial species numbering close to 200. The species are distributed widely throughout the world. The taxa are extremely diverse in form and are adapted to a wide range of ecological habitats. These vary from salt-tolerant annual species that grow at sea level to ones adapted to xerophytic desert conditions. There are perennials with deep taproots found growing in arid desert habitats; also at progressively higher elevations until you reach species at very high elevations growing under alpine conditions (Grant; Can. J. Genet. Cytol. 7:457-471, 1965).

Lotus corniculatus, is widely distributed in Europe and was recognized by Linnaeus. In eastern North America, L. corniculatus, has emerged from an introduced weed in the 1920's to become a highly successful forage crop (Grant and Marten 1985). Two other species, L. tenuis and L. uliginosus, are also grown to some extent and more recently, some hybrids and allopolyploids of species found within the L. corniculatus group have received attention. Interest in the origin of the tetraploid L. corniculatus has generated considerable research since Dawson's classical paper in 1941. The Symposium has made a significant contribution to the resolution of this topic. Drs. Ross and Jones (M.D. Ross, Abteilung fur Forstgenetik und Forstpflanzenzuchtung der Universitat Gottingen F.R.G. and W. T. Jones, Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North, N.Z.) report on tannin production, self-incompatibility, phenolic content and Rhizobium specificity of species in the Lotus corniculatus group. Their study virtually excludes the possibility that L. corniculatus arose through autopolyploidy of L. tenuis (which agrees with the previous studies on phenolic content of the species and hybrids by Harney and Grant 1965 and Somaroo and Grant 1971, 1972) or L. alpinus. Ross and Jones suggest that L. corniculatus arose through hybridization of L. alpinus and/or L. tenuis (probably as a female parent) with L. uliginosus (probably as the male parent), followed by chromosome doubling in the hybrid.

Grant reported on some of the cytogenetic features that have been found in the genus Lotus. Chromosome numbers are known for 108 species out of the approximately 200 described for the genus. This represents an increase of 32 species since 1965 (A Chromosome Atlas and Interspecific Hybridization Index for the Genus Lotus, Can. J. Genet. Cytol.7: 457-471). Chromosome numbers are now known for 76 Eurasian species, 30 North American and 2 Australian; 71 are diploid, 12 have both diploid and tetraploid cytotypes, and 25 species are tetraploid. Thus since 1965, chromosome numbers for 32 new species have been reported. This represents a 29.6% increase in the number of species for which chromosome numbers have been determined since 1965. However, at this rate - one new species per year - it will require almost another century for the chromosome numbers to be determined for the remaining species of the genus. A basic chromosome number of 5 has also been discovered, making basic chromosome numbers of 5, 6 and 7 for the genus. Of the 12 taxa with both diploid and tetraploid cytotypes, 7 have cytotypes with haploid numbers of both 6 and 12, and 5 taxa have cytotypes with haploid chromosome numbers of both 7 and 14. No polyploid taxa have been reported to date in North America. The only two Australian species are both tetraploids with basic numbers of 7.

Grant has shown that interspecific hybridization has resulted in the phenomena of cytomixis (Nettancourt and Grant 1964) and also B chromosomes (Somaroo and Grant 1971, Small, Grant and Crompton 1984). Both phenomena have occurred in progeny when L. alpinus (2x) was one of the parents.

Karyotype analyses, cytophotometric and chiasma frequency measurements have been carried out on L. corniculatus and closely related diploid species. A recent study (Therrien and Grant, Cytologia 49: 27-32, 1984) on quadrivalent frequency in L. corniculatus indicated a lower quadrivalent frequency in this species than previously reported, suggesting some selection for increased diploidization. DNA values (obtained through cytophotometry) correlated well with total complement lengths (Cheng and Grant 1973). Grant suggested that classical karyotype analyses may not be a suitable method to investigate the parentage of L. corniculatus because of chromosomal repatterning which has occurred during the evolutionary development of the closely related diploid species.

Prof. D. A. Jones (S. G. Compton, S. G. Beesley and David A. Jones: Department of Plant Biology and Genetics, University of Hull, Hull HU6 7RX, England) reported on an experimental investigation of the responses of insects and molluscs to the polymorphic character "cyanogenesis" in leaves and flowers of Lotus corniculatus. Prof. Jones had previously shown that HCN appeared to be a defense mechanism in that molluscs feed preferably on acyanogenic plants and that there is a relationship between densities of molluscs and the distribution of the cyanogenic form. Insects (3 species of grasshoppers, earwigs, butterflies, broom moths, sawflies) and molluscs, were given a choice between feeding on cyanogenic and acyanogenic leaves or flowers. In general, the grasshoppers were reluctant to feed on the leaves of L. corniculatus but when starved showed only a slight preference for the acyanogenic form. In contrast earwigs readily ate L. corniculatus, but had a distinct preference for the acyanogenic form. In the case of flowers, the grasshoppers, as well as the earwigs, preferred the acyanogenic forms. The larvae of the 2 species of butterflies ate the L. corniculatus leaflets irrespective of HCN phenotype. Adults of Phyllopius roboretanus and P. viridiaeris both preferred acyanogenic leaves and flowers. Larvae of Hypera plantaginis showed no preference for the acyanogenic morph. In the case of some molluscs, conditioning the phenotype showed that after six days, the degree of aversion to cyanogenic petals was significant in those individuals maintained on acyanogenic leaves. The conclusion was that cyanogenesis indicates that a plant is potentially unpalatable to the general herbivore, and thus acts as a feeding inhibitor and not a toxin.

In a comparison of microhabitats investigated in a section of a chalk quarry where the vegetation ranged from pioneer species growing on almost bare chalk through to a mature grassland assemblage, Prof. Jones stated that leaf cyanogenic and dark keeled plants occurred at higher frequencies in grassland areas than in the more open conditions typical of earlier successional stages at the quarry. He concluded that variation in herbivore activity was unlikely to be a major factor influencing the pattern of distribution of cyanogenic individuals in the quarry.

D. Cartier and S. Blaise (Université Paris-Sud, Orsay, France) reported on geographical distribution, ecology and cyanogenesis polymorphism in L. corniculatus/L. alpinus from SW France. A study of 30 populations of L. corniculatus ($2n = 4x = 24$) / L. alpinus ($2n = 2x = 12$) proved the plants to be polymorphic for cyanogenesis. The highest frequencies of HCN^+ individuals were generally found upon carbonate, in open vegetation (in contrast to the previous reported study by Prof. Jones) and at high altitude (sharp separation at 1650 m). However, populations from various substrata in the upper valley of the Var consistently had far fewer cyanogenic plants than the colonies further to the South. The authors supposed that this distribution pattern might be influenced by the occurrence of ancestral populations of L. alpinus at Mt. Ventoux and Montagne de Lure.

In another study, Krystyna M. Urbanska (Dept. Geobotany, Swiss Federal Institute of Technology, 38 Zurichbergstrasse, 8044 Zurich, Switzerland) reported on the polymorphism of cyanogenesis in L. alpinus from the alpine vegetation belt in the Swiss Alps and showed cyanogenesis to be strongly influenced by the substratum type. Cyanogenic plants (AcLi) containing both cyanogenic glucosides and the corresponding beta-glucosidase occurred only in 7.9% upon acidic silicate, but represented 56% upon carbonate. Acyanogenic plants Acli (glucoside present but lacking enzyme) represented only 1.9% from silicate but corresponded to nearly half of the material from carbonate (48.1%); acLi individuals (lacking glucoside, enzyme present) occurred in 37.8% upon silicate, their frequency being much lower upon carbonate

(8.7%); plants with neither glucoside nor enzyme (acli) were well represented upon both substrata (silicate, 60.2%; carbonate, 43.2%). Thus populations growing in acidic silicaceous soils at high altitudes are essentially different from colonies occurring upon carbonate soil when they carry the dominant allele (Ac) for the locus controlling the biosynthesis of cyanoglucoside. Thus, not only cyanogenic individuals, but glucosidic plants have an advantage upon carbonate soil. It is conceivable that the production of cyanoglucosides represents an important element in the life strategy of alpine plants inhabiting carboniferous soils in extreme alpine ecosystems.

Rhodanese is a sulphurtransferase which catalyzes in vitro the formation of thiocyanate from cyanide and thiosulphate or some other sulphur donor. Far less is known about its occurrence and activity in plants than in mammals. One assumed function in vivo concerns cyanide detoxification. Its metabolic activity in leaf extracts of L. corniculatus was reported on by Jan Derk G. Smit and K. M. Urbanska (Dept. of Biochemistry and Dept. Geobotany, S.F.I.T., Zurich, Switzerland). To distinguish between the four cyanogenic types, viz. AcLi (cyanogenic plants with both cyanoglucosides and the appropriate beta-glucosidase), Acli (acyanogenic with cyanoglucosides but without enzyme, acLi (acyanogenic without cyanoglucosides but with enzyme) and acli (acyanogenic with neither glucosides nor enzyme), differential tests with sodium picrate paper were carried out on plants from various natural habitats in Switzerland. Enzymatic thiocyanate formation was easily detected from leaf extracts. The differences seemed to be correlated with the polymorphism of cyanogenesis: cyanogenic plants AcLi showed much higher rhodanese activity than the acyanogenic types Acli and acLi. The results suggest a possible mechanism of cyanide detoxification in cyanogenic plants.

Flavonoid compounds are genetically determined markers that have the potential to reflect a highly integrated response of the plant to its environment and may serve as biochemical indicators of a given ecological niche. There is a diversity of flavonoid patterns in L. corniculatus and J. Reynaud and M. Jay (Laboratoire de Phytochimie, Université Claude Bernard, Lyon I, France) presented a classification of some populations of L. corniculatus based on their flavonoid characters. Plant material was collected from 24 natural sites from two different geographic regions in France: the Massif Central (surrounding Saint-Etienne) and the southern Alps (Briançon). Polyamid thin-layer chromatography was used to study the diglycoside/monoglycoside (D/M) ratio, the result of genetically controlled glycosyltransferase activity. In the case of the Massif Central plants, there was an increase in the D/M ratio which was significantly correlated with altitude (2.3 to 14.8 between 700 to 1,350 m). In the populations in the Alps, a significant decrease of the D/M ratio corresponded with an increase in altitude. A thermal gradient is generally associated with altitudinal variation, but since the altitudes in the Massif Central are all relatively low they considered water supply as the factor influencing the ratio. They found Lotus plants at lower altitudes (700 to 1,000 m) had the lowest D/M ratios and were inhabited by hygrophilic associations of plants, whereas plants at higher altitudes (1,100 to 1,350 m) had high D/M ratios and corresponded to drier soils. In the

Alps, the metabolic balance was very different and temperature was considered to play the most important role in the wide altitudinal range (1,200 to 2,800 m). In the Alps, ancestral populations probably survived the last glaciations in distant refuges, but in the course of post-glacial extension at low and middle altitudes, the diglycosylation pathway was advantageous. In conclusion, 1) diglycosylation seemed to be favored in stable environments
(in both regions); low diglycosylation might characterize either unstable or stressed environments in both regions. 2) Assuming that the Alps was recolonized from refuge sites (such as Mont Ventoux), there were two evolutionary trends: in the most advantageous ecological situations, the colonization was by tetraploids apparently better competitors. Diploids occurred mostly in disturbed or stressed sites above 2,000-2,200 m. These plants in the Alps are still able to adapt as shown by their regulation of polyphenolic metabolism, different from the diploids from Mont Ventoux.