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The origin of *Lotus corniculatus*

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Summary. Earlier students of the origin of *Lotus corniculatus* suggested that this tetraploid species arose as an autotetraploid of the closely related diploid species *L. tenuis* or *L. alpinus*. More recent studies suggested that *L. alpinus* and *L. japonicus* could be ancestral forms. The present study of tannin content, phenolic content, cyanide production, morphology, cytogenetics, *Rhizobium* specificity and self-incompatibility in the *corniculatus* group virtually excludes the possibility that *L. corniculatus* could have arisen through autopolyploidy of *L. tenuis* or *L. alpinus*, and suggests that *L. corniculatus* arose through hybridization of *L. alpinus* and/or *L. tenuis* (probably as female parent) with *L. uliginosus* (probably as male parent), followed by chromosome doubling in the hybrid.

Key words: *Lotus corniculatus* – *L. uliginosus* – *L. tenuis* – *L. alpinus* – Evolution – Hybridization – Allopolyploid

Introduction

The origin of the tetraploid species *Lotus corniculatus* has long interested students of plant evolution.

Dawson (1941) found tetrasomic inheritance of cyanide production in *L. corniculatus*, suggesting that the two parental genomes were closely related. On predominantly morphological grounds Dawson concluded that *L. corniculatus* had probably originated as an autotetraploid of *L. tenuis* or its prototype. A similar conclusion was reached by Wernsman et al. (1964), based on chromosome studies. However, an artificial autotetraploid of *L. tenuis* was found to differ from *L. corniculatus*, and did not cross with it (Tomé and Johnson 1945). This cross has since been obtained (e.g. De Latour et al. 1978), but the two species can hardly be regarded as interfertile. True autotetraploid species are held to be very rare, and many proposed autotetraploids (including *L. corniculatus*) are considered to be of interspecific hybrid origin (Stebbins 1971). Larsen (1954) held that *L. alpinus* and *L. corniculatus* resembled each other more closely than any other species of the *corniculatus* group, suggesting that *L. corniculatus* may have arisen from autopolyploid *L. alpinus*. However, since that time many other diploids closely related to *L. corniculatus* have been studied (Harney and Grant 1964 b). Jaronowski and Wojciechowska (1963) held on cytological grounds that *L. tenuis* was probably not involved in the origin of *L. corniculatus*. Somaroo and Grant (1971 a) obtained artificial tetraploids from interspecific hybrids between diploid members of the *corniculatus* group, and crossed 12 such hybrids with *L. corniculatus*. The highest percentage of successful crosses (85% of pollinations successful) was obtained with tetraploid *L. japonicus* × *L. alpinus*, so that on these and on morphological grounds the authors concluded that these two species could be involved in the origin of *L. corniculatus*. This conclusion was supported also by studies of meiosis (Somaroo and Grant 1971 b).

Despite the many studies that have been made, there appears to be no generally accepted hypothesis for the origin of *L. corniculatus*. The purpose of this paper is to give a hypothesis based upon evidence from as many origins as possible, but where the biochemical and physiological work is emphasized, as this seems to give the most critical information.

Tannins, phenolics, self-incompatibility and *Rhizobium* specificity

These physiological and biochemical characters seem to be particularly useful in eliminating potential parent species of *L. corniculatus*. Before going into details, however, it is desirable to consider an assumption made here, namely that both parent species of *L. corniculatus* are members of the *corniculatus* group, a group of more or less sibling species closely related to *L. corniculatus*. For the maternal parent this assumption can readily be defended because *Rhizobium*/plant interactions may be highly specific and are apparently maternally inherited (Pankhurst and Jones 1979, see below), but for the paternal parent the situation is less clear, since other studies e.g. in wheat have shown that markedly distinct species can contribute to an allopolyploid (Stebbins 1971). Thus the possibility that a very distinct species, not a member of the *corniculatus* group, may have contributed to *L. corniculatus* cannot be entirely ruled out.

Lotus corniculatus is well known to be a very variable species, e.g. morphologically (Ball 1968), in degree of self-incompatibility (Bubar 1958), and in its polymorphism for HCN and leaf tannins (Ross and Jones 1983). Such variability is in accord with the presumed hybrid ancestry of this species. Table 1 shows that, on the available evidence, only *L. uliginosus* could have contributed leaf tannins to *L. corniculatus.* The tannin polymorphism in *L. corniculatus* suggests that the second parent species is tannin negative. The third column of the Table suggests that *L. tenuis* or *L. alpinus* contributed the incompatibility system, and the fourth column favours *L. alpinus* over *L. tenuis* as the second parent species, since *L. tenuis* could not have contributed the leaf phenolic U1, for which *L. corniculatus* is polymorphic. However, only one *tenuis* strain was tested, and an unknown *tenuis*-like diploid had this phenolic (Harney and Grant 1964b), so that further studies could well show that it is present in *L. tenuis* also. Twelve strains of *L. corniculatus* had the unknown phenolic, and four did not (Harney and Grant 1964b).

The last column in Table 1 gives the interactions of two *Rhizobium* strains with species of the *corniculatus* group. The interactions with *Rhizobium* strain 2 allow the tested species of the *corniculatus* group to be classified into two subgroups, namely *L. uliginosus,* which forms root nodules ineffective in fixing nitrogen, and the others (including *L. corniculatus, L. tenuis* and *L. japonicus*), which form effective nodules. *Rhizobium* strain 1 confirms this classification, but the results for effectiveness are reversed. The hybrid *L. tenuis* (female) ×*L. uliginosus* (male) gives the same result as *L. corniculatus* and *L. tenuis,* whereas the reciprocal hybrid gives an intermediate reaction, but nearer to that shown by *L. uliginosus.* Clearly there is a strong tendency to maternal inheritance of *Rhizobium* specificity, and any cross between *L. uliginosus* and *L. tenuis* which may have given rise to *L. corniculatus* has presumably occurred with *L. tenuis* as female parent. Similarly, if *L. apinus* and *L. uliginosus* were involved in the original cross, it seems more probable that *L. alpinus* should have been the female parent. This is despite the fact that artificial crosses between self-incompatible and self-compatible species are usually much more successful when the self-compatible species is the female parent (Lewis and Crowe 1958). For example De Latour et al. (1978) obtained the cross *L. uliginosus*×*L. tenuis* using embryo transplants, but were unable to obtain the reciprocal. Since then, however, R. M. Greenwood (personal communication) has obtained the reciprocal cross using semi-natural methods involving bee pollination, so that it can no longer be regarded as unlikely that a self-incompatible species should have been the original female parent. This conclusion is supported by the results for flower colour (see "Supporting evidence").

Supporting evidence

In addition to the above, there is some biochemical and morphological evidence which supports the conclusions made in the previous section. The results for HCN (Table 2, column 2) are consistent with the proposed hypothesis that *L. uliginosus* and *L. tenuis* or *L. alpinus* could have given rise to *L. corniculatus,* but like many other results, do not yield decisive evidence. Among the results for phenolics (columns 3–7), those for delphinidin exactly parallel those to tannins, whereas the others can be taken as representing the large body of undecisive evidence in the literature. Taken together, however, the results for these other phenolics do tend to favour *L. alpinus* over *L. tenuis.* The apparently essential role played by *L. uliginosus* in the origin of *L. corniculatus* is confirmed by the results for chromatogram band 14. The results for flower shade in the hybrids of *L. uliginosus* and *L. tenuis* suggest maternal interhitance, and confirm the suggestion based on *Rhizobium* work that *L. tenuis* could have been the female parent in any cross with *L. uliginosus* which could have given rise to *L. corniculatus.* The flower-colour results of Grant et al. (1962) were made independently of the others in the table, and unfortunately do not include a comparison with *L. corniculatus* and *L. tenuis.* The last column in the table gives the ratio of leflet breadth to length, and thus reflects the leaflet shape, which is one of the important taxonomic characters used for this group. *L. uliginosus* had broad leaves, *L. tenuis* narrow leaves, and *L. corniculatus* is intermediate but variable in this respect. This is also seen in the table, where *L. tenuis* has the lower ratios of around 0.35, *L. uliginosus* 0.61, and their hybrids are intermediate, and similar to the ratio of 0.43 found for *L. corniculatus* (De Latour et al. 1978). For diploids, Bent (1962) obtained breadth/length ratios of 0.16 for *L. tenuis,* 0.42 for *L. uliginosus,* 0.26 for the hybrid, and 0.28 for *L. corniculatus.* Other characters used for distinguishing among these three species are the presence of stolons, and whether the stems are hollow or solid. The two *L. uliginosus* parents studied by De Latour et al. (1978) had many stolons and hollow stems, the single *L. tenuis* parent, like

Table 1. Tannin production, self-incompatibility, phenolic content and *Rhizobium* specificity in the *corniculatus* group

Species or hybrid	Leaf tannin	Self-incompatibility	Leaf phenolic U1[a]	*Rhizobium* strain: 1, 2
corn	+–±	+[b]	+–	i 5
ten (2×)	–	+	–	1 5
ten (4×)[c]	–	+		
ulig (2×)	+	–	–	5 1
ulig (4×)[c]	+	–		
ulig (4×)[c] × *ten* (4×)[c]	+			E 3
ten (4×)[c] × *ulig* (4×)[c]				i 5
alp (2×)	–	+[d]	+	
alp (4×)		+		
jap (2×)	–	–[e]	+	5
(*jap* × *alp*) (4×)[c]		–	+	
filic (2×)	–	–	+	5
schoell (2×)	–	–	+	5
kryl (2×)	–	–	+	5
borb (2×)			–	
unknown (2×), *tenius*-like			+	

corn = *L. corniculatus, ten* = *L. tenius, ulig* = *L.uliginosus, alp* = *L. alpinus, jap* = *L. japonicus, filic* = *L. filicaulis, schoell* = *L. schoelleri, kryl* = *L. krylovii* and *borb* = *L. borbasii.* The name *L. uliginosus* Schkuhr is used here for plants previously referred to under the name *L. pedunculatus* Cav

\+ Means that all plants of a sample had the character in question, – means that no plant had that character, and ± means that a sample was polymorphic for that character. For *Rhizobium* specificity, the effectiveness of the *Rhizobium* strains CC814s (strain 1) and NZP2213 (strain 2) in fixing nitrogen was measured on a scale ranging from 1 (ineffective) to 5 (highly effective) (Pankhurst and Jones 1979). In other tests with these *Rhizobium* strains, interactions were simply classed as ineffective (given as i) or effective (given as E) (W. T. Jones, unpublished)

The Table is based on the following publications: De Latour et al. (1978); Bent (1962); Somaroo and Grant (1971a); Bubar (1958); Zandstra and Grant (1968); Harney and Grant (1964a, b); Pankhurst and Jones (1979); Grant et al. (1962); Urbanska-Worytkiewicz and Wildi (1975); Urbanska-Worytkiewicz et al. (1979) and Ross and Jones (1974)

[a] U1 represents an unknown leaf phenolic (Harney and Grant 1964b)

[b] Variable degrees of self-incompatibility were found (Bubar 1958; Bent 1962; Zandstra and Grant 1968; De Latour et al. 1978)

[c] Artificial tetraploid

[d] This species was first reported as self-sterile (de Nettancourt and Grant 1964), and later as self-compatible (Somaroo and Grant 1971a). The latter report is apparently incorrect, and the species is self-incompatible (W. F. Grant, personal communication; Urbanska-Worytkiewicz et al. 1979)

[e] Previously reported as self-sterile (de Nettancourt and Grant 1964), but is self-compatible (Somaroo and Grant 1971a; W. F. Grant, personal communication)

L. corniculatus, had no stolons and solid stems, and the hybrids had no (2 cases) or few (1 case) stolons and solid stems. They strongly resembled *L. corniculatus.* Again these results support the possible origin of *L. corniculatus* from a hybrid between *L. uliginosus* and *L. tenuis.* Some of the most important evidence for and against the involvement of the three most likely species in the ancestry of *L. corniculatus* is given in Table 3.

Discussion

It is generally accepted that *L. tenuis* is morphologically very close, and *L. uliginosus* less close to *L. corniculatus.* Thus of the nine morphological characters used by Dawson (1941) for differentiating among these three species, seven characters were similar in *L. corniculatus* and *L. tenuis,* but in the remaining two characters *L. corniculatus* differed from *L. tenuis* in the direction of *L. uliginosus.* Thus a possible influence of *L. uliginosus* is detectable at the morphological level. For phenolics, however, *L. uliginosus* is close to *L. corniculatus,* whereas *L. tenuis* is very distinct (Harney and Grant 1964b).

It is of interest to consider which characters could have contributed to the evolutionary success of *L. corniculatus.* This species is a generalist, since it is both drought resistant (Charlton et al. 1978) and able to grow in wet places (Zandstra and Grant 1968). *L. alpinus* is of course a specialist, *L. tenuis* is variously described as growing in dry places (Clapham et al. 1952) or on poorly-drained heavy or saline soils (Zandstra and Grant 1968), and *L. uliginosus* lacks drought resistance (Charl-

Table 2. Additional biochemical characters, and morphology in the *corniculatus* group

Species or hybrid	Additional biochemical characters							Flower shade (yellow)	Leaflet breadth/ length
	HCN	D	Q	C	pC	S	14		
corn	+–±	+	+	+	+	+–	+	light	0.43
cauc[a]	–	+	+	+	–	+	+		
ten (2×)	+±	–	–	–	–	–	–	light	0.36
ten (4×)[b]	+							light	0.34
ulig (2×)	–	+	+	+	+	+–	+	deep	
ulig (4×)[b]	–	+	–	+	+	+	–	deep	0.61
ulig (4×)[b]× *ten* (4×)[b]	+							deep	0.37–0.48
ten (4×)[b]× *ulig* (4×)[b]								light[c]	
F_2 of above cross[b]								light[c]	
alp (2×)	+–±	–	+	+	+	+	–	deep[d]	0.43
jap (2×)	+	–	+	+	+	+	–	deep[d]	0.57
jap × *alp*			+	+	+	+	–	deep[d]	0.46
filic (2×)	±	–	+	+	+	–	–	light[d]	0.33
schoell (2×)	±–	–	+	+	+	+	–	light[d]	0.52
kryl (2×)	+–±	–	+	+	+	+	–	pale[d]	0.33
borb (2×)	–	–	–	–	–	–	–		
unknown (2×), *tenuis*-like		–	–	–	–	–	–		

cauc = *L. caucasicus.* Other species abbreviations and the meanings of the signs +, –, and ± are the same as in Table 1

D = delphinidin, Q = quercetin, C = cyanidin, pC = p-Coumaric acid, S = sinapic acid, and 14 stands for a band which appeared on developed chromatograms of leaf extracts (Harney and Grant 1964a)

The table is based on the following publications: Grant et al. (1962); Somaroo and Grant (1971a); Harney and Grant (1964a, b, 1965); Grant and Sidhu (1967); De Latour et al. (1978); Urbanska-Worytkiewicz and Wildi (1975) and Phillips (1968)

[a] Tetraploid, regarded in Flora Europaea as conspecific with *L. corniculatus*

[b] Artifical tetraploid

[c] W. T. Jones (unpublished)

[d] Grant et al. (1962)

Table 3. Summary of evidence for and against the participation of the three proposed species in the ancestry of *Lotus corniculatus.* The evidence refers only to species in the *corniculatus* group

Species	Evidence
L. uliginosus	For: only known possible supplier of leaf tannins, delphinidin, or chromatographic band 14. Several of the characters which distinguish it from *L. corniculatus* are recessive in *tenuis* × *uliginosus* hybrids, which strongly resemble *L. corniculatus.* Suitable leaf shape Against: no evidence
L. alpinus	For: one of two possible suppliers of self incompatibility. Has leaf phenolic U1 Against: Leaf shape possibly unsuitable
L. tenuis	For: one of two possible suppliers of self incompatibility. Leaf shape suitable Against: does not have leaf phenolic U1 (but only one strain tested)

ton et al. 1978), and grows best on continuously moist soils (Zandstra and Grant 1968). Presumably the large leaflets of *L. uliginosus* are associated with its lack of drought resistance. During selection for drought resistance in a hybrid between *L. tenuis* or *L. alpinus* and *L. uliginosus* one would expect selection for narrower, smaller leaflets, and such selection could account for the close morphological resemblance between *L. tenuis* and *L. corniculatus. L. alpinus* leaflets are short, not much longer than they are broad (Urbanska-Worytkiewicz and Wildi 1975). In addition to its advantage as a generalist, *L. corniculatus* may owe its success to its having inherited two defence mechanisms against herbivores, namely cyanide from *L. tenuis* or *L. alpinus* (Jones 1962) and tannin from *L. uliginosus* (Feeney 1968). Tannin is also effective against fungi (Bell et al. 1965). A related question we may ask is why should *L. corniculatus* apparently have arisen from the cross *L. tenuis* or *L. alpinus* as female by *L. uliginosus* as male, rather than from the reciprocal cross? Presumably such crosses occurred in both directions, and were probably more often successful when *L. uliginosus* was the female parent. It seems that the cross with *L. uliginosus* as male parent had a selective advantage, and one possible advantage could lie in the *Rhizobium*-plant interaction.

The hypothesis that *L. alpinus* or *L. tenuis* and *L. uliginosus* contributed to the origin of *L. corniculatus* does not exclude the possibility that yet other species may have contributed. *L. corniculatus* may even have had a multiple origin involving different species in different regions, as in *Biscutella laevigata* (Manton, cited by Stebbins 1950). This is all the more possible because the genus *Lotus* is still not well understood, and apparently contains at least one unknown tannin-containing diploid (Forde and De Latour 1978).

Stebbins (1971) presented a scheme for identifying the parent species of a polyploid. Ideally, the diploid relatives should be hybridized with each other, and with the polyploid. Although many hybrids have been obtained between relatives of *L. corniculatus,* the crucial ones with respect to tannins and self incompatibility have apparently not yet been crossed with

L. corniculatus itself. Additional biochemical data are also desirable. Nevertheless, the data presented here do provide a strong indication of the origin of *L. corniculatus.*

Conclusions

It is proposed that *L. corniculatus* arose from the cross *L. tenuis* and/or *L. alpinus* as female with *L. uliginosus* as male. The hypotheses that *L. corniculatus* is an autopolyploid of *L. tenuis* (Dawson 1941) or *L. alpinus* (Larsen 1954) can be rejected because *L. corniculatus* has leaf tannins, whereas *L. tenuis* and *L. alpinus* apparently do not. The possibility that *L. japonicus* and *L. alpinus* could have been involved (Somaroo and Grant 1972) cannot be rejected, but it is unlikely that these two species alone could have given rise to *L. corniculatus,* since neither species has leaf tannins.

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