Biochemical Anomaly in Flower Extracts of Interspecific Hybrids between Lotus Species

Abstract. *Chromatographic analyses of unhydrolyzed flower extracts of two* Lotus *hybrids and of two individuals from an F_2 population have revealed the presence of a substance which was not present in the extracts of the parental species. This hybrid substance has not been identified, but its occurrence might be explained as the result of gene interaction in heterozygous individuals.*

In a chromatographic study of the incidence and inheritance of phenolic substances in taxa of the genus *Lotus* (Leguminosae), a substance was found in crude extracts of flowers of two interspecific hybrids between species closely related to *L. corniculatus* L. (*1*), which was not present in flower extracts from the parental species. A substance was also located on the chromatograms, at the same R_F as the substance from the two interspecific hybrids, from extracts of flowers of two individuals from an F_2 population which, in this instance, was not present in flower extracts from the parental species nor in extracts from the F_1 plant. This compound is considered to be comparable with the "hybrid substances" found by Alston and Turner (*2*) during chromatographic studies of the flowers of natural hybrid swarms of *Baptisia laevicaulis* × *B. viridis*. A similar phenomenon has been reported in the flowers of an amphidiploid of *Collinsia concolor* and *C. sparsifolia* which yielded four pigments not detected in the flowers of the parental species (*3*).

Table 1. The incidence of compound 3 in unhydrolyzed flower extracts of interspecific hybrids obtained from crosses between certain species of the *Lotus corniculatus* group*.

	Interspecific hybrids	Compound 3†
E717	*L. japonicus* × *alpinus*	—
E602	*L. japonicus* × *filicaulis*	—
E612	*L. japonicus* × *krylovii*	—
E905	*L. japonicus* × *schoelleri*	—
E125	*L. japonicus* × *tenuis*	—
C64	*L. krylovii* × *japonicus*	—
C8	*L. krylovii* × *schoelleri*	—
C95	*L. schoelleri* × *krylovii*	—
E442	*L. tenuis* × *filicaulis*	—
C347	*L. alpinus* × *japonicus*	+
E1061	*L. schoelleri* × *japonicus*	+

* Unhydrolized flower extracts of the parental species, including *L. corniculatus*, did not contain compound 3. † Dash (—) indicates compound absent; (+) present.

Fresh flowers were extracted overnight in 95 percent ethanol containing 1 percent HCl and the extracts applied directly to Whatman No. 1 chromatograph paper. The chromatograms were developed, by descending chromatography, in a solvent consisting of 15 parts glacial acetic acid and 85 parts water (*4*). After drying, the developed chromatograms were examined both in visible and in ultraviolet light. Initially, flowers were analyzed from ten individual plants of *L. japonicus* and *L. corniculatus* that were being cultivated under different environmental conditions: in an experimental field plot, a cold frame, a greenhouse, and a growth chamber. The plants varied in maturity and general vigor. Since, in all instances, the phenolic content of the flowers was the same for each plant of the species under investigation, it was considered that the phenolic content of the flowers from any one plant of a known species would be indicative of that species. Subsequently, flowers from only three or four plants for each species were used and extracted individually. No intraspecific variability in the phenolic content of the flowers was observed. For some of the first generation hybrids, only a single plant of a particular cross was available for analysis.

Extracts from the flowers of *L. corniculatus* L. (B280) and of six closely related diploid species of the *L. corniculatus* group, namely, *L. japonicus* (Regel) Larsen (B129), *L. filicaulis* Dur. (B37), *L. alpinus* Schleich. (B77), *L. krylovii* Schischk. and Serg. (B86), *L. schoelleri* Schweinf. (B87), and *L. tenuis* Waldst. et Kit. (B222), contained eight identical compounds. Although none of these compounds have been identified as yet, their appearance on the chromatograms, in visible and ultraviolet light, indicates that one is an anthocyanin, two are isoflavones and three are flavonols or flavonol glycosides. With one exception, the extracts from the flowers of the interspecific hybrids possessed the same phenolic constituents as those of the parental species. Extracts of flowers of the exceptional hybrid, *L. japonicus* × *L. filicaulis* (E602), lacked the eighth compound which was present in extracts of both *L. japonicus* and *L. filicaulis*.

What is more remarkable, however, was the discovery of a compound, designated as compound 3, in flower extracts of the interspecific hybrids, *L. schoelleri* × *L. japonicus* (E1061) and *L. alpinus* × *L. japonicus* (C347), which was not found in the extracts of the flowers of any of the parental species (Table 1). In addition, compound 3 was also found in flower extracts of two plants of an F_2 population of ten plants of the cross *L. japonicus* × *L. krylovii* (E613). This "hybrid substance" (compound 3) appeared on the chromatograms as a purple band in ultraviolet light at R_F 0.20. Its presence could not be verified by spraying with *p*-nitroaniline, indicating that it may be some compound other than a phenol.

At present, it is only possible to provide a tentative explanation for the "hybrid substance" in the interspecific hybrids and in the F_2 individuals. Further studies on the inheritance, as well as the identification of the compound, are required. However, it may be surmised that the hybrid substance (compound 3) in the interspecific hybrids occurs as a result of gene interaction in heterozygous individuals. Although the compound was absent from flower extracts of the reciprocal crosses *L. japonicus* × *L. schoelleri* (E905) and *L. japonicus* × *L. alpinus* (E717), and this might lead to the supposition that the "hybrid substance" in this instance was due to some type of maternal effect, this does not explain the occurrence of compound 3 in two plants of the F_2 population or its absence in the parental species and in the interspecific hybrid. Although the unidentified "hybrid substance" is considered to be identical in all the aforementioned plants, it is possible that it may be a different compound in the interspecific hybrids and in the F_2 individuals. Should this be so, then one might suggest that its occurrence in the hybrids is due to genic expression in a foreign cytoplasm, and that the occurrence of a totally different compound in the F_2 individuals is due to polygenic action.

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References and Notes

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