PRESLIA (PRAHA)
38: 229—236, 1966

Chemotaxonomy and Genesis of Protein Characters with Special Reference to the Genus *Phaseolus*

Chemotaxonomie a genese bílkovinných znaků se zvláštním zřetelem k rodu *Phaseolus*

Josef Kloz, Eva Klozová and Věra Turková

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha 6, Flemingovo 2

Received December 20, 1964

Abstract — There has been shown on the experimental material (especially on several species of the genus *Phaseolus*) the possibility of using the protein characters as a further criterion for solving some taxonomical and genetical problems. It has been demonstrated, that on the studied material there exist positive relations between the protein characters and the graft-affinity, geographical origin and crossability. It has been further demonstrated that the development of the protein characters in the course of further generations following the interspecific crossing is controlled by specific laws. It has been further argued on the basis of a confrontation of the specificity of protein characters with the validity of certain biological laws on the used material, that the protein characters constitute a kind of "common denominator" for the given laws. In this way there has been demonstrated the objective validity of results obtained by means of serological methods on plant materials.

It is the foremost objective of systematic botany to reconstruct the natura system in such a way that it would include all taxons, whether wild or cultivated, in an arrangement reflecting their actual phylogenetic kinship. This reconstruction of the natural system is probably more complicated than it would appear at first sight. It is true that Keck (1959) wrote in his treatise on the perspectives of systematic botany that the flora of the temperate zone will be brought to a final stage of description within 30—40 years, that of the tropical region within some 80 years and that of the Cryptogamae within about 200 years but we can agree with his estimate only insofar as descriptions and rough classification of individual species is concerned; there will always be enough work in the reconstruction of the natural system, there will always be things to correct and to make more precise.

Systematic botany—and we have in mind here the natural system—represents the result of our entire knowledge about plants and of all related fields of science, and as every scientific discipline it reflects the temporal vogue and should be treated historically rather than as a dogma and absolute truth.

Since Linné's time systematic botany has been above all a morphological and geographic discipline. It should be stressed at this point that morphology as a classical method will always form its basis. On the other hand, it is quite natural that data obtained by morphological description and analysis must agree with data obtained by other branches of experimental taxonomy, physiology, genetics, cytogenetics, biochemistry.

It must be assumed and a number of papers tend to prove it that the morphological evolution of species is connected with the chemical evolution of their contents, with regular changes in the type of their metabolism. A representative paper of this group would be that by Alston, Mabry

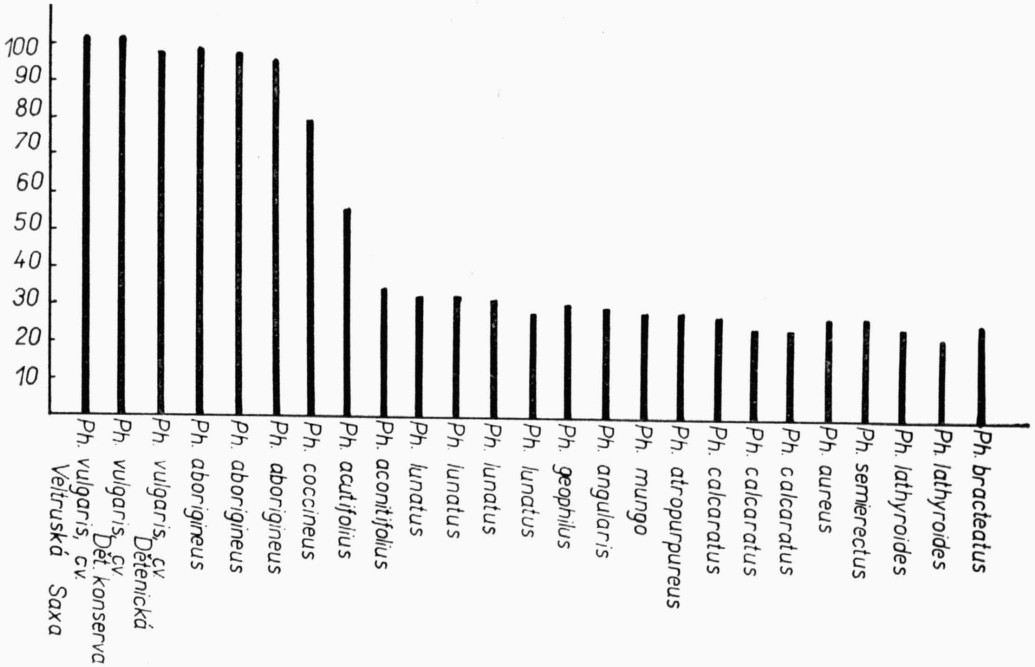
et TURNER (1963) who used two-dimensional chromatography of low-molecular metabolic intermediates to demonstrate that the chromatographic patterns are species-characteristic and that on crossing species undergo predictable changes. ALSTON et TURNER (1963) also summarize the present state of knowledge in this branch of chemotaxonomy in their "Biochemical Systematics", similarly as SWAIN (1963) in his "Chemical Plant Taxonomy" or HEGNAUER (1962) does in his several volumes of "Chemotaxonomie der Pflanzen".

Even if no final judgment can be pronounced about the importance of the individual chemical components as species characteristics, it seems likely that each character taken alone (and this holds for any type of trait, whether morphological or chemical) is only of limited importance as a species characteristic, with respect to the unequal taxonomical width, with respect to the unequal taxonomical value of single characters. Only the most complete set of all characters can yield an objective picture about the species and its phylogenetic and ontogenetic state. From this point of view all the partial contributions to the data available should be considered as we shall hardly ever have enough of them. Perhaps a concentration of effort and suitable division of labour on an international scale would help here.

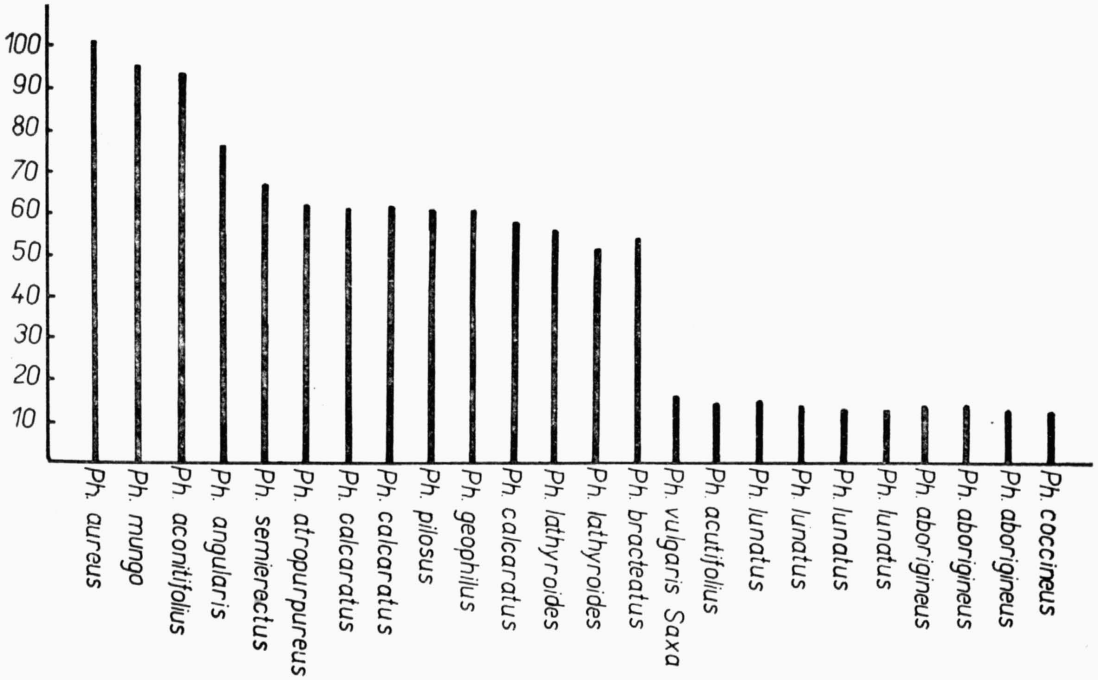
It is evident that nucleic acids together with proteins have a special importance in this connection. Both are the most important components of living matter and are often designated as primary metabolic components. Even if no sharp boundary can be drawn between primary and secondary metabolism—it is still fainty certain that nucleic acids together with proteins have a special importance even as species characteristics. According to the data contained in presently available papers it appears that universal taxon specificity is possessed by these high-molecular components of living matter. When the individual protein characters are examined it may be seen that convergence is very rare or perhaps non-existent, unlike the morphological or secondary metabolic characters. Thus the species and organ differentiation at the macroorganism level is associated with species and organ differentiation of structure of its nucleic acids and proteins, i.e. with changes on the level of biologically important macromolecules. The work of a number authors e.g. on the structure of hemoglobin shows that the hemoglobins of closely related animal species are characterized by infrequent deviations in the amino-acid sequence, these represent only a fraction of a percent of all aminoacids composing the hemoglobin chains of the individual animals species. Such deviations are more frequent with more distantly related species.

At present in chemotaxonomy, methods permit us to study more readily the proteins as species characteristics. There exist a number of physico-chemical, biochemical and biological methods for testing proteins as plant characters. These include separation into components on different carriers and on different physical principles (ion-exchange chromatography, molecular sieves, electrophoresis, in particular that using various gels as carriers), enzymological methods, and immunological tests applied in different modifications and combinations. Immunological methods have progressed considerably since the time of Mez and at present appear to be most expedient for studying the specificity of native proteins. This is true in spite of some limitations and possibilities of artifacts or rather asystematic reactions which have been elucidated in the plant material by MORITZ (1964) and which are probably due to the fact that serological methods do not permit the investigation of whole protein molecules but rather only the immunologically active, i.e. terminal determinants which represent but a small section of the whole protein molecule. This decreases the number of possible combinations as compared with the original large molecule. Another limitation lies in the individuality of animals used for the preparation of antisera which are then instrumental in detecting either qualitatively or quantitatively the specific proteins.

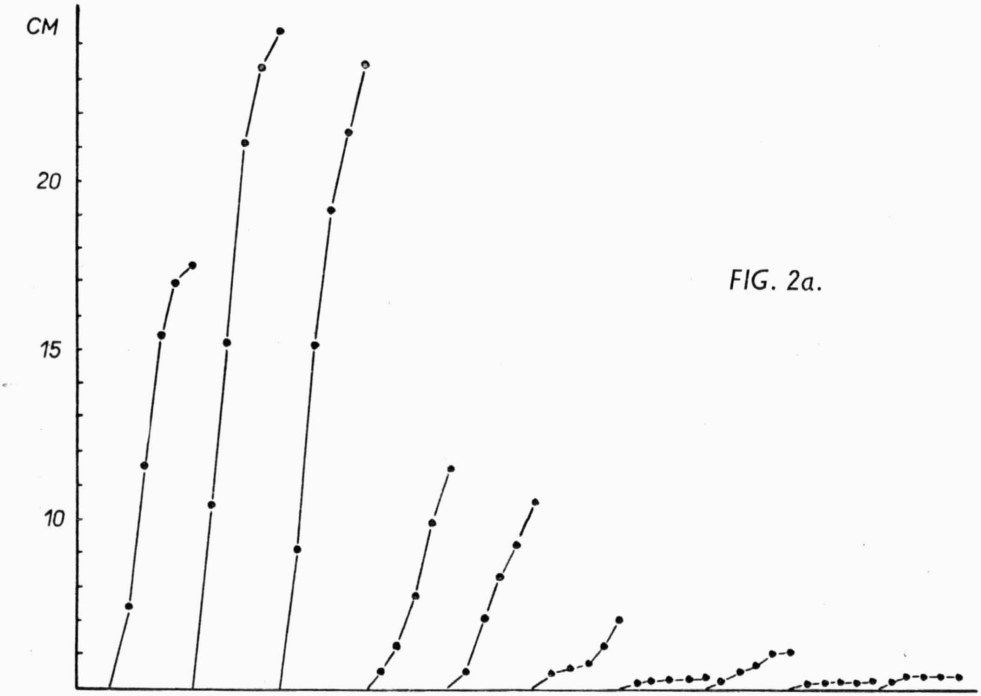
Let us mention now a few examples from our own work to demonstrate that in spite of the above-mentioned limitations the protein characters followed by

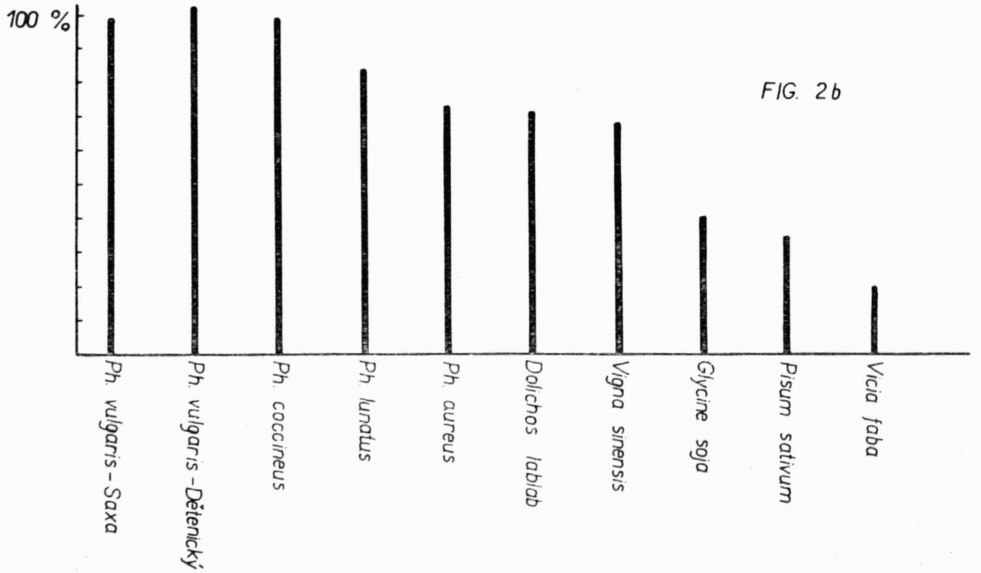


1a Intensity of serological reactions between the antiserum against seed proteins of *Phaseolus vulgaris* and seed proteins of the various other species.



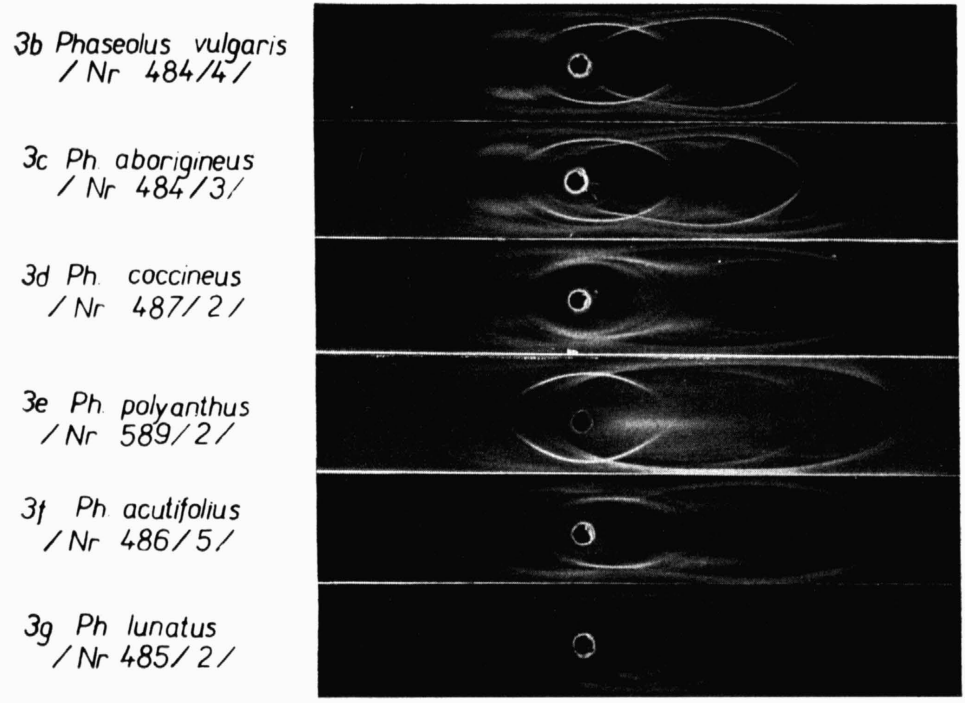
1b Intensity of serological reactions between the antiserum against seed proteins of ***Phaseolus aureus*** and seed proteins of the various other species.



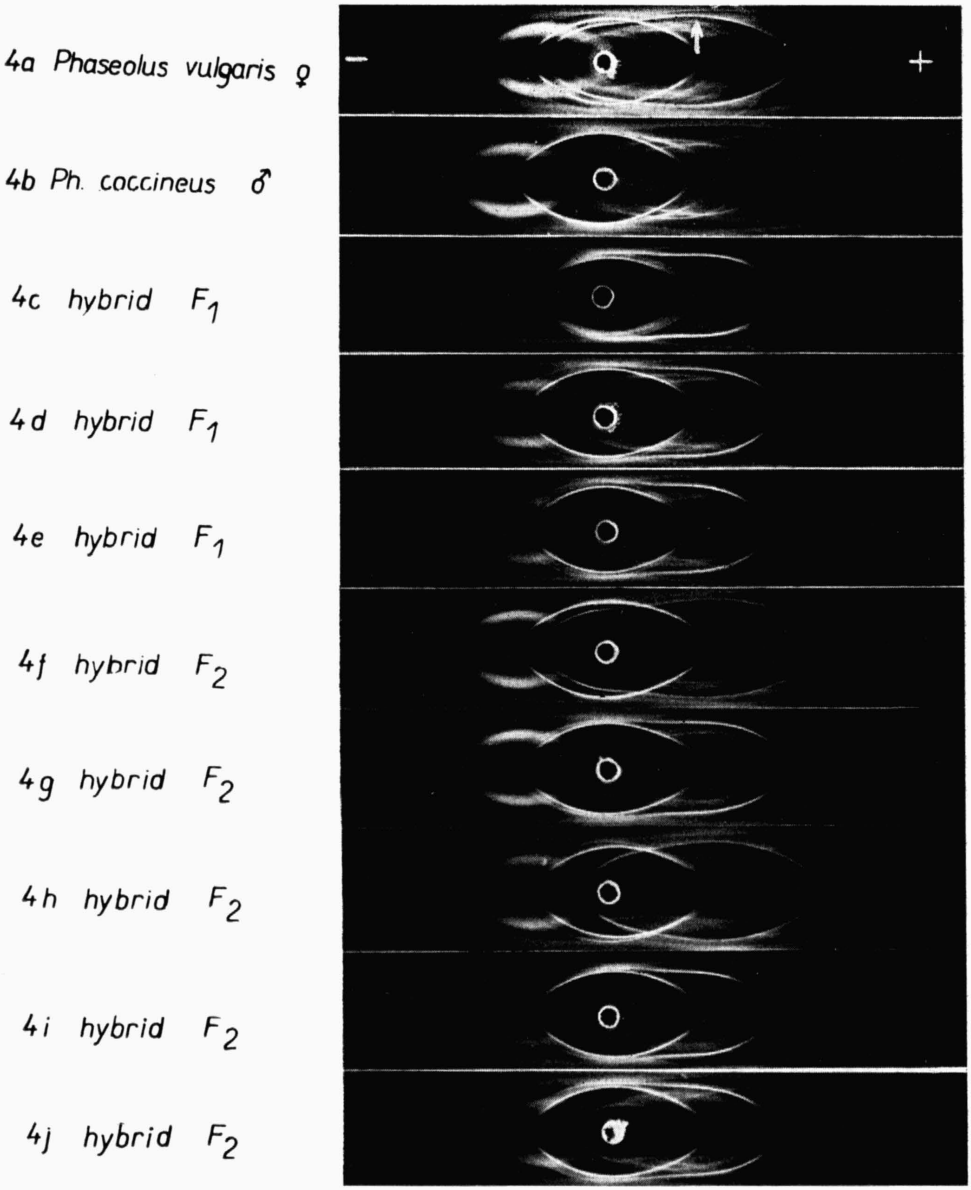


2a Increase in size of the scions of *Phaselus vulgaris* cv. Saxa over a five-week period after grafting on stocks of various species.

2b Relationship between the species *Phaseolus vulgaris* (cv. Saxa) and other species demonstrated by means of the intensity of serological reactions.



3b—g Immunoelectrophoreograms of protein characters (albumin fractions of cotyledon) of: 3b *Phaseolus vulgaris* (Nr. 484/4), 3c *Phaseolus aborigineus* (Nr. 484/3), 3d *Phaseolus coccineus* (Nr. 487/2), 3e *Phaseolus polyanthus* (Nr. 589/2), 3f *Phaseolus acutifolius* (Nr. 486/5), 3g *Phaseolus lunatus* (Nr. 485/2). The proteins in Figs. 3b—3g were detected with an antiserum against albumin fraction of *Phaseolus vulgaris* cotyledons.



4a–j Immunoelectrophoreograms of the cotyledon albumins of: 4a *Phaseolus vulgaris*, 4b *Phaseolus coccineus*, 4c *Phaseolus vulgaris* × *Phaseolus coccineus* F_1, 4d *Phaseolus vulgaris* × *Phaseolus coccineus* F_1, 4e *Phaseolus vulgaris* × *Phaseolus coccineus* F_1, 4f *Phaseolus vulgaris* × *Phaseolus coccineus* F_2, 4g *Phaseolus vulgaris* × *Phaseolus coccineus* F_2, 4h *Phaseolus vulgaris* × *Phaseolus coccineus* F_2, 4i *Phaseolus vulgaris* × *Phaseolus coccineus* F_2, 4j *Phaseolus vulgaris* × *Phaseolus coccineus* F_2. The proteins in Figs. 4a—4j were detected with an antiserum against cotyledon proteins of *Phaseolus vulgaris*.

serological methods can well indicate kinship and represent thus a relatively objective species characteristics. Species of the genus *Phaseolus* have been used predominantly. This material served to compare kinship relations with some biological or geographical facts.

1. Some kinship relations between *Phaseolus* species at our disposal should be brought up first as they were investigated by serological comparison of their protein characters. The characters of seeds (cotyledons) of the following species were compared: *Phaseolus vulgaris* subsp. *aborigineus* BURKART, *Ph. aconitifolius* JACQ., *Ph. acutifolius* A. GRAY, *Ph. angularis* (WILLD) W. F. WIGHT, *Ph. atropurpureus* MOC. E. SESSE, *Ph. aureus* (ROXB.), *Ph. bracteatus* NEES a. MART., *Ph. calcaratus* ROXB., *Ph. coccineus* L., *Ph. geophilus* BURK., *Ph. lunatus* L., *Ph. mungo* L., *Ph. semierectus* L., *Ph. vulgaris* L. subsp. *vulgaris*, The protein characters of these species were compared with those of *Phaseolus vulgaris* as is shown in Fig. 1a and with protein characters of *Phaseolus aureus* as is shown in Fig. 1b. This last named graph reveals that the group of East Asian species, including *Phaseolus aureus*, *Ph. mungo*, *Ph. angularis*, *Ph. calcaratus*, *Ph. aconitifolius*, gradually pass over to species of the tropical zone, beginning with India all the way to tropical America—here belong *Phaseolus atropurpureus*, *Ph. bracteatus*, *Ph. geophilus*, *Ph. semierectus*. And a separate, rather isolated group of American endemites originating from American elevated plateous, including *Phaseolus vulgaris*, *Ph. aborigineus*, *Ph. coccineus*, *Ph. acutifolius* and *Ph. lunatus* can be found at the end of the graph.

Fig. 1b thus permitted to compare the protein characters of all the species mentioned with those of *Phaseolus aureus*. Fig. 1a then shows the same but in comparison with the proteins of *Phaseolus vulgaris*. *Phaseolus vulgaris* may be seen to be most closely related to the American endemites in the sequence as corresponds to the views of present systematics, with the possible exception of *Phaseolus lunatus* whose classification with respect to *Phaseolus coccineus* has not been unequivocal. Then follows a more or less undifferentiated group of the above—mentioned East Asian and tropical *Phaseolus* species. In this respect our results are in good agreement with geographical facts.

2. In another experiment the kinship relations as demonstrated by serological investigation of the protein characters were compared with the graft affinity of these species in the family *Viciaceae*: *Phaseolus vulgaris* L. subsp. *vulgaris*, *Ph. coccineus* L., *Ph. lunatus* L., *Ph. aureus* ROXB., *Dolichos lablab* L., *Vigna sinensis* (STICKM.) SAVI ex HASSK, *Glycine soja* SIEB. et ZUCC., *Pisum sativum* L., *Vicia faba* L. The species *Phaseolus vulgaris* was compared with all the above species both with regard to graft affinity and to serological relationships. The experiment was so arranged that scions of *Phaseolus vulgaris* were grafted on stock of the same species as well as that of other species. In a similar way, the serological kinship between *Phaseolus vulgaris* and all other species was investigated. The graft affinity was investigated according to increment of scions within 5 weeks of grafting. The grafting was carried out in several repetions, a total of some 300 plants being treated. The results are shown in Fig. 2a and Fig. 2b. Fig. 2a shows the increments of scions removed from *Phaseolus vulgaris* and grafted on the various species mentioned above. Fig. 2b shows the kin relations demonstrated serologically as they exist between *Phaseolus vulgaris* and other species mentioned. The vegetative parts of seedlings were used here as material for obtaining protein characters. It follows from both figures that a positive correlation exists between the degree of similarity of

protein characters and the graft affinity in this particular material. In this way the objectivity of the results obtained serologically seems to be at least partly corroborated. It can be concluded that in this material the same degree of similarity between genomes was demonstrated both with respect to graft affinity and to protein compatibility.

3. In the next part of the work described here the kin relations between the individual species of *Phaseolus* were compared with their mutal crossability as far as it is known. In this experiment we again proceeded from the results on the kin relations demonstrated serologically as mentioned sub (1) and as shown in Figs. 1a and 1b.

Let as first consider the crossability of the individual species with *Phaseolus vulgaris* since for that species most information is available. The facts known from the literature are shown in Table 3a: *Phaseolus vulgaris* can be crossed very readily with *Phaseolus aborigineus*, fairly readily with *Phaseolus coccineus* (if *Phaseolus vulgaris* is mother but only with difficulty vice versa), only unwillingly with *Phaseolus acutifolius* (the hybrids mentioned by the authors in Table 3a were obtained only by cultivating embryos in vitro), rather unwillingly with *Phaseolus lunatus* (intervarietal hybrids of both species and in vitro cultivation were applied).

3 a Crossability between the species *Phaseolus vulgaris* and other species of the same genus in accordance with literature.

	crossability		
Ph. vulgaris × *Ph. aborigineus*	+++	very good	(Burkart + Brücher 1953, Rudorf 1958)
Ph. vulgaris × *Ph. coccineus*	++	good	(Tschermak 1901, Lamprecht 1948, Kroh 1962 et al.)
Ph. vulgaris × *Ph. acutifolius*	+	bad	(Honma 1956)
Ph. vulgaris × *Ph. lunatus*	+	bad	(Honma + Heeckt 1959)

There is thus again a positive correlation between the degree of similarity of protein characters and crossability of this material. It would be probably possible to assess in this way the crossability between species of other genera, as well. This, too, might be tackled more successfully on an international scale. (We are so confident of the reliability of the method as applied to *Phaseolus* that the observation on crossing of *Phaseolus vulgaris* and *Phaseolus aureus* [STRAND 1943] should be probably revised).

On the other hand, the results on the relationship between crossability and kinship of protein characters were confirmed on other material, as well. We have subsequently studied the protein characters of *Phaseolus polyanthus* GREENMANN. It was shown that this species is closely akin to *Phaseolus vulgaris* and that we could attempt planed crossing of these two species. The similarity between the protein characters of the *Phaseolus vulgaris* group including *Phaseolus polyanthus* has not yet been expressed quantitatively but rather by qualitative analysis of immunoelectrophoresis as shown in Figs. 3b–g. It follows from the immunoelectrophoreograms of cotyledon albumins that e.g. *Phaseolus vulgaris* and *Phaseolus polyanthus* are more closely related than *Phaseolus vulgaris* and *Phaseolus lunatus*. We began therefore with crossing the first two species and obtained a fairly high percentage of germinable hybrid seeds. In the meantime a report on successful crossing of the two species at Gainesville, Florida, has been published (1961).

A hybridization of *Phaseolus aureus* with *Phaseolus mungo* has been described which again corresponds to the degree of kinship in the *Phaseolus aureus* group.

4. Let us now consider briefly the genesis of some protein characters after hybridization between *Phaseolus vulgaris* and *Phaseolus coccineus* as it reflects the relationship between morphological and protein characters.

At first it was necessary to find suitable interspecific characteristics differentiating between *Phaseolus vulgaris* and *Phaseolus coccineus*. They were found in the albumin fraction of cotyledons. The immunoelectrophoreograms shown in Fig. 4a, 4b and others reveal proteins detected with a suitable antiserum against *Phaseolus vulgaris* cotyledon albumins. It should be stressed that in all these cases each individual elctrophoretic analysis has been carried out in a small part of the cotyledon of a single seed. The remainder of the seed including vegetation primordia was always sown out and thus it was possible to compare the habit with the protein characters of a single plant and its offspring.

May we call the attention to the most striking feature designated by the arrow in Fig. 4a at *Phaseolus vulgaris*. In *Phaseolus coccineus* this feature is lacking. The immunoelectrophoreograms in Figs. 4c, 4d and 4e show the spectra of protein characters of hybrids of the F_1 generation (i.e. from seeds harvested on mother *Phaseolus vulgaris* pollinated by father *Phaseolus coccineus*). In this case we can thus safely distinguish between the nonhybrid seeds from the hybrid ones even before sowing. Figs 4c—e show that the F_1 generation hybrids are in their protein characters intermediate between the two parents and about balanced which is in agreement with morphological interspecific features. Figs. 4f—j show immunoelectrophoreograms of the protein characters of the F_2 generation hybrids. There is an apparent splitting of characters and we encounter types resembling the mother as well as the father and even transitional types. A similar behaviour is observed with the interspecific morphological features of plants in the F_2 generation. The relations between the morphological and protein characters (relation of form and content) are not so close as it would appear at first sight (the generally intermediate character of the F_1 generation) and will be studied further. It follows from the immunoelectrophoresis, however, that the genesis of protein characters in distant hybrids is governed by similar laws as the genesis of morphological features.

The experimental material presented here was intended to demonstrate that proteins as components of plants can serve as equally satisfactory taxon characteristics as morphological and other features and that the laws of their genesis need not be at variance with the laws of physiology and genetics. It can be even said that in this case the proteins represent here a type of common denominator for physiological and genetic, as well as systematic characters.

Souhrn

Současná systematika a taxonomie je charakterisována komplexnějším přístupem k řešení otázek souvisících s rekonstrukcí přirozeného systému. I když klasické morfologické hledisko zůstane vždy základem, přinesla např. cytogenetika již dosti positivních výsledků a v poslední době i chemotaxonomie.

V naší práci si všímáme bílkovin, které spolu s nukleovými kyselinami jsou primárními produkty metabolismu a jsou i primárními charakteristikami taxonů. A právě v uvedené práci dokazujeme možnost použití bílkovinných znaků sledovaných expeditivními serologickými metodami pro řešení taxonomických i jiných otázek.

1. Sledovali jsme 15 taxonů rodu *Phaseolus*, jejichž specificita bílkovinných znaků odpovídá i jejich geografickému původu. Můžeme tedy i na základě chemické příbuznosti odlišit od sebe skupiny taxonů geograficky odlišitelných jako americké, asijské a tropické.

Z hlediska bílkovinných znaků jsou jasnější příbuzenské vztahy např. u *Phaseolus vulgaris* - *Ph. polyanthus* - *Ph. coccineus* - *Ph. lunatus*; dřívější názory na tyto vztahy nebyly vždy jednotné.

2. Roubová afinita (srůstavost roubu s podnoží) je v rámci sledovaného materiálu (3 druhy rodu *Phaseolus* a 5 druhů z blízkých rodů čeledi *Viciaceae*, tedy patrně v rámci určité vyšší systematické jednotky) též v souhlase s podobností bílkovinných znaků.

3. Vzájemná křižitelnost mezi druhy rodu *Phaseolus*, pokud je známa, je rovněž v korelaci s podobností jejich bílkovinných znaků. Byla tu možná i prognosa křižitelnosti na základě sledování specificity bílkovinných znaků.

4. A konečně byla sledována genese bílkovinných znaků v dalších generacích po křížení druhů *Phaseolus vulgaris* L. × *Ph. coccineus* L. První filiální generace (F_1) hybridů je v bílkovinných znacích intermediérní povahy. V druhé filiální generaci se objevuje v bílkovinných znacích štěpení na řadu jedinců s různým stupněm matro- i patroklinity.

Bílkovinné znaky jsou tedy za vhodných experimentálních podmínek poměrně objektivní charakteristikou příbuzenských vztahů mezi taxony.

References

Alston R. E., Mabry T. J. et Turner B. L. (1963): Perspectives in Chemotaxonomy. — Science 1/142, 3592 : 545—552.

Alston R. E. et Turner B. L. (1963): Bichemical Systematics. — New Jersey USA.

Burkart A. et Brücher H. (1953): Phaseolus aborigineus Burkart, die mutmassliche andine Stammform der Kulturbohne. — Züchter 23/3 : 56—72.

Hegnauer R. (1962): Chemotaxonomie der Pflanzen. — Basel u. Stuttgart.

Honma S. (1956): A Bean Interspecific Hybrid. — J. Heredity 47 : 217—220.

Honma S. et Heeckt O. (1959): Interspecific hybrid between Ph. vulgaris and Ph. lunatus. — J. Heredity 50 (5) : 233—237.

Keck D. D. (1959): The Future of Systematic Botany. — Systematic Zoology 8 : 76—82.

Kroh M. (1962): Vergleichende Untersuchungen an Phaseolus coccineus — Selbstungen und Kreuzungen zwischen Ph. vulgaris und Ph. coccineus. — Z. Pflanzenzücht. 47/3 : 201—216.

Lamprecht H. (1948): Zur Lösung des Artproblems. Neue und bisher bekannte Ergebnisse der Kreuzung Phaseolus vulgaris L. × coccineus L. und reziprok. — Agri. Hort. Genet. 6 : 87—145.

Manwell C., Boker C. M. A. et Childers W. (1963): The Genetics of Hemoglobin in Hybrids. — I. A Molecular Basis for Hybrid Vigor. — Comp. Biochem. Physiol. 10 : 103—120.

Moritz O. (1964): Some Special Features of Serobotanical Work. — Taxonomic Biochemistry and Serology 15 : 275—290.

Rudorf W. (1958): Genetics of Phaseolus aborigineus Burk. — Proceed. X. Internat. Congr. Genet. II., 243.

Strand A. B. (1943): Species Crosses in the Genus Phaseolus. — Proc. Amer. Soc. Hort. Science 42 : 569—573.

Swain T. (1963): Chemical Plant Taxonomy. — London and New York.

Tschermak E. (1901): Weitere Beiträge über Verschiedenwertigkeit der Merkmale bei Kreuzung von Erbsen und Bohnen. — Z. landwirtsch. Versuchsw. Österr. 4 : 641—735.

Ann. Report of Agricult. Exp. Stations, Univ. of Florida, 30 : 399, 1961.