Theor. Appl. Genet. 57, 225-232 (1980)

© by Springer-Verlag 1980

Investigations on the Relations Within the Family Papilionaceae on the Basis of Electrophoretic Banding Patterns

G. Wolff
Institute of Genetics, University of Bonn (Federal Republic of Germany)

Summary. Gel electrophoretic investigations were made on the seed albumins of several members of the family Papilionaceae. Relationships were found with taxa of a lower order i.e. between mutants, varieties and subspecies. More distantly related ones, for example species of the same genus or species of different genera, did not show similarities. Thus, it was concluded that the albumin banding pattern is only suitable for studying phylogenetic and taxonomic problems if the material under investigation is not too distantly related.

Key words: Electrophoresis – Proteins – Leguminosae – Taxonomy – Evolution

Introduction

When studying evolutionary problems, the question which often arises is just how much morphological characteristics alone are of importance, and just how much chemical traits can be helpful in classification. Even today the importance of chemotaxonomy is debated. Chemical differences and concordances between and within a group of taxa might be misleading if certain presuppositions are not considered. An important point to consider is the natural variation caused by environmental and ontogenetic factors which might cover up the genetically caused deviations. Therefore, the material to be investigated in this respect should be at the same level of development and should have been grown under similar conditions. In any case it would be useful if chemical characteristics were not the only criteria for characterization, but could be used in combination with other traits, mostly morphological but to some extent also cytological (for literature cf. Harborne et al. 1971; Frielinghaus 1976; Smith 1976; Ladizinsky and Hymowitz 1979).

The present paper compares the electrophoretically separated albumin patterns of several legumes belonging to different taxonomic groups within the Papilionaceae. The material investigated were ripe seeds. This material was chosen because seeds of different plants can be best compared because of their uniform physiological state. As far as mutants are concerned, the seeds were harvested from plants grown under similar conditions.

According to the actual definition new species predominantly arise by mutation whereby more or fewer genes are involved. Because of this it is proposed that forms which are more closely related differ in a smaller number of mutated genes than those which are less closely related. To study such a problem it is believed to be more useful to choose a character that is more or less a direct product of genes. As such, seed albumins – comprising predominantly enzymes – seem to be a conveniant character for these analyses as they reflect in a certain way the manifold genetic constitution of an individual. This presupposed, genetic variation then should be expressed in a changed protein pattern. In extreme, the mutation of a single gene, if affecting the seed proteins, might be reflected in a small but distinct change in the respective protein pattern. Furthermore, we should presume that in systematics, individuals that are closely related, show a more similar protein pattern than those that are phylogenetically less related.

In the present paper, mutants of the variety 'Dippes gelbe Viktoria' of *Pisum sativum*, subspecies of the genus *Pisum*, and species of different genera within the Papilionaceae are compared on the basis of their albumin pattern. Similarities and discordances in the respective pattern might give us hints on the relations and on the phylogenetic development within the Papilionaceae. Moreover, these investigations should show us, just how useful a tool electrophoresis is in taxonomy.

Materials and Methods

A. Material

Mutants

Seeds of 11 mutants of Pisum sativum variety 'Dippes gelbe Viktoria' were used from our mutants' collection in Bonn. Most of

0040-5752/80/0057/0225/$ 1.60

the mutants differ in one gene, except mutants 46C and 38E in which two genes are mutated.
- mutants with bracts: 34G and 2648,
- mutants with increased number of stipules: 39 and 3091,
- mutants with a bifurcated axis: 46C, 157, 239CH and 1201A,
- early flowering mutants: 38E and 46C,
- mutants with narrow leaflets: 122 and 2218.

Seeds of Pisum species and subspecies (the material provided by Dr. Blixt, Plant Breeding Institute, Weibullsholm, Sweden). The material was classified according to the system proposed by Lehmann (1954).

Pisum formosum (Stev.) Boiss.
Pisum fulvum Sibth. et Sm.
Pisum elatius (M.B.) Stev.
Pisum abyssinicum A.Br.
Pisum sativum L. sens. lat. Gov. ssp. asiaticum Gov.
Pisum sativum L. sens. lat. Gov. ssp. transcaucasicum Gov.
Pisum sativum L. sens. lat. Gov. ssp. sativum var. arvense (L.) Alef.
Pisum cinereum } not in Index
Pisum tibetanicum } Kewensis
Pisum abyssinicum A.Br. var. vavilovianum Gov. (provided by the "Zentralinstitut für Genetik und Kulturpflanzenforschung" in Gatersleben, DDR).

Seeds of Vicia species and subspecies (from Gatersleben, DDR)

Vicia faba L. ssp. faba var. faba svar. faba
Vicia faba L. ssp. minor (Peterm. em. Harz) Rothm. var. minor svar. minor
Vicia faba L. ssp. minor (Peterm. em. Harz) Rothm. var. minor svar. tenuis Murat.
Vicia amoena Fisch
Vicia cracca L.
Vicia dalmatica A. Kern
Vicia johannis Tamamsch.
Vicia lathyroides L.
Vicia macrocarpa (Moris) Bertol.

Seeds of various species within the Leguminosae (from various Botanical Gardens in Germany)

Baptisia australis (L.) R. Br.
Coronilla varia L.
Galega officinalis L.
Genista tinctoria L.
Glycine max (L.) Merr.
Lathyrus pratensis L.
Onobrychis viciifolia Scop.
Phaseolus vulgaris L.
Spartium junceum L.
Trifolium pratense L.

B. Methods

Extraction, Separation and Determination of Seed Albumins

Albumins were extracted following the method described by Danielsson (1949). Albumins were separated by use of a flat-gel-electrophoresis on a 10% acrylamide gel, with a Tris/Glycine buffer (pH 8,9) in the upper buffer vessel and a Tris/HCl buffer (pH 8.1) in the lower one. 6 samples were separated on the same gel. A control sample was assayed on each gel. Gels were stained with a solution of 0.05% Coomassie Blue in 12.5% TCA. Esterases were identified after separation of the albumin samples under the same conditions as described above with α-naphthyl-acetate as substratum and Fast Blue RR salt. Immediately after staining the gels were photographed (Fig. 1). According to the original gels and the respective pictures the R_p-values were determined and drawings were made. Evaluation of the banding pattern in detail was difficult in some cases because some bandings were very faint and their determination was thus uncertain. In single cases therefore it is possible that the one or other faint banding in a pherogram is lacking, though in reality it is present. Furthermore, in same cases it was impossible to determine whether a broad band was composed of two or three subbands. Therefore, it is probable that a double band in one pherogram is identical with a broad band in the other. Because of these difficulties the evaluations without exception were done by the same person to make the subjective error as small as possible.

As to the drawings of the protein patterns, various breadths of the single bands and their intensity represent roughly a reproduction of the situation in the original pherograms.

In the course of our investigations we changed the physical conditions of our electrophorese apparatus because of a new voltage supplier. This is why identical bands in two pherograms of the same sample show different R_p-values (Figs. 2, 4, 9).

Results

A fundamental problem that arises in connection with investigations on albumin patterns is just how far the protein pattern is influenced by environmental factors. Our own investigations in this direction have shown that environment has no influence on the albumin pattern (Coomassie), if genetically identical material of *Pisum sativum* grown in different vegetation periods is analysed. Yet some minor quantitative variations are found within the esterase pattern. Because of this, we can proceed from the fact that differences found within the material under investigation are due to genetic alterations, as far as the Coomassie pattern is considered. The esterases, on the other hand, are only relevant in this connection if qualitative characteristics are used for conclusions.

The results obtained are arranged in four parts according to the various taxonomic groups of plants that were analysed:
- Mutants of *Pisum sativum* var. 'Dippes gelbe Viktoria',
- Species and subspecies of the genus *Pisum*,
- Species of the genus *Vicia*,
- Species of various genera within the Papilionaceae.

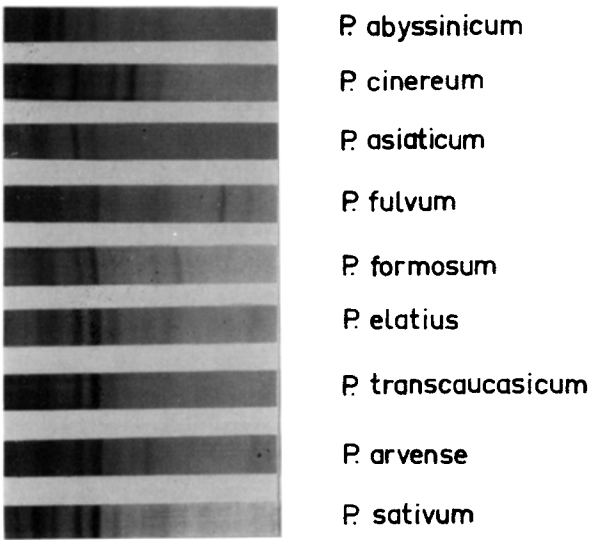

Fig. 1. Seed albumin pattern of several *Pisum* forms; original photograph

Mutants of Pisum sativum

Genetically this group of mutants is very homogeneous, and the genetic situation is fully known to us. In each case (except mutants 46C and 38E, where two genes are involved) only a single gene is mutated. Initially the material was selected with regard to some morphological deviations. Because of the pleiotropic action of a mutated gene, it was supposed that some of these genes involved influence also the seed proteins of the mutants.

In figure 2 the albumin patterns of 11 mutants and their parental line are given. Compared to the parental line, each mutant shows a somewhat different distribution of the bands indicating in each case an influence of the mutated gene on seed albumins. The differences seem to be due to additions and deletions of single bands and to quantitative differences. Just how far shiftings of the bands caused by deviations in loading of specific proteins are involved, cannot be decided so far. For this, more extensive investigations on the identification of special enzymes have to be made.

In spite of these differences an overall similarity between the patterns is visible. At R_p 0.35 two distinct bands are visible which are not changed in any of the mutants' pattern. They are not influenced by any of the mutated genes involved. In the upper third of the pherograms, two groups of bands are discernible: Slow running ones in a very low position (< Rp 0.1), which are very narrow, and somewhat faster running ones, which are thicker than the former. Several of these bands are found in each pattern, though sometimes with quantitative deviations. On the other hand, several bands are added or removed according to the genetic situation of the material analysed. Similar conditions are found in the group of bands with Rp values greater than 0.4.

To characterize the protein pattern further, it is necessary to identify special enzymes within the albumin fraction. Until now only the unspecific esterases have been investigated. Figure 3 represents the pattern of these enzymes within some of the mutants. Here too, the distribution of the bands shows a more or less clear similarity. A group of slow and fast running bands is typical for this distribution scheme.

Species and Subspecies of Pisum sativum

The next step within our investigations was to analyse *Pisum* species and subspecies. The discussion about the relations within the genus *Pisum* is still going on and no consensus about the number of *Pisum* species has been found. Therefore, the analysis of the protein pattern might give some further hints in this direction.

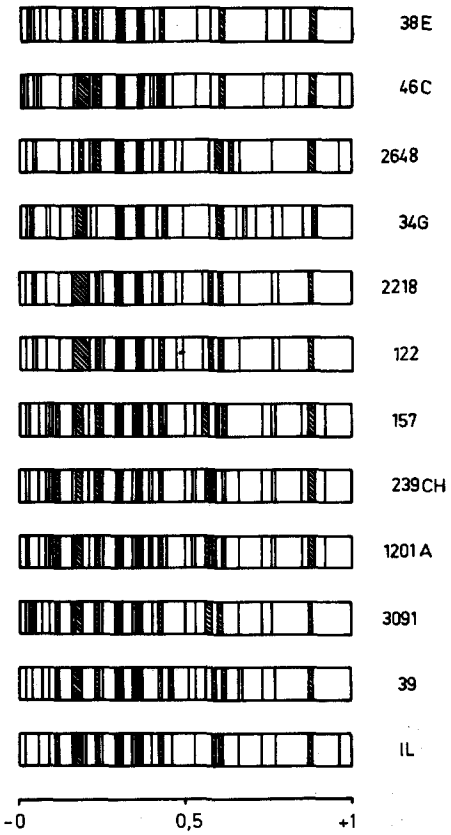

Fig. 2. Seed albumin pattern of eleven mutants of *Pisum sativum* and their initial line (IL)

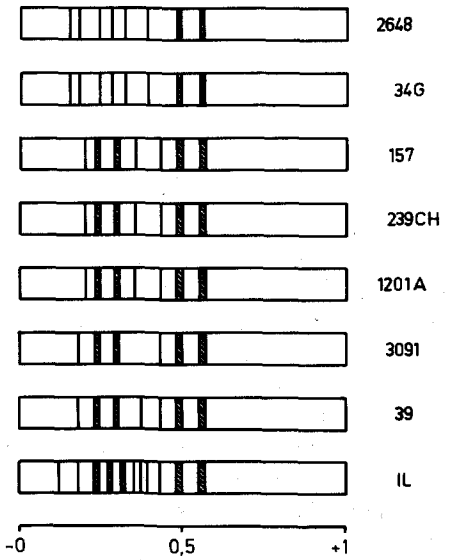

Fig. 3. Esterase pattern of seven mutants of *Pisum sativum* and their initial line (IL)

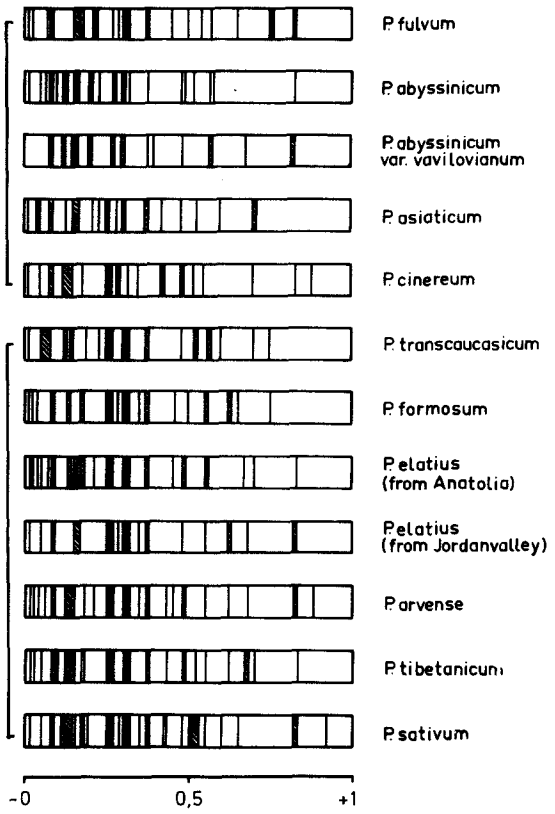

Fig. 4. Seed albumin pattern of several species, subspecies, and varieties of the genus *Pisum*

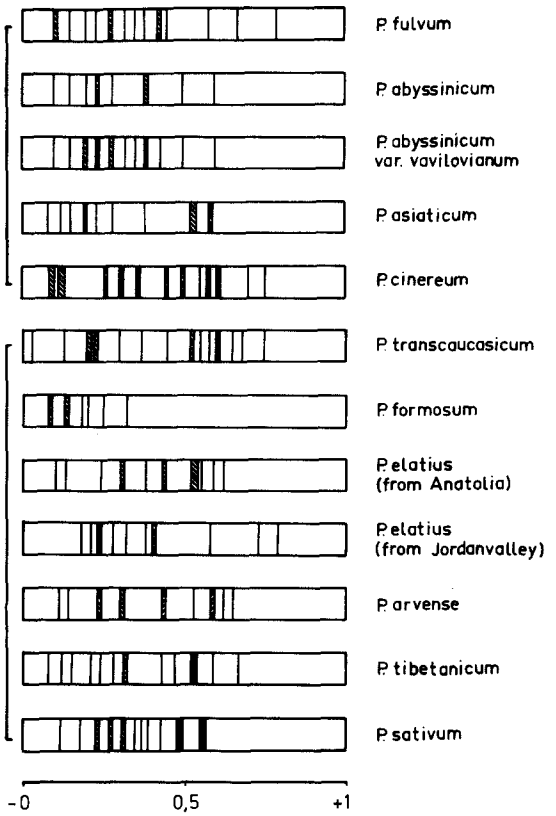

Fig. 5. Esterase pattern of several species, subspecies, and varieties of the genus *Pisum*

Figure 4 represents the albumin pattern of different *Pisum* 'species'. According to the protein pattern we can divide the material into two groups:

group 1 (fig. 4, lower part):	group 2 (fig. 4, upper part):
Pisum sativum	*Pisum cinereum*
Pisum tibetanicum	*Pisum asiaticum*
Pisum arvense	*Pisum abyssinicum*
Pisum elatius	*Pisum fulvum*
Pisum formosum	
Pisum transcaucasicum	

A characteristic pattern of *Pisum* seed albumins seems to be the two dark blue bands (Coomassie) in the upper part of the pherograms (Rp 0.3-0.35). These bands were found in all mutants (Fig. 2) and are clearly visible in the pherograms of Figure 4, lower part. In the patterns of Figure 4, upper part, these bands are not found. The concordance of the lower part pattern is comparably large. Several similarities in the protein bands can be recognized but the overall banding pattern, that was found within the pattern of the mutants is not detectable. The greater genetic diversity of the material studied becomes relevant in this respect.

The patterns of *Pisum sativum, P. tibetanicum* and *P. arvense* are similar, especially the area in the upper part of the pherograms. Other *Pisum* forms show a greater diversity in this region. The banding pattern of the lower region is characterized by a number of bands which occupy the same position in each case. Quantitative deviations are especially predominant here. Just how far the same position is an indication for the same protein can only be decided after extensive investigations on specific enzymes have been made. On the whole, the results point to a considerable similarity between the *Pisum* forms of this group. From this we can conclude that a certain genetic resemblance exists between these forms.

Of interest are the differences, which are found between both the *elatius* forms. Apparently, the genetical situation is not identical, and both might be ecological races.

The protein pattern of the second group presents far greater variability than the first one. First of all, the clear dark bands are not to be found. Furthermore, the number of identical bands (i.e. those with the same Rp-value) is smaller. Apparently the relations in these cases are less close between one another than in the first group. The great agreement between both the *abyssinicum* forms (the agreement is far greater than in case of *Pisum elatius*) points to a closer genetic relation between these plants.

Considering the esterase pattern (Fig. 5), this grouping is not detectable. Each genotype has its own distribution of bands. Apparently the esterases are not so useful in characterizing peas taxonomically. The esterase pattern of *Pisum elatius* and *abyssinicum* points as a whole in the same direction as the albumin pattern. As to *abyssinicum* the patterns are comparable, while the *elatius*-patterns are considerably different.

Species of Vicia

The genus *Pisum* is proposed to comprise one, two or at maximum six species, according to various systematics; all the other forms found within *Pisum* represent subspecies or only varieties. Therefore, we should expect in this case a more uniform or similar protein pattern. The results obtained confirm this assumption in general. With *Vicia* a greater interspecific diversity is probable because predominantly distinct systematic species were chosen for our analyses in these cases. Similarities between the rather closely related genera *Pisum* and *Vicia* are not to be found, as a comparison of Figures 4 and 6 reveals. Considerable genetic differences between genera become fully evident. Compared to this, the genetic diversity between species of the same genus is smaller. Nevertheless, any similarity within the albumin pattern of this group is covered up. Similarities between species of the same genus and between species of different genera are in no way greater. Resemblances are detectable (as within the *Pisum* forms) in the same species of different origin (*Vicia cracca* or *johannis*). The respective patterns reveal that the genetic situation is not identical, therefore, varieties or ecological races must be under discussion in these cases. In case of *Vicia* (Fig. 8) it can be shown once more that in forms of a lower taxonomic rank (f. ex. subspecies) species-resemblances are detectable. The three subspecies of *Vicia faba* exhibit similarities. Regarding the esterases (Fig. 7), an overall resemblance is not visible. A similar diversity as in the total protein pattern is found, whereby, however, same species from different origins show certain concordances within the pattern.

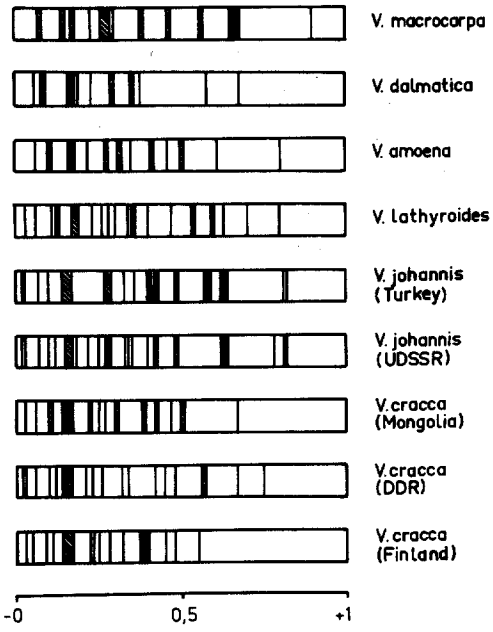

Fig. 6. Seed albumin pattern of several species of the genus *Vicia*

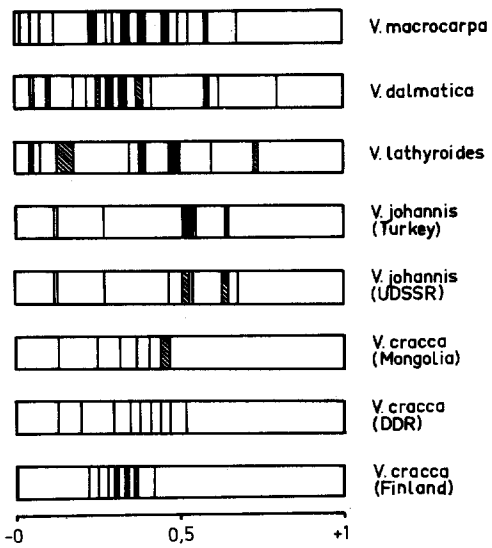

Fig. 7. Esterase pattern of several species of the genus *Vicia*

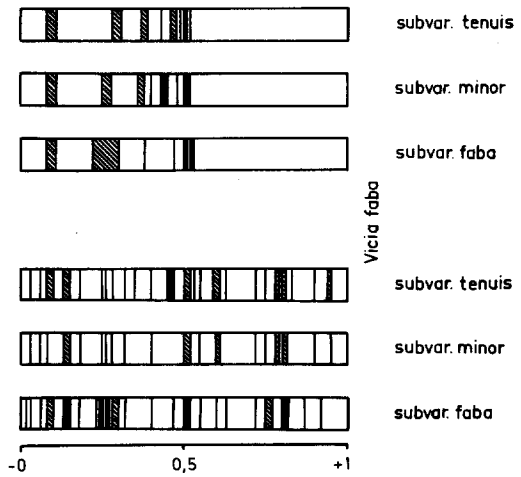

Fig. 8. Seed albumin (lower) and esterase pattern (upper part) of three subspecies of *Vicia faba*

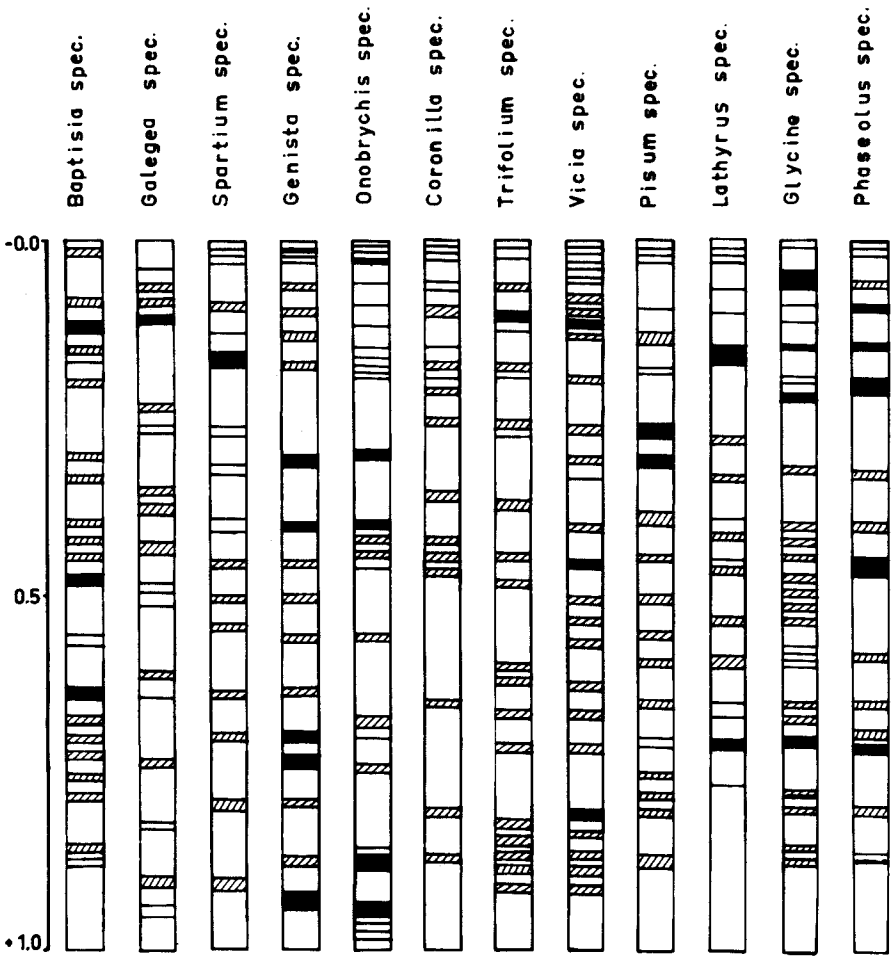

Fig. 9. Seed albumin pattern from several species of different genera within the Leguminosae

Species of Various Genera

The last group computes species from different genera. Clear differences between all the albumin patterns of these can be recognized from Figure 9. Relations between closely related species, for example between *Pisum, Vicia* or *Lathyrus*, cannot be found. Apparently the genetic diversity between the genera has become so pronounced that similarities no more exist now. Analyses of the esterases were not made.

Discussion

The work reported in this paper was done with three viewpoints:

– Usefulness of the electrophoretic banding pattern of the seed albumins with regard to the relations within the members of the family Papilionaceae is discussed.

– An application of this subject should make it possible to judge the usefulness of the method in connection with evolutionary problems.

– Investigations within the genus *Pisum* shall give a further elucidation of the open questions in connection with taxonomy.

As was already pointed out, seed albumins seem to be useful material for investigations in chemotaxonomy in two respects: Investigations on chemotaxonomy require plant material that is in a physiologically identical state. Such identity is possible in ripe seeds. Furthermore, while working upon a genetical problem it is useful to consider substances that are more or less the direct products of genes. Albumins, being predominantly enzymes, are such substances. Genetically caused variations within these proteins should be directly expressed in changed electrophoretic behaviour. According to the actual meaning of the word, systematics is a scale for the genetic resemblances within a group of individuals. This means: Genera, species, varieties and so on differ in a more or less large number of genes according to their position within the

systematic hierarchy. In the case of close relatives fewer genes are different than in case of distantly related ones, and this should become evident from greater similarity in the actual gene products. Fewest differences should be expressed in mutants of the same species where only single genes are changed while between species of different genera the differences should be numerous.

Because the albumin fraction is known to be composed predominantly of enzymes, it is useful not to restrict the investigations on the total protein pattern but to analyse special enzymes as well. From the great number of enzymes within the seeds, only unspecific esterases have been considered so far. The analysis of these enzymes shows that environment is not without influence. We expect that investigations on other enzymes probably will reveal further environmentally caused differences. From these results, we can conclude that the total albumin pattern so far is more useful in chemotaxonomy because environmental influences are not expressed.

A single mutated gene (this is the case in nearly all mutants mentioned in Fig. 2) can affect the albumin pattern. Each mutant exhibits in detail a somewhat different pattern as compared to that of the initial line. Besides these differences, an overall similarity in the distribution pattern of the albumins and esterases is found in each pherogram. Thus, the results within the group of mutants are conflicting. The number of alterations within the albumin spectrum is amazingly high if one considers that only a single gene is involved in each case. Besides, we must presume that mutations occur which have no effect on the seed albumins. If a single gene has such a pronounced effect, then we must expect that a greater number of genes could change the pattern so drastically that no more similarity would be detectable. The group of *Pisum* forms of Figure 4 is composed of species, subspecies, and varieties of the species *Pisum sativum*. Thus, they possess, at least partly, a close relationship to the former group of peas. The complete overall distribution pattern that is characteristic for the mutants is no longer visible. We can distinguish two groups of *Pisum* forms (Fig. 4): The first one without exception has two clear dark bands while the second one lacks these. Especially because of these bands we presume that the first group of peas is more related to our *Pisum sativum* form than the second one, though the similarities within the overall pattern are not too great.

The relations within the genus *Pisum* are not perfectly known. Because *Pisum* is one of the oldest agriculturally used plants, especially in the Mediterranian area, the Near East, and Iran, and because hybridization and mutation are extremely widespread in this genus, the origin and phylogenetic relations cannot easily be detected. Several attempts were made to solve the problems, predominantly on the basis of morphological traits.

Linné attributes two species, *sativum* and *arvense*, to the genus *Pisum*. Later, Lamprecht (1966) proposed that *Pisum* is monospecific (*Pisum sativum*) and that all the other variations belong to taxa of lower rank. Davis (1970), on the other hand, accepts two species: *Pisum sativum* and *P. fulvum*. Lehmann (1954), in his most detailed paper, proposes six original species: *Pisum sativum, P. abyssinicum, P. elatius, P. formosum, P. fulvum,* and *P. syriacum*. Przybylska's investigations (1977) found *Pisum elatius, P. syriacum,* and *P. sativum* to be closely related because no distinct differences between these three forms were found. *Pisum cincereum, P. abyssinicum* and *P. fulvum*, on the other hand, showed distinct differences in electrophoretic pattern, therefore being proposed as different taxa.

According to our findings, *Pisum sativum, P. arvense, P. tibetanicum, P. elatius, P. transcaucasicum* and *P. formosum* in general – though differences exist – show good concordances indicating close phylogenetic relations. In our opinion this excludes, any of these forms being separate species. They all are subspecies or varieties of the species *Pisum sativum*. The degree of relations between one another cannot be decided so far. Of interest is the fact that *Pisum formosum* belongs to this group, which according to Govorov (1937) and Davis (1970) represents a separate genus (*Vavilovia*) being the link between the genera *Pisum* and *Lathyrus*. Our results are not in agreement with this. On the basis of seed albumin pattern this special position cannot be attributed to this *Pisum* form.

The second group of *Pisum* forms exhibit differences from the *sativum*-group and also between one another. These differences are as pronounced as they are between species of the same genus (cf. *Vicia, Lathyrus*). Davis (1970) accepted *Pisum fulvum* as a separate species, an opinion to which our results are in concordance.

Concerning *Pisum abyssinicum* two views exist: *Pisum abyssinicum* was regarded as a close relative of *P. fulvum* (Govorov 1937). We did not find special similarities between these forms and cannot support this opinion. Lehmann (1954) proposed that *P. abyssinicum* in addition to *P. sativum*, summarize the attributes of all agriculturally used varieties. Furthermore he accepted *Pisum abyssinicum* as a separate species, an opinion that is in agreement with our results. The same author described *Pisum asiaticum* and *P. transcaucasicum* as subspecies of *Pisum sativum*. This indicates a more or less similar degree of relation of both these forms towards *Pisum sativum*. It is evident from our results (Fig. 4) that *Pisum transcaucasicum* is more closely related to *Pisum sativum* than *Pisum asiaticum*. Therefore, *Pisum asiaticum* should be placed farther from *P. sativum*, for example as a separate species.

The esterase pattern of the *Pisum* forms (Fig. 5) do not contribute further elucidation to this problem. The clear differences between the first and second group of *Pisum* forms (Fig. 4) have shown that with increased genetic

dissimilarity the albumin pattern becomes more divergent. Species no longer show similarities. This clearly can be seen within the group of species of *Vicia*. Each pattern is distinctly different. As for our results, statements on genetic relations are impossible in this hierarchic group. Also as expected, no similarities exist between species of different genera, not even between the closely related genera *Vicia* and *Lathyrus*.

One viewpoint of this paper was to judge the usefulness of electrophoretic seed albumin pattern for taxonomic purposes. So far our results clearly demonstrate that electrophoretic seed albumin pattern of Leguminosae do not show similarities, if one moves away from closely related forms. This means that there are no relations between the albumin patterns and the taxonomic position of the species studied. Therefore it seems impossible to use the mentioned criteria for chemotaxonomy.

So far, our findings are in contrast to the results published by Fox et al. (1968) on Leguminosae. They found species similarities within a genus and also similarities between species of genera which are grouped together on morphological grounds. This discrepancy might be explained by the comparable small number of species that were analysed by these authors. In the case of *Avena* (Dass 1972) and *Gossypium* (Cherry et al. 1971), similarities as well as dissimilarities between species of the same genus were found. Other authors published papers on this topic which dealt predominantly with closely related forms (Adriaanse et al. 1969, Larsen 1967, Ladizinsky 1975, McDaniel 1970). All these papers also show that interesting results in connection with varieties and cultivars are available but that the usefulness of the method under discussion is doubtful in connection with more distantly related forms.

Literature

Adriaanse, A.; Klop, W.; Robbers, J.E. (1969): Characterization of *Phaseolus vulgaris* cultivars by their electrophoretic pattern J. Sci. Food Agric. **20**, 647-650

Cherry, J.P.; Katterman, F.R.H.; Endrizzi, J.E. (1971): Comparative studies of seed proteins of species of *Gossypium* by gel electrophoresis. Evolution **24**, 431-447

Danielsson, C.E. (1947): Seed globulins of the Gramineae and Leguminosae. Biochem. J. **44**, 387-400

Dass, H.C. (1972): Analysis of species relationships in *Avena* by thinlayer chromatography and disc electrophoresis. Canad. J. Genet. Cytol. **14**, 305-316

Davis, P.H. (1970): Flora of Turkey, Vol. III. Edingburgh: University Press

Fox, D.J.; Thurman, D.A.; Boulter, D. (1964): Studies on the proteins of seeds of the Leguminosae I. Albumins. Phytochemistry **3**, 417-419

Frielinghaus, G. (1976): Möglichkeiten und Grenzen der Chemotaxonomie. Wiss. Arbeit im Rahmen der ersten Staatsprüfung für das Lehramt an Gymnasien, Univ. Bonn

Govorov, L. (1937): Erbsen, Flora of Cultivated Plants **4**, 231-336

Harborne, J.B.; Boulter, D.; Turner, B.L. (1971): Chemotaxonomy of the Leguminosae. London, New York: Acad. Press

Ladizinsky, G. (1975): Seed protein electrophoresis of the wild and cultivated species of selection *Faba* or *Vicia*. Euphytica **24**, 785-788

Ladizinsky, G.; Hymowitz, T. (1979): Seed protein electrophoresis in taxonomic and evolutionary studies. Theor. Appl. Genet. **54**, 145-151

Lamprecht, H. (1966): Die Entstehung der Arten und höheren Kategorien. Wien, New York: Springer

Larsen, A.L. (1967): Electrophoretic differences in seed proteins among varieties of soybean, *Glycine max* (L.) Merill. Crop. Sci. 7, 311-313

Lehmann, Ch.O. (1954): Das morphologische System der Saaterbsen (*Pisum sativum* L. sens lat. Gov. ssp. *sativum*). Züchter **24**, 316-337

McDaniel, R.G. (1970): Electrophoretic characterization of proteins in *Hordeum*. J. Hered. **61**, 243-247

Przybylska, J.; Blixt, St.; Hurich, J.; Zimniak-Przybylska, Z. (1977): Comparative studies of seed proteins in genus *Pisum*. Genet. Polon. **18**, 28-37

Smith, P.M. (1976): The Chemotaxonomy of Plants. Contemporary Botany (Eds. Barrington, E.J.W., Willies, A.J.) London: Edward Arnold

Received November 1, 1979
Accepted January 25, 1980
Communicated by O.E. Nelson

Dr. G. Wolff
Institut für Genetik
Universität Bonn
Kirschallee 1
D-5300 Bonn (Federal Republic of Germany)