MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXIX, No. 1, Article 4 (Biology), 1962

Taxonomic Study of the Genus *Acer*, with Special Reference to the Seed Proteins

II. Analysis of Protein

By

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(Received July 28, 1961)

A plant seed contains many kinds of proteins. Some of the properties of the plant, such as the genealogical and the physiological ones, are preserved within each protein. The serodiagnostic methods have been used for the phylogenetical study of plants since about 1910. The genealogical system obtained from the serodiagnosis was often quite different from the morphological system. It is, however, difficult to show the cause of difference between the serodiagnostic system and the morphological one. The cause was sometimes explained as follows: the phylogenetical relationships cannot be shown by the serodiagnostic system on account of that the proteins are changeable in accordance with their environments. This explanation has not been proved as regards the proteins. The proteins in a plant may be changeable in quantity, but not easily so in quality.

There are three means used to reveal the characters of proteins: physico-chemical method, chemical analysis and immuno-chemical method.

To gain the information of the cubic structure of a protein molecule, the physico-chemical method is superior to the chemical analysis. Besides, for the investigation of the cubic structure of a protein molecule, it may also be useful to measure the following items: the molecular weight, the electric charge, the molecular shape, the number of dissociative bases, the rate of dissociation and characters accompanied with the transformation of the protein molecule, etc. But the chemical analysis is superior to the method mentioned above in taking out structural amino acids one by one according to the combinational order. Though these two methods are useful to know the general structure, they are still insufficient for visualizing each structure of protein and for getting the information of the relationships among species, unless each protein is purely refined.

Detecting the specificity of every fractions of the proteins is useful for the phylogenetical study. The detailed data are obtainable from the examination

of the behaviours of the proteins in certain solvent; for example, the differential saltingout, the agarography and the turbidometric titration, and also from the electrophoretic studies of labeled nucleotides and the immunochemical method.

The immunochemical method is superior to the physico-chemical and the chemical methods in dealing with the protein mixture as it is, and also is superior in the strictness of the specificity of species. But the immunochemical method has difficulties in the direct examination of the structure of a protein. Another weak point is that the antigenic properties of protein are not always adequate for the experiments and the antibodies are not always uniform. Sometimes different sorts of proteins show similar diagnoses, because small similar antigenic parts of two different protein molecules strongly act upon the diagnosis. Moreover, it is required to count the number of common properties of the proteins, for example, to count the number by the absorption method.

For the phylogenetic study of the plant groups the writer wants to get information as to what extent they resemble or differ from each other in their proteins. As the writer can treat only the living plants with this method, the genealogical and the systematic relations among the plant groups may be suggested only to a certain degree. It is highly desirable to conduct in future analyses of small amount of proteins, amino acids and nucleotides in fossils. In analysing the chemical components of plants, a great deal of material is usually necessary.

The writer investigated the seed proteins of the genus *Acer* by means of the turbidometric titration and the electrophoresis on filter paper. From the results obtained he presumes the affinities of proteins among species. In this experiment, he examined the variation by comparing one individual with another. Then the writer proceeded to study the affinity among the plant groups.

Salting out of Protein

Seed protein solution becomes turbid by adding salt solution (for instance, saturated aqueous solution of ammonium sulphate) and leaving it still, in view of that the protein is partly salted out. Various volumes of the protein solution are mixed with various volumes of the salt solution, The mixtures become turbid according to salt concentrations. The quantities of proteins salted out (or the quantities of proteins not salted out) are measured for each mixture. Differences of the quantities of salted out proteins are calculated for every difference between the two neighbouring grade of salt concentration. The difference for each salt concentration is recorded. This method investigating the protein fractions was used already; DERRIEN (1952), STEYN-PARVE and VAN DEN HOUT (1953), VENTURA and HOLLANDA LIMA (1957) and others particularly investigated the protein fractions by making many steps of the salt concentration. They measured the quantities by the absorption at 276 mμ. AMIRKHANOVA *et al.* analysed the protein quantitatively by means of centrifuging the protein

fractions which were salted out (or precipitated) by the salt of heavy metal. These methods need plenty of protein.

The present writer, apart from them, has studied since 1951 the methods of fractionating a small quantity of protein by the following two methods.

The one is the turbidometric titration. In this method, the salt solution is mixed bit by bit into a small quantity of the protein solution to raise step by step salt concentration of the solution. At the same time, the quantity of protein salted out in every step is measured by the turbidity of the solution. By this process the turbidometric titration curve of the protein fractions is obtained. Then the increment of turbidity (ΔT) against the increment of salt concentration (ΔC) is derived from the curve. The diagram of $\Delta T/\Delta C$ to the salt concentration has the same meaning as the diagrams of VENTURA and the other authors above mentioned. In other words, this diagram shows the fractions of the protein.

The other method is the agarography. In this method, the seed protein solution is mixed into agaragar sol of about 1 per cent. A sheet of agaragar gel which is 1 mm in thickness and 100 mm in length is made from the sol. A vessel of about 10 cm in height is filled up with distilled water. Salt is diffused from the bottom of the vessel. Thus the salt solution with the gradient of salt concentration is made in the vessel. In the vessel the sheet of agaragar gel is hanged. The turbidity is measured from the bottom of the agaragar gel sheet, namely, from the high salt concentration to the low salt concentration. The protein fractions contained in this sheet can be known by the former calculating method.

The sheet of the agaragar gel is hanged for an hour, and then the sheet is taken out and put into the saturated solution of that salt. Then after about two hours, in the agaragar sheet appear white lines. The number of the white lines is equivalent to the number of the protein fractions. The position of a white line corresponds to the position of a salt concentration in which the fraction is first salted out.

K_2HPO_4–KH_2PO_4 buffer was used mainly as the solvent of the seed protein. The salt solution mainly used as the precipitant was ammonium sulphate aqueous solution, of which 4 M was standardized. And its dilutions were used in accordance with the object of each experiment.

Reexamining the experiment using the different matter as the precipitant or the solvent, the protein fractions are known more precisely.

Repeating the experiments for another material of the same kind in the same solvent, a certain component is found at the same salt concentration in each experiment. The reexamination of the above two materials in the other solvent increases the reliability of the identification.

Experiment on the Specificity of Seed Protein

The next experiment was planned for the investigation of the variation of

seed protein as the preliminary experiment on the affinity among plant groups.

The results of the experiments on several pure lines of cultivated *Brassica rapa* group are as follows. The seeds were analysed one by one, and the results of each seed were compared.

Each seed of a pure line has the same components of protein fractions. Among the lines, each component of the protein was somewhat different in quantity. Generally, the components were same in the quality, but a few components were different. As for the hybrid lines, the seed of F_1 had most of its parental components. The seeds of F_2 apparently showed combination or segregation of some protein components. The seeds obtained from the market, generally considered as refined, had in general many same protein components and a few different ones. Averaging these results of the analyses, general composition of the components was found.

The results of the comparative study of seed proteins obtained from *Brassica rapa*, *B. oleracea* and *B. napus* are as follows. Concerning the composition of the components, the seed protein of *B. napus* seems to consist of the components of *B. rapa* and *B. oleracea*. Cytogenetically, *B. napus* is proved to be composed of *B. rapa* and *B. oleracea*.

The writer arrived at the same conclusion from the experiment by means of antigen-antibody reaction. It has been found that anti-*B. napus* serum precipitates the antigens from *B. rapa* and *B. oleracea*, but also somewhat precipitates many other antigens from *Raphanus sativus*. By means of the absorption method, most of the anti-*B. napus* antibodies are absorbed by *B. rapa* antigens and *B. oleracea* antigens. The remainder of anti-*B. napus* antibodies are precipitated by *B. napus* antigens. This result suggests that the seed proteins of *B. napus* are mainly composed of those of *B. rapa* and *B. oleracea*. (In this experiment, the remainder of anti-*B. napus* antibodies partially precipitates the other antigens of related species.)

The writer tested the salting out experiment on the seeds of several species of *Pinus* and *Abies*. He recognized that the endosperm of each species was specifically distinct.

In *Benincasa cerifera*, there are many cultivated lines. The writer analysed the seed globulin fractions of the six lines of *Benincasa cerifera* at Dr. H. SUGIYAMA's request. The six lines are scarcely distinguished from each other by the slight differences in the size and shape of fruit and seed. These lines are divided into two groups by the slight difference in the main component of seed globulin fractions. The difference was ascertained by the comparative experiment using various solvents. The two groups respectively correspond to the following two groups, which are defined by the shape of the seed: the one has the seeds with fimbrillate elevated margin and the other has the entire seeds. Otherwise, the two groups obscurely differ from each other in the shape of the calyx tube. But there are intermediate forms between the two. The two groups do not correspod to the two groups defined by the shape or the

waxiness of the fruit. Thus the difference in seed protein corresponds to the certain morphological differences but not always.

Experiment on the genus *Acer*

Material and method: Seed proteins extracted from the embryo cotyledon of *Acer* were investigated by means of the turbidometric titration. The seed proteins were analysed in each seed. And the results were carefully compared.

The solvent for protein is composed of 1 M of ammonium sulphate and 0.4 M of sodium chloride aqueous solution, and buffered with 0.25 M of K_2HPO_4–KH_2PO_4 to pH 7.0. Before the proteins dissolved, carbonic acid gas and air in the solvent are dispelled. Using this solvent, the seed proteins of *Acer* are dissolved, though BÄRNER (1927) wrote that the seed protein was not dissolved in 1 per cent of sodium chloride aqueous solution.

Four M of ammonium sulphate aqueous solution was used as a precipitant. Solution of the seed proteins (0.001 per cent, 1.5 cc) is accurately measured, and is taken into a glass cell. Calculated volume of the ammonium sulphate solution is added through a microburette. After they are stirred for a few minutes, the turbidity of the mixture is measured. Then the next calculated volume of the ammonium sulphate solution is added to the mixture. By repeating this procedure at regular intervals, the protein is salted out step by step.

As the protein solution is diluted by adding ammonium sulphate solution, the turbidity measured at every step of the titration is adjusted on a fixed volume basis. The calibrated values of turbidity (T) are figured against the concentration of ammonium sulphate (C). The increments of turbidity to the increments of concentration of ammonium sulphate ($\Delta T/\Delta C$) are figured to C. This figure is named "turbidometric titration diagram". The number of peaks on this curve corresponds to the number of the components of the protein. Most of the seed protein fractions are salted out between 40 per cent (V/V) saturation (1.6 M) and 70 per cent (V/V) saturation (2.8 M) of the ammonium sulphate aqueous solution. Within this salt concentration about 25 components of each individual are found. These components of seed protein in each seed are carefully compared with each other among individuals or among species.

Results: The diagrams of turbidometric titration of *A. rufinerve* are not in accord with the variation in the length of pedicels nor with the slight variation in the folding manner of the cotyledons, etc. (Fig. 9–1).

In the diagrams of *A. mono* there is considerable fluctuation of the quantity of each component. The fluctuation decreases between 55 per cent (V/V) saturation and 70 per cent (V/V) saturation of ammonium sulphate. And it increase especially between 50 per cent (V/V) saturation and 55 per cent (V/V) saturation.

Two trees of *A. buergerianum* were compared. The one has small fruits with the patent wings and obtusely keeled locules, and usually has some

undivided leaves beside three lobed leaves. The other one has larger fruits with parallel wings and acutely keeled locules, and has rarely undivided leaves. According to the writer's experiments, the quantity of the component salted out at the concentration of 56.3 per cent (V/V) saturation was most abundant in the former, while in the latter the quantity (of the component salted out) at 53.2 per cent (V/V) saturation was most abundant. As for the tree morphologically intermediate between the two, roughly intermediate quantities are observed concerning both components. The diagrams of turbidometric titration of the two different trees have 17 components in common with each other out of 19. Of the other 4 components, 2 of them were common to some of the other individuals and the remaining 2 were characteristic components of the two individuals respectively (Fig. 9-3).

From the interspecific comparison of the turbidometric titration diagrams it was found that the nature of seed protein was nearly fixed for each species (Figs. 9-1~12).

Comparing the diagrams of *A. spicatum* (from N. America) and *A. ukurunduense* (from Japan), there are 5 specific components in each species and 19 common components. The main components of both seed proteins are salted out between 46 per cent (V/V) saturation and 46.5 per cent (V/V) saturation of ammonium sulphate. In their morphological characteristics, too, these two species are related to each other (Fig. 9-4).

Comparing the diagrams of *A. campestre* and *A. miyabei*, there are 13 common components. And also in the morphological characters these two species are related to each other, though they are separately distributed in Europe and Japan (Fig. 9-5).

Within *A. palmatum*, the range of morphological variation is wide. The groups which are distinguished by the difference of the turbidometric titration diagram correspond to subsp. *matsumurae*, subsp. *amoenum* and subsp. *palmatum*. In subsp. *palmatum*, two subgroups may be distinguished by the diagram of the protein. These two subgroups roughly correspond to the subgroups divided by the size of flower and leaf.

Among *A. palmatum*, *A. sieboldianum*, *A. japonicum*, *A. shirasawanum*, *A. tenuifolium*, *A. pseudosieboldianum* and *A. circinatum*, the turbidometric titration diagrams of each species greatly resemble each other. In these species more than 80 per cent of seed proteins are salted out between 60 per cent (V/V) saturation and 65 per cent (V/V) saturation of ammonium sulphate. In the morphological characteristics, too, these species are closely related to each other (Fig. 9-6).

As for *A. glabrum*, *A. argutum*, *A. barbinerve*, *A. tetramerum*, *A. stachyophyllum*, *A. rufinerve*, *A. nipponicum*, *A. kawakamii*, *A. crataegifolium*, *A. micranthum*, *A. tschonoskii*, *A. ukurunduense* and *A. spicatum*, there are gradual differences along this sequence concerning the components of the seed proteins. The first 11 species of this sequence were included in *Intrastaminalia* PAX sensu KOIDZUMI,

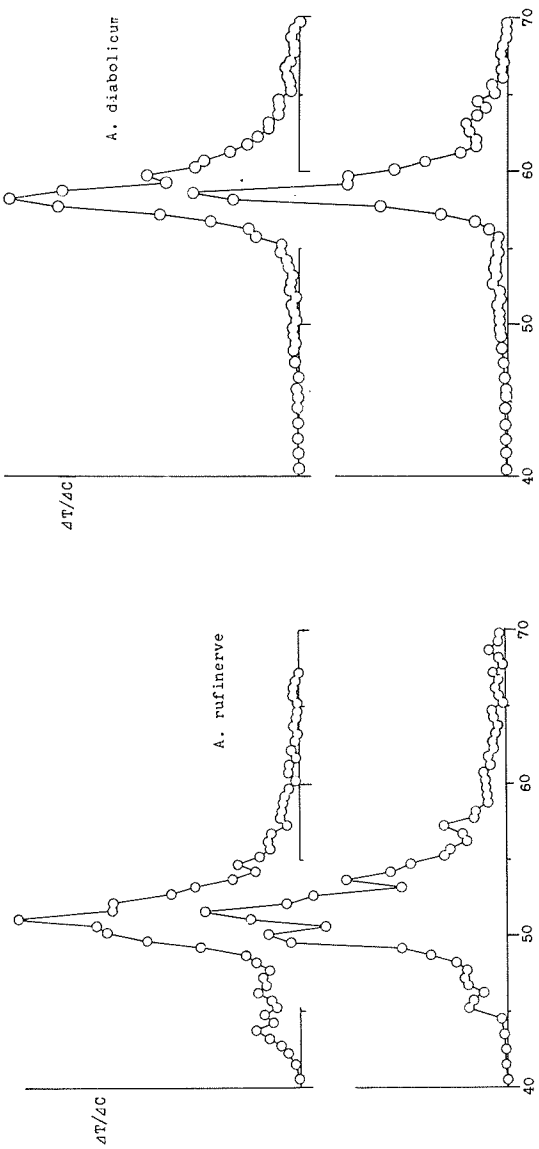


Fig. 9-1. Intraspecific variation of seed protein of *A. rufinerve*.

Fig. 9-2. Intraspecific variation of seed protein of *A. diabolicum*.

Fig. 9 (1-12). Increment of turbidity at various concentration of ammonium sulphate. ΔT shows the difference before and after each step of titration. ΔC is the variance of the concentration of ammonium sulphate.

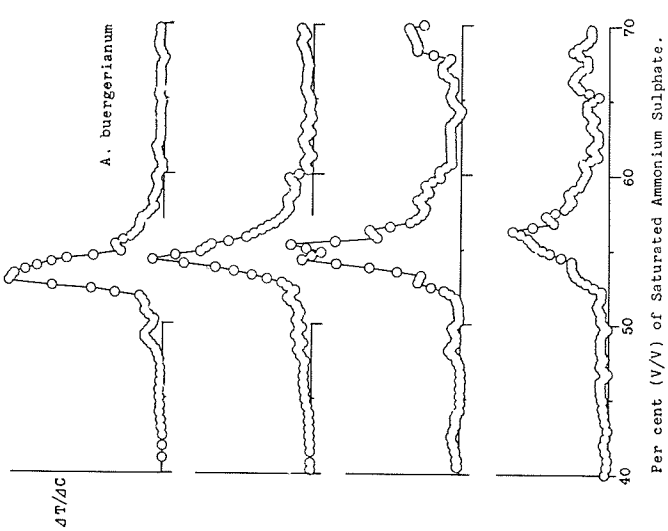


Fig. 9-3. Intraspecific variation of seed protein of *A. buergerianum*.

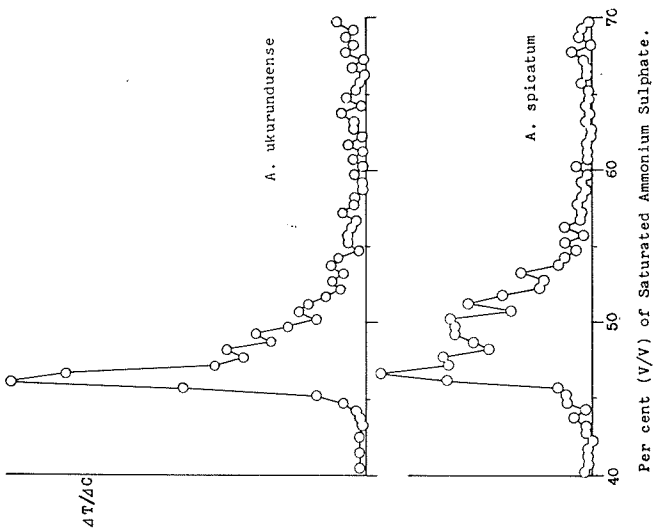


Fig. 9-4. Comparison of seed protein. *A. ukurunduense*—Japan. *A. spicatum*—N. America.

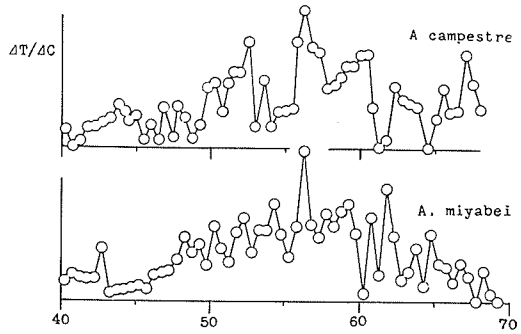


Fig. 9-5. Comparison of seed protein. *A. campestre*—Europe. *A. miyabei*—Japan.

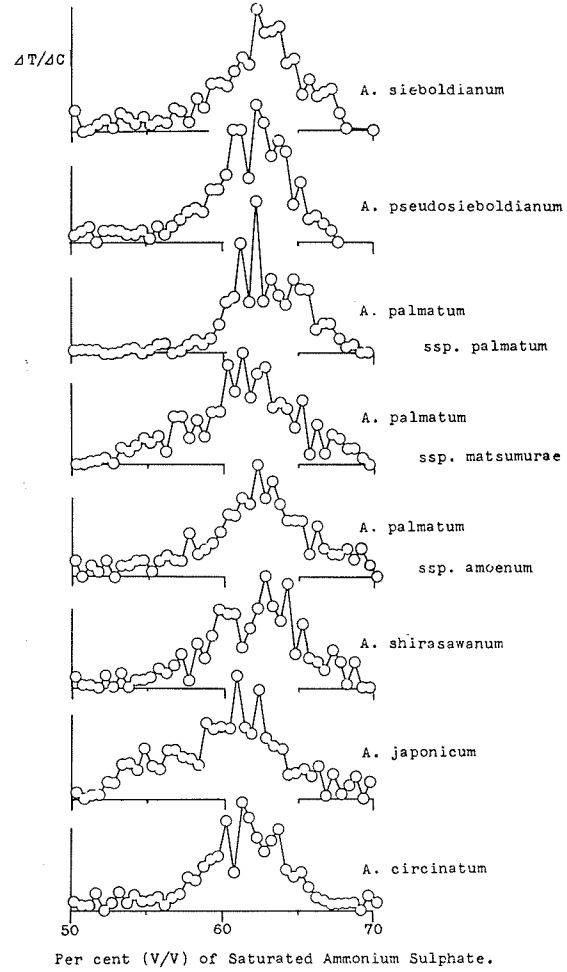


Fig. 9-6. Comparison of seed protein.

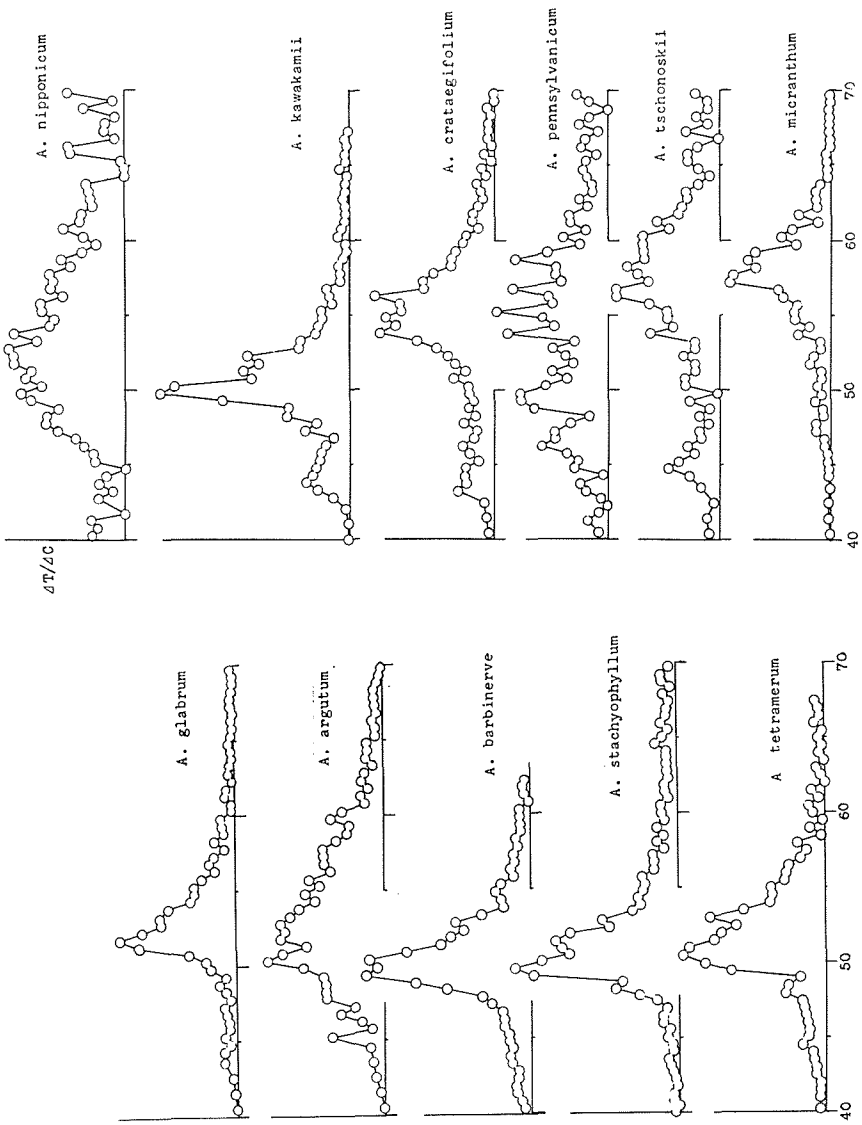


Fig. 9-8. Comparison of seed protein.

Fig. 9-7. Comparison of seed protein.

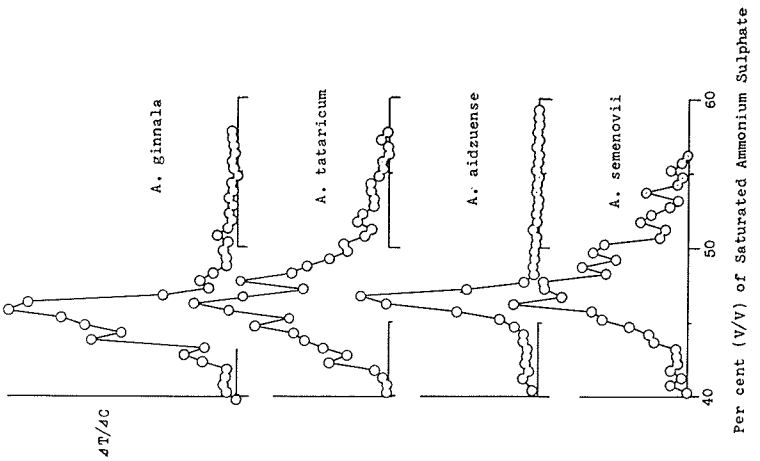


Fig. 9-9. Comparison of seed protein.

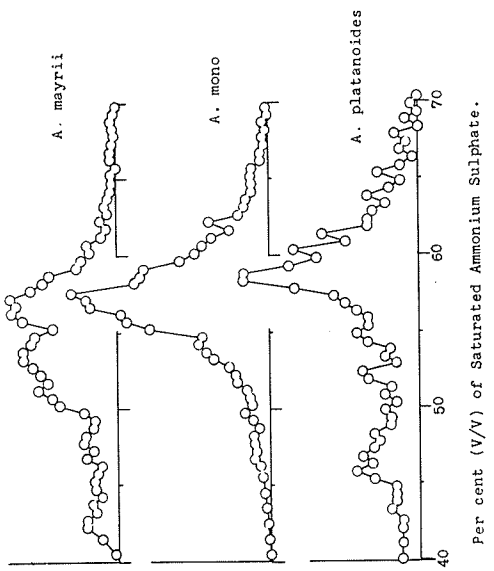


Fig. 9-10. Comparison of seed protein.

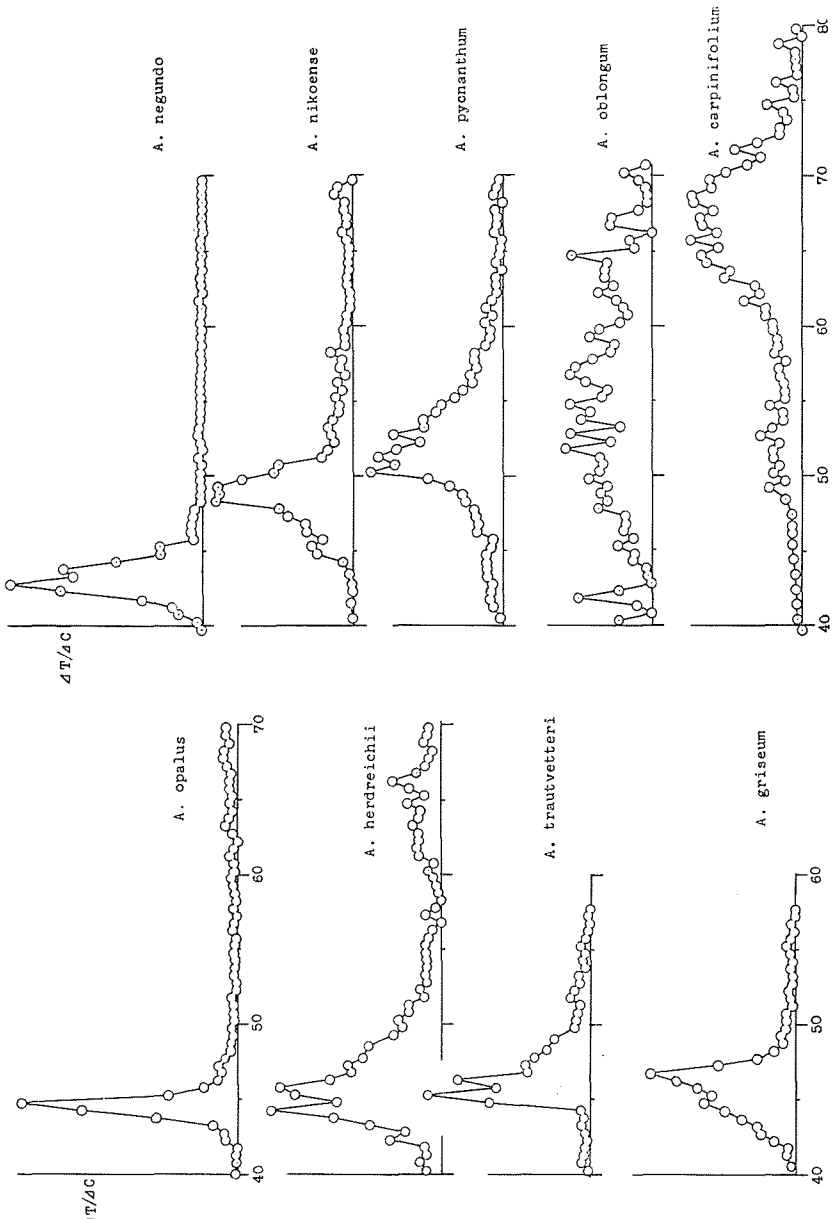


Fig. 9-11. Comparison of seed protein.

Fig. 9-12. Comparison of seed protein.

and the two remainders in the section *Spicata* of *Extrastaminalia* (Fig. 9-7, 9-8 and 9-4).

Acer crataegifolium and *A. micranthum* have 15 common components. In both species, more than 80 per cent of seed proteins are salted out between 52 per cent (V/V) saturation and 62 per cent (V/V) saturation of ammonium sulphate.

Acer crataegifolium and *A. rufinerve* are closely related to each other morphologically, and both have 10 common components. But in *A. rufinerve*, more than 80 per cent of seed proteins are salted out between 47.5 per cent (V/V) saturation and 55 per cent (V/V) saturation of ammonium sulphate. And also more than 80 per cent of seed proteins of *A. glabrum*, *A. argutum*, *A. tetramerum*, *A. barbinerve*, *A. stachyophyllum* and *A. pycnanthum* are salted out between 47.5 per cent (V/V) saturation and 55 per cent (V/V) saturation of ammonium sulphate. Among these species, *A. glabrum* and *A. argutum* have 12 common components, and *A. argutum*, *A. tetramerum*, *A. barbinerve*, and *A. stachyophyllum* have more than 12 common components. But *A. pycnanthum* *A. glabrum*, *A. argutum* and *A. rufinerve* have less than 7 common components. *Acer glabrum*, *A. argutum* and *A. rufinerve* have more than 11 common components.

As for the concentration of ammonium sulphate, there is a range in which most of the seed proteins is salted out. Among the species not so closely related to, whose ranges of salting out are the same, there are generally some different components within those ranges.

These results suggest that the number of the common components of the seed proteins accords with the morphological affinity.

Diagram of protein affinity: The following signs are used in this work. For indicating the degree of affinity between species *A* and *B*, "*a*" is chosen as a number of components of species *A*, "*b*" is chosen as a number of components of *B*, and "*p*" is chosen as a number of components which are common to *A* and *B*. Then the rate of difference and resemblance of the components between *A* and *B* is $(a+b-2p)/p$. The smaller the value of the rate is, the greater is the resemblance of the components between *A* and *B*. The value of $(a+b-2p)/p$ is concerned with the number of the components between *A* and *B*, and is not concerned with the quantity of each components. The value may be called the affinity of the components between *A* and *B*.

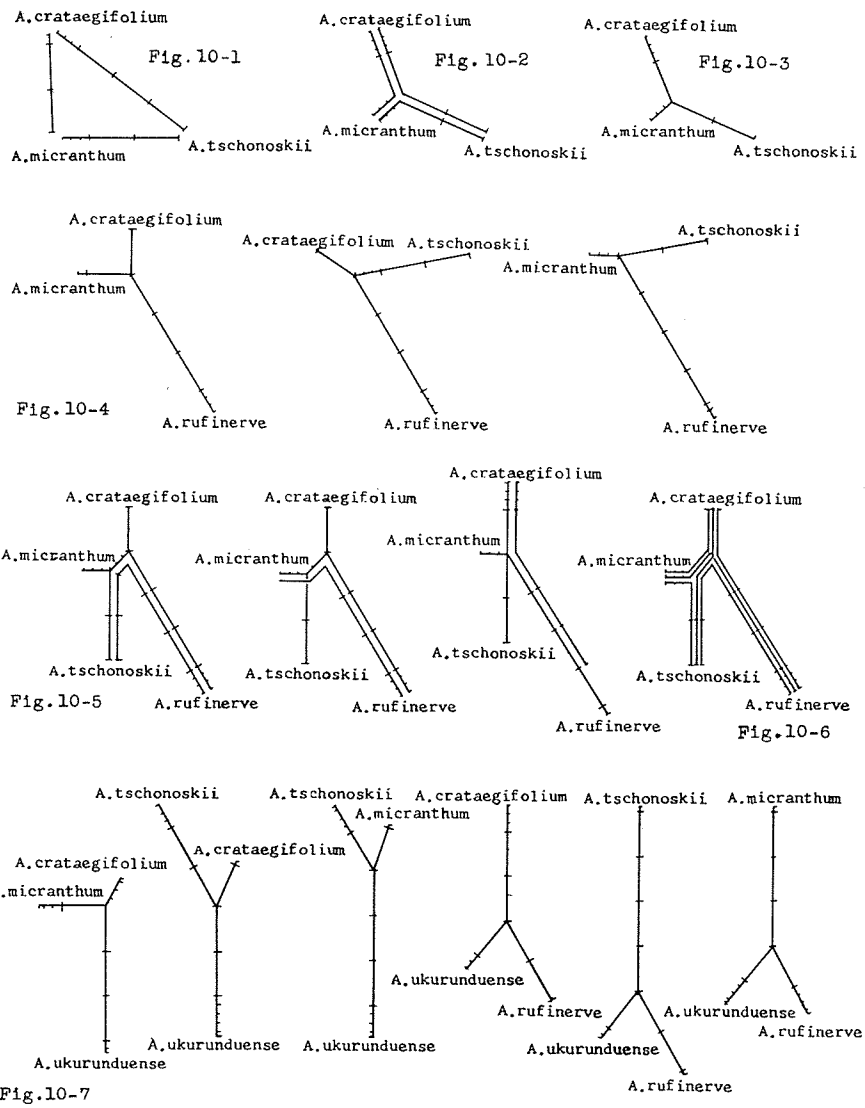
Taking a length for a substitution of the value, the relation of the affinities of the components among experimented species can be shown on a diagram.

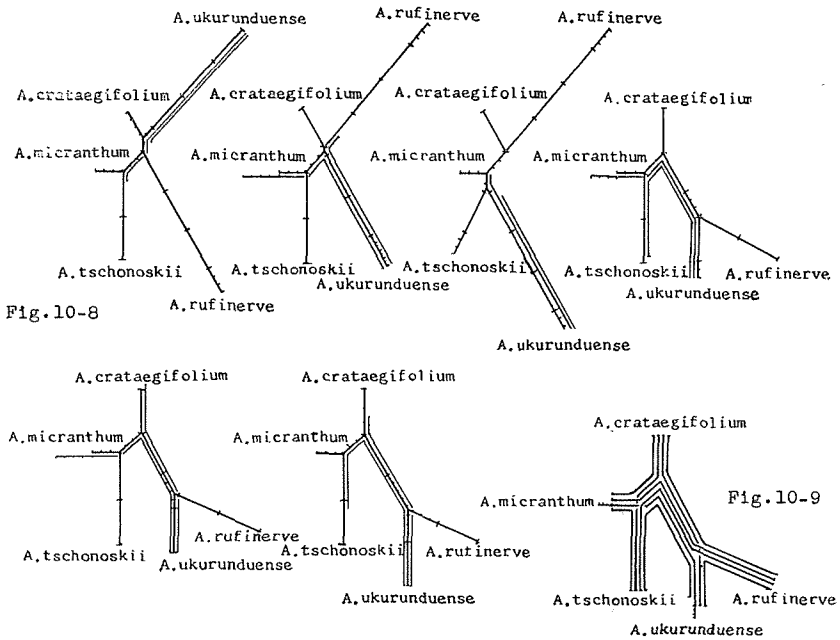
The affinities of the components among the three species, *A. crataegifolium*, *A. tschonoskii* and *A. micranthum*, can be depicted as shown in Fig. 10-1. In this figure, only the lengths of the segments are concerned with relations among the species, and the relative position of the segments has no meaning. Therefore the expression of Fig. 10-1 can be transformed to Fig. 10-2 or to Fig. 10-3.

The affinities among *A. rufinerve* and two of above three species are depicted as shown in Fig. 10-4. Adding Fig. 10-3 to Fig. 10-4, the affinities among these

four species are shown in Fig. 10-5. Among these three diagrams in Fig. 10-5, the right one is not accepted because the length of segment between *A. crataegifolium* and *A. rufinerve* is discrepant. And the remaining two are accepted. These two are the same.

When each diagram has discrepancy to some extent, a diagram with the





least discrepancy is accepted. Figure 10-6 is delineated by the same manner used in Fig. 10-2.

The affinities among *A. ukurunduense* and two of the above four species are depicted as shown in Fig. 10-7. Adding Fig. 10-5 to Fig. 10-7, the affinities among these five species are shown in Fig. 10-8. Among six diagrams in Fig. 10-8 (though the total number of the calculable combinations is 36), the fourth diagram from the left is accepted. Figure 10-9 is delineated by the same manner used in Fig. 10-2.

The relation of the lengths among all experimented species is shown in Fig. 11. This figure roughly shows the affinities of the protein components among all experimented species.

The expression concerning the relation among different species of a genus, as shown in Fig. 11, may be applicable to some extent to that among different lines of a species. The diagram of the relations of components among individuals of a species shows some netty parts.

In this figure, the affinity between two species is expressed by the total length of the segments which connect the two species at the shortest course.

Distribution of the common components of protein: In this article, a component is called by the relative volume of saturated ammonium sulphate solution (total volume: 100), in which the component is salted out. For example, 46.80 means a protein component salted out at 46.80 per cent (V/V) saturation of

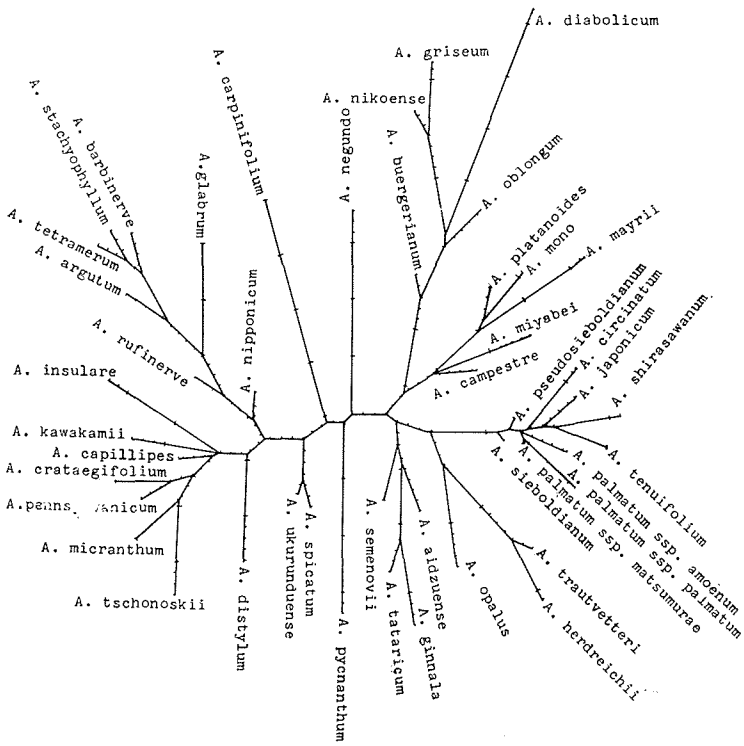


Fig. 11. Diagram of protein affinity.

ammonium sulphate.

Each component of protein is distributed on this figure, as follows.

Components 43.8, 46.3, 50.1, 66.2 and 68.8 are found nearly in all experimented species; 61.5 is found in *A. negundo*, *A. pycnanthum*, *A. carpinifolium*, *A. ukurunduense*, *A. spicatum*, *A. crataegifolium*, *A. pennsylvanicum*, *A. micranthum*, *A. tschonoskii*, *A. insulare*, *A. capillipes*, *A. kawakamii*, *A. distylum*, *A. nipponicum*, *A. rufinerve*, *A. glabrum*, *A. argutum*, *A. tetramerum*, *A. barbinerve*, *A. stachyophyllum*; 45.0, 53.7 and 64.9 are found in the above mentioned species except *A. negundo*; 42.5, 47.2 and 67.5 are in *A. crataegifolium*, *A. pennsylvanicum*, *A. micranthum*, *A. tschonoskii*, *A. insulare*, *A. capillipes*, *A. kawakamii*, *A. distylum*, *A. nipponicum*, *A. rufinerve*, *A. glabrum*; 42.8 and 59.3 are in *A. negundo*, *A. herdreichii*, *A. trautvetteri*, *A. ginnala*, *A. aidzuense*, *A. tataricum*, *A. semenovii*, *A. palmatum*, *A. japonicum*, *A. circinatum*, *A. shirasawanum*, *A. sieboldianum*, *A. pseudosieboldianum*, *A. tenuifolium*, *A. campestre*, *A. miyabei*, *A. platanoides*, *A. mono*, *A. mayrii*, *A. buergerianum*.

Generally, the shorter the sum of the segments among the species, the

greater the number of the common components. For instance, 46.8, 52.3, 53.3, 54.8, 55.8, 57.0, 62.2, 65.3 and 67.2 are found only in *A. palmatum*, *A. japonicum*, *A. circinatum*, *A. shirasawanum*, *A. sieboldianum*, *A. pseudosieboldianum* and *A. tenuifolium*; 42.8, 45.8, 48.3, 55.3 and 54.0 are found in *A. ginnala*, *A. aidzuense*, *A. tataricum*, *A. semenovii*, *A. herdreichii*, *A. trautvetteri* and *A. opalus*; 41.2, 55.0, 56.5, 60.3 and 65.8 are found in *A. crataegifolium*, *A. pennsylvanicum*, *A. tschonoskii*, *A. micranthum* and *A. kawakamii*.

Only six components out of about ninety components are found in two groups separated remotely from each other by the several intermediate segments. And these six components are found only in a few species. For instance, 69.8 is found in *A. buergerianum*, *A. oblongum* and *A. circinatum*, 69.3 is found in *A. opalus* and *A. platanoides*, 60.8 is found in *A. nikoense*, *A. diabolicum* and *A. carpinifolium*, 59.9 is found in *A. argutum* and *A. carpinifolium*, 46.8 is found in *A. griseum* and *A. argutum*, 43.3 is found in *A. crataegifolium* and *A. oblongum*.

Thus all intermediate segments between the two species roughly represent the sum of the common differences.

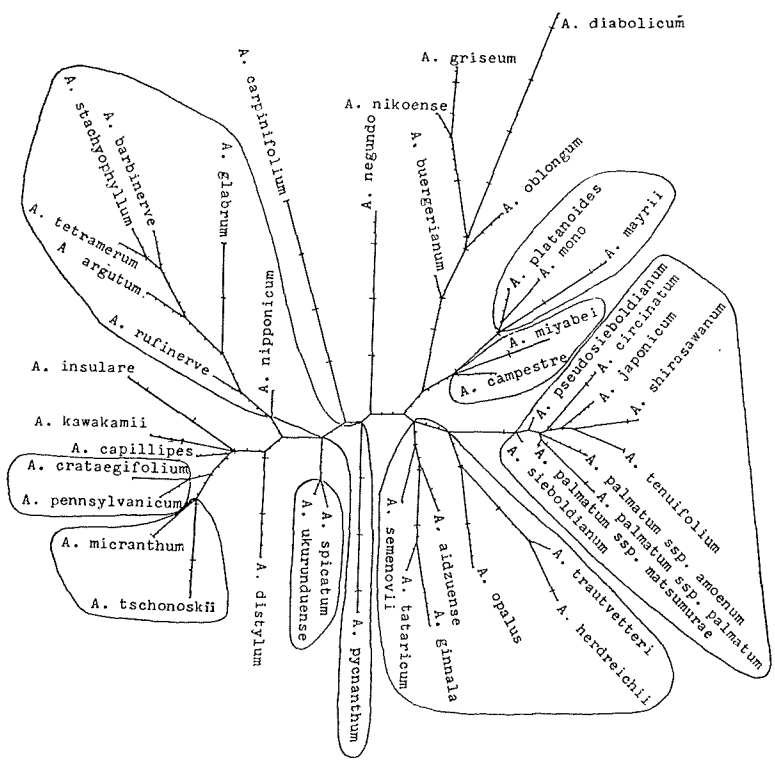


Fig. 12. The range of salt concentration.

Therefore in other words, the writer was able to summarize the relation among all experimented species in Fig. 11 with a few discrepancies.

The range of the salt concentration: Each group of Fig. 12 includes the species in which more than 80 per cent of its proteins are salted out in the same range of the concentration of ammonium sulphate solution. Similar groups are formed also by means of electrophoresis on filter paper concerning the cataphoretic values of each protein.

Thus the range of the concentration of salting out is usually the same among the species having many components in common. But the range is sometimes the same among the species having a few common components (e. g., between *A. opalus* and *A. ginnala* or between *A. pycnanthum* and *A. glabrum*). This is regarded as the accidental coincidence or parallelism.

Comparison between the Diagram of Protein Affinity and the Taxonomic System

In Fig. 11, the shorter the segments between two given species, the closer the affinity of protein between the two. Here the writer compares the affinity of protein with the taxonomic affinity. For this purpose, the writer encircles on this diagram those species which have been grouped by REHDER (Fig. 13).

Comparison of this diagram with the REHDER's system (in Manual of Cultivated Trees and Shrubs, 1940): Sect. *Platanoidea* of REHDER includes *A. platanoides*, *A. miyabei*, *A. mono* and *A. mayrii*.

In this diagram, *A. platanoides*, *A. mono* and *A. mayrii* are closely related to one another by the protein components, and connected with short segments, and may be included in a line as a compact group. Thus the grouping accords with the taxonomic system. In other words, the affinity of the protein components shows the taxonomic affinity among the species.

In this diagram, *A. miyabei* is closely related by the protein components to *A. campestre* of sect. *Campestria.*

Section *Campestria* of REHDER includes *A. campestre* and *A. opalus*. This section is distinguished from *Platanoidea* by the fruits with convex, strongly veined nutlets. But *A. campestre* has nutlets not so strongly convex nor strongly veined as the other members of this section. The latter may be removed from *Campestria*, forming series *Monspessulana* of sect. *Platanoidea.*

In the writer's conception, *Campestria* is closely related to *Platanoidea*, differing from the latter in the slightly convex nutlets and in the obtuse leaf-lobes, forming a series of the sect. *Platanoidea* as already mentioned by POJARKOVA.

Acer miyabei is taxonomically very closely related to *A. campestre*, and should be included in series *Campestria.*

Thus the affinity of the protein components shows the taxonomic affinity between *A. campestre* and *A. miyabei* as expressed by the short segments.

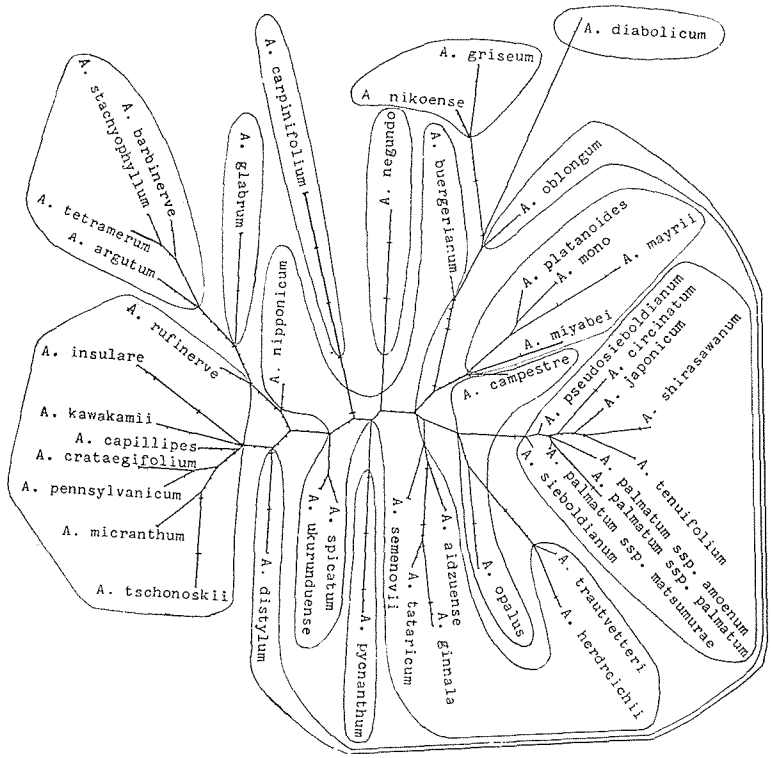


Fig. 13. Comparison of Fig. 11 with REHDER's system.

Series *Monspessulana* is rather remotely related by the protein components to sect. *Platanoidea*.

Section *Saccharina* of REHDER has no data in this diagram.

Section *Spicata* of REHDER includes *A. herdreichii*, *A. trautvetteri*, *A. buergerianum*, *A. ginnala*, *A. tataricum*, *A. semenovii*, *A. aidzuense*, *A. spicatum*, *A. ukurunduense*, and *A. nipponicum*. In this diagram, *A. spicatum* and *A. ukurunduense* are very closely related by many common components of protein. The protein affinity coincides with the taxonomic affinity regarding the relation between the two.

Acer ginnala, *A. tataricum*, *A. aidzuense* and *A. semenovii* are very closely related by the protein affinity and may be included in a compact group. Thus, protein affinity accords with the taxonomic affinity, regarding the relation among those species.

Acer herdreichii and *A. trautvetteri* are very closely related. The protein affinity between the two accords with the taxonomic affinity.

While, the above three groups and *A. buergerianum* and *A. nipponicum* are

separated from each other by the differences of protein components. Their protein affinities do not accord with the taxonomic affinities proposed by REHDER.

Section *Spicata* of REHDER is characterized by the extrastaminal disc, convex and strongly veined nutlets and andro-polygamous flowers in panicle.

Acer spicatum and *A. ukurunduense* are intrastaminal in the writer's conception, because the discs are lobulated. The scales of winter buds are two-paired. The nutlets of both the species are plane, and finely veined.

Acer nipponicum is intrastaminal. The scales are two-paired. The nutlets are convex and obscurely veined.

Acer distylum is classified in sect. *Integrifolia* according to REHDER. But this species is related to *A. nipponicum* by the intrastaminal disc, two-paired bud scales, obscurely veined convex nutlets and crenulate leaves.

The writer includes in sect. *Spicata A. spicatum, A. ukurunduense, A. nipponicum* and *A. distylum,* and excludes the other species. These four species are related to each other by the protein affinity, except *A. distylum* which is a little more closely related to *A. capillipes* of sect. *Macrantha* than to *A. nipponicum.*

Thus, the protein affinity accords with the taxonomic affinity with this one exception.

Acer ginnala, A. tataricum, A. aidzuense and *A. semenovii* are extrastaminal. Their winter buds have many scales. Their nutlets are plane and strongly veined. The leaves are three lobed or undivided and irregularly serrated. The writer includes these species in sect. *Trilobata* according to the opinion of POJARKOVA.

Thus the protein affinity coincides with the taxonomic affinity proposed by POJARKOVA.

Acer trautvetteri and *A. herdreichii* have extrastaminal discs. Their winter buds have many imbricate scales. Their nutlets are convex and have thick obscure veins.

The writer includes the two species in sect. *Gemmata* according to POJARKOVA.

Acer buergerianum has extrastaminal disc. The winter buds have many scales. The nutlets are keeled convex. The leaves are three lobed or undivided, entire or remotely serrated.

The writer includes this species in sect. *Integrifolia* and distinguishes it as a series of *Integrifolia.*

Thus REHDER's sect. *Spicata* is divided into several distinct sections in this work.

Section *Palmata* of REHDER includes *A. circinatum, A. palmatum, A. sieboldianum, A. japonicum, A. pseudosieboldianum, A. shirasawanm* and *A. tenuifolium.* In the diagram these species are very closely related to each other by the protein components and may be included in a line as a compact group. Thus

the protein affinity coincides with the taxonomic affinity.

Section *Glabra* of REHDER includes *A. glabrum*. In the diagram, *A. glabrum* has no closely related species, and is remotely related to *A. argutum*, *A. barbinerve*, *A. tetramerum* and *A. stachyophyllum*, and will be discussed later.

Section *Integrifolia* of REHDER includes *A. oblongum* and *A. distylum*. In this diagram, *A. oblongum* and *A. distylum* are separated from each other by many segments, in other words differing from each other in many protein components.

In this work *A. distylum* is excluded from sect. *Integrifolia* and included in sect. *Spicata*.

As mentioned above, *A. distylum* has an intrastaminal disc. The winter buds have two-paired scales. The nutlets are convex. The leaves are undivided, three nerved and crenulate. While *A. oblongum* has an extrastaminal disc. The winter buds have many scales. The nutlets are keeled convex. The leaves are entire. The two species were chosen by the shape of leaf and the andro-polygamous panicle.

In this article the writer includes *A. oblongum* and *A. buergerianum* in sect. *Integrifolia* as already mentioned. The two species are closely related to each other by the protein.

Thus the protein affinity accords with the taxonomic affinity as explained by the writer.

Section *Indivisa* of REHDER includes *A. carpinifolium*. In this diagram, *A. carpinifolium* is isolated from the other species by many different protein components.

The disc is intrastaminal. The winter buds have many scales which are very different from those of the other species. The nutlets are plane, having fine parallel nerves. The leaves are undivided, acutely and finely biserrate, and have many parallel side-veins.

The writer regards this section as one of the three subgenera of *Acer*.

Thus the protein affinity accords with the taxonomic affinity.

Section *Macrantha* of REHDER includes *A. crataegifolium*, *A. pennsylvanicum*, *A. rufinerve*, *A. capillipes*, *A. tschonoskii*, *A. micranthum*, *A. kawakamii* and *A. insulare*. In this diagram these species are closely related to each other by protein components, except for *A. rufinerve* which is a little remotely related.

Section *Macrantha* is characterized by the intrastaminal disc, winter buds with two paired scales, nutlets concave on one side, the accumbent cotyledons, and by the simple raceme.

Acer rufinerve has the nutlets convex on both sides and the incumbent cotyledons.

By the protein affinity, *A. rufinerve* is closely related to *A. nipponicum*. Both *A. rufinerve* and *A. nipponicum* have the convex nutlets and incumbent cotyledons.

The writer put *A. rufinerve* in sect. *Macrantha*, instead of including it in

sect. *Spicata* with *A. nipponicum*, because of the simple unisexual raceme. Here is the intricate relation, rarely found in this diagram.

Section *Arguta* of REHDER includes *A. argutum*, *A. barbinerve*, *A. tetramerum* and *A. stachyophyllum*. In this diagram, these species are closely related to each other by the protein components, forming a compact group. Thus the protein affinity coincides with the taxonomic affinity.

Acer glabrum of sect. *Glabra* is related to sect. *Arguta* in the intrastaminal disc, winter buds with two-paired scales, the male inflorescence frequently lateral and by the female inflorescence frequently bracteolate. But *A. glabrum* has the keeled convex nutlets, and sometimes male flowers in the female inflorescence.

So the writer includes *A. glabrum* in sect. *Glabra*, series *Glabra*. Section *Glabra* is the earlier synonym of sect. *Arguta*. Section *Arguta* of REHDER is treated in this article as sect. *Glabra*, series *Arguta*.

Section *Lithocarpa* of REHDER includes *A. diabolicum*. In this diagram, *A. diabolicum* is isolated from the other species of *Acer*.

Section *Rubra* of REHDER includes *A. pycnanthum*. In this diagram *A. pycnanthum* is isolated from the other species of *Acer*.

Section *Trifoliata* of REHDER includes *A. nikoense* and *A. griseum*. In this diagram the two species are closely related to each other by the protein components, forming a compact group.

Section *Negundo* of REHDER includes *A. negundo*. In this diagram *A. negundo* is isolated from the other species of *Acer*.

Thus, in sects. *Lithocarpa*, *Rubra*, *Trifoliata* and *Negundo*, the protein affinity accords with the taxonomic affinity.