日本産ツクバネソウ連の種子のアイソザイム

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: Some Seed Isozymes in the Japanese Paridae

井原正昭：日本産ツクバネソウ連の種子のアイソザイム

Enzyme polymorphism has been known in various organisms, which provided a new facet of evolutionary studies (e.g., AYALA 1976). In comparison with anatomy and/or gross morphology, electrophoretic means enables us to study direct products of genes, so that it is possible to have an insight into the organic evolution.

This paper describes four isozymes observed in seeds of *Paris* and *Trillium*, viz., alcohol dehydrogenase (EC. 1.1.1.1 ; ADH), acid phosphatase (EC. 3.1.3.2 ; Acp), peroxidase (EC. 1.11.1.7 ; Px) and 6 phosphogluconate dehydrogenase (EC. 1.1.1.44 ; 6PGD).

Materials and Method

Sources of materials Seeds were collected either from natural populations or at nursery gardens of University of Tokyo at Koishikawa and Nikko. Original localities of the plants are listed in Table 1.

Electrophoresis A 10 % starch gel electrophoresis was employed for the study as described elsewhere (IHARA & ENDO 1981).

Enzyme assays Methods of respective enzyme assays were carried out as follows with a minor modification in each case: ADH after SCHWARTZ and ENDO (1966), Acp after ENDO *et al* (1971), Px after ENDO (1978) and 6PGD after BENDER and OHNO (1968).

Table 1. Original localities of the materials investigated

Species	Localities
T. kamtschaticum	ca. 1 km SE of Kuzakai sta., Miyako line of JNR ; Iwate-ken.
T. tschonoskii	ca. 4 km E of Torisawa sta., Chuo line of JNR ; Yamanashi-ken.
T. smallii	ca. 1 km NW of Hosoo, Nikko, Tochigi-ken ; about half way of S folk of the Daiya River.
P. tetraphylla	ca. 1 km S of Chuzenji bus-stop, Lake Chuzenji, Tochigi-ken.
P. verticillata	Nikko Botanical Garden, Univ. of Tokyo, Nikko, Tochigi-ken.

Results and Discussion

1. *Alcohol dehydrogenase* As shown in Figure 1, the Japanese trilliums show 5 bandmorphs, viz., a fast moving band in *T. kamtschaticum* PALL., 3 slow moving bands in *T. tschonoskii* MAXIM. and a fast, an intermediate and a slow moving band in *T. smallii* MAXIM. Genetics of these bandmorphs are reported previously (IHARA & ENDO 1981); thus, the fast moving isozyme in *T. kamtschaticum* is coded by $Adh^{F(K)}/Adh^{F(K)}$; in *T. tschonoskii* these three bands are composed of two homodimers and one heterodimer coded by $Adh^{S}1^{(T)}$ and $Adh^{S}2^{(T)}$ which exist in a state of "fixed heterozygosity" because of no segregation in meiosis. Fixed heterozygosity is also seen in *T. smallii*, in which the monomer of the fast moving isozyme is yielded approximately 1/10 less than that of the slow moving isozyme, resulting less intense activity of the heterodimer (FS dimer) as expected from random association of these two

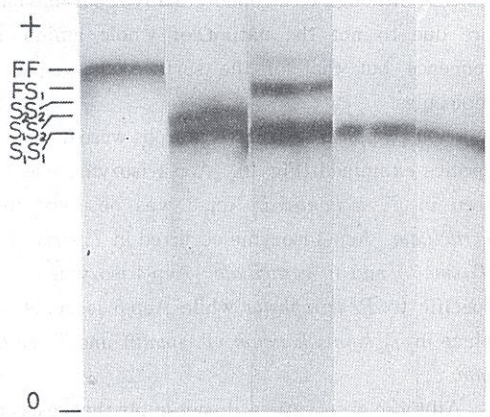

Fig. 1. Zymograms showing seed alcohol dehydrogenase isozymes in the Japanese Paridae. Electrophoretic run was done at 250 V for 4 hr in a refrigerator (8°C). Zymograms are arranged from left to right as follows: *T. kamtschaticum*, *T. tschonoskii*, *T. smallii*, *P. verticillata* and *P. tetraphylla*. Abbreviation: +, anode ; o, origin ; F, S_1 and S_2, fast and slow moving monomers.

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subunits of the isozyme, viz., $(3S^f + 3U^s)^2$ where S^f and U^s are monomers coded by $Adh^{F(S)}$ and $Adh^{S(U)}$, respectively. In other words, it is needed for the explanation of the zymograms to postulate the operation of a regulator gene which controls the yield of the S^f monomer.

The electromorphs of *Paris verticillata* M. v. BIEB. and *P. tetraphylla* A. GRAY were slow moving ones, corresponding to those observed in trilliums, viz., the S_1 band of *T. tschonoskii* and the S band of *T. smallii*.

Recently, it was found that the slow moving isozymes are also seen in the seed alcohol dehydrogenase of both the pedicellate- and sessile-flowered North American species, viz., *T. erectum* L, *T. cernuum* L, *T. flexipes* RAF., *T. ovatum* PURSH., *T. sessile* L, *T. cuneatum* RAF., *T. luteum* HARBISON and *T. stamineum* HARBISON. (IHARA unpublished data). This evidence entails why the slow moving isozymes are so conservative in such distantly separated species, although electrophoretically separable bands are due to not the nature of whole amino acid sequence but that of the surface charge of the molecules.

2. *Acid phosphatase* 5 electromorphs were detected in species examined (Fig. 2). Acp-1 isozyme was only seen in *T. tschonoskii*. Acp-2 was observed in *P. verticillata*. Acp-3 isozyme occurred in *T. tschonoskii*, *T. smallii* and *P. verticillata*. Acp-4 isozyme may be specific to *P. tetraphylla* while Acp-5 isozyme took place in *T. kamtschaticum*, *T. smallii* and *P. verticillata*.

All these observations do not imply that respective isozymes occur exclusively in each species as described above. Thus, different occurrence of these isozymes would be expected in different developmental stages of the seeds as well as in different organs. Gentics of these isozymes are not clear, so that it is uncertain how many loci contribute to bring about these 5 isozymes.

3. *Peroxidase* With the present enzyme assay, 3 isozymes were seen cathodally and 2 isozymes which are common to all the species investigated were detected anodally (Fig. 2). The anodal isozymes are not displayed in the figure. Px-A_1 isozyme was seen in *P. tetraphylla* whereas Px-A_2 isozymes were detected in both species of *Paris*. Px-A_3 was common to trilliums exclusively.

Since peroxidases are either monomeric or dimeric in plant species (e.g., HAMILL & BREWBAKER 1969; SHAHI *et al* 1969), these isozymes may be governed by three different loci. Plant peroxidases are known to be highly polymorphic (e.g., SHEEN 1970) unlike the present materials. In this connection it may be noteworthy that the present assay sometimes fails to detect peroxidase activities in dicotyledon even when the activities are detected by the use of Benzidine as a dye coupler (IHARA unpublished data). Therefore it is possible that fairly homomorphic occurrence of peroxidase isozymes in the present study is due to the enzyme assay employed here. But direct and more sophisticated analyses are needed for further insight of the problem.

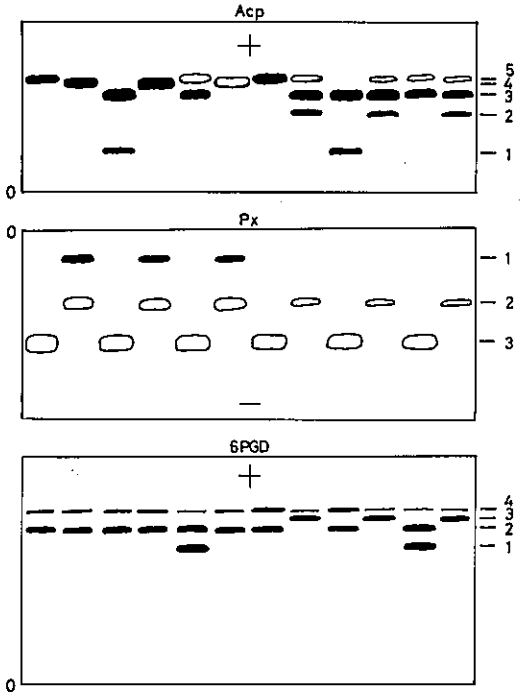

Fig. 2. Zymograms displaying seed isozymes of acid phosphatase (Acp), peroxidase (Px) and 6 phosphogluconate dehydrogenase (6PGD) in the Japanese Paridae. The electrophoresis was conducted as ADH. Respective bands are arranged from left to right as follows: *T. kamtschaticum* (k), *P. tetraphylla* (tt), *T. tschonoskii* (ts), tt, *T. smallii* (s), tt, k, *P. verticillata* (v), ts, v, s and v. Abbreviation: + and −, anodal and cathodal position: o, origin.

4. *6-Phosphogluconate dehydrogenase* 4 isozymes were detected (Fig. 2). 6-PGD-1 isozyme occurred in *T. smallii*. 6-PGD-2 isozymes were seen in all three

trilliums and *P. verticillata*. 6-PGD-3 isozyme was detected only in *P. verticillata* while 6-PGD-4 isozymes were observed in all species examined. Genetics of these isozymes are not known.

Summing up these observations as shown in Table 2, a comparison of these isozymes is made from the view point of enzyme polymorphism. There are two extreme and contrasting views for the biological causes of enzyme polymorphism: One attributes the occurrence of the polymorphism to random fixation of neutral or nearly neutral alleles raised by mutation (KIMURA & OHTA 1971), and the other to balancing selection. To resolve the neutralism-selectionism controversy, several ideas have been proposed; relationships between polymorphism and the subunit structure of enzymes (HARRIS *et al* 1977), between enzyme heterozygosity and quaternary structure (WARD 1977), between polymorphism and subunit size of the enzymes (EANES & KOEHN 1978), and others.

Of 4 enzymes examined in the study, those except for acid phosphatase are dehydrogenases, and probaly they are essential for surviving during the dormant stages of seeds because of rather anaerobic conditions in soil. Some acid phosphatases like ATPase may also be needed for energy transfer. In such cases any new alleles raised by mutation, providing new forms of enzymes ultimately, may hardly contribute for their carriers to survive, viz., to suffer severe natural selection. On the contrary, if these enzymes are entirely neutral to natural selection, the number of isozymes expected would relate to the differences in their molecular weight essentially, and the number of isozymes classified into respective categories in the Table 2 would be at random. The present data is insufficient to discriminate either cases. Yet, it is at least probable that respective enzymes investigated here have been their own ways of molecular evolution irrespective of morphological diversification in their carriers. This aspect has been pointed out from time to time in theories of molecular evolution (cf. NEI 1975), which suggests that our views of the organic evolution may be reorganized in future. More information is needed to this end.

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Table 2. A comparison of enzyme polymorphism in *Trillium* and *Paris*

Enzymes	Subunit structure*	MW* (x 10^3)	No. isozymes detected	No. isozymes common to all species	No. isozymes common to both genera	No. isozymes specific to one species	Others
ADH	dimer	~ 60	5	0	1	1	3
Acp	mono/dimer	~100	5	0	2	3	0
Px	mono/dimer	~ 45	5	2	0	1	2
6 PGD	dimer	~ 50	4	1	1	2	0

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摘　要

デンプンゲル電気泳動法を用いて，日本産ツクバネソウ連に属する5種の種子のアイソザイムを，アルコール脱水素酵素（ADH），酸性ホスファターゼ（Acp），パーオキシダーゼ（Px），6－グルクロン酸脱水素酵素（6PGD）の4酵素種について調査した。遺伝様式が解明されているアルコール脱水素酵素のアイソザイムについては，未発表のデーターも加えて，特にSS型アイソザイムがかなりの種で見られる事を述べた。

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○ 続　**宮城の自然をたずねて**——海浜・湖沼の植物——宮城植物の会編著　第一法規出版株式会社（107　東京都港区南青山2－11－17，振替口座東京3－133197）発行（昭和56年12月），B24取版，232頁，定価1,900円（〒300円）

先に刊行された"宮城の自然をたずねて——野山の植物——"では，宮城県の代表的な高山である蔵王山と仙台市の裏山である太白山・佐保山をえらんで，丘陵地から高山帯に生育する樹木・草本100種を取り上げて紹介されたが，本書はそれに引続き，海浜として仙台市の海岸部に位置する蒲生潟周辺をえらび，そこに生育する海岸植物を，湖沼として県北の渡り鳥の渡来地として名高い伊豆沼を取り上げ，そこに見られる水生植物を紹介している。

編者は，自然に親しんだり，ふるさとを意識したりするには，そこに生活している植物と会話できることが必要で，植物と会話ができるためには，そこに生活している植物の名前を知ることが早道であると述べて居られる。本書を通じて，全国的に破壊が進む海岸植物・水生植物に対する愛護の心が育てられるならば真に幸である。　（里見信生）

○ **種子の本**（続）　（古池　博）　Hiroshi HURUIKE: Further Notes on the Handbooks of Seeds

前回に続いて，種子の同定に役立つ参考書で，比較的入手しやすい本を紹介したい。

⑪ 沼田　真　編：1981。種子の科学—生態学の立場から—，研成社　東京。

種子生態学談話会の活動を背景にしてうまれた好著で編者をふくめて7名の研究者が執筆されている。副題の通り，種子生態学の現在の到達点を平易に紹介することに焦点がすえられているが，第7章は種子図譜となっており，笠原安夫氏が担当されている。口絵には96種の草本種実（雑草や人里植物が多い）の走査型電子顕微鏡写真があり，本文にその解説がのせられている。また，氏が明らかにされた種実の表皮細胞構造の40型の一覧表がある。走査型電子顕微鏡の特性（焦点深度の深さと分解能の良さ）が十分，発揮されている。本書でも強調されている通り，「植物を見る第三の目」としての役割が納得でき，将来における発展方向の一つが説得力をもってしめされている。もっと多数の種類についての，この方法による種子図譜が，一般に公にされることを期待するものである。

⑫ 長田　武正：1981。原色野草観察検索図鑑，保育社。

361種の野草について精密な図がのせられているが，そのほとんどに種実の図解がある。従来の図鑑類の種実図に比較して，大きくかつ精密な点に特徴がある。

⑬ CORNER, E. J. H. 1976。 The Seeds of Dicotyledons. I, II, Cambridge University Press. London。

これは二巻からなる大著で，第一巻には本文が，第二巻には図版がのせられている。著者の関心は，双子葉植物の正しい系統分類にある。双子葉植物の分類は従来，花を重視してきたが，これは誤っている可能性があり，種子こそ，本質的な意義をもっていると著者は強調する。各科ごとに大きくて精確な種子の解剖図と記載がのせられているが，これは，種子の進化にもとずいて，新らしく，著者によって示された，分類体系の根拠となるデータである。この著作を読むと，従来からの種子の分類が，人為分類の方法によっていたのに対して，系統分類の方法によって代えられる方向にあることが理解できる。