Theor. Appl. Genet. 59, 169-175 (1981)

Origin and Differentiation of *Aegilops triuncialis* L. as Determined by Esterase Isozyme Analysis.*

Y. Nakai
Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Kyoto (Japan)

Summary. Banding patterns of esterase isozymes in *Aegilops triuncialis* (2n = 28, genome formula C^uC^uCC) and its putative parental species, *Ae. umbellulata* (2n = 14, C^uC^u) and *Ae. caudata* (2n = 14, CC), were studied by the gel isoelectric focusing method using pH 6-8 carrier ampholite. Zymogram phenotypes of both parents were quite uniform. Seven zymogram phenotypes (designated as phenotypes 1 to 7) were found among the 260 strains of *Ae. triuncialis* examined. Of these phenotypes, phenotype 1 was identical to the zymogram phenotype produced by the ancestral species, *Ae. umbellulata*, and bands considered to have been derived from *Ae. caudata* were absent in this phenotype. Phenotype 3 had all bands of both parents. The other phenotypes differed greatly from phenotype 3. Therefore, phenotype 3 was considered to be most primitive of the 7 types, and the *Ae. triuncialis* strains which showed phenotype 3 to be the most primitive of the strains examined. If *Ae. triuncialis* originated as a hybrid between *Ae. umbellulata* and *Ae. caudata*, the zymogram phenotype must have been phenotype 3, in which the isozymes of both parental species are present. Whether the phenotypes other than type 3 were due to introgressive hybridization could not be verified, but they were considered in this article to be a consequence of a rearrangement of chromosomes.

Key words: Esterase isozymes – *Aegilops triuncialis*

Introduction

Aegilops triuncialis L. (2n = 28, genome constitution C^uC^uCC) is a tetraploid and is assumed to have originated as a hybrid between the two diploid species, *Ae. umbellulata* Zhuk. (2n = 14, C^uC^u) and *Ae. caudata* L. (2n = 14, CC) (Kihara 1940). This species has a wide range of distribution from the Western Atlantic coast to the Near East and Central Asia (Fig. 1; Eig 1929; Zohary 1965). Eig (1929) divided this species into two subspecies, i.e., ssp. *eu-triuncialis* and ssp. *orientalis*, from morphological characteristics. Ssp. *eu-triuncialis* includes two varieties, 'typica' and 'constantinopolitana', and ssp. *orientalis* consists of three varieties, 'assyriaca', 'persica' and 'anathera'. This indicates that there is great intraspecific variation in *Ae. triuncialis*.

Earlier studies in esterase isozymes have also suggested that *Ae. triuncialis* is intraspecifically more variable than other tetraploid *Aegilops* species, though there is little variation in its putative parental species (*Ae. umbellulata* and *Ae. caudata*) (Nakai and Tsunewaki 1971; Nakai 1973). The materials used at that time were limited. Two artificially synthesized CCC^uC^u strains, one of which was produced by Sears (1939) and the other by Kondo (1940), have shown the zymogram phenotypes being different from each other. The Kondo strain showed a phenotype that was a sum of both parental species, but the Sears strain did not. The latter greatly differed in this regard from the former (Nakai 1972).

Based on these previous works, further electrophoretic studies on many *Ae. triuncialis* and its putative parental species, *Ae. umbellulata* and *Ae. caudata* were conducted. The two artificially synthesized strains were also used to compare these strains with those of *Ae. triuncialis* and its putative parents. Four additional zymogram phenotypes were newly found. Based on the results obtained, the relationship of zymogram phenotypes between the two synthesized strains and the natural *Ae. triuncialis* strains, as well as possible geographical regions of origin of this tetraploid species are discussed.

Materials and Methods

Materials

In this experiments, strains of *Aegilops triuncialis* L. and those of its putative parental species, *Ae. umbellulata* Zhuk. and *Ae.*

* Contribution from the Laboratory of Genetics, Faculty of Agriculture, Kyoto University No. 432

0040-5752/81/0059/0169/$ 1.40

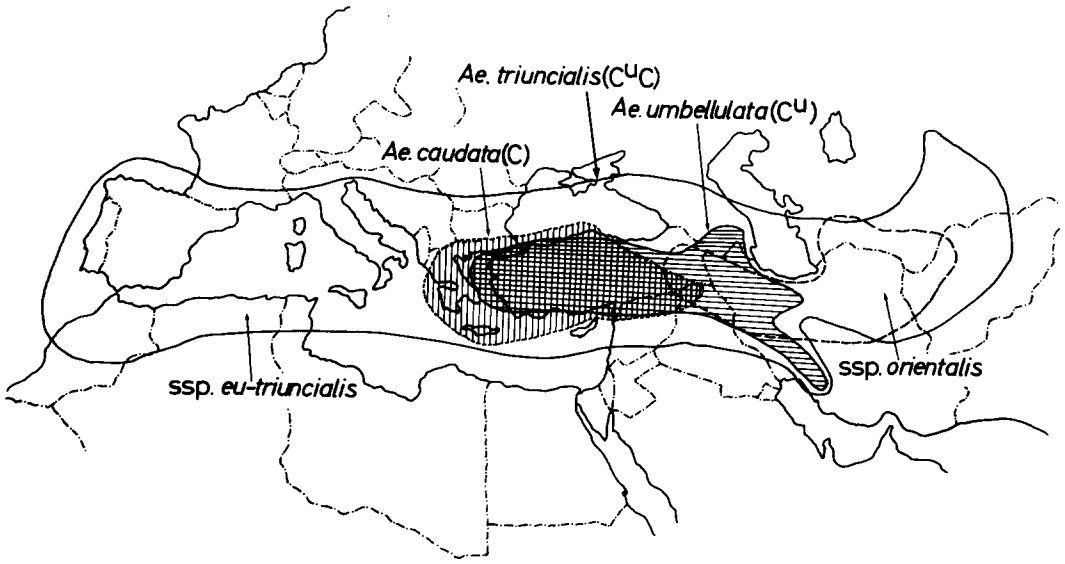

Fig. 1. Geographical distribution of *Ae. triuncialis* and its parental species, *Ae. caudata* and *Ae. umbellulata* (Eig 1929; Zohary 1965)

caudata L. were used. Of 260 strains of *Ae. triuncialis* examined, 95 were collected in Afghanistan and Iran by the Kyoto University Scientific Expedition to the Karakoram and Hindukush (abbreviated as KUSE) in 1955, 61 in Syria, Turkey and Greece by the Botanical Mission of University of Kyoto to the Eastern Mediterranean countries (BMUK) in 1959, 35 in Iraq by the Botanical Expedition of Kyoto University to Mesopotamia (BEM) in 1970, and 59 in Turkey by the Kyoto University Scientific Expedition to Eastern Turkey (KUET) in 1976. These materials were all obtained from Prof. Dr. M. Tanaka, Plant Germ-plasm Institute, Kyoto University. The remaining 10 strains are as follows: Five strains from U.S.S.R. were kindly provided by Dr. V. Jaaska, Institute of Zoology and Botany, Academy of Sciences of Estonian S.S.R.; one Bulgarian strain was a kind gift from Dr. I. Panayotov, Institute for Wheat and Sunflower, Bulgaria; and four Rumanian strains were collected by Mrs. M. Matsumura. Two artificially synthesized CCC^uC^u strains, i.e., the Kondo and Sears strains, were also examined and were designated as K-CCC^uC^u and S-CCC^uC^u, respectively, in this paper. Of 33 strains of *Ae. umbellulata* examined, 32 were from Turkey, obtained by the BMUK and KUET, and one was from Transcaucasus, provided by Dr. V. Jaaska. Of 29 strains of *Ae. caudata* examined, 19 were from Turkey, one from Syria, and nine were from Greece by the BMUK and KUET.

Methods

To analyze the esterase isozymes, the gel isoelectric focusing method was performed, as described previously (Nakai 1973). About 20 mg (one grain) of seed from each strain was homogenized in a glass mortar in 1 ml of 0.05 M potassium phosphate buffer at pH 7.0. The homogenate was centrifuged at about 20,000 × *g* for 15 min at 0°C. The supernatant (0.3 ml) was placed on polyacrylamide gel containing a carrier ampholite with a pH range of 6.0 to 8.0. The anode vessel on the top and the cathode vessel on the bottom were filled with 0.02M HCl and 0.02M ethylendiamine, respectively. The electric current was stabilized at 200 volts and this condition was maintained for 3 hr. After electric focussing, the gels were removed from the tubes, soaked in 1/15M phosphate buffer at pH 7.0 for 2-3 min, and stained with 0.1% Fast Blue RR salt and 0.01% α-naphtyle acetate (w/v) in the same buffer. The homology of the esterase band was estimated by a mixture (1:1 by weight) of esterase extracts and marker proteins.

Results

Esterase Zymograms of Ae. triuncialis

Seven different esterase zymogram phenotypes were identified among 260 strains of *Ae. triuncialis* examined, and were designated as phenotypes 1 to 7 (Fig. 2). Of these types, phenotypes 3, 4 and 5 were identical to those of three phenotypes, i.e., *typica* type (phenotype 3), Rumanian type (phenotype 4) and *persica* type (phenotype 5) which were previously reported (Nakai 1972). The other four phenotypes had not been observed before this present work. It was not clear whether phenotype 6 or 7 was a homozygotic expression of each gene which controls a corresponding band on the gel, as strains with such a type might not be a pure line. Three major bands, 3E, 4E and 6E (band nos. 3, 4 and 6 in Fig. 2-1, respectively), were observed in phenotype 1. Phenotype 2 showed three major bands, 4′E, 7E and 11E, in addition to the three bands which characterize phenotype 1 (Fig. 2-2). Another major band 14E, as well as 3E, 4E, 6E, 7E and 11E, was present in phenotype 3 (Fig. 2-3). Visual distinction between bands 4E and 4′E was occasionally very difficult because these two bands had very close isoelectric points. Phenotypes 4, 5, 6 and 7 clearly differed from the other phenotypes. Phenotype 4 had two addi-

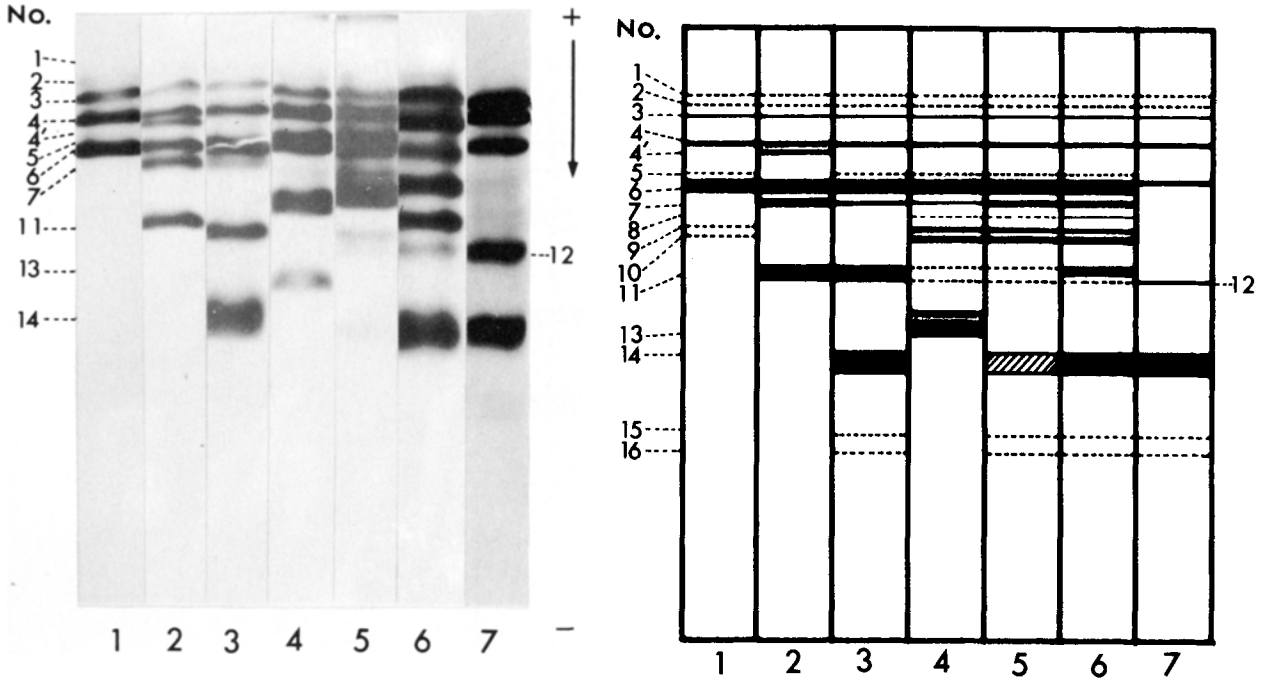

Fig. 2. Photograph of esterase zymograms and a schematic drawing of the bands for *Ae. triuncialis*. *1* type 1 (*Ae. triuncialis* var. 'typica' BMUK 6814); *2* type 2 (var. 'typica' BMUK 6830); *3* type 3 (var. 'typica' No. 1); *4* type 4 (var. 'typica' Rumania A); *5* type 5 (var. 'persica'); *6* type 6 (var. 'typica'); *7* type 7 (var. 'typica')

tional major bands, 9E and 10E, as compared with phenotype 3 (Fig. 2-4), but band 14E was absent. Band 14E of phenotype 5 was not as deeply stained as that of phenotype 3 (Fig. 2-5). Phenotype 6 was similar to phenotype 5, and they merely differed from each other in the intensity of some bands (Fig. 2-5, 6). In phenotype 7 the major bands, 7E, 9E, 10E and 11E, were missing though these were present in phenotype 5 (Fig. 2-7).

The *Ae. triuncialis* strains which were electrophoretically examined in the present study comprised four out of five varieties existing in nature (Table 1). Phenotypes 1 and 3 were found in var. 'typica' and var. 'constantinopolitana'. Phenotypes 2, 6 and 7 were observed only in var. 'typica'. All of the four varieties of *Ae. triuncialis* contained phenotypes 4 and 5. Among the seven phenotypes, phenotype 5 (52.3%) was the most prevalent.

Esterase Zymograms of Ae. umbellulata and Ae. caudata

All of the 33 strains of *Ae. umbellulata* showed the same zymogram phenotype which consisted of three highly active bands, 3E, 4E and 6E (Fig. 3A, B-1). This phenotype

Table 1. Number of *Ae. triuncialis* strains having various phenotypes of esterase zymograms

Variety	No. of strains tested	Zymogram phenotype						
		1	2	3	4	5	6	7
Ssp. *eu-triuncialis*								
typica	224	9	1	28	49	118	8	11
constantinopolitana	13	1	0	7	1	4	0	0
Ssp. *orientalis*								
assyriaca	15	0	0	0	6	9	0	0
persica	8	0	0	0	3	5	0	0
Total	260	10	1	35	59	136	8	11
(%)	(100)	(3.8)	(0.4)	(13.5)	(22.7)	(52.3)	(3.1)	(4.2)

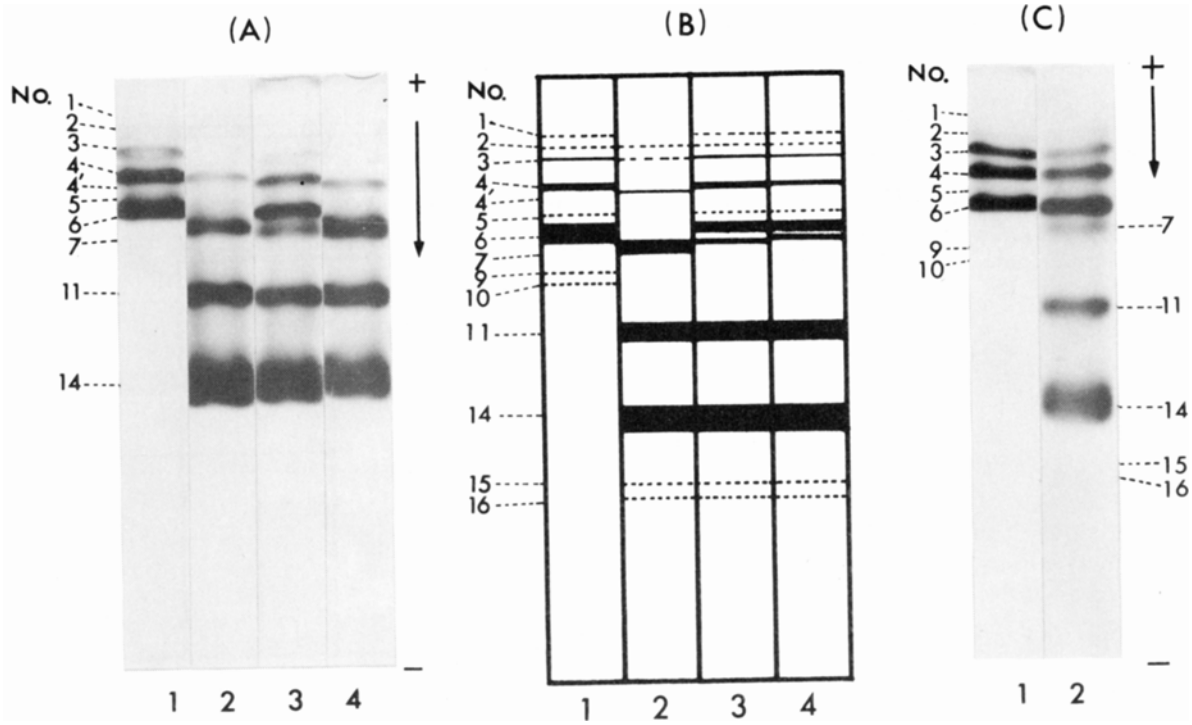

Fig. 3A-C. Electrophoretic patterns of esterase isozymes of synthesized *Ae. triuncialis* and parental species. A, B *1 Ae. umbellulata*; *2 Ae. caudata*; *3* mixture of seed extracts (1:1 by weight); and *4* synthesized *triuncialis* K-CCC^{u}C^{u}. C *1* Sear's strain (S-CCC^{u}C^{u}); *2* Kondo's strain (K-CCC^{u}C^{u})

was identical with phenotype 1 of *Ae. triuncialis*. Also, all of the 29 strains of *Ae. caudata* showed the same zymogram phenotype which consisted of four major bands, 4′E, 7E, 11E and 14E (Fig. 3A, B-2). A 1:1 mixture of the protein extracts of *Ae. umbellulata* and *Ae. caudata* produced all the isozyme bands of the two species, and the banding pattern from this mixture exactly coincided with that of phenotype 3 of *Ae. triuncialis* (Fig. 3A, B-3).

Esterase Zymograms of Two Synthesized CCC^{u}C^{u} Strains

The esterase zymogram phenotype of S-CCC^{u}C^{u} is composed of three highly active bands (3E, 4E and 6E) and five minor bands (1E, 2E, 5E, 9E and 10E) (Fig. 3C-1). Its banding pattern was found to be identical with that of phenotype 1 produced by some *Ae. triuncialis* strains. K-CCC^{u}C^{u} produced four highly active bands (4E, 6E, 11E and 14E), one moderately active band (7E) and five minor bands (1E, 2E, 3E, 15E and 16E) (Fig. 3C-2). This phenotype is exactly the same as phenotype 3 of *Ae. triuncialis*, and all the major and intermediate bands contributed by *Ae. umbellulata* and *Ae. caudata* were present in this phenotype.

Geographical Distribution of the Seven Zymograms in Ae. triuncialis

Data on the geographical distribution of the seven zymogram phenotypes are summarized in Table 2. Of the materials examined, the localities of 176 strains are illustrated in Figures 4 and 5 along with the routes of the expeditions. It can be seen that samples from the Near East produced phenotype 4 or 5, samples from Transcaucasus produced phenotype 4 only, and Iraq strains showed phenotypes, 1, 3, 4 and 5 (Fig. 4). Figure 5 presents the geographical distribution of the zymogram phenotypes in Turkey, Greece and Syria, where the greatest variation of the esterase isozymes was revealed. Phenotype 1 was sporadically found in western Turkey, but phenotype 2 was found in one region, i.e., in Denizli, Turkey. Many strains that produced phenotype 3 were collected in the western part of Turkey (Fig. 5). All Greek strains also showed phenotype 3, and one Syrian strain showed phenotype 5 (Fig. 5).

Discussion

Studies on the zymogram phenotypes of the esterase isozymes confirmed that *Ae. triuncialis* had originated as a

Table 2. Number of *Ae. triuncialis* strains collected in various countries and having different esterase zymograms

Country	No. of strains tested	Zymogram phenotype						
		1	2	3	4	5	6	7
Afghanistan	32	0	0	0	21	11	0	0
Iran	63	0	0	0	12	51	0	0
Iraq	35	2	0	7	3	23	0	0
Turkey	114	8	1	23	16	47	8	11
Syria	1	0	0	0	0	1	0	0
U.S.S.R.	5	0	0	0	3	2	0	0
Greece	5	0	0	5	0	0	0	0
Bulgaria	1	0	0	0	1	0	0	0
Rumania	4	0	0	0	3	1	0	0
Total	260	10	1	35	59	136	8	11

hybrid between *Ae. caudata* and *Ae. umbellulata*. Based on the results obtained, it can be said that the most primitive type of *Ae. triuncialis* is phenotype 3, in which all the bands contained by both parental species were present. Nakai et al. (1969) previously suggested that the K-CCC^uC^u strain might produce some new isozyme (hybrid isozymes) as the result of a cross between *Ae. caudata* and *Ae. umbellulata*. But, zymogram phenotypes of a 1:1 protein mixture (by weight) of the two parental species and the K-CCC^uC^u strain were phenotype 3, and no hybrid isozymes were produced under the present experimental conditions. Hence the phenotype 3 strains seems to be an original type. Nevertheless, among the 260 strains examined, only 14% showed phenotype 3 and the remain-

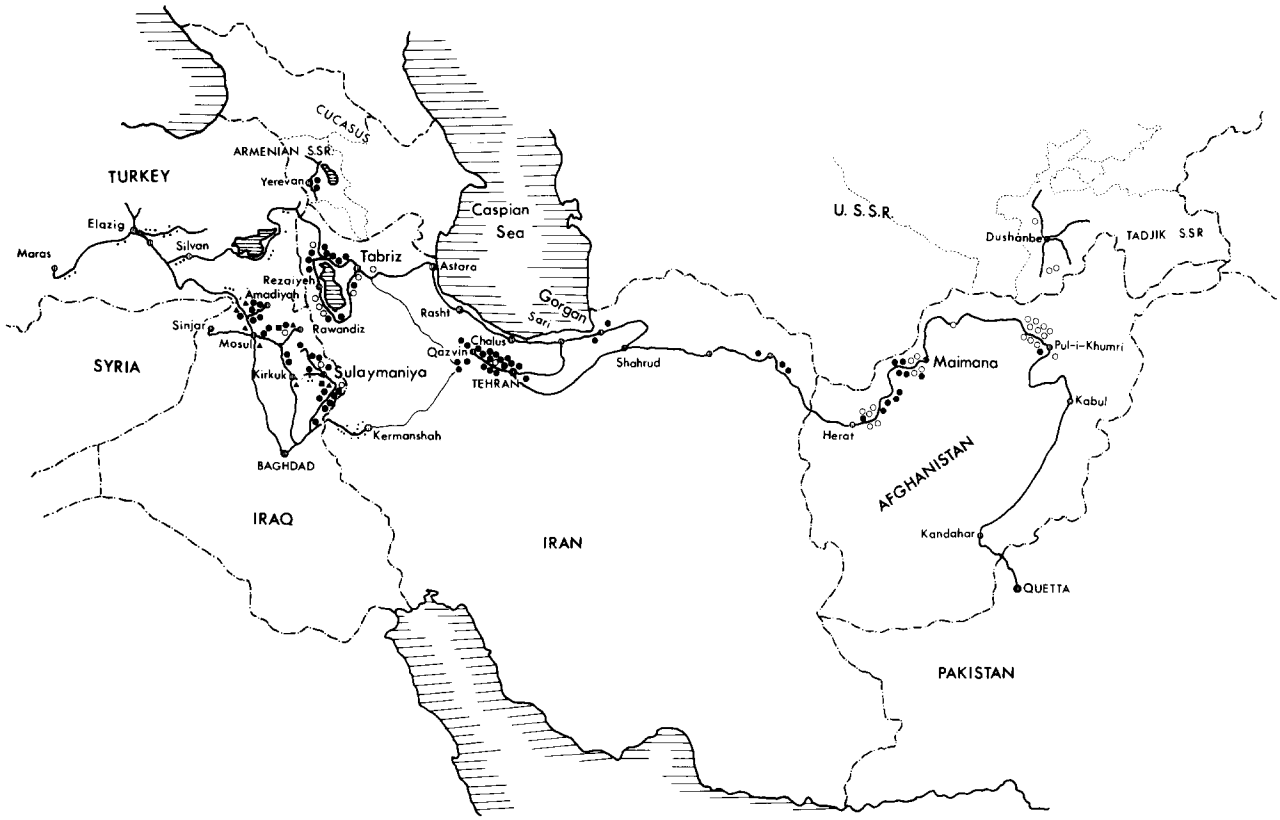

Fig. 4. Geographical distribution of *Ae. triuncialis* strains having various esterase zymograms along the expedition routes of KUSE (1955) and BEM (1970). The materials in U.S.S.R. were obtained from Dr. V. Jaaska. The small circle represents no tested sample. Explanation of symbols; ■ type 1, ▲ type 3; ○ type 4; ● type 5

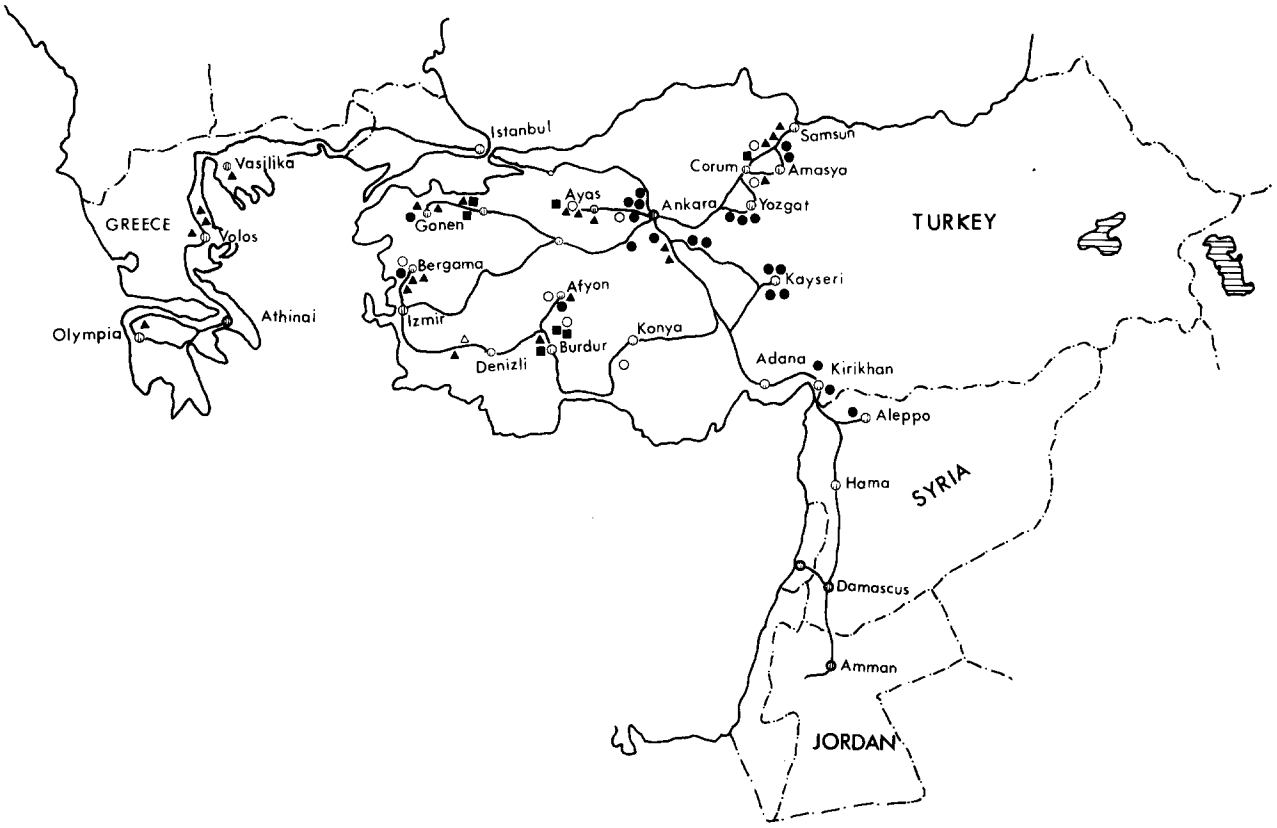

Fig. 5. Geographical distribution of *Ae. triuncialis* strains having various esterase zymograms in Syria, Turkey and Greece (BMUK 1959). Explanation of symbols: ■ type 1; △ type 2; ▲ type 3; ○ type 4; ● type 5

ing 86% of the strains were variants of this type. To explain why those variants have occurred, we point out the following three possibilities: i) Different strains of parental species (*Ae. caudata* and *Ae. umbellulata*) may have some different zymogram phenotypes. Different combinations of those parental strains would result in variants of *Ae. triuncialis*. ii) By introgressive hybridization a gene flow(s) may have taken place from the other polyploid *Aegilops* species to *Ae. triuncialis*. iii) The rearrangement of chromosomes in *Ae. triuncialis* may also have taken place after its synthesis, leading to the modification or loss of the genes involved.

In the present study no zymogram variation was found in *Ae. caudata* nor *Ae. umbellulata*. Zymograms of both parents seem to be quite uniform. Therefore, the first possibility may be rejected, although more samples must be tested to verify this decision. Concerning the second, Zohary and Feldman (1962) and Zohary (1965) have shown that *Ae. triuncialis* is crossable with other tetraploid species, i.e., *Ae. ovata* ($C^uC^uM^oM^o$), *Ae. columnaris* ($C^uC^uM^cM^c$), *Ae. variabilis* ($C^uC^uS^vS^v$) and *Ae. biuncialis* ($C^uC^uM^bM^b$), under natural conditions. They considered that there was a gene flow(s) among the tetraploid species by introgressive hybridization. Zymogram phenotypes of *Ae. triuncialis*, *Ae. columnaris* and *Ae. variabilis* had some bands in common, and those common bands were derived from the genes of *Ae. umbellulata* (Nakai and Tsunewaki 1971). This shows that the characteristics of the C^u genome has remained remarkably more constant than the other genomes in the tetraploids. Strains with phenotypes 4 or 5 which differed from the primitive type (phenotype 3) had wide ranges of geographical distribution. The bands which were considered to have been derived from the genes of *Ae. umbellulata* were present in these phenotypes, but few of those derived from the genes of *Ae. caudata* were observed (Nakai and Tsunewaki 1971). In other words, *Ae. caudata* contributes the bands not normally present in phenotypes 4 or 5 of *Ae. triuncialis*. It is not clear whether phenotypes 4 or 5 has occurred through introgressive hybridization but its possibilities cannot be totally rejected.

Among the three possibilities indicated above, the third one seems to be most reasonable. Nakai (1972) previously reported that the K-CCC^{u}C^u strain had all the bands of both parents, and the S-CCC^{u}C^u strain differed from the K-CCC^{u}C^u strain. The present study showed that the zymogram phenotype of the S-CCC^{u}C^u strain was similar to phenotype 1 of *Ae. triuncialis*. The mixture

of the extracts of the strains of *Ae. caudata* and *Ae. umbellulata* which were used by Sears for the synthesis of S-CCC^{u}C^u, produced a zymogram identical with phenotype 3. This means that the strains of *Ae. caudata* and *Ae. umbellulata* which were used by Sears were not different from other strains of *Ae. caudata* and *Ae. umbellulata* with regard to their esterase zymogram phenotypes. These results suggest that the gene(s) for those esterase isozymes formerly present in the C genome were lost or modified during the period of about 40 years after the synthesis of S-CCC^{u}C^u. Detailed observations of the chromosome pairing in the two synthesized strains was not carried out in this study. But, according to Kihara and Kondo (1943) and Kihara (1963), one or more multivalents and some univalents were always observed in the synthesized *triuncialis*. This suggests that the zymogram of S-CCC^{u}C^u (phenotype 1) might have been produced as a result of rearrangement of chromosomes. Similary, it can be suggested that phenotypes 4 or 5 and the other types excluding phenotype 3 were produced by structural changes of their chromosomes. Judging from the geographical distribution of the zymogram phenotypes of *Ae. triuncialis* and their putative parents a possible geographical region of origin of phenotype 3 may be assumed to be in the areas from western region of Turkey to northern Iraq.

Acknowledgement

I am grateful to Dr. M. Tanaka, Dr. V. Jaaska and I. Panayotov for supplying seed stocks of the strains used in this study. I also wish to thank Dr. K. Tsunewaki, Professor of Laboratory of Genetics, Kyoto University, for his kind guidance and Dr. S. Tsuji, Laboratory of Genetics, Kyoto University, for his critical reading of the manuscript.

Literature

Eig, A. (1929): Monographisch-kritische Übersicht der Gattung *Aegilops*. Repertorium speciarum novarum regni vegetabilis Beihefte 55, 1-128

Kihara, H. (1940): Anwendung der Genomanalyse für die Systematik von *Triticum* und *Aegilops*. Jpn. J. Genet. 16, 309-320 (in Japanese)

Kihara, H. (1963): Interspecific relationship in *Triticum* and *Aegilops*. Seiken Ziho 15, 1-12

Kihara, H.; Kondo, N. (1943): Studies on amphidiploids of *Aegilops caudata* × *Ae. umbellulata* induced by colchicine. Seiken Ziho 2, 24-42 (in Japanese)

Kihara, H.; Yamashita, K.; Tanaka, M.; (1965): Morphological, physiological, genetical and cytological studies in *Aegilops* and *Triticum* collected from Pakistan, Afghanistan and Iran. Results of the KUSE to the Karakoram and Hindukush, 1955. 1, 1-118

Kondo, N. (1940): Colchicine treatment in *Secale, Haynaldia* and *Aegilops*. Plant and Animals 8, 69-70 (in Japanese)

Nakai, Y. (1972): Isozymes of *Aegilops triuncialis* and its parental species. Wheat Info. Serv. 33-34, 15-19

Nakai, Y. (1973): Isozyme variations in *Aegilops* and *Triticum*, II. Esterase and acid phosphatase isozymes studied by the gel isoelectrofocusing method. Seiken Ziho 24, 45-73

Nakai, Y.; Teramura, T.; Tsunewaki, K. (1969): Variation in esterase of *Triticum* and *Aegilops* analyzed by the gel isoelectrofocusing technique. Jpn. J. Genet. 44, 259-270 (in Japanese)

Nakai, Y.; Tsunewaki, K. (1971): Isozyme variations in *Aegilops* and *Triticum*, I. Esterase isozymes in *Aegilops* studied using the gel isoelectrofocusing method. Jpn. J. Genet. 46, 321-336

Sears, E.R. (1939): Amphidiploids in the *Triticinae* induced by colchicine. J. Hered. 30, 38-43

Zohary, D. (1965): Colonizer species in the wheat group. In: The Genetics of Colonizing Species (eds. Baker, H.G.; Stebbins, G.L.), pp. 403-423. New York: Acad. Press

Zohary, D.; Feldman, M. (1962): Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. Evolution 16, 44-61

Received October 7, 1980
Communicated by R. Riley

Dr. Y. Nakai
Laboratory of Genetics
Faculty of Agriculture
Kyoto University
Kyoto, 606 (Japan)