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Protein variation and inter-specific relationship of four spider mites of Tetranychus (Acari:Tetranychidae) in Taiwan【Research report】

台灣的四種葉蟎的蛋白質變異及種間關係【研究報告】

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Abstract

Isozyme patterns of individual mites were visualized by enzyme-specific staining after electrophoretic separation on native acrylamide gels. Tetranychus piercei can easily be identified by its isozyme pattern. Total protein composition of a single mite can also be separated on an one-dimensional SDS polyacrylamide gel and visualized by silver staining. Both isozyme and major protein composition analyses showed that T. kanzawai, T. truncatus, and T. urticae are closely related to one another, and that they are distinct from T. piercei. Although they are morphologically similar, the intra-specific genetic distances calculated from the major protein composition data are smaller than inter-specific ones. The congruence of traditional taxonomic justification and protein difference indicate that the phenogram constructed by protein data may help determined the phylogeny.

摘要

單隻葉蟎的異構酶型式可經由聚丙烯醯胺膠體電泳後，以酵素特異染色法顯示出來。其中，皮爾斯氏葉蟎很容易由其異構[酉每]型式鑑定出來。單隻葉蟎的蛋白質組成則可經由SDS聚丙烯醯胺膠體電泳及銀染法顯示出來。異構酶及主要蛋白質組成兩種分析方法，都顯示神澤葉蟎、偽二點葉蟎與二點葉蟎彼此間親緣關係很近，而距皮爾斯氏葉蟎較遠。雖然這些葉蟎種類形態相似，但由主要蛋白質組成計算出的種內遺傳距離比種間小，和傳統分類所得結果一致，因此這種由蛋白質差異所得的表型關係可能反映其親緣關係。

Key words: genetic variation, isozyme, phenogram, species identification, Tetranychus

關鍵詞: 遺傳變異、異構酶、表型關係、種類鑑定、葉蟎。

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Protein Variation and Inter-specific Relationship of Four Spider Mites of *Tetranychus* (Acari: Tetranychidae) in Taiwan

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ABSTRACT

Isozyme patterns of individual mites were visualized by enzyme-specific staining after electrophoretic separation on native acrylamide gels. *Tetranychus piercei* can easily be identified by its isozyme pattern. Total protein composition of a single mite can also be separated on an one-dimensional SDS polyacrylamide gel and visualized by silver staining. Both isozyme and major protein composition analyses showed that *T. kanzawai, T. truncatus,* and *T. urticae* are closely related to one another, and that they are distinct from *T. piercei.* Although they are morphologically similar, the intra-specific genetic distances calculated from the major protein composition data are smaller than inter-specific ones. The congruence of traditional taxonomic justification and protein differences indicate that the phenogram constructed by protein data may help determine the phylogeny.

Key words: genetic variation, isozyme, phenogram, species identification, *Tetranychus*

Introduction

There are 16 species in the genus *Tetranychus* found in Taiwan. These spider mites are polyphagous and injure many crops such as strawberry, mulberry, tea, pear, etc. Although body lengths are smaller than 1 mm, they cause serious agricultural loss. Four of them, *Tetranychus kanzawai* Kishida, *T. piercei* McGregor, *T. truncatus* Ehara, and *T. urticae* Koch, are economically

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important pests on this island (Lo and Chau, 1976; Chang and Chen, 1984). In general, different pest species respond differently to pesticides. To identify the predominant species in the field is of particular importance to improve pest management. These spider mites are distinguished mainly by the males' aedeagal characters (Pritchard and Baker, 1955), and it is harder to identify the species using female individuals because the females are very similar in appearance and lack discriminant morphological characters. Moreover, sex ratios of the spider mites can be skewed by their population density and host quality (Boudreaux, 1963; Wrench and Young, 1978; Hamilton *et al.*, 1986) and hence, a population may contain just females which makes identification extremely difficult. Therefore, a rapid and simple method for identifying the species by individual females is demanded when males are not available in a population.

Electrophoretically detectable variations are useful genetic markers for distinguishing small arthropods, such as mites, as well as for analyzing their population genetic structure. Isozyme analyses have been applied for studying the population relationships in *Tetranychus*. Ward *et al.* (1982) first detected intra- and inter-specific variation in malate dehydrogenase (MDH) allozymes in 7 populations of 3 *Tetranychus* species from the Central Valley of California in the United States. *T.* (*Tetranychus*) *urticae* Koch and *T. turkestani* (Ugarov and Nikolski) share no MDH electromorphs identical in motility to those of *T.* (*Armenychus*) *pacificus* McGregor, which has been placed in a different subgenus. Sula and Weyda (1983) revealed high intrapopulation variability by using nonspecific esterases among several populations of the two-spotted spider mite, *T. urticae*, in southern and western Bohemia of the Czech Republic. Grafton-Cardwell *et al.* (1988) distinguished these 3 *Tetranychus* species by using 2 isozymes, phosphoglucose isomerase (PGI) and MDH, whereas esterases in *T. turkestani* are only partially differentiated from *T. urticae* and *T. pacificus*. Gotoh *et al.* (1991) also detected esterase differences between non-diapausing and diapausing individuals of the hawthorn mite, *T. viennensis*, in Japan. Goka and Takafuji (1992), Gotoh *et al.* (1993), and Hinomoto and Takafuji (1994) studied the population structure of the two-spotted spider mite by analyzing PGI allozyme variability. In this study, we investigated several isozyme patterns and genetic variation of spider mites in Taiwan in order to be able to identify species by a single female mite.

The inter-specific relationship among members of these *Tetranychus* species has generally been neglected due to their small size and similar external morphologies. In this study, we further reveal that the major proteins of a single mite can be separated on the basis of molecular weight and visualized by sensitive silver stain on one-dimensional SDS-polyacrylamide gel (SDS-PAGE). Since different *Tetranychus* populations apparently showed different electrophoretic patterns of major protein composition on the gel, genetic distances and a phenogram were then constructed according to this information.

Materials and Methods

Mites

One purpose in this study was to find out whether total protein comparison could be a reliable method to reveal phylogenetic relationships. Four

important and common *Tetranychus* species in Taiwan, *T. kanzawai, T. piercei, T. truncatus,* and *T. urticae,* were chosen to test the method. Eighteen populations of these 4 species of spider mites were collected in different seasons and locations in Taiwan including Taipei City, Luku of Nantou County, Lishan and Wuling of Taichung County, and Tienwei of Changhua County (Table 1). In addition, the k14 stock of *Tetranychus kanzawai* Kishida was mass cultured on multiflora bean (*Phaseolus coccineus* L.) in the laboratory for preliminary enzyme tests. The species was determined by examining the aedeagal structures of males. Because of the morphological similarities between females of different species, females were recognized to be the same species as males collected from the same colony on a leaf. Adult females were individually stored in a -70 ℃ deep freezer until used for protein assay. Both isozyme and total protein electrophoreses were analyzed by using the homogenate of a single adult female to avoid the possible variance caused by the different developmental stage expression.

Table 1. Sources of 4 *Tetranychus* species

Population	Location[a]	Host plant
T. kanzawai		
k1	Nankang	*Phaseolus* sp.
k2	Taipei	*Rosa* sp.
k6	Luku	*Camellia sinensis*
k7	Luku	*Crassocephalum rabens*
k8	Luku	*Ampelopsis bravipedunculata*
k9	Tienwei	*Dolichos lablab*
k12	Taipei	*Rosa* sp.
k13	Taipei	*Manihot utilissima*
k14		*Phaseolus coccineus*
T. piercei		
p1	Lishan	*Rubus* sp.
p2	Lishan	Cucurbitaceae
T. truncatus		
t1	Luku	*Pueraria lobata*
t4	Taipei	*Jatropha curcas*
T. urticae		
u0	Lishan	Cucurbitaceae
u1	Lishan	*Pyrus serotina*
u2	Lishan	*Phaseolus vulgaris*
u3	Lishan	*Oxalis corymbosa*
u4	Wuling	*Prunus mume*
u5	Lishan	*Trifolium repens*

[a] Nankang, Taipei City; Lishan, Taichung County; Luku, Nantou County; Taipei, Taipei City.

Isozymes analyses

Adult females were homogenized individually in 3 μl deionized water on a piece of parafilm. After mixing with 3 μl of tracking solution (0.5% bromophenol blue and 50% glycerol), the mixture was loaded onto a 0.75 mm thick polyacrylamide gel with 5% stacking gel (pH 6.8) and 7.5% resolving gel (pH 8.8). The gel was run with Tris-glycine buffer (pH 8.3) at 4 ℃ until the dye front reached the end of the gel. The substrate-specific staining was performed according to Ayala *et al.* (1972). More than 10 isozymes were screened for distinguishing one or more of the 4 mite species and/or their population variation. However, only 6 isozymes, including esterase (EST, EC 3.1.1.-), alkaline phosphatase (APH, EC 3.1.3.1), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), malate dehydrogenase (MDH, EC 1.1.1.37), phosphoglucomutase (PGM, EC 5.4.2.2), and phosphoglucose isomerase (PGI, EC 5.3.1.9), were visualized by using a single adult female. EST was stained with a mixture solution of 0.0278% sodium dihydrogen phosphate·dihydrate, 0.0536% disodium hydrogen phosphate·12 hydrate, 0.008% α-naphthyl acetate, 0.01% β-naphthyl acetate, and 0.05% Fast Garnet GBC. APH was stained with a mixture solution of 0.05 M Tris-HCl buffer (pH 8.6) (DH buffer), 0.6% magnesium chloride, 0.6% manganous chloride, 5% polyvinyl pyrrolidine, 1% α-naphthyl acid phosphate, and 1% Fast blue BB. G6PDH was stained with a mixture solution of 0.5 M Tris-HCl buffer (pH 7.1), 0.02% nitro blue tetrazolium (NBT), 0.02% nicotinamide adenine dinucleotide phosphate (NADP), 0.02% glucose-6-phosphate, 0.025% ethylene diamine tetra-acetic acid (EDTA), and 0.005% phenazine methosulphate (PMS). MDH was stained with a mixture solution of DH buffer, 0.02% NBT, 0.025% NAD, 0.05% L-malic acid, and 0.005% PMS. PGM was stained with a mixture solution of 0.6% glucose-1-phosphate, 0.2% magnesium chloride, 0.01% NADP, 0.02% NBT, 0.8 unit glucose-6-phosphatase per ml, and 0.005% PMS. PGI was stained with a mixture of 0.1 M Tris-HCl (pH 7.1), 0.02% NBT, 0.01% NADP, 0.2% magnesium chloride, 0.02% fructose-6-phosphate, 0.025% EDTA, 0.8 unit glucose-6-phosphatase per ml, and 0.005% PMS.

Total protein electrophoresis

Each individual mite was crushed in 3 μl deionized water on a piece of parafilm. After mixing with 3 μl of 2 × sample buffer (12.5 mM Tris (pH 6.75), 20% glycerol, 4% SDS, 10% β-mercaptoethanol, and 0.2% bromophenol blue solution), the mixture was transferred into an eppendorf tube and heated in a boiling water bath for 10 min. The solution was loaded onto a well of a 0.75 mm thick SDS-polyacrylamide gel with 5% stacking gel (pH 6.8) and 7.5% resolving gel (pH 8.8). Gel was run at 10 mA per gel with Tris-glycine buffer (pH 8.3) at 4 ℃ until the dye front reached the end of the gel. After that, the gel was fixed in 50% methanol, washed with deionized water, oxidized with 1% glutaraldehyde and 0.5% sodium thiosulfate, washed with deionized water, stained with ammoniacal silver stain (8% silver nitrate, 0.36% sodium hydrate, and 1.6% ammonia), and treated with a mixture solution of 0.5% citric acid, 7.6% formaldehyde, and 15% methanol until the appearance of protein bands. After staining, the gel was rinsed with water, and stopped with a mixture solution of 0.5% citric acid and 0.1% methylamine (Merril *et al.*, 1981). Finally, the gel was fixed with 50% methanol and then dried.

Phenetic Analysis

The protein bands were scanned with a densitometer (LKB Company) and the 20 most abundant proteins of each mite sample were chosen for comparison. Based on protein differences between 2 populations detected by SDS-PAGE, genetic distances (d*ij*) were calculated by Nei's equation (Nei, 1978):

$$dij = -\ln \left(\Sigma X_{ki} X_{kj} / \Sigma X_{ki} \; \Sigma X_{kj} \right)^{1/2}$$

with the NTSYS program (Rohlf, 1987), where *i* or *j* is the mite population and *k* is the protein band (or allele). From the n × n matrix of genetic distance, a phenogram was constructed following the clustering method of the unweighted pair-group method using arithmetic averages (UPGMA).

Results

Nonspecific esterases showed high intra- and inter-populational variability. MDH, PGM, and PGI, which revealed the same single band among these 4 species, could not distinguish them. Since neither a too high nor a too low enzyme variation is useful, only 2 of them, namely, APH and G6PDH, are adequate for the comparison of these mite species. Both APH and G6PDH have 3 patterns in the 4 mite species, respectively (Fig. 1). *T. kanzawai* (sample size N=5), *T. truncatus* (N=3), and *T. urticae* (N=4) all have the same APH^A pattern, whereas that of *T. piercei* is either an APH^B (N=1) or APH^C (N=1) pattern. G6PDH zymograms of *T. kanzawai* (N=109) and *T. urticae* (N=20) were $G6PDH^B$. Most individuals of *T. truncatus* examined showed also $G6PDH^B$ (N=20) though a few individuals revealed either $G6PDH^A$ (N=1) or $G6PDH^C$ (N=2). Whereas, the zymogram of *T. piercei* was $G6PDH^C$ (N=43) (Table 2).

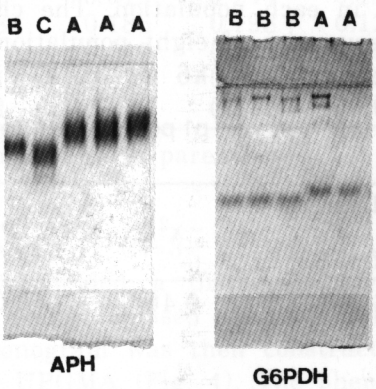

Fig. 1 Zymograms of alkaline phosphatase (APH^A, APH^B, and APH^C) and glucose-6-phosphate dehydrogenase ($G6PDH^A$, and $G6PDH^B$).

Table. 2. Types of alkaline phosphatase (APH) and glucose-6-phosphate dehydrogenase (G6PDH) in *Tetranychus* species in Taiwan

Population	Enzyme[a] APH	G6PDH
T. kanzawai		
k6	–[b]	B(18)
k7	–	B(20)
k8	–	B(18)
k9	–	B(19)
k12	A(1)	B(1)
k13	A(1)	B(1)
k14	A(3)	B(32)
T. urticae		
u0	–	B(1)
u1	A(1)	B(3)
u2	A(1)	B(8)
u3	–	B(3)
u4	A(1)	B(4)
u5	A(1)	B(1)
T. truncatus		
t1	A(2)	B(19),C(2)
t4	A(1)	A(1),B(1)
T. piercei		
p1	B(1)	C(25)
p2	C(1)	C(18)

[a] Sample size in parentheses.
[b] Not done.

In the *T. piercei* p1 population, electrophoretic analysis of esterase revealed that there were 5 esterase zones (EST-1 to EST-5); however, 2 of them interpreted as *Est-3* and *Est-4* (Fig. 2) are polymorphic. *Est-3* was apparently a dimeric-enzyme locus with 2 codominant alleles, *F* (fast, frequency: 0.09) and *S* (slow, 0.91), since each individual mite expresses either one band representing a homozygote (*FF* or *SS*) or 3 bands representing a heterozygote (*FS*). The *Est-4* locus, which codes for a monomeric enzyme, also contains 2 codominant alleles, *F* (fast, 0.36) and *S* (slow, 0.64), and the heterozygote (*FS*) expresses double bands. Neither the frequencies of genotypes of *Est-3* (N=72) nor of *Est-4* (N=38) deviated from the Hardy-Weinberg expectations (Table 3).

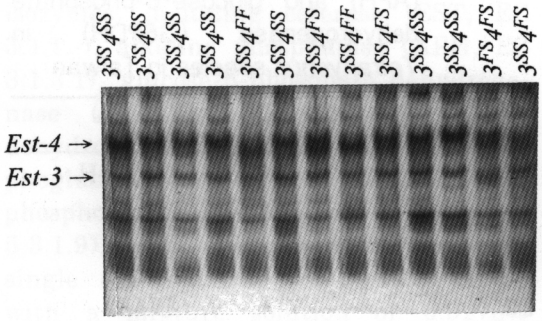

Fig. 2 Dimeric *Est-3* and monomeric *Est-4* loci of the *T. piercei* p1 population. Genotypes (for example: $3^{SS}4^{SS}$) of these 2 loci from each spider mite are labeled above each lane.

A preliminary study showed that less than 5 protein bands on the electrophoretic gel from each individual female mite could be visualized with Coomassie blue staining. However, by using the sensitive silver staining method, at least 30-40 protein bands could be recognized. The latter method was accordingly adopted for protein staining. With the aid of a densitometric scanning, the 20 most abundant protein bands of an individual spider mite were chosen and marked with black spots on SDS-gel (Fig. 3). Each protein band was interpreted as a character in the NTSYS calculation, and thus 57 characters were recognized. The character state "1" represents the presence of a protein band, whereas the state "0" represents the absence of it. Both quality and quantity of proteins were used as criteria for deciding their character states. Although some proteins were shown in minor amounts, they were not coded as positive character state "1" if not included in the 20 most abundant ones. Then, the frequency of character state "1" was scored in each population. The character frequencies of eight populations, k1 (N=9), k2 (N=6), k6 (N=3), k7 (N=3),

Table 3. Genotype frequencies of *Est-3* and *Est-4* loci in the *T. piercei* p1 population fitted to Hardy-Weinberg expectations

Locus	Genotype	Obs. no.	Exp. no.	Degrees of freedom	x^2	*P*
Est-3	(N=72)			1	0.46	>0.05
	FF	1	0.55			
	FS	11	11.90			
	SS	60	59.55			
Est-4	(N=38)			1	3.84	>0.05
	FF	2	4.64			
	FS	23	17.71			
	SS	13	15.64			

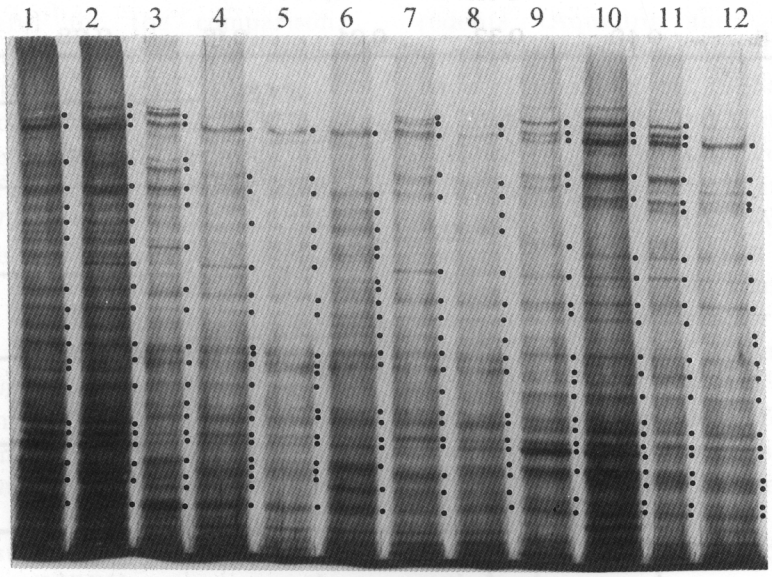

Fig. 3. Protein profiles of 3 *Tetranychus* species. Lanes 1-2: *T. kanzawai;* 3-9: *T. piercei;* 10-12: *T. urticae.* The 20 most abundant protein bands were marked with spots in SDS-polyacrylamide gel with the help of densitometer scanning.

Table 4. Matrix of genetic distances calculated from the differences of total proteins in 4 spider mite species, *Tetranychus kanzawai* (k1, k2, k6, k7, and k8), *T. truncatus* (t1), *T. piercei* (p1), and *T. urticae* (u1), with Nei's equation (1978).

	k1(9)[a]	k2(6)	k6(3)	k7(3)	k8(3)	t1(3)	p1(3)	u1(3)
k1								
k2	0.180							
k6	0.265	0.192						
k7	0.319	0.244	0.130					
k8	0.186	0.198	0.121	0.118				
t1	0.317	0.310	0.259	0.349	0.246			
p1	0.399	0.470	0.406	0.506	0.447	0.367		
u1	0.370	0.382	0.260	0.347	0.211	0.305	0.420	

[a] Sample size in parentheses

k8 (N=3), t1 (N=3), p1 (N=3), and u1 (N=3), yielded a distance matrix by using Nei's unbiased distance (Table 4). A phenogram was then constructed by using UPGMA (Fig. 4). The phenogram showed that *T. kanzawai* populations were clustered into 2 subgroups, 2 Taipei populations (k1 and k2) and 3 Nantou populations (k6, k7, and k8). Furthermore, all these 5 populations formed one larger group separate from the other species. The most closely related species are *T. kanzawai* and *T. truncatus*. *T. urticae* is the next closely related one to the former group. *T. piercei* joins them in the dendrogram with a larger genetic distance.

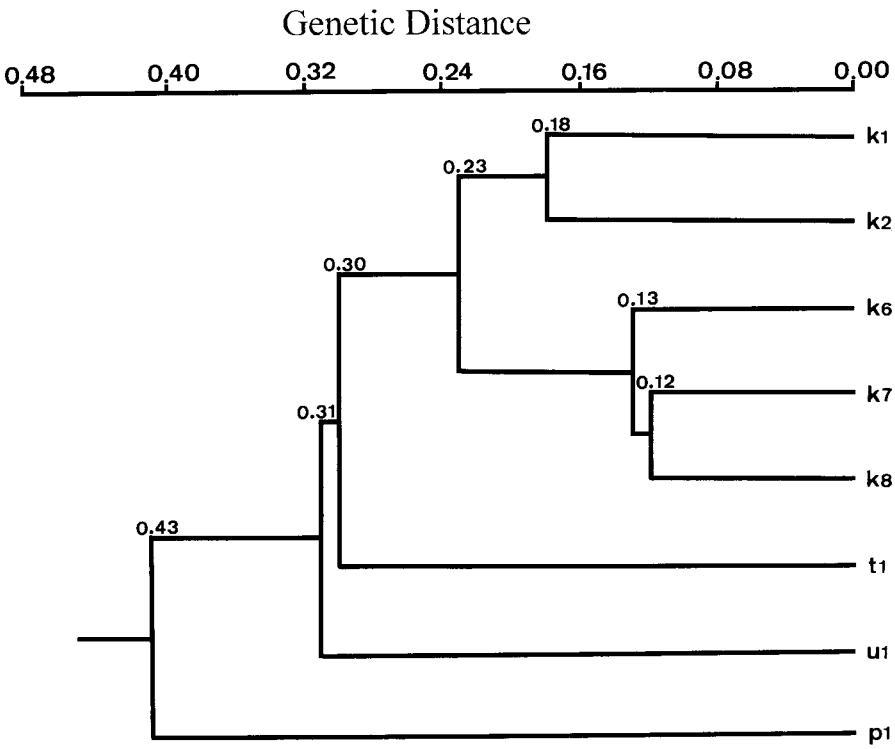

Fig. 4. The dendrogram of 4 spider mite species, *Tetranychus kanzawai* (k1, k2, k6, k7, and k8), *T. truncatus* (t1), *T. piercei* (p1), and *T. urticae* (u1) based on the genetic distances of Table 4 and constructed by the UPGMA clustering method.

Discussion

A phenogram constructed on the basis of overall similarity can still possibly reflect phylogenetic relationship as long as the characters used have a genetic basis and the rate of accumulation of differences is constant. By total protein comparison, what we see is the accumulation of mutations after species divergence instead of the difference of speciation genes. To test the reliability of this method, we adopted 5 populations of *T. kanzawai* as control. The results showed that the genetic distances of intra-specific populations in *T. kanzawai* were indeed smaller than those of inter-specific ones. This means that these analytic procedures can group conspecific populations together without mixing them with other species. Again, populations from the same locations also showed smaller genetic distances than those from different locations for the same species. This reliability indicates that the topology of these 4 species may reflect their phylogenetic relationship.

No information of inter-specific relationships among these 4 species by either morphological or other criteria has been published before. Therefore the relationships among them reconstructed by the major protein data can not be compared with phylogeny reconstructed by other methods. Our isozyme analysis was used as a separate data set to supply limited information which

could be useful for the comparison. After surveying variations of some enzymes in these 4 mite species, 2 enzymes, we determined that APH and G6PDH showed adequate variation and would be useful for species comparison. *T. kanzawai, T. truncatus,* and *T. urticae* have the same APH zymograms, and *T. piercei* can be easily identified by either the APH or G6PDH pattern from a single female. G6PDH showed that *T. urticae* is more closely related to *T. kanzawai* than to *T. truncatus,* but in the phenogram of total protein composition *T. kanzawai* and *T. truncatus* are the most closely related group. However, most *T. truncatus* individuals have the same G6PDH patterns as the other 2 species, and the genetic distances obtained from protein data among the 3 species are similar. The *T. kanzawai, T. truncatus,* and *T. urticae* triad was not solved in this study and isozyme patterns can not be used as an identification tool for these 3 species. Both isozyme patterns and major protein composition showed that *T. kanzawai, T. truncatus,* and *T. urticae* are closely related to each other, and they are distantly related to *T. piercei.*

If the phenogram can reflect phylogeny, there are 2 advantages for doing phenetic analysis of mite species by this method. First, it is fast and simple, and therefore a large number of samples can be analyzed in a short time. Second, the ability to analyze the protein composition of a single mite allows intra-populational comparison, and further studies of population genetics. The two dimensional electrophoresis (2DE) of mites lacks these 2 advantages because a single mite is not enough to be analyzed. By using 2DE, total protein differentiation has been widely used to study phenetic relationships of a variety of organisms such as rodents (Aquadro and Avise, 1981), mosquitoes (Imajoh, 1981), and *Drosophila* (Ohnishi *et al.* 1983a; Ohnishi *et al.,* 1983b; Ohnishi and Watanabe, 1984) . However, the protein content of an individual spider mite is insufficient to be analyzed by 2DE. At least 100 female citrus red mites (*Panonychus citri*) or 70-100 adult female *Schizotetranychus* were required for a 2DE analysis (Osakabe and Sakagami, 1993; Osakabe *et al.,* 1993). Therefore, our silver staining method for the total protein composition of a single mite is a good candidate for the analysis of inter-specific relationship and population genetics in mites and other small arthropods.

MDH and PGI shared the same and homogeneous types in these 4 spider mite species in Taiwan and could not be used analyzing the genetic relationships of these species, whereas *T. pacificus* can be distinguished from *T. urticae* and *T. turkestani* in California by the same 2 enzymes (Ward *et al.,* 1982; Grafton-Cardwell, 1988). Both Goka and Takafuji (1992) and Hinomoto and Takafuji (1994) showed that dimeric PGI locus in Japanese populations of the two-spotted spider mite were polymorphic with *F* and *S* alleles. Gotoh *et al.* (1993) showed that there were 5 alleles shown on the locus in the cucumber strain of the two-spotted spider mite whereas only one allele in the tomato strain in the Netherlands. The lower variability of the 2 enzyme loci in Taiwanese populations of the spider mites than those of either the Japanese or the Dutch populations are likely due to either sampling error or a past bottleneck in Taiwanese populations, resulting in a reduced number of alleles and in a lower amount of overall heterozygosity (Nei *et al.,* 1975). To know whether Taiwanese populations

are really of low genetic variation, it is necessary to collect more samples for the analysis of these 2 enzymes.

The results of the nonspecific esterases showed high genetic variability within and between populations. The high variabilities in the nonspecific esterases were consistent with the results of the studies such as Sula and Weyda (1983), Grafton-Cardwell *et al.* (1988), and Gotoh *et al.* (1991). Nonspecific esterases showed too high variability to be used as a proper candidate for the distinction of these mite species. However, the enzymes can be useful for studying population structure of mites. Ward *et al.* (1982) found that within collecting locations (fields 600 m^2 in area), the genotype frequencies did not detectably deviate from the Hardy-Weinberg expectations in populations of either *T. urticae* or *T. turkestani* by using the MDH-2 locus as a genetic marker. By using the PGI locus as a genetic marker, Hinomoto and Takafuji (1994) found that the genotype frequencies fitted the Hardy-Weinberg equilibrium within single leaflets. However, genotype frequencies of a sub-population, which consists of several leaflets, deviated from the Hardy-Weinberg equilibrium. The *T. urticae* and *T. turkestani* populations (Ward *et al.*, 1992) were in an open field where long-distance dispersal of mites by the aid of wind would be more frequent than *T. urticae* populations in a vinyl-house studied by Hinomoto and Takafuji (1994). The genotypic frequencies of the 2 esterase loci (Table 3) of *T. piercei* p1 population, which was collected from a *Rubus* plant in an open field, also confirmed the above prediction.

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台灣的四種葉蟎的蛋白質變異及種間關係

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摘　要

單隻葉蟎的異構酶型式可經由聚丙烯醯胺膠體電泳後，以酵素特異染色法顯示出來。其中，皮爾斯氏葉蟎很容易由其異構酶型式鑑定出來。單隻葉蟎的蛋白質組成則可經由 SDS 聚丙烯醯胺膠體電泳及銀染法顯示出來。異構酶及主要蛋白質組成兩種分析方法，都顯示神澤葉蟎、僞二點葉蟎與二點葉蟎彼此間親緣關係很近，而距皮爾斯氏葉蟎較遠。雖然這些葉蟎種類形態相似，但由主要蛋白質組成計算出的種內遺傳距離比種間小，和傳統分類所得結果一致，因此這種由蛋白質差異所得的表型關係可能反映其親緣關係。

關鍵詞：遺傳變異、異構酶、表型關係、種類鑑定、葉蟎

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