Genetic Changes in the Population Structure of the Two-Spotted Spider Mite, *Tetranychus urticae* KOCH (Acari: Tetranychidae), on Vinyl-House Strawberries¹

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Two alleles were detected at the phosphoglucosomerase (PGI) locus of the two-spotted spider mite, *Tetranychus urticae* KOCH. For clarifying the temporal change in genetic structures of the mite population, we introduced two laboratory-selected strains of the mites which were homozygous for PGI alleles onto vinyl-house strawberries. The results revealed that mites initially tended to form breeding colonies on individual leaflets, but with an increase in their density, they dispersed all over the plants. As a result, the population became genetically homogeneous as a whole.

Key words: allozyme, breeding colony, population structure, *Tetranychus urticae*, Wright's *F*-statistics

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* KOCH, has a large genetic diversity within populations in some genetic traits. For example, TAKAFUJI et al. (1991) showed that populations in the intermediate latitudes of Japan consist of individuals with a variety of diapause capacities. Such intra-population variations are likely to be derived partly from their mating and dispersal behavior: adult males guard quiescent females and copulation soon takes place after the emergence of the females (HELLE and OVERMEER, 1973). Dominant dispersers are adult females (HUSSEY and PARR, 1963), which however tend to stay on leaves where they have colonized unless resource depletion occurs (KONDO and TAKAFUJI, 1985). These observations imply that females will have a high possibility of mating with their brothers or close relatives, and populations tend to consist of a number of small breeding colonies.

Allozyme variability analyses have been a useful tool for clarifying the genetic structure of mite populations. WARD et al. (1982) showed that the inbreeding coefficient calculated from the frequencies of malate dehydrogenase (MDH) allozymes was very close to zero in seven populations of three species of spider mites (*T. urticae*, *T. turkestan*i and *T. pacificus*), suggesting that random mating over a wide area occurred in each of the populations. Conversely, our previous study using phosphoglucosomerase (PGI) allozymes (HINOMOTO and TAKAFUJI, 1994), which analyzed the genetic structure of a population of *T. urticae* on

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Fig. 1. The arrangement of strawberry plants in the vinyl-house. Thirty adult females of strains F and S were introduced onto the plants B2 and B4, respectively.

strawberries in a vinyl-house, showed the mites tend to form their breeding colonies on single leaflets of strawberry plants. However, we did not monitor changes in the genetic structure during a longer population episode. In this study, we introduced two homozygous strains for PGI allozymes of *T. urticae* onto strawberries in a vinyl-house for clarifying the temporal change in genetic structures of the population.

MATERIALS AND METHODS

Mite strains. Mites were collected at Tohaku, Tottori Prefecture, in western Japan, on August 27, 1990 from Japanese pear trees. They were kept on detached bean leaves (*Phaseolus vulgaris* L.) placed on wet cotton at 25°C–16L8D. GOKA and TAKAFUJI (1992) and HINOMOTO and TAKAFUJI (1994) detected two alleles, “F” and “S”, at the PGI [EC 5.3.1.9] locus in *T. urticae*. Thus, there were three types of band patterns; homozygous of fast migrating enzyme (*F/F*) and that of slow migrating enzyme (*S/S*), and heterozygous of these two alleles (*F/S*). By consecutive sib-matings over several generations, we selected 2 homozygous strains “F” and “S”.

Introduction of mites. We planted 15 strawberry plants. Each was planted in a flower pot (21 cm in diameter) and kept in a vinyl-house at the Kyoto University campus (Fig. 1). Each flowerpot was buried to four fifths in height under the soil. The plants were treated with acaricide and insecticide to exclude any mites and insects. This treatment was done about 4 weeks before mites were introduced. Creased vinyl walls (INOUE, 1990) were set up around the group of flowerpots to prevent the immigration of any other mites. On April 15, 1992, when the plants had 5 to 6 leaves, we introduced 30 adult females of the strain “F” onto a leaf of the plant coded “B2” and those of the strain “S” onto “B4” (Fig. 1).

Mite sampling and electrophoresis. We sampled 3 leaves at random from each of the 15 plants and counted the number of adult females on them at about 10 day intervals during 70 days of a population episode. On the 6th and 7th samplings, when the number of mites had reached a high level, we collected adult females from 2 of the 3 leaves of each plant. They were preserved at –20°C for electrophoresis. We used individual adult females for polyacrylamide vertical slab gel electrophoresis and monitored their PGI genotypes, following the method used by GOKA and TAKAFUJI (1992).

Genetic study. Each of the sampled leaves consisted of three leaflets, and therefore the mites collected belonged to four levels of sub-population, i.e., a leaflet, a leaf, a plant, and the whole field (a group of 15 plants). Based on the electrophoretic data, we calculated frequencies of genotypes and alleles for each sub-population at the four levels of sub-

Fig. 2. Changes in the density of adult females of *T. urticae* per leaf on 15 strawberry plants.

Fig. 3. Spatial distribution of adult females of *T. urticae* on 15 strawberry plants. Solid circles represent the plants on which mites were present.

population. We then carried out a χ^2 -test for goodness of fit between observed genotype frequencies and the HARDY-WEINBERG equilibrium. Furthermore, the fixation index (F_{ST}) (WRIGHT, 1978) was calculated at each of the levels from the equation:

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

where H_T is the expected heterozygosity for the whole population, and H_S is an average of expected heterozygosities for each sub-population. The F_{ST} measures the genetic differentiation in allele frequencies among sub-populations. This index ranges between 0 and 1, and the greater the value, the larger the genetic differentiation.

RESULTS

The density of adult females remained low during the first 42 d after introduction (Fig. 2), and their distribution was restricted to B2 and B4 on which they were introduced, and a few plants adjacent to them (Fig. 3). Thereafter, it rapidly increased (Fig. 2), and by day

Table 1. Genotype and allele frequencies at the whole population level

Days after introduction	Observed genotype frequencies (Expected genotype frequencies)				Allele frequencies	
	<i>F/F</i>	<i>F/S</i>	<i>S/S</i>	Total	<i>F</i>	<i>S</i>
47	58 (51.8)	28 (40.3)	14* (7.8)	100	0.72	0.28
68	102 (96.5)	61 (71.4)	18 (13.2)	181	0.73	0.27

* Significantly ($p < 0.05$) deviated from the HARDY-WEINBERG equilibrium.

Table 2. No. (%) of sub-populations which deviated from the HARDY-WEINBERG equilibrium

Level of sub-population	Day 47		Day 68	
	No. of total sub-populations	No. (%) of sub-populations which deviated from the HARDY-WEINBERG equilibrium	No. of total sub-populations	No. (%) of sub-populations which deviated from the HARDY-WEINBERG equilibrium
Leaflet	17	1 (5.9)	57	0 (0.0)
Leaf	9	0 (0.0)	25	0 (0.0)
Plant	6	0 (0.0)	13	0 (0.0)
Whole	1	1 (100.0)	1	0 (0.0)

Fig. 4. Proportion of sub-populations in which only either of the two alleles was found (monomorphic sub-population) at each sub-population level. The open and solid bars show the data on days 47 and 68, respectively.

68 the mites had dispersed onto all of the 15 plants (Fig. 3). These results are consistent with the findings that *T. urticae* has a low tendency to disperse unless host plants heavily deteriorate (INOUE and SUGIURA, 1984; KONDO and TAKAFUJI, 1985).

At the whole field level, the genotype frequencies on day 47 were significantly deviated from the HARDY-WEINBERG equilibrium, whereas those on day 68 were not (Table 1). On day 47, only in a single sub-population at the leaflet level genotype frequencies deviated

Fig. 5. The fixation index (F_{ST}) at three levels of sub-population. The open and solid bars show the data on days 47 and 68, respectively.

Fig. 6. The relation between the density of adult females per leaf, and the fixation index (F_{ST}) which was calculated regarding leaflets as sub-populations and leaves as the whole populations.

from the HARDY-WEINBERG equilibrium, and there were no such sub-populations at the leaf and plant levels (Table 2). At the leaflet level, the proportions of sub-populations in which only either of the two alleles was found (monomorphic sub-population) were 35% and 42% on day 47 and 68 respectively (Fig. 4). Furthermore, the values of F_{ST} , the fixation index, tended to be higher on day 47 than on day 68 at all levels (Fig. 5).

On day 68, the proportion of monomorphic sub-populations was also relatively high at the leaflet level, but it decreased drastically at the leaf level and was much lower than the value on day 47 (Fig. 4). Further, at the plant level, no monomorphic sub-population was observed. On day 68 the F_{ST} at the leaflet level was higher than that at the leaf and plant levels (Fig. 5).

If we regard leaves as the whole populations and leaflets as the sub-populations, then the F_{ST} indicates the genetic differentiation between leaflets belonging to the same leaf. Figure 6 shows that the higher the mite density per leaf, the lower the value was of F_{ST} for each leaf sub-population. This result clearly indicated that the gene flow between leaflets

Fig. 7. The allele frequencies at the plant level. Solid and open areas show the proportions of *F* and *S*. Dotted circles represent the plants onto which mites were initially introduced.

occurred more frequently as mite density increased. This tendency was also shown at higher levels. For example, the 8 plants where mites were colonized had a variety of allele frequencies on day 47, while most plants had similar frequencies on day 68 (Fig. 7).

DISCUSSION

SLATKIN (1987) reviewed the relationship between gene flow and the geographic structure of natural populations. He stressed that gene flow plays an important role for genetic homogenization among populations, therefore estimating how much gene flow occurred is necessary to know the genetic structure of populations which are geographically apart. In the review, he showed two types of methods for estimating the amount of gene flow. One was the "direct method," in which dispersal distance and breeding success of dispersers were observed. The other was the "indirect method," in which allele frequency differentiation among populations was determined for estimating gene flow. The fixation index, F_{ST} , was useful for estimating gene flow in the indirect method, and it has been used in several studies. However, populations discussed in these studies were distant from each other. For example, KORMAN et al. (1993) estimated the gene flow between populations of the tobacco budworm, *Heliothis virescens* (F.) collected from areas in several counties as well as states in the United States.

Our studies (HINOMOTO and TAKAFUJI, 1994 and the present study) show that the indirect method using allozyme variability as a marker is very useful for also studying the genetic structure of a single population in a small area. We used only PGI alleles as genetic markers, while KORMAN et al. (1993) used 13 enzyme loci for estimating the values of F_{ST} . It is desirable to use many loci because there is a possibility of fitness differentiation among loci or alleles. However, in this study, no difference in allele frequency at the whole field level was detected between days 47 and 68, suggesting that during this period no difference occurred in fitness between the two alleles. This fact confirms that PGI alleles can be used as genetic markers for analyzing the genetic structure of mite populations.

On day 47, genotype frequencies significantly deviated from the HARDY-WEINBERG equilibrium at the whole level, suggesting that non-random mating had occurred by this

Fig. 8. Schema showing the process of changes in genetic structure of a mite population.

time. In addition, the proportion of monomorphic sub-populations was highest at the leaflet level, and the F_{ST} was also relatively high. These results suggest that mites tend to form breeding colonies on single leaflets and gene flows between such colonies are restricted due to their infrequent dispersal. This fact is consistent with the results of our previous study (HINOMOTO and TAKAFUJI, 1994). However, the present analysis suggests that a gene flow occurred, due to mite dispersal in response to an increased injury on plants, at levels higher than leaflets between days 47 and 68. This fact is supported by much lower proportions of monomorphic sub-populations as well as lower levels of F_{ST} at leaf and plant levels on day 68 than on 47.

The result of the present study shows that *T. urticae* initially forms many isolated breeding colonies, but the genetic structure of the population changes during the population episode in which the spatio-temporal structure of the population changes over a period. The process of this genetic change in population structure is summarized in Fig. 8. First, a few mated females colonize empty leaflets, and they form a population on the leaflets by repeating sib-matings or matings with close relatives over several generations. During this period,

these breeding colonies have small genetic diversity, while genetic differentiation between colonies is large. However, as population density increases and the leaflets deteriorate, females begin to disperse rapidly onto other leaflets where other mites may be present. If the genotypes of the founders and colonizers are different from each other, then genetically polymorphic colonies are formed and the differentiation between colonies becomes smaller. This gradually leads to the genetically homogeneity of the whole population.

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