
SHORT COMMUNICATIONS

Electrophoretic Differentiation of F₁ Hybrids of the Sibling Species *Panonychus osmanthi* EHARA et GOTOH and *P. citri* (McGREGOR) (Acari: Tetranychidae)¹Yasuki KITASHIMA and Tetsuo GOTOH²*Laboratory of Applied Entomology and Zoology,
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Panonychus osmanthi EHARA et GOTOH, which is closely related to *P. citri* (McGREGOR), infests garden trees such as *Osmanthus* spp., while *P. citri* occurs on evergreen trees such as citrus and Japanese holly, *Ilex crenata* THUNB. (KITASHIMA and GOTOH, 1995; EHARA and GOTOH, 1996). The Japanese pear, *Pyrus pyrifolia* var. *culta* (MAKINO), is a common host for *P. osmanthi* and *P. citri*, and these mite species frequently migrate to pear orchards from nearby orange osman and holly trees, respectively (e.g., GOTOH and KUBOTA, 1997). Since interspecific crosses produce viable but sterile F₁ hybrid females in the laboratory (KITASHIMA and GOTOH, 1995), there is a possibility that interspecific hybridization occurs in pear orchards where the two species coexist. However, it is difficult to discriminate F₁ hybrids from their parent species by morphological characters, because the difference between females of the two species is small. The electrophoresis of enzymes can overcome this problem, as it is superior to morphological characters in detecting intraspecific variation, separating sibling species and identifying closely related species (MENKEN and ULENBERG, 1987; GOTOH and ISHIKAWA, 1992; GOTOH and TAKAYAMA, 1992; GOKA and TAKAFUJI, 1997). Electrophoretic analyses for detecting interspecific hybrids have been carried out on fruit flies, *Rhagoletis*

spp. (SMITH et al., 1993), and parasitoids, *Torymus* spp. (IZAWA et al., 1996), and F₁ individuals were successfully differentiated from the parents in these species. In the present study, we analyzed interspecific variation in esterase (Est) zymograms in order to establish practical criteria for verifying F₁ hybrids produced from crosses between *P. osmanthi* and *P. citri*.

MATERIALS AND METHODS

Mites. More than 100 female adults of *P. osmanthi* were collected from the orange osman, *Osmanthus fragrans* var. *aurantiacus* MAKINO, in Chiyoda (36°09'N–140°14'E), Ibaraki, on 8 Sept. 1996, and females of *P. citri* were collected from the Japanese holly on 8 Sept. 1996 at an adjacent site (Chiyoda). OSAKABE (1984) reported that the activity of esterase bands in *P. citri* was different among mites collected from different host plants. Therefore, to eliminate any influence that the ingredients of host plants might have on the banding patterns, each population was maintained on leaf discs of the Japanese pear for two generations after collection. Thereafter, female adults of each population were stored at –80°C until esterase zymogram analysis. F₁ hybrid females produced from crosses between *P. osmanthi* and *P. citri* on Japanese pear leaves were also stored at –80°C 5 days after they matured.

Measurement of the length of the C3 and L3 setae. Although it is difficult to discriminate closely related mite species by morphological characters of females, the females of *P. osmanthi* can be distinguished from those of *P. citri* by using average lengths of two dorsal setae (EHARA and GOTOH, 1996): in the females of *P. osmanthi*, the 3rd dorsolateral hysterosomal setae (L3) are ca. 30 µm shorter than the 3rd dorsocentral hysterosomals (C3), while in the females of *P. citri*, the L3 setae are slightly shorter than, or as long as the C3 setae. To determine whether these morphological characters are useful for discriminating F₁ hybrids from their parental species as well as discriminating the two species, the lengths of the L3 and C3 setae of the two species and the hybrid females obtained from crosses between *P. osmanthi* and *P. citri* were measured. Adult females were taken from each culture and mounted in HOYER's medium. Measurements were made under a phase-contrast microscope with a micrometer eyepiece.

Electrophoresis. Electrophoresis (native PAGE) was

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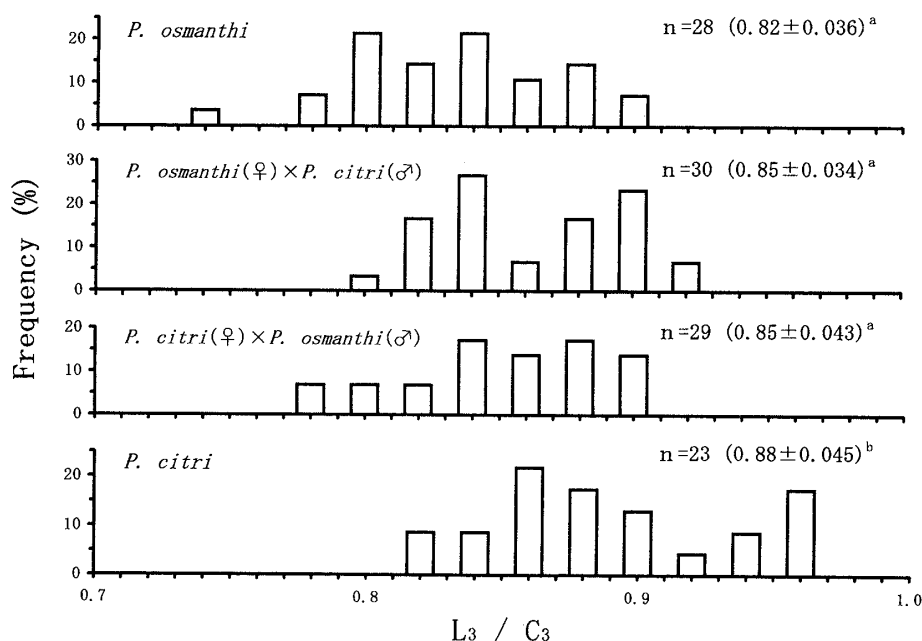


Fig. 1. Frequency distributions of the L3/C3 ratios of female in *P. osmanthi*, *P. citri* and the hybrids of *P. osmanthi* and *P. citri*. Mean $\pm$ S.D. in a column followed by different letters are significantly different ($p < 0.05$; TUKEY-HSD test).

carried out as described by GOKA and TAKAFUJI (1992, 1997): frozen females were individually homogenized in 10 μ l of 32% (w/v) sucrose with 0.1% Triton X-100 and 0.002% bromophenol blue. The gels were 1 mm thick, 145 mm wide and 160 mm high, and contained Triton X-100 (concentration 0.05% in the separating gels and 0.1% in the stacking gels). The concentration of acrylamide was 10% in the separating gels and 2.5% in the stacking gels. Electrophoresis was carried out at a constant current of 20 mA/gel at 5°C for 10 h. The electrode buffer was 25 mM Tris–192 mM glycine, pH 8.6. For the detection of non-specific esterases, the gels were incubated for 40 min at 30°C in 0.02% of α -naphthyl acetate in 0.1 M phosphate buffer (pH 6.5) which contained 1% acetone, and then the gels were soaked for 1 h in 0.2% Fast Blue BB salt solution. Forty to 56 individuals of each of *P. osmanthi*, *P. citri* and their hybrids were used for the analysis of esterase zymograms.

RESULTS AND DISCUSSION

The frequency distributions of the ratios of the L3 seta length to the C3 seta length (L3/C3) of *P. osmanthi*, *P. citri* and their hybrids are shown in Fig. 1. The average L3/C3 ratio in *P. osmanthi* was signifi-

cantly smaller than that in *P. citri* ($p < 0.05$; TUKEY-HSD test after angular transformation). However, the distribution of the L3/C3 ratios of *P. osmanthi* overlapped that of *P. citri*. The L3/C3 ratios of F₁ hybrid females obtained from the reciprocal crosses between *P. osmanthi* and *P. citri* were intermediate between those of the parents, but the ratios completely overlapped those of the parents. Thus, it is not possible to identify *P. osmanthi*, *P. citri* and their hybrids based on this morphological character.

No variation was observed among the zymograms of individuals of either species, but *P. osmanthi* could be discriminated from *P. citri* by the esterase zymograms (Fig. 2). The α -Est 4, 5 and 6 loci in *P. osmanthi* were conspicuously located on the cathodic side of the α -Est loci in *P. citri*. Furthermore, the α -Est 3 locus in *P. osmanthi* appeared slightly on the anodic side of the α -Est locus in *P. citri*, but the difference was extremely small. Therefore, frequently we were unable to detect any difference in the α -Est 3 locus between the two species.

Hybrid females obtained from the cross between *P. osmanthi* and *P. citri* could be discriminated from their parents by the α -Est 1 locus (Fig. 2). Two or sometimes three bands, which appeared near the origin of the separating gel, were distinctive of all hybrid fe-

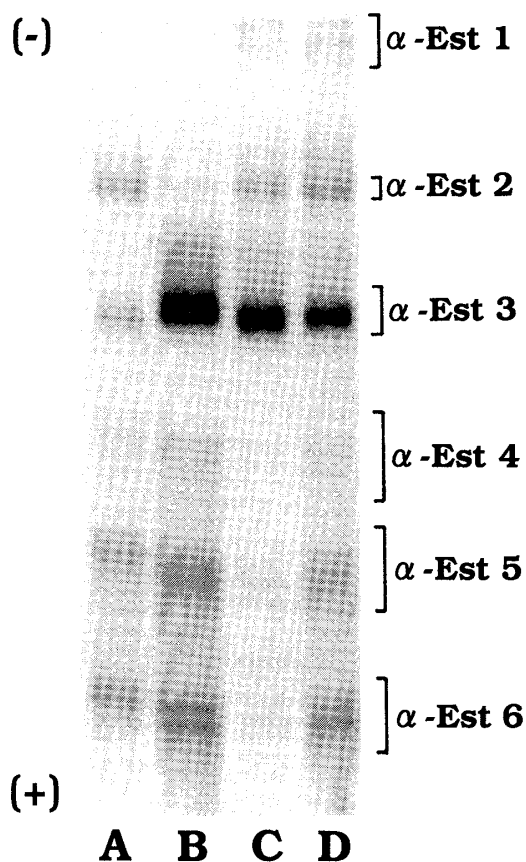


Fig. 2. Esterase zymograms of *P. osmanthi* (A), *P. citri* (B), hybrids from the *P. osmanthi* female $\times$ *P. citri* male cross (C), and the reciprocal cross (D).

males, although the function of the gene at this locus is not known. Thus, we could clearly distinguish among *P. osmanthi*, *P. citri* and their hybrid females using esterase zymograms.

OSAKABE and KOMAZAKI (1996) reported that *P. osmanthi* (= the *Osmanthus* population of *P. citri*) could be discriminated from *P. citri* as well as their hybrids using the α -Est 3 locus, although they named it the α -Est 1 locus. However, much practice is needed to discriminate the banding patterns of the α -Est 3 locus in *P. osmanthi*, *P. citri* and their hybrid females, because the differences are extremely small (see also

Fig. 2). Accordingly, the usefulness of the α -Est 3 locus is relatively limited from an applied point of view. In contrast, the present study indicated that the α -Est 1, 4, 5 and 6 loci are better markers for easily differentiating *P. osmanthi*, *P. citri* and their hybrid females. Thus, electrophoretic analysis using several loci of esterase zymograms appears to be a practical technique for verifying F_1 hybrid females produced from natural crossings between *P. osmanthi* and *P. citri* in field populations.

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