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Esterase variation and some biological characteristics of two Turkish Trichogramma (Hymenoptera: Trichogrammatidae) populations

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Abstract. In this study, esterase variation was investigated for one *Trichogramma turkestanica* Meyer and two *Trichogramma brassicae* Bezdenko populations collected from Southeast Turkey. Depending on the banding patterns there were slower (Est1) and faster (Est2) bands as two different isozymes were determined. *T. turkestanica* had a different banding pattern than *T. brassicae*. At the same time, numbers of parasitized eggs and female and male offspring, and adult longevity were determined for each population. There were no significant differences between cultures except adult longevity. Some morphometric characters of cultures were also measured, comprising body length, flagellum length, head width, the longest seta of the flagellum, hind tibia and hind wing length. All characters were similar between the two *T. brassicae* populations and were significantly different from *T. turkestanica*.

Key words: *Trichogramma*, esterase, electrophoresis, biological characteristics.

Introduction

Trichogramma spp. (Hymenoptera: Trichogrammatidae) are minute parasitoids of insect eggs, particularly Lepidoptera. As such, they are widely used in biological control initiative. Two of the most important aspects of successful biological control are the correct identification of the species to be used, and choosing the most appropriate species for the situation of concern. Knowing biological characters such as longevity and parasitization rate is also very important for achieving success in biological control programs (Oliveira et al. 2003).

Within the genus *Trichogramma*, there are approximately 200 described species worldwide (Pinto, 1998). Identification of these parasitoids is problematic because of their small size and low interspecific morphological variation. Most *Trichogramma* species have high intraspecific variation in morphological characters and the problem of distinguishing between closely related species has prompted the use of other methods such as physiological, molecular, ecological, reproductive and behavioral characters. There are a large number of studies on the identification of *Trichogramma*, but earlier studies depend on morphological characteristics for distinguishing species within this genus. However, the changing taxonomy of the genus makes it difficult to compare the biological and morphological differences among strains of *Trichogramma* reported in earlier works with their present taxonomic status.

Morphological characters such as body color, number and size of body hairs can be variable depending on body size, season, rearing temperature and host. Nagarkatti & Nagaraja (1977) reported that the use of male

genitalia in identification of *Trichogramma* species was useful, and has since become a widely used and important character. Reproductive compatibility studies and morphological characters such as wing venation and antennal structure can also be used to support putative identifications (Knutson 1998). Vargas & Cabello (1985) distinguished *T. cordubensis* Vargas & Cabello, by using male genitalic and antennal characters. In contrast *T. minutum* Riley and *T. platneri* Nagarkatti can be distinguished only by using crossing experiments (Pinto & Stouthamer 1994). However, it is impossible to identify female *Trichogramma* by using male-specific characters. A type of parthenogenesis, thelytoky, is a common phenomenon in *Trichogramma* with virgin females producing daughters only (Stouthamer et al., 1999). This presents a major problem for identification of completely parthenogenetic forms where males are not present.

Enzymatic analysis, especially esterase electrophoresis is a useful technique that can easily distinguish variation within the genus. Allozyme electrophoresis is a technique that has proved useful for determining the genetic variation of many organisms (Gomez 1998), and can be useful for distinguishing closely related species in cases where it may be impossible to separate them using morphological characters (Pintureau 1993). In *Trichogramma*, esterase electrophoresis has been the allozyme technique most widely used (Pintureau 1993).

In the present work, the variation of esterase isozymes of different *Trichogramma* cultures on an esterase zymogram was detected by electrophoresis, and the reproductive compatibility and morphometric variation among cultures were measured.

Materials and methods

Parasitoid material: *Trichogramma* species (*T. brassicae* and *T. turkestanica*) and populations used in the study and their collection date, site, and host are presented in Table 1. They were collected from Adana, Southeast of Turkey, in the eggs of European corn borer, *Ostrinia nubilalis* Hubner (Lepidoptera: Crambidae) parasitised by *Trichogramma* spp. in maize fields in 2006 and were identified by using ITS2 sequence analysis (Stouthamer et al. 1999). Parasitoids were reared at 25°C and 70% RH on eggs of *Ephestia kuehniella* Zeller in laboratory conditions (Oztemiz 2007). Adult parasitoids were stored at -20°C before being homogenized for the electrophoretic study. Voucher specimens have been deposited in the insect museum unit of the Plant Protection Research Institute in Adana, Turkey.

Electrophoretic studies: Vertical polyacrylamide gel electrophoresis was carried out according to the technique described by Pintureau (1993). Living adults of each population was homogenized in a 20 µl mixture of 10% sucrose solution and borate buffer (pH: 7). The homogenates were centrifuged and the supernatant syringed into wells in the gel. Electrophoresis was carried out at 4°C at constant amperage (20 mA) on a native polyacrylamide vertical gel (8%). Tris glycine (pH: 8.3) was used as a running buffer. Gel was stained for esterase at 37°C for 20 minutes. Fast Blue RR in 0.1 M phosphate buffer (pH: 7) containing α-naphtyl acetate (dissolved in acetone) was used for staining.

Biological studies: Ten replicates of each culture were used for comparison of longevity and parasitization rates of cultures. Female *Trichogramma* were fed honey and their vitality was recorded daily until all had died. Female wasps were placed individually in glass tubes and 50±5 host eggs were given to each wasp for parasitization. Eight days after eggs were given to the parasitoids, the numbers of parasitized eggs were scored. After parasitoids emerged from the host eggs, numbers of female and male were recorded for each culture.

Crossing studies: Reciprocal crosses were performed between the two species. *T. brassicae* (Tb) and *T. turkestanica* (Tt) were crossed each other (Tb1♀ x Tb2♂, Tb1♂ x Tb2♀, Tb1♀ x Tt1♂, Tb1♂ x Tt1♀, Tb2♀ x Tt1♂ and Tb2♂ x Tt1♀).

Morphological studies: Male antennal features i.e. the length of flagellum (FL), the longest seta of flagellum (LSF); and body length (BL), head width (HW), length of hind tibia (HT) and length of hind wing (HWL) were measured in 10 individuals using light microscopy. For light microscopy male wasps were mounted on a microscope slide according to Knutson (1998).

Statistical analysis: Biological and morphological data were analyzed using one way analysis of variance (ANOVA) and the differences between groups were tested at $P<0.05$ significance level using Tukey's honestly

significant difference (HSD) test as implemented in SPSS 13.0 for Windows.

Results

The number of parasitized eggs and total emergence, female and male emergence are presented in Fig. 2 and Table 2. The difference in parasitism (F=0.068; df=2; P=0.935), total emergence (F=0.072; df=2; P=0.930), female emergence (F=0.002; df=2; P=0.998) and male emergence (F=2.798; df=2; P=0.079) were not significant between groups. Figure 3 shows the female adult longevity of populations. *T. turkestanica* survived only one day longer compared with others.

In morphological studies, body length, flagellum length, the longest seta at the flagellum and hind wing length except head width and hind tibia length were not significantly different between *T. brassicae* whereas *T. turkestanica* was differentiated from other groups according to these features. Head width and hind tibia length were significantly different among all groups (Fig. 4, Table 3).

In crossing experiments, only *T. brassicae* populations were reproductively compatible, *T. brassicae* and *T. turkestanica* were reproductively incompatible (Tb1♀ x Tt1♂, Tb1♂ x Tt1♀, Tb2♀ x Tt1♂ and Tb2♂ x Tt1♀).

Isozyme patterns of the cultures are shown in Fig.1. There are two banding groups, slower

Table1. *Trichogramma* species and populations used in the study

Species	Locality	Original Host	Date of Collections
T. brassicae	N 37° 6′ 17.0″ E 35° 6′ 39.8″ Elevation: 86m	*Ostrinia nubilalis*	September 2006
T. brassicae	N 37° 13′ 34.0″ E 35° 5′ 37.0″ Elevation: 173m	*Ostrinia nubilalis*	September 2006
T. turkestanica	N 37° 6′ 41.2″ E 35° 6′ 43.6″ Elevation: 95m	*Ostrinia nubilalis*	September 2006

Table 2. Number of parasitized eggs and emergent offspring in one *T. turkestanica* and two *T. brassicae* cultures. Parasitization, total emergence, and female and male emergence were not significantly different between cultures (P>0.05)

Population	Parasitization	Total Emergence	Female Emergence	Male emergence
Tb1	4.475±0.284	3.533±0.756	3.282±0.685	1.630±0.397
Tb2	4.527±0.881	3.403±1.009	3.288±0.992	1.312±0.295
Tt	4.430±0.436	3.428±0.621	3.303±0.562	1.353±0.274

Table 3. Comparing some characteristics of *T. brassicae* with *T. turkestanica*. (µm±S.D)

Population	BL	HW	FL	LSF	HT	HWL
Tb1	342.7±0.823^{a}	169.9±0.737^{a}	189.6±1.429^{a}	98.6±0.699^{a}	135.8±1.751^{a}	305.9±0.567^{a}
Tb2	342.6±0.699^{a}	166.2±1.316^{b}	188.5±0.527^{a}	99.2±0.788^{a}	134.0±0.942^{b}	305.9±0.567^{a}
Tt	344.8±0.632^{b}	158.9±0.875^{c}	180.0±0.816^{b}	97.0±0.471^{b}	130.4±0.516^{c}	304.7±0.483^{b}

(Est1) and faster (Est2) bands. *T. turkestanica* was differentiated from *T. brassicae* according to its banding pattern.

Discussion

It is important to know the biological characteristics of *Trichogramma* before using them in biological control programs. Some parameters such as sex ratio and longevity can be used in selecting the most suitable species of *Trichogramma*. We found no significant differences in the number of parasitized eggs between *T. turkestanica* and *T. brassicae* (F=0.068; df=2; P=0.935). In contrast, Haile et al. (2002) found significant differences in parasitization potential between two strains of *T.* sp. nr. *mwanzai* Schulten and Feijen and *T. bournieri* Pintureau and Babault but there was no significant differences in adult longevity between these strains. In the study, *T. turkestanica* survived only one day longer compared with *T. brassicae* populations (Fig.3). Morphological

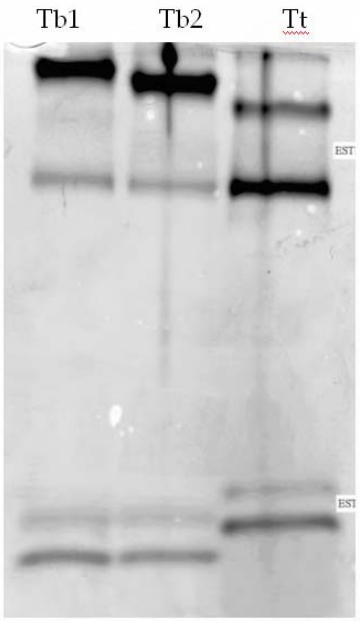

Figure 1. Esterase zymogram of *T. brassicae* and *T. turkestanica* (Tb1: First *T. brassicae* population, Tb2 Second *T. brassicae* population, Tt: *T. turkestanica*), slower (Est1) and faster (Est2) bands.

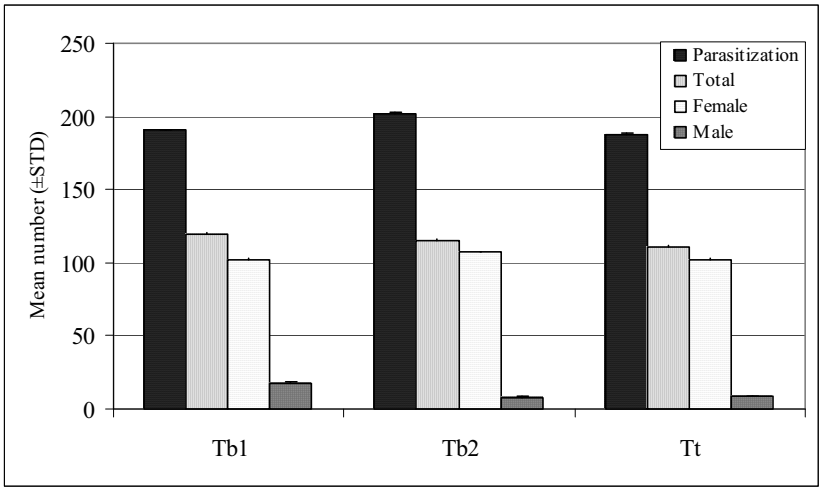

Figure 2. Number of parasitized eggs and emergent offspring in one *T. turkestanica* and two *T. brassicae* cultures.

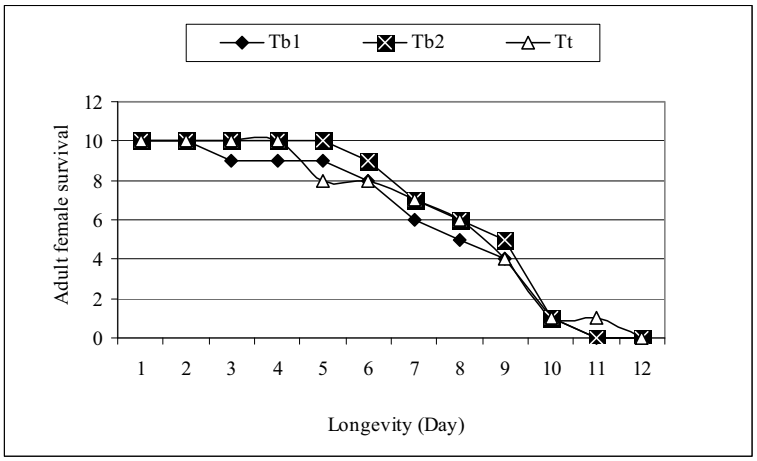

Figure 3. Adult female survival of *T. brassicae* and *T. turkestanica.*

data showed that the two *T. brassicae* populations were not able to be differentiated according to these characters, with the exception of head width and hind tibia length (Table 3). On the other hand, *T. turkestanica* was statistically significantly ($P<0.05$) different in body length, flagellum length, the longest seta at the flagellum and hind wing length from *T. brassicae*. So *T. turkestanica* can be easily differentiated from *T. brassicae* populations according to all morphological characters (Fig.4).

In the study, we determined four electrophoretic bands in the esterase enzyme system (Fig.1). Two faster and two slower activity bands for each group were detected. The banding patterns of *T. turkestanica* differed from *T. brassicae*, the two populations of which had the same banding patterns and same morphological features as each other. Therefore, it was determined that the two *T. brassicae* populations that were reproductively compatible also had the same isozymes. Compatible cultures produced 20% or greater level of female progeny (Pinto et al. 2003).

Ram et al. (1995) showed that esterase banding patterns of *Trichogramma* strains from Moldavia and Slovakia were differentiated by using mobility and intensity of bands. Miura et al. (1990) found two high and two low activity bands for *T. chilonis* Ishii while only one high and two low activity bands for *T. ostriniae* Pang and Chen. They used a single wasp of each species for identification. Differences of activity of bands on the gel derived from the size of adults used for homogenization. Isozymes of different species showed different banding patterns. We detected two high and two low activity bands for each culture like *T. chilonis.* In another study, esterase zymograms of *T. evanescens* Westwood strains containing six bands were obtained (Ram et al. 1995). The host and rearing conditions used in this study were different from ours. Smith & Hubbes (1986) reported that the isoenzymogram of *T. minutum* Riley was not affected by temperature, but, isoenzyme patterns did differ according to host. Basso et al. (1999) showed that there were eight esterase bands in vertical gel for *T. pretiosum* Riley strains of which only one band showed any variation. For *T. exiguum* Pinto & Platner four to six bands were determined and most of them were variable. Our banding patterns differ from theirs but we used same number of individuals descending

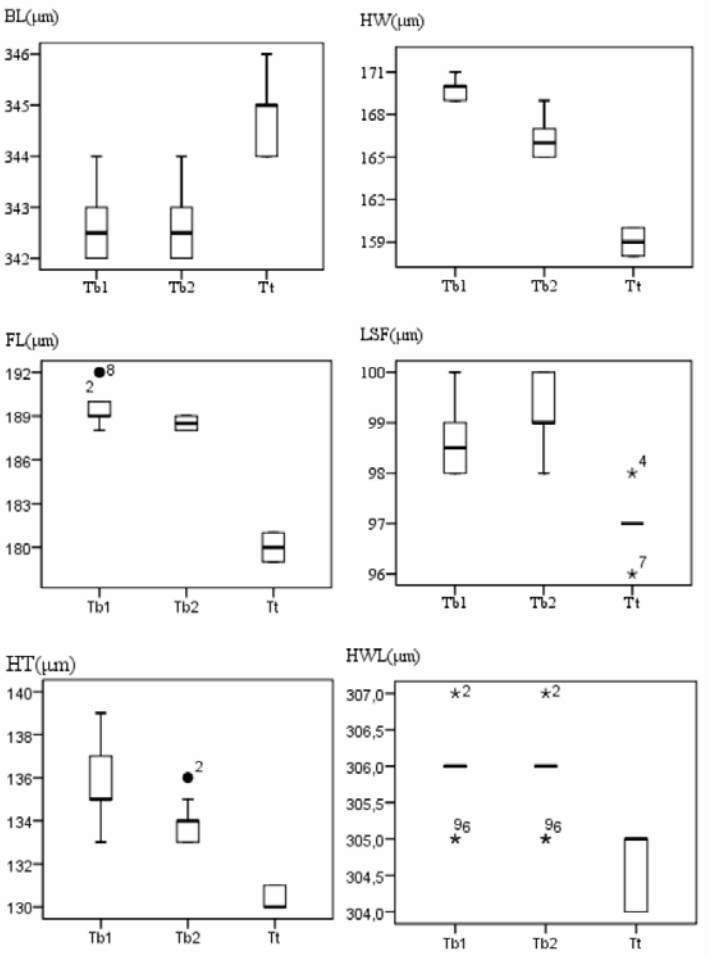

Figure 4. Comparing some characteristics of *T. brassicae* with *T. turkestanica*. BL (body length), HW (head width), FL (flagellum length), LSF (longest seta at flagellum), HT (hind tibia), HWL (hind wing length). The unit of measurements is micrometer (µm).

from a virgin female. Pintureau & Babault (1981) used esterases and malate dehydrogenases for the characterization of *T. evanescens* and *T. maidis* Pint. & Voeg. strains.

In conclusion, esterase isozymes of two *Trichogramma* species; *T. turkestanica* and *T. brassicae* were determined. Differences in mobility of bands indicated different isozymes. Esterase bands proved to be a useful technique for discrimination between *T. turkestanica* and *T. brassicae*. The numbers of parasitized eggs and emergent female and male offspring were not significantly different (P>0.05) between the three populations. Adult female survival of cultures showed no difference between populations. *T. turkestanica* was different from other

groups according to morphological characters of except head width and hind tibia length (Table 2). These differences were statistically significant ($P<0.05$) according to Tukey's HSD tests. Cultures that were reproductively compatible had the same banding patterns and had the same morphological features.

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