Genetic divergence of *Trichogramma aurosum* Sugonjaev and Sorokina (Hymenoptera: Trichogrammatidae) individuals based on ITS2 and AFLP analysis

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Keywords
Trichogramma aurosum, AFLP, biological control, ITS2 region, parasitoids, rDNA

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Received: November 15, 2006; accepted: October 3, 2007.

doi: 10.1111/j.1439-0418.2007.01245.x

Abstract

Taxonomy and phylogeny of members of the genus *Trichogramma* is often critical because of the fact that proper species discrimination can only be achieved by male morphology. Cryptic species, particularly when only females are available (in case of parthenogenetic species or strains), are common in this genus with consequences for practical purposes like biocontrol, unless males can be obtained after antibiotic treatment. The internally transcribed spacer 2 region of the ribosomal DNA was used to assess the identity of *Trichogramma aurosum* Sugonjaev and Sorokina individuals collected on eggs of *Nematus tibialis* Newman (Hymenoptera: Tenthredinidae) from different locations in Middle Europe. Amplified products were identical in length (ca. 450bp), sequences showed a high percent similarity (>96%), and no cryptic species could be detected in the samples. In contrast, a comparison with *T. aurosum* populations from the USA showed values between 86% and 90%. Additional studies are needed to clarify the relationship between US and European populations. Furthermore, amplified fragment length polymorphism (AFLP) analysis was conducted with *T. aurosum* wasps collected at 25 different European locations. One hundred and twenty-three AFLP fragments could be detected using three different AFLP primer combinations of which 98% were polymorphic in more than one individual. An analysis of genetic distances based on the obtained AFLP markers indicated the existence of some genetic variability between the European *T. aurosum* individuals and allowed a grouping according to their geographic origin. This study represents the first successful application of the AFLP marker technique to such tiny insects as *Trichogramma* species.

Introduction

Trichogramma spp. (Hymenoptera: Trichogrammatidae) are minute parasitoid wasps (<1mm in length) that parasitize eggs of several insect orders, especially members of the order Lepidoptera. Several *Trichogramma* species are mass-reared and released for biological control purposes at present (Smith 1996). However, correct identification of *Trichogramma* spp. is crucial for successful biological control programmes (Smith and Hubbes 1986; Stouthamer et al. 1999). Classical taxonomy of *Trichogramma* relies on morphological features, especially on the structure of male genitalia, which is a time-consuming process and requires a skilful specialist (Pinto 1999). However, a large number of species show

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remarkable morphological homogeneity and, on the top of it, thelytokous populations/species that usually carry *Wolbachia* symbionts present difficulties for taxonomy based on male characters (Stouthamer et al. 1999; Querino and Zucchi 2002; Almeida de and Stouthamer 2003). Yet, stable bisexual (arrhenotokous) *Trichogramma* lines can be obtained from *Wolbachia*-cured species after treatment with antibiotics (Grenier et al. 2002).

DNA fingerprinting techniques for the analysis of genetic variation have become an important approach in taxonomy as well as in genetic and evolutionary studies of insect species (Loxdale and Lushai 1998). The most frequently used DNA markers include restriction fragment length polymorphisms (RFLPs) of mitochondrial or nuclear DNA, DNA fingerprinting of microsatellite or minisatellite sequences, and random amplified polymorphic DNA (RAPD) analysis of nuclear DNA. Another fingerprinting technique called *a*mplified *f*ragment *l*ength *p*olymorphisms (AFLP) was developed, which is based on selective amplification of a subset of DNA fragments generated by restriction endonucleases (Vos et al. 1995). Comparing with RFLP and RAPD, it exhibits a higher resolution and information content, has a good reproducibility, it requires only a low amount of genomic DNA (Reineke et al. 1998) and can be used with stored, dry or old samples (e.g. museum samples; Loxdale and Lushai 1998). However, it requires a complete digestion of intact, high-molecular weight genomic DNA.

Few studies have so far investigated the molecular genetics of *Trichogramma* spp. (Smith and Hubbes 1986; Laurent et al. 1998; Stouthamer et al. 1999). Since *Trichogramma* individuals are minute in size compared with other insects, little amount of genomic DNA is obtained (Reineke et al. 1998), which makes investigations with molecular techniques based on restriction digest of total genomic DNA such as RFLPs difficult. The importance of properly matching the correct *Trichogramma* species or strain to the appropriate pest situation has been discussed extensively (Stouthamer et al. 1999). Several studies reported cases of misidentification of natural enemies in initially unsuccessful biological control projects (Wajnberg 1994). On the other hand, the ability to differentiate between populations from different geographical origins would help to better understand the differences found within and/or in the population before selecting a proper candidate wasp strain for mass production and subsequent field release. Sequence analysis of the internal transcribed spacer region (ITS2) has gained increasing importance in the identification of cryptic *Trichogramma* species, in studying differentiation within populations as well as for the reconstruction of phylogenetic relationships between closely related species (Stouthamer et al. 1999; Ciociola et al. 2001; Pinto et al. 2002). ITS2 sequences have been shown to be a suitable tool to separate closely related species, such as *Trichogramma deion* Pinto and Oatman, *Trichogramma kaykai* Pinto and Stouthamer, *Trichogramma sathon* Pinto, *Trichogramma pratti* Pinto, *Trichogramma pretiosum* Riley, *Trichogramma interius* Pinto, and *Trichogramma oleae* Voegelé and Pointel (Stouthamer et al. 1999). Genetic analysis of the ITS2 thus allows to distinguish species or subspecies that may be difficult or impossible to differentiate on the basis of morphological or phenotypic characteristics.

This work aimed at differentiating *Trichogramma aurosum* (Sugonjaev and Sorokina 1976) individuals collected at various locations in middle Europe by two different molecular approaches. *Trichogramma aurosum* is a holarctic species belonging to the *exiguum* section (Pinto 1999). It is widely distributed in eastern and western Europe as well as in North America (Sorokina 1993), with no evidence existing in the literature towards an introduction of *T. aurosum* from eastern Europe into western Europe or into North America. Hosts of *T. aurosum* belong either to the order Hymenoptera (i.e. *Nematus tibialis* Newman) or Lepidoptera (i.e. *Cydia pomonella* L.), which indicates that *T. aurosum* could be a strong potential candidate for biological control of insect pests of both orders. Here, we amplified and sequenced the ITS2 region of *T. aurosum* individuals and analysed genetic differences between European and American genotypes. In addition, the AFLP technique was applied to assess the genetic variability among 25 *T. aurosum* individuals collected at different localities in Germany and in six other European countries. Hence, this study should provide information about the genetic differentiation in this wasp species on a European scale.

Materials and Methods

Collection of *Trichogramma* individuals

Field trips and collection from the field were done during summer 2002 and 2003 by collecting parasitized (blackened) eggs of *N. tibialis* on leaves of *Robinia pseudoacacia* L. trees in several locations in the German Federal Republic and its neighbouring countries (Table 1). Parasitized eggs were transferred to

Table 1 List of collected *Trichogramma aurosum* individuals, their geographic locations with latitude and longitude and time of collection

T. aurosum code	Collection place	Latitude	Longitude	Time of collection
Ta4	Germany: BaWü, Stuttgart	48°42′N	9°13′E	July 2001
Ta5	Germany: BaWü, Heilbronn	49°08′N	9°13′E	June 2001
Ta6	Germany: BaWü, Hochberg	47°58′N	9°31′E	August 2001
Ta7	Germany: BaWü, Neckargmünd	49°27′N	8°29′E	August 2001
Ta8	Germany: BaWü, Heidelberg	49°24′N	8°43′E	August 2001
Ta9	Germany: BaWü, Mannheim	49°29′N	8°27′E	August 2001
Ta10	Germany: Hesse, Worms	49°39′N	8°21′E	August 2001
Ta11	Germany: Saxony, Moritzburg	51°09′N	13°41′E	September 2001
Ta12	Germany: RhP, Mainz	50°0′N	8°16′E	July 2002
Ta13	Germany: Bavaria, Munich	48°08′N	11°35′E	July 2002
Ta14	Germany: Bavaria, Munich	48°09′N	11°30′E	July 2002
Ta15	Germany: NRW, Gevelsberg	51°18′N	7°19′E	August 2002
Ta16	Germany: BaWü, Freiburg	47°59′N	7°50′E	August 2002
Ta17	Germany: BaWü, Singen	47°45′N	8°50′E	August 2002
Ta18	Germany: BaWü, Ulm, Eselsberg	48°44′N	8°00′E	August 2002
Ta19	Germany: Lower Saxony, Göttingen	51°32′N	9°55′E	August 2002
Ta20	Germany: Berlin, Schöneberg	52°28′N	13°22′E	August 2002
Ta22	Austria: Vienna, Schönbrunn castle	48°11′N	16°18′E	July 2003
Ta23	Austria: Vienna, Botanical Garden	48°11′N	16°22′E	July 2003
Ta26	Luxembourg, Luxembourg	49°36′N	6°07′E	July 2003
Ta27	Luxembourg, Luxembourg	49°36′N	6°07′E	July 2003
Ta28	Belgium, Brussels, Hallepoort	50°50′N	4°21′E	July 2003
Ta30	France: Paris, Quai d' Orsay	48°42′N	2°10′E	August 2003
Ta31	France: Paris, Porte Dauphine	48°51′N	2°15′E	August 2003
Ta33	The Netherlands, Amsterdam	52°22′N	4°53′E	August 2003
Ta34	Denmark, Copenhagen	55°41′N	12°34′E	August 2003

the laboratory, where *Trichogramma* individuals were allowed to hatch. The collected strains were maintained for further laboratory experiments (Samara 2005) on eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), in a climatic cabinet at ca. 25°C, 85% RH and 18:6h L:D photoperiod. After parasitization of *E. kuehniella* eggs, wasps were collected and stored in 100% ethanol for subsequent genetic studies. Male specimens of each strain were mounted onto Canada balsam following Platner et al. (1999), and identified to species level using the terminology of Pinto (1999). Voucher specimens of all *T. aurosum* strains are deposited at the collection of the Department of Entomology, University of Hohenheim.

DNA extraction

DNA was extracted from single males of each location following a modified cetyltrimethyl ammonium bromide protocol; with an additional polyethylene glycol precipitation (Reineke et al. 1998). The resulting DNA pellet was resuspended in 50μl buffer (10mM Tris–HCl; 1mM EDTA; pH 8.0) overnight and DNA concentration was measured in a UV spectrophotometer (GeneQuant II, Amersham Biosciences, Freiburg, Germany). DNA samples with concentrations of at least 15ng/μl were used for further analysis.

Table 2 List of amplified fragment length polymorphism primers and their sequences used in the present study

Primer	Code	Sequence 5′–3′
*Eco*RI adaptor		CTCGTAGACTGCGTACC
		CATCTGACGCATGGTTAA
*Mse*I adaptor		GACGATGAGTCCTGAG
		TACTCAGGACTCAT
*Eco*RI primer		
*Eco*14	E14	GACTGCGTACCAATTCAAG*
*Eco*15	E15	GACTGCGTACCAATTCACA*
*Eco*16	E16	GACTGCGTACCAATTCACC*
*Mse*I primer		
*Mse*10	M10	GATGAGTCCTGAGTAACA
*Mse*22	M22	GATGAGTCCTGAGTAAT
*Mse*23	M23	GATGAGTCCTGAGTAATGT
*Mse*28	M28	GATGAGTCCTGAGTAAGCT
*Mse*33	M33	GATGAGTCCTGAGTAATAG
*Mse*34	M34	GATGAGTCCTGAGTAAGGA

*Cy-5 fluorescently labelled primer.

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Table 3 Pair-wise distances of *Trichogramma aurosum* individuals based on sequence analysis of the internally transcribed spacer 2 region obtained by clustal v (weighted)*

	Ta10	Ta28	Ta11	Ta16	Ta19	Ta8	Ta5	Ta15	Ta34	Ta26	Ta12	Ta9	Ta13	Ta7	Ta31	Ta4	Ta17	USI	USII	Ta23	Ta33	T.pr	T.bou	T.ca	T.ev	T.ex	Swiss
Ta10	***	97	97	97	97.3	97	97	97	96.6	96.8	97	97	97	98.3	97	97	96.3	86.8	90.3	96.3	96.6	70.3	30.8	66.5	66.7	69.7	96.1
Ta28	0.5	***	98.8	100	98.8	100	100	100	99.5	99.8	97.6	100	100	96.1	97.6	100	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta11	0.5	0	***	98.8	97.3	98.8	98.8	98.8	98.3	98.5	98.8	98.8	98.8	96.1	98.5	98.8	98.1	89.1	85.9	98	96.8	69.2	29.9	70.3	66.9	68.1	97.5
Ta16	0.5	0	0	***	98.8	100	100	100	99.5	99.8	97.6	100	100	96.1	97.6	100	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta 19	0.5	0	0	0	***	98.8	98.8	98.8	98.3	98.5	97.6	98.8	98.8	97.8	97.6	98.8	96.8	87	89.8	98	98	69.8	32	70.7	65.9	69.5	97.5
Ta8	0.5	0	0	0	0	***	100	100	99.5	99.8	97.6	100	100	96.1	97.6	100	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta5	0.5	0	0	0	0	0	***	100	99.5	99.8	97.6	100	100	96.1	97.6	100	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta15	0.5	0	0	0	0	0	0	***	99.5	99.8	97.6	100	100	96.1	97.6	100	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta34	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	***	99.8	97.1	99.5	99.5	95.6	97.1	99.5	97.3	85.5	89.5	99.8	97.3	68.7	30.1	70.4	65.8	67.9	98.3
Ta26	0.7	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	***	97.3	99.8	99.8	95.8	97.3	99.8	97.1	85.7	89.8	99.5	97.1	69	30.3	70.7	66	68.1	98.5
Ta12	0.5	0	0	0	0	0	0	0	0.5	0.2	***	97.6	97.6	96.6	99.8	97.6	99.3	85.7	89.8	96.8	96.6	69.2	30.3	70.7	67.1	68.4	96.3
Ta9	0.5	0	0	0	0	0	0	0	0.5	0.2	0	***	100	96.1	97.6	100	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta13	0.5	0	0	0	0	0	0	0	0.5	0.2	0	0	***	96.1	97.6	100	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.7	0.7	0.5	0.5	***	96.6	96.1	95.8	87	89.8	95.3	97.1	70.6	29.2	71.1	66.2	69.2	95.8
Ta31	0.5	0	0.2	0	0	0	0	0	0.5	0.2	0.2	0	0	0.7	***	97.6	99	85.7	89.5	96.8	96.4	68.7	30.3	70.9	66.3	68.4	96.3
Ta4	0.5	0	0	0	0	0	0	0	0.5	0.2	0	0	0	0.5	0	***	96.8	86	89.8	99.3	96.8	69.2	30.3	65.5	66.3	68.1	98.8
Ta17	1.2	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.2	0.5	0.7	0.7	0.7	1.5	1	0.7	***	84.9	89.3	97.1	96.9	68.4	30	70.2	66.6	67.9	95.6
USI	1.1	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.6	1.3	1.3	1.3	1.3	1.6	1.3	1.3	1.9	***	94.5	85.2	86.2	66.8	31.9	67.5	69.9	70.8	85.7
USII	1.3	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.9	1.6	1.6	1.6	1.6	1.9	1.6	1.6	2.1	1.3	***	89.3	89.5	66.6	34.3	73.6	68.8	68.1	90.1
Ta23	1.3	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.2	0.5	0.7	0.7	0.7	1.2	0.7	0.7	0.5	1.9	2.1	***	97.1	68.4	29.8	70.2	65.5	71.3	98
Ta33	1.2	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.2	0.5	0.7	0.7	0.7	1.2	1	0.7	0.5	1.9	1.9	0.5	***	68.7	29.5	69.6	65	69.7	95.6
T.pr	11.5	11.5	11.8	11.5	11.8	11.5	11.5	11.5	12.1	11.8	12.3	11.5	11.5	11.8	12	11.5	13.3	12.5	13.2	12.5	12.6	***	30.8	62.1	63.9	60.2	69.2
T.bou	58.7	57.4	58.1	57.4	57.7	57.4	57.4	57.4	57	57.5	58.6	57.4	57.4	58.1	58.6	57.4	58.8	56	56.6	57.5	58.8	68.3	***	31.5	25.6	35.8	34.9
T.ca	38.9	38.9	38.6	38.9	38.8	38.9	38.9	38.9	39	38.9	38.4	38.9	38.9	39.4	38.8	38.9	38.9	35.2	34.9	38.9	39.6	45.2	91.3	***	59.8	62.7	65.8
T.ev	20.5	21.5	21.4	21.5	21.5	21.5	21.5	21.5	22.2	21.9	21.3	21.5	21.5	21.1	21.6	21.5	22.3	17.3	16.4	22.5	22.3	25	68.2	43.8	***	64.2	66.6
T.ex	9.3	9.5	9.7	9.5	9.5	9.5	9.5	9.5	9.8	9.5	9.7	9.5	9.5	9.8	9.4	9.5	10.3	8.5	9.3	10.1	10.3	16.9	55.7	34.9	20.2	***	68.1
Swiss	0.5	0	0	0	0	0	0	0	0.5	0.2	0	0	0	0.5	0	0	0.7	1.3	1.6	0.7	0.7	11.5	57.4	38.3	21	9.2	***

*Percent similarity is denoted in upper triangle and percent divergence in lower triangle.

Analysis of the ITS2 region

Polymerase chain reaction (PCR) of ITS2 of the ribosomal DNA was performed using the specific forward 5′-TGTGAACTGCAGGACACATG-3′ and reverse 5′-GTCTTGCCTGCTCTGAG-3′ primers (Stouthamer et al. 1999). PCR products were electrophoresed and excised from the agarose gel. Negative controls lacking template DNA were included in all experiments. The samples were purified with a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sent for sequencing (GATC Biotech AG, Lake Constance, Germany). The sequences were aligned using the Clustal V algorithm (Higgins and Sharp 1989) as implemented in the program MEGALIGN version 5.03 (DNAStar Inc., Madison, MA, USA). A phylogenetic tree was produced using the same software. As reference taxons, the following ITS2 sequences deposited in the GenBank database were used: two populations of *T. aurosum* from the USA (accession nos. AF408667 and AF408668) and one from Switzerland (accession no. AY146649); the following species also belonging to the *exiguum* section: *T. pretiosum* (accession no. AF282233), *T. cacoeciae* Marchal (accession no. AY146442), *T. evanescens* Westwood (accession no. AF043618), *T. exiguum* Pinto and Platner (accession number AY 182768), and a morphologically distant-related species of the *parkeri* section, *T. bourarachae* Pintureau and Babault (accession no. AF043625).

AFLP reactions and data analysis

AFLP analysis was used to analyse genetic diversity between European *T. aurosum* individuals. AFLP analysis was performed according to a modified protocol of Reineke and Karlovsky (2000), using Cy-5 fluorescently labelled *Eco*RI primers for selective amplifications. The sequences of AFLP adaptors and primers, respectively, are given in Table 2. Amplification products were loaded on a 5% denaturing polyacrylamide gel and separated for 1.5h at 42V/cm in an ALFexpress II Automatic Sequencer (Amersham). As a size standard, ALFexpress size marker (Amersham Biosciences) was run at the beginning and end of the gel lanes.

Using ALFEXPRESS image evaluation software (Amersham Biosciences), a tiff image of the gel was collected and AFLP digital images patterns were analysed using GEL COMPARE 4.0 software (Applied Maths, Belgium). GEL COMPARE assigned bands using band search filters according to Reineke et al. (1999). A band position tolerance value of 0.1% of the total length of the pattern was used for band comparison. Using these parameters, the same AFLP reactions run on different gels were grouped together with genetic similarities of 96–100%. A binary matrix of the presence (1) or absence (0) of AFLP band was created, and statistics on genetic diversity were computed following Lynch and Milligan (1994) and using the program AFLP-SURV version

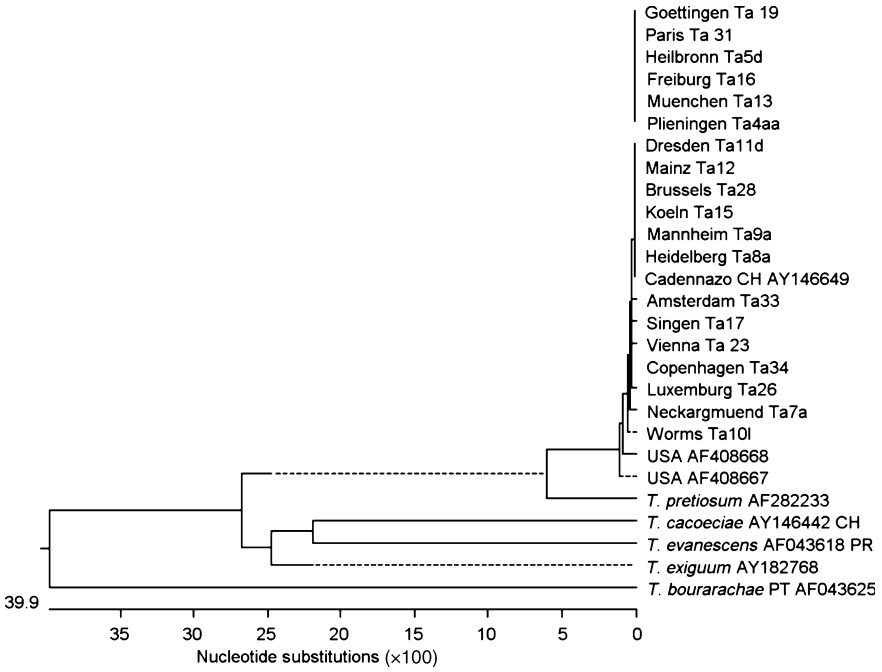

Fig. 1 Phylogenetic tree based on an analysis of the internally transcribed spacer 2 region of *Trichogramma aurosum* individuals from different European and American localities and sequences from other *Trichogramma* species deposited in the database based on Clustal V alignment. ITS2 sequences of European *T. aurosum* individuals are deposited in GenBank under the following accession nos: Ta4aa: EF710754; Ta5: EF710755; Ta7: EF710756; Ta8: EF710757; Ta9: EF710758; Ta10: EF710759; Ta11: EF710760; Ta12: EF710761; Ta13: EF710762; Ta15: EF710763; Ta16: EF710764; Ta17: EF710765; Ta19: EF710766; Ta23: EF710767; Ta26: EF710768; Ta28: EF710769; Ta31: EF710770; Ta33: EF710771; Ta34: EF710772.

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1.0 (Vekemans 2002). To account for the dominant nature of AFLP markers, allele frequencies were calculated using a Bayesian method with uniform prior distribution of allele frequencies. Based on genetic distances between pairs of individuals, a dendrogram was constructed using the unweighted pair group methods of arithmetic averages (UPGMA) with the help of the program MEGA3.1 (Kumar et al. 2004).

Results and Discussion

Internally transcribed spacer 2

Molecular methods have been proposed to help in the identification of *Trichogramma* species (Smith and Hubbes 1986; Pinto et al. 1993, 2002; Vanlerberghe-Masutti 1994; Sappal et al. 1995; Laurent et al. 1998). DNA sequence analysis of the ITS2 of the ribosomal gene complex, showing little variation within species, was more recently used (Orrego and Agudelo-Silva 1993; Stouthamer et al. 2000; Pinto et al. 2002). Most of the variation was restricted to the number of microsatellite repeats. Overall, the molecular approach based on ITS2 sequence analysis was proved to be a reliable method for identifying *Trichogramma* species (Stouthamer et al. 1999; but see Stouthamer et al. 2000). In our study, the amplified products of the ITS2 region of all European *T. aurosum* individuals showed the same size on an agarose gel (ca. 450bp, not shown). Sequence analysis of the ITS2 region (411nucleotides) of these populations revealed a high degree of homology (>96%) (Table 3, Fig. 1; GenBank accession nos EF710754–EF710772). However, when the ITS2 sequences were compared with those of two *T. aurosum* strains from USA, homology ranged from 86% to 90% (Fig. 1). Yet, the *aurosum* clade was depicted as monophyletic, and it formed a second group with the other four species of the *exiguum* section (*T. pretiosum*, *T. cacoeciae*, *T. evanescens*, and *T. exiguum*). This second group was clearly separated from the more distant-related *T. bourarachae*. The obtained homology values for sequences of *T. aurosum* raise the question, whether European and US populations belong to the same, or to very close distinct species. Pinto (1999) states that North American specimens are morphologically very similar to the type series from Russia and adds: 'Dr. A.P. Sorokina, one of the author of this species, examined North American material and agrees (*in litt.*) that Palaearctic and Nearctic populations should be considered conspecific'. In our opinion, additional studies including both morphological and molecular characters are necessary to clarify the relationship between European and US populations in detail.

Amplified fragment length polymorphism

As the AFLP technique displays presence or absence of restriction fragments rather than length differences, an initial survey was conducted for a few number of *T. aurosum* strains with 18 *Eco*RI–*Mse*I primer combinations either with one, two or three selective nucleotides at their 3′-end (Table 2). For some *T. aurosum* individuals, amplification of reproducible AFLP banding patterns was not always possible, which is likely to be because of very low DNA amounts obtained from single wasps (see e.g. Fig. 2, lanes 2 and 9). These individuals were thus excluded

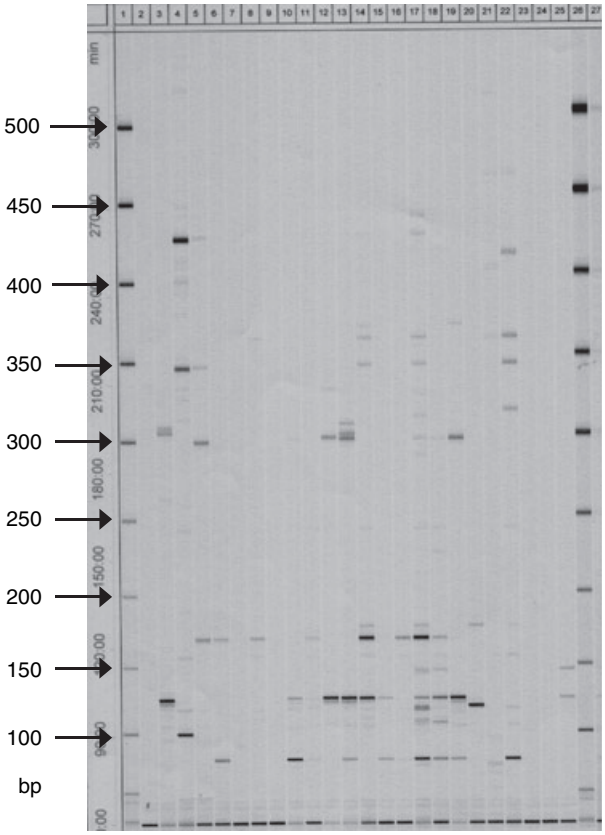

Fig. 2 AFLP fingerprints generated from genomic DNA of 31 *Trichogramma aurosum* individuals from seven different geographic origins using primer combination E16/M34 (E+ACC/M+GGA). Lanes 2–7 *T. aurosum* from Austria; lanes 8–11 from Luxembourg; lanes 12–15 from Belgium; lanes 16–21 from France; lane 23 from The Netherlands; lanes 24–25 from Denmark. Lanes 1 and 26 size marker, fragment sizes are indicated on the left.

from the subsequent AFLP analysis. As a result, three AFLP primer combinations (*Eco*16/*Mse*33; *Eco*16/*Mse*34; *Eco*14/*Mse*33) were chosen which showed a clear and reproducible banding pattern and were subsequently used for generating AFLP fingerprints in 25 *T. aurosum* individuals from different European localities (Fig. 2). The total number of fragments recovered from the wasps by the presence of markers was 123 with a mean number of fragments of 29 per individual. AFLP fragment size ranged from 43bp to 398bp.

Based on AFLP patterns, a matrix was constructed in which each individual was characterized by the presence or absence of a band at the respective position. From this matrix a genetic distance coefficient was calculated between each pair of individuals and results were graphically presented as a cluster analysis (Fig. 3). Two individuals collected at the same location in Germany, Munich (Ta13 and Ta14) showed the lowest distance values and were thus grouped close together in cluster analysis. Moreover, UPGMA cluster analysis resulted in two distinct groups of the 25 *T. aurosum* individuals, with all individuals from Germany and Austria forming one group and wasps from Denmark, France, and the Benelux countries forming the second group (Fig. 3). This grouping indicates the existence of genetic variability between these European *T. aurosum* wasps and is thus an important first step towards the identification of populations of this potential biological control agent. In addition, we could use this information for defining best practices

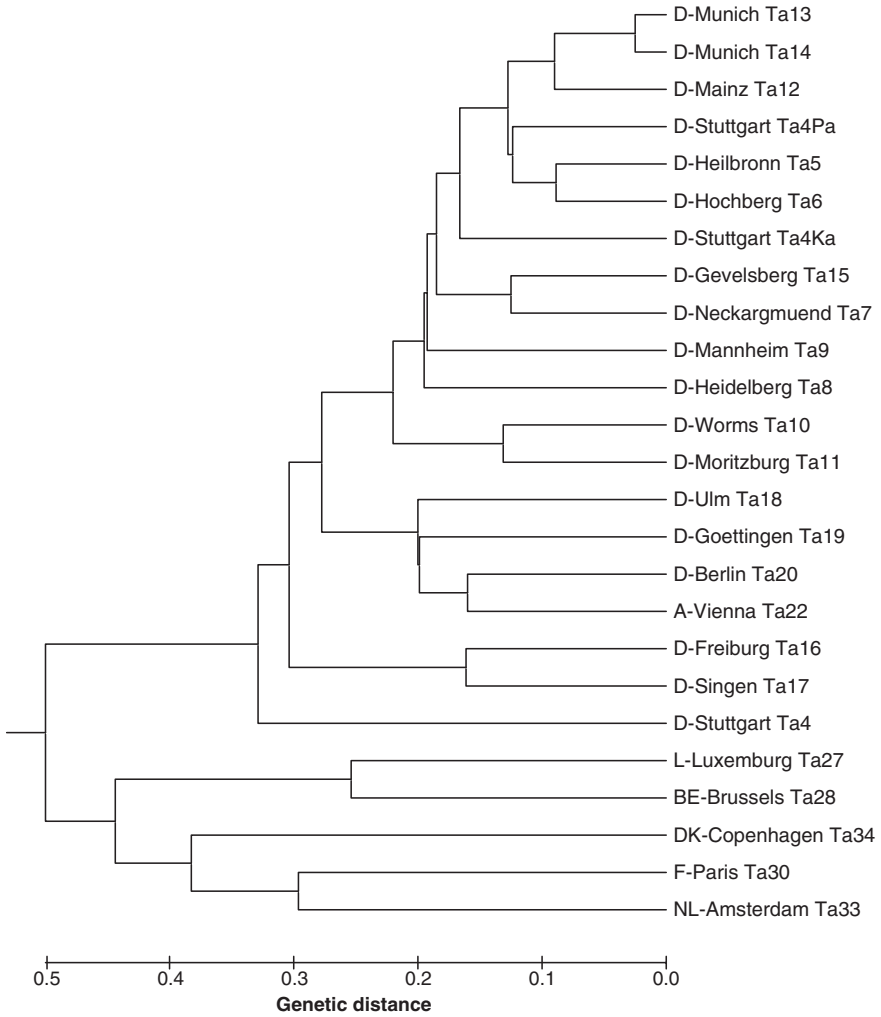

Fig. 3 Unweighted pair group methods of arithmetic averages dendrogram of 25 *Trichogramma aurosum* individuals from different European origins showing genetic distances based on arithmetic fragment length polymorphism data of 123 polymorphic markers.

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for the collection, breeding, and genetic management of these wasps being mass-reared for the release as a biocontrol agent.

Successful AFLP analysis requires a minimum amount of ca. 100ng high-molecular weight DNA, as smaller quantities of DNA often result in poor amplification profiles (for review see Meudt and Clarke 2007). Our study is the first report of the successful application of the AFLP marker technique to such a tiny insect species. We also encountered some problems in amplifying reproducible AFLP products from some individuals (see above). This study nonetheless shows, that AFLP markers have a high potential to reveal genetic diversity and represent a highly useful and important tool for population genetic studies in this important biological control agent.

Acknowledgements

The authors would like to thank the German Academic Exchange Service (DAAD, A/00/19004) for the financial support of this research. Thanks are extended to T. Qubbaj for his help in the field collection, R. Siegel for maintaining the lab culture of *E. kuehniella* and B. Breitinger for technical assistance. We are also indebted to two anonymous reviewers for their valuable comments on an earlier draft of the manuscript.

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