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Biology, Behaviour and Genetic Diversity of *Trichogramma aurosum* Sugonjaev and Sorokina (Hymenoptera: Trichogrammatidae)

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This work is dedicated to my parents, my husband and my children

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CONTENTS

Abbreviations

a/c	acceptance : contact ratio
AFLP	amplified fragment length polymorphisms
bp	base pair
d	day
DD	degree day
D_t	doubling time
H	hour
ha	hectare
IGR	insect growth regulators
ITS2	internal transcribed spacer 2
L:D	light : dark period
l_x	number of survivors at a given age
M	molar (mol / l)
min	minute
m_x	age specific fertility
R_0	net reproductive rate
RH	relative humidity
r_m	intrinsic rate of natural increase
SIT	sterile insect technique
spp.	species
T_c	cohort generation time
U	unit of enzyme activity
vs.	versus
λ	finite rate of increase

CHAPTER 1

1. GENERAL INTRODUCTION

1.1 Codling moth life history and distribution

The Codling moth, *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae), is a major pest on pome fruits (Madsen and Morgan 1970, Blomefield 1989). Although it has generally spread around the world, along with the cultivation of apples and pears, it has not been reported from China, Korea and Japan (Chen and Tseng 1992, Bajwa and Kogan 1997, Figure 1-1). Larvae are known to be polyphagous and can feed not only on apples, but also on pears (Howell et al. 1992), cherries (Moffitt et al. 1992), nectarines (Curtis et al. 1991), prunes (Yokoyama and Miller 1999), and even walnuts (Vail et al. 1993). Infestation rates have been reported to reach 95% when no sufficient control methods were applied (Anonymous 2002b). *Cydia pomonella* larvae bore into fruit and feed until their larval development is complete, sting and bore into the fruit flesh and presence of frass at the entry site reduces the marketable fruit. Moreover, Jackson (1979) recorded that 80% of Codling moth eggs are deposited on leaves, which suggests that larvae may also feed on them.

The Codling moth hibernates as fully-grown larva in a silken cocoon, usually located under loose bark on the tree trunk and limbs. Cocoons may be also found in brush, on posts, in cracks in the soil, and on harvesting crates (Barnett et al. 1991). The last larval instar (L_5) transforms into a pupa and later into a greyish-brown moth. Colour and markings of the wings are characterized by crisscrossed light grey lines and a bronze- or copper-coloured patch near the outer margins of the forewings. The moth is 8 mm long when it is at rest with its wings folded, and has a 17 mm wingspan (Barnett et al. 1991). The first moths of the season usually appear as the last petals fall from the apple blossoms. The peak of moth emergence occurs 4 to 12 days later, depending on weather conditions (Anonymous 2000). The last moths of the hibernating brood may not appear until six or seven weeks after petal fall. About three days after emergence, the female moths begin to lay single eggs on the fruit and leaves. Each female lays an average of 50 to 60 eggs (Almatni 2003). When temperatures are below 16 °C, few eggs are laid and there is little moth flight activity (Barnett et al. 1991). The egg is tiny, 1.2 mm in diameter, whitish, flattened disc-shaped, and almost transparent. First instar larvae hatch after 8 to 14 days (Anonymous 2000). The newly hatched larva is yellowish-white with a black head; it immediately begins crawling to seek for a fruit on which to feed (Barnett et al. 1991). On the fruit, the larva crawls seeking a rough area, such as the calyx end or scab spot, in which to make an easier entrance into the fruit. The larval stage lasts about three weeks undergoing five distinctive instars (Williams and Mc Donald 1982). The fully-grown larva is 12 mm long, white with a pinkish tinge on the upper surface, and has a brown head (Anonymous 2000). When nearly full-grown, the larva leaves the fruit seeking a suitable place to spin a cocoon (Barnett et al. 1991). Number of generation per year differs from one

year to another according to the climatic conditions (Shel'deshova 1967). Temperature is considered as the most limiting factor for generation development and detraining number of generations per year (Croft and Hoyt 1983).

Figure 1-1. Geographical distribution of the Codling moth, *Cydia pomonella* (Bajwa and Kogan 1997).

1.2 Control methods

1.2.1 Chemical control

Among the strategies that have been followed to control this pest, chemical control was widely used (Madsen and Morgan 1970). During the fifty's of the last century, DDT was the main insecticide used to control the Codling moth, until the pest developed resistance against it (Fisher 1960). Then many organophosphates, carbamates, pyrethroids and other compounds were employed to control the larvae (Oatman and Libby 1965, Barnes 1959, Varela et al. 1993). The proper timing of insecticide applications is critical if they are to be effective against Codling moth; they must be applied just as eggs are hatching. Once the caterpillar has gone into the fruit or nut, it is protected from pesticides (Ohlendorf 1999). The most effective way to time insecticide applications is based on the calculation of degree-days. Meanwhile the use of broad-spectrum chemical insecticides have harmed many non target indifferent and beneficial insects, in addition to the residual effect of insecticides on the environment (Cossentine and Vincent 2002). On the other hand, different classes of insecticides and growth regulators (IGRs) have shown great promising results for controlling this insect. They include the benzoylphenylurea compounds such as diflubenzuron, triflumuron, chlorfluazuron, (Mulder and Gijswijt 1973). Important characteristics of these IGRs are their activity at low dosage, relatively short persistence, their very low toxicity to mammalians and their relatively good selectivity (Anderson and Elliott 1982).

1.2.2 Mating disruption technique

Mating disruption technique using pheromones and additives was used for the first time in 1973 (Cardé and Minks 1995). This technique is based on interrupting the partner-finding process of Codling moth males by application of an unphysiologically high concentration of the respective sex-pheromone. This synthetically produced female sex-pheromone (codlemone) covers the natural pheromone gradient of the calling female (confusion technique). In consequence, the male is not able to find and mate females. Thus, the number of larvae is reduced, since eggs deposited are not fertilized, and the population size of subsequent generations will be reduced (Barrett 1995).

Whereas the confusion technique requires wide area application of pheromones, the attract-and-kill method follows mass-trapping by spot applications of pheromones. The idea behind mass-trapping is to attract all the male moths in the area into sticky traps or insecticide-treated traps. Once trapped, males are no longer available for mating. Unmated females cannot produce viable eggs. In order to obtain the best results, all the males in the area must be caught before they can mate (Charmillot 2000). Therefore, mass-trapping should be attempted in trees isolated by at least 100 m from other trees harbouring Codling moth (especially apple, pear, and walnut trees). It is also recommended to use other control methods in combination with pheromone traps (Hofer and Brassel 1992, Cardé and Minks 1995, Hapke 2003, Hapke et al. 2001).

Both mating disruption techniques reveal best results when the density of the target population is low (~ 1,000 larvae / ha or 2-3 overwintering larvae / tree). Statistical probability of eventual encounters of males and females rises with increasing population density of the target population.

1.2.3 Sterile insect technique

Sterile insect technique (SIT) is another successful control method used to suppress or eradicate the insect population by introducing sexually sterile insects into the natural population, in which they compete with the wild / native insects. Male moths are rendered sterile by either physical factors such as exposure to high or low temperature or exposure to X- or gamma (γ)-ray irradiation (Bloem et al. 1999). On the other hand, Wang et al. (2001) reported that exposure to radio frequency heating (microwave) for 3 min resulted in 100% mortality of Codling moth larvae. Afterwards sterile males are released in quantities such that the ratio of sterile to wild fertile males was deliberately reduced to 10:1 (Proverbs 1969). When the females mate with the sterile males the resulting eggs are not fertilized, causing 90-98% reduction in the pest population (Arneson 1996). SIT was widely used as part of control of the Codling moth and other insect pests in many countries like Canada (Proverbs and Newton 1966), USA (Butt et al. 1970), Western Europe (Wildbolz and Mani 1975), Australia and New Zealand.

1.2.4 Biological control

The Codling moth is attacked by a considerable number of natural antagonists (Blommers 1994, Glen 1982). However, these beneficial species have never been considered the primary control method in commercial orchards. This is because Codling moth can cause major damage, even when present at very low densities. For example, a female moth could lay 30-70 eggs depending on the weather conditions, each individually placed close to an apple fruit (Barnett et al. 1991). This is sufficient for the hatching larvae to cause economic damage, even when the moth population is as low as one female per several trees. Natural enemies are rarely effective in controlling pest populations at these low densities. Classical biological control of Codling moth was attempted in New Zealand early in the 20th century by the introduction of natural enemies from overseas, such as *Ascogaster quadridentata* Wsml. (Hymenoptera: Braconidae: Cheloninae) and *Liotryphon caudatus* Ratzeburg (Hymenoptera: Ichneumonidae). Although these parasitoids successfully established on high populations of Codling moth on neglected apple trees, they have made only a minor contribution to control in commercial orchards.

1.2.4.1 Microbial control

Bacillus thuringiensis (Berliner) (abbr. Bt) is a spore forming bacterium that produces crystalline proteins, which are toxic to many insect taxa. At present, Bt shows little promise for Codling moth control. It was reported that Bt is ineffective against Codling moth because the larvae feed only very few Bt-contaminated surface material and mainly consume the fruit flesh which is not contaminated with Bt (Andermatt et al. 1988, Almatni and Jamal 2003). Another entomophagous pathogen for the Codling moth is *Cydia pomonella* granulovirus (CpGV), which was originally identified in Mexico from Codling moth in 1963 (Tanada 1964). The virus was propagated in Codling moth larvae and sprayed on apple trees in the field. It showed effective and quick control of larvae causing about 98–99% mortality (Falcon et al. 1986). It affects only Codling moth and does not interfere with activity of natural enemies. However, recently decreased susceptibility of *C. pomonella* towards CpGV has been reported, forcing research and farmers to find practical alternatives in biocontrol (Fritsch et al. 2005)

Different entomopathogenous fungi were found to attack the Codling moth. The most infective are *Spicaria farinosa* (Fries) Vuillemin, *Metarrhizium anisopliae* (Metchnikoff) Sorokin, *Aspergillus flavus* Link, *Verticillium lecanii* Zimmerman, *Fusarium oxysporum* Schlecht, *Beauveria brongniartii* (Saccardo) Petch (Pristavko et al. 1975), *Beauveria globulifera* (Spegazzini) Picard, and *Beauveria bassiana* (Balsamo) Vuillemin. The last pathogen reduces the Codling moth populations in the field when it attacks the overwintered larvae (Arkhipova 1965, Hagley 1971). Another approach in microbial control is the use of the entomopathogenous nematode *Steinernema feltiae* Filipjev, which attacks the immature stages of the Codling moth. It has been found in the USA (Dutky and Hough 1955), Czech

Republic (Weiser 1955), and Mexico (Poinar 1979). The use of this nematode for suppression of overwintering Codling moths appears to be promising during the winter months. Its application to the trunks and main branches of apple trees can suppress the moth population to 60% (Dutky 1959).

1.2.4.2 Predators

Birds are one of the most important natural predators of the Codling moth (Falcon and Huber 1991), as well as bats, spiders (Dondale et al. 1979), insects, and some mite species. All of them can feed on both Codling moth eggs and young larvae (before entering the fruit). Codling moths may also be attacked by insects from different orders such as Neuroptera, Thysanura, Heteroptera, and some Coleoptera (Glen 1982, Jaynes and Marucci 1947).

1.2.4.3 Parasitoids

The most important parasitoids of Codling moth belong to the Hymenoptera, but some dipterous species have also been found to parasitize this pest. All life stages can be parasitized except the adult. Several types of parasitoids have been encountered, e.g. egg parasitoids, larval parasitoids (either endo- or ectoparasitic, Glen 1982), ectoparasitoids of cocooned prepupae, as well as larval-pupal endoparasitoids (Rosenberg 1934). A good example of such parasitoids is represented by *A. quadridentata*. This egg-larval parasitoid lays his eggs into the eggs of the Codling moth. The adult parasitoid wasp does not emerge until the following spring, because it is adapted to its host and hibernates with its host-larva (Suckling et al. 2002). Also, several species of the gregarious egg parasitoids *Trichogramma* spp. (Trichogrammatidae) have been employed for attempts at controlling this pest.

1.3. *Trichogramma* spp.

The gregarious egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are tiny wasps that attack eggs of more than 200 insect pests, including borers, webworms, loopers, leafworms, fruitworms, cutworms, Codling moth, bollworms, and armyworms (Knutson 1998). They are the most widely used natural enemies worldwide with several species being mass-produced and sold by a number of commercial companies (Smith 1996). Five species of *Trichogramma* have been reported to be potentially useful for control of the Codling moth. They include *T. platneri* Nagarkatti (Mills et al. 2000), *T. minutum* Riley, and *T. pretiosum* Riley (Yu et al. 1984) in the nearctic region, while *T. dendrolimi* Matsumura and *T. cacoeciae* (Marchal) (also erroneously identified as *T. embryophagum* Hartig) have been tested in the palearctic region (Hassan 1993). Mills et al. (2000) reported 60% reduction of damage in California walnut and apple orchards through releases of *T. platneri*. In Germany, both *T. cacoeciae* and *T. dendrolimi* have been experimentally released achieving 40-60% reduction of pest damage (Hassan et al. 1993). There is, however, necessity to seek for additional candidate species, since substantial reduction in pest damage does not

necessarily mean that percent damage at the end of the growing period will be below the economic threshold. Furthermore, a clear tendency towards exploiting the potential of indigenous species is evident because 1) they are believed to be better adapted to the local climatic conditions (Hassan 1994) and 2) safety of releasing exotic *Trichogramma* spp. has become an important topic (van Lenteren et al. 2003).

1.3.1 Trichogramma aurosum Sugonjaev and Sorokina

Trichogramma aurosum Sugonjaev and Sorokina is a holarctic species that occurs naturally in Middle Europe, the former USSR (Lopatina 1983; Livshits and Mitrofanov 1986), and in the USA (Pinto et al. 2002). It was collected in Germany for the first time in 2000 from eggs of the locust sawfly *Nematus tibialis* Newman (Hymenoptera: Tenthredinidae) on *Robinia pseudoacacia* L. In preliminary host preference experiments it was shown that this species prefers eggs of the Codling moth over other lepidopteran hosts. Therefore, it may be a potential candidate antagonist for attempts at controlling *C. pomonella* in apple orchards. Although this species was described by Sugonjaev and Sorokina in 1975 (Sorokina 1993), only studies on its distribution (Lopatina 1983; Livshits and Mitrofanov 1986) and systematics (Pinto 1999; Pinto et al. 2002) have been carried out in the former USSR and in the USA, respectively. Among other hosts, *T. aurosum* has been also retrieved from eggs of *C. pomonella* in both mentioned regions. From 2001 to 2003, a wide collection of this species was conducted in the German Federal Republic and on selected sites in six European countries from eggs of *N. tibialis* on *R. pseudoacacia*, in order to obtain different strains for pre-introductory research. It is generally accepted that this is necessary before a species or a strain can be recommended for release. Pre-introductory research includes assessment of biological (i.e. life table characteristics, influence of abiotic factors), behavioural parameters (i.e. host age and host preference), as well as intraspecific genetic variability. Natural enemies for inundative and seasonal inoculative biological control should possess a good adaptability to climatic extremes and various habitats, searching efficiency, host specificity, host discrimination, ability to kill the host or use it for reproduction, reproductive capacity, and environmental safety.

1.4 Host selection

According to Flanders (1937), Vinson (1976), and Gordh et al. (1999), successful parasitism is divided into 5 steps: host habitat location, host location, host acceptance, host suitability and host regulation. The first three of these steps are referred to as host selection. Host suitability is concerned with factors affecting the development of a parasitoid within potential hosts. Following the host finding and host selection process, host suitability is a final step in the host parasitoid relationship toward successful parasitism (Pak et al. 1990), while host preference under controlled conditions is important in the risk assessment of biological control agents (Mansfield and Mills 2004). Host selection depends on several factors, e.g.

environmental factors, host factors, chemical and physical parameters (Vinson 1976). Shape, size, age of the host (Vinson 1976, Gordh et al. 1999), and host chemicals (Takasu and Nordlund 2001) can influence the host selection behaviour. Consoli et al. (1999) found that egg chorion thickness and hardness may affect the host selection behaviour of *Trichogramma*, also resulting in different drilling time by *T. galloi* Zucchi and *T. pretiosum* Riley when they drill eggs of the same chorion thickness. Additionally, the structural integrity of the egg chorion (which is the weight needed to penetrate the egg) was an important factor limiting successful oviposition by *T. platneri* in larger host eggs such as *Actias luna* Linnaeus and *Bombyx mori* Linnaeus (Mansfield and Mills 2002). Chorion thickness of more than 20 μm has not been penetrated by *Trichogramma* (Quednau 1955).

1.5 Host age selection

The reproductive ability of a female parasitoid is controlled by host factors such as host age, size and physical defences (Godfray 1994). Host age affects host preference and host suitability of parasitoids (Pak et al. 1986). Also, some species of parasitoids can control the sex ratio of their progeny in response to host age (Pak and Oatman 1982), while others do not (Nakamura and Noda 2001). For some parasitoids, host age has been shown to have a significant effect on offspring size (Husni et al. 2001), whereas it can have a greater effect on sex allocation by other parasitoids such as *Oomyzus sokolowskii* Kurdjumov (Nakamura and Noda 2001). Hence, studying the effect of host age and size on parasitoid reproductive characters will not only allow to better understand the ecological strategies of parasitoids, but it will also be helpful to select them for augmentative control. Many studies have shown that host stage at parasitism affects parasitoid host acceptance behaviour especially oviposition behaviour, while some parasitoid tend to reject the host egg after either drumming or drilling due to the unsuitability of the egg age or content (Marston and Ertle 1969, Pak et al. 1986). After contacting the host, the following three behavioural events have been described for *Trichogramma* spp. (1) drumming with the antennae, (2) ovipositor penetration and oviposition, and (3) host feeding. Ovipositor penetration and oviposition can be, depending on the host, hardly distinguishable. After oviposition, parasitoids withdrew their ovipositor and females may eventually start host feeding. Some of them repeat ovipositor penetration followed by host feeding two or three times. After contacting and drumming the host, some females abandon the host immediately without trying to oviposit. Acceptance and suitability of various hosts in non-choice situations is no evidence that the wasps will parasitize certain host species under natural conditions, while preference studies in the laboratory can be a predictor for behaviour under natural conditions (van Dijken et al. 1986).

1.6 Fertility life tables

Fertility life tables are a powerful technique to study the population dynamics of insects (Southwood 1978), to enable the development of biological control programmes (Pratissoli and Parra 2000), and to evaluate natural enemies, because they provide a detailed description of age specific mortality of individuals in the population (Zhang et al. 2001). The parameters usually estimated from fertility life tables are the net reproductive rate (R_0), the intrinsic rate of increase (r_m), which is a measure of the growth rate of a population per female (Pak and Oatman 1982); the mean cohort generation time (T_c), the doubling time (Dt), and the finite rate of increase (λ) (Southwood 1978; Maia et al. 2000; Nagarkatti and Nagaraja 1978). Net reproduction rate varies according to variation in temperature (Pratissoli and Parra 2000), where values are reduced at high temperatures. This could be either due to the production of both males and females (Cabello and Vargas 1988) or to the increase of flight activity resulting in a faster dispersion (Pak and van Heiningen 1985).

1.7 Adaptation to temperature

Temperature has a major influence on both activity and metabolic processes in poikilotherm organisms such as insects (Suverkropp et al. 2001). As for *Trichogramma* spp. and their use for biological control, candidate species should be adapted to adverse abiotic conditions (Pak 1988). This is important for two reasons: Firstly, the parasitoids are normally distributed in the field as immature stages (Knutson 1998), and hence, they must be able to successfully develop even under unfavourable conditions. Secondly, it is necessary that emerged adults search for hosts and parasitize them independent of whether abiotic conditions are extreme or not. Tolerance of immature stages of *Trichogramma* species / strains to high or low temperature extremes has been subject of several studies, e.g. for *T. cordubensis* Vargas and Cabello (Garcia and Tavares 1994), *T. maidis* Pintureau and Voegelé (= *T. brassicae* Bezdenko) (Bigler et al. 1988), *T. turkestanica* Meyer (Hansen 2000; also as *T. evanescens* Westwood by Schöller and Hassan 2001), *T. maidis* (= *T. brassicae*), *T. pretiosum*, and *T. semblidis* Aurivillius, (Pak 1988), *T. cacoeciae*, *T. dendrolimi* Matsumura, and *T. principium* Sugonjaev and Sorokina (Sakr 2003), *T. confusum* Viggiani (= *T. chilonis* Ishii, Nagarkatti and Nagaraja 1978), *T. minutum* Riley (Smith and Hubbes 1986a). Generally, most of the literature reports that longevity and development time from egg to adult is negatively correlated with temperature and positively with parasitism (Prasad et al. 2002). Emergence rate and female fecundity is only affected by low temperature (Smith and Hubbes 1986a, Consoli and Para 1995), while sex ratio is reduced at high temperatures (Consoli and Para 1995).

1.8 Genetical studies

During the past years, a variety of PCR – based molecular methods have drawn attention for use in molecular entomology, molecular ecology and molecular genetics, such as RAPD, SCAR and AFLP. Attention toward the use of DNA fingerprinting techniques is growing for insect taxonomy, biodiversity, evolution, ecology and behaviour (Hoy 1994, Loxdale and Lushai 1998, Stouthamer et al. 1999). Most of DNA fingerprinting techniques uses PCR for detection of DNA fragments from a specific DNA sample (Laurent et al. 1998). The choice of the best fingerprinting technique should require no prior investments in terms of sequence analysis, primer synthesis, or characterization of DNA probes (Loxdale and Lushai 1998). The internal transcribed spacer 2 (ITS2 of ribosomal DNA) has been shown to be a promising region for identification of cryptic *Trichogramma* species and to distinguish between closely related species, subspecies, and populations (Pinto et al. 2002). AFLP technique is drawing more attention as super fingerprinting technique, as it only need few amount of genomic DNA, can be used with dry, old or stored samples, and above all due to its high reproducibility, high resolution and high information. It produces a larger number of polymorphism per PCR reaction. AFLP technique is involved in human, animal, plant and insect genetic. Recently it is widely used for creating genetic maps and genetic transformation, determining the relatedness within and between populations, genetic diversity and molecular phylogeny studies.

1.9 Aim of the study

Pre-introductory research includes assessment of species or strain biology (i.e. life table characteristics, influence of abiotic factors), studying behavioural parameters (such as host-age and host-preference), as well as examining the genetic variability within and between populations. To assess the usefulness of natural enemies, intra- and/or interspecific biological studies are necessary depending on whether populations of a single species or those of at least two species are considered as potential candidates. Comparison of longevity, fecundity, and sex ratio is regarded to have higher importance for intraspecific studies (Smith and Hubbes 1986b), while successful development on potential hosts, i.e. host suitability, and competition relationships need to be assessed for interspecific studies (Pak and Oatman 1982; Pratissoli and Parra 2000). Host acceptance also is part of our present study, in which the parasitoid has to decide which host is most suitable for the development of its progeny. The aim of this work was to examine the host preference for different host species, when females have the option to choose between two different host species and when they have no choice to select between the host species.

Due to the gaps in knowledge about the biology of *T. aurosum*, the objectives of this work were to study:

1. Characterisation of genetic diversity.
2. Effect of the abiotic factors (e.g. temperature) on the development and reproduction
3. Reproduction potential on both natural and factitious hosts.
4. Construction of fertility life tables
5. Host recognition and acceptance
6. Host suitability, preference and age selection.
7. Behavioural observation of the learning capacity.

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CHAPTER 2

2. GENERAL MATERIALS AND METHODS

2.1 Field trips and parasitoid collection

Field trips were carried out to collect the parasitoids from different localities in the German Federal Republic and neighbouring countries. The parasitoids were recovered from parasitized (blackened) eggs of the locust sawfly *Nematus tibialis* (Hymenoptera: Tenthredinidae) on leaves of *Robinia pseudoacacia* (Table 2-1). Thirtytwo strains of *T. aurosum* were reared for the laboratory experiments.

2.2 Host rearing

2.2.1 Codling moth

Pupae of the Codling moth, *C. pomonella,* were kindly provided by the Institute of Biological Control of the Biologische Bundesanstalt für Land- und Fortswirtschaft, Darmstadt (BBA). After adult emergence they were collected and transferred into oviposition cages covered with plastic folia as oviposition sites. Adults were fed with water and honey. Freshly laid eggs were collected daily and kept in a refrigerator at *ca*. 7 °C. Eggs not used for the experiments were used to maintain a stock culture in the laboratory. Every week 5-10 plastic folia with deposited moths eggs were transferred into a petri-dish lined with a wet filter paper on the bottom and introduced into an incubator at 25 °C, 85% RH and 18:6 L:D photoperiod. After 3-5 days the eggs hatched into larvae, which were subsequently transferred onto a rearing medium (preparing the media in appendix). The larvae feed on the medium content and develop into pupae then into adult.

2.2.2 Mediterranean flour moth

Culture of the factitious host species Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), was maintained in the laboratory according to the method described by Cerutti et al. (1992). Every day 50-100 eggs were transferred into a plastic container (18 x 13 x 7 cm^3; 1,000 ml) filled with 100 g oatmeal and kept in a growth chamber at 25 °C, 85% RH and 18:6 h L:D photoperiod. Eggs developed into larvae, which, after larval hatch, feed on the medium mixture (containing oatmeal, semolina and wheat germ) and develop into pupae, then into adult. After 6-8 weeks the newly hatched adults were then transferred daily into ovipositing cages as described by Cerutti et al. (1992). Adults were fed on diluted honey. Freshly laid eggs were collected daily and those used for the preparation of the egg cards were first sterilised either by freezing for 4 h at –20 °C or UV light for 30 min and then kept in a refrigerator at *ca.* 7 °C. Sterilisation of the host eggs is essential, since the larvae are cannibalistic.

2.2.3 Other lepidopterous hosts

Pupae of the Cotton leaf worm, *Spodoptera littoralis* Boisd., the African bollworm *Helicoverpa armigera* Hübner, the Turnip moth *Agrotis segetum* Schiff., the Grapevine moth *Lobesia botrana* Den. and Schiff, required for the experiments were supplied either by private or institutional companies (BBA, Forschungsanstalt Geisenheim, AMW Nützlinge, Bioconctrol AG-Switzerland) and adults and eggs were reared and maintained in the laboratory at 25 °C, 85% RH and 18:6 h L:D photoperiod. When the adults hatched they were collected and transferred into oviposition cages. Adults were fed on diluted honey. Laid eggs were collected daily and stored in the refrigerator at *ca.* 7 °C.

2.4 Parasitoids

After the emergence of the parasitoids, laboratory colonies were started and maintained on eggs of *E. kuehniella*. Males of each collection were slide-mounted following Platner et al. (1999), and identified using the terminology of Pinto (1999). Voucher specimens of all strains are deposited in the collection of the Dept. of Applied Entomology, Institute of Phytomedicine, University of Hohenheim. A strain is thereinafter defined as the progeny emerged from all eggs collected at the same site on the same day. Strains were placed in culture tubes (70 mm height, 20 mm diam) closed with a plastic lid, which had a small hole for aeration. The tubes were kept in a climatic cabinet at ca. 25 ± 0.5 °C, 85 ± 5% RH and 18:6 h L:D photoperiod during pupal development of the parasitoids. To feed emerged adults, a droplet of honey was placed in the tube prior to or upon their emergence. Emerged parasitoids were provided with fresh host eggs on an ‘egg card’. Egg cards were prepared by sprinkling host eggs on a drop of Arabic gum on a piece of paper index card (ca. 50 x 15 mm).

Table 2-1 List of the collected *T. aurosum* strains, their locations, latitude, longitude and time of the 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° 31E	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° 21E	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° 19E	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° 22E	August 2002
Ta21	Germany: Hesse, Erlenbach am Main	49° 39 N	8° 44 E	September 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
Ta24	Austria: Vienna, City park	48° 12 N	16° 22 E	July 2003
Ta25	Austria: Vienna, Rothensiedel	48° 12 N	16° 20 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
Ta29	Belgium, Brussels, Main Station	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
Ta32	France: Paris, Champ de Mars	48° 51 N	2° 17 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

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CHAPTER 3

3. HOST AGE SELECTION BEHAVIOUR

3.1 ABTRACT

Host age selection of several German strains of *Trichogramma aurosum* Sugonjaev and Sorokina was examined in laboratory choice tests under direct observation for 90 min., in order to select candidate strains for attempts at controlling the Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae). Experiments were conducted at room temperature by exposing combinations of two host ages (zero vs. four and one vs. five-days old) to a single female wasp. Host age did not appear to affect the wasps parasitization behaviour, although some strains spent longer time drilling on old eggs (four and five days old) compared to fresh ones (zero and one day old). This not necessarily means that they preferred fresh eggs over old ones, since both type of hosts were parasitized in the choice test. Possibly an increased mechanical resistance of the chorion of older eggs was responsible for the prolonged drilling time. Mean drumming time was independent of host age. Drilling time made more than 80% of the mean handling time of all strains tested for all host ages, followed by resting and walking.

Keywords: *Trichogramma aurosum*, egg parasitoids, host age, choice test, host selection, parasitization behaviour, *Cydia pomonella*.

3.2 INTRODUCTION

The use of natural enemies like predators, parasitoids and microorganisms to suppress populations of insect pests is the major aim of biological control. The most widely used beneficial organisms worldwide belong to the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) (Smith 1996). This genus comprises more than 200 nominal species that are primary egg parasitoids (Pinto 1999). Cosmopolitan in distribution, *Trichogramma* spp. occupy habitats that range from sea level to high arboreal. Although these extremely tiny wasps (*ca*. 500 µm) attack mainly eggs of lepidopterous species, they have been collected from well over 200 species belonging to > 70 families and eight orders.

Trichogramma females have to find and accept a host to complete their reproductive cycle. Once they have physically contacted a potential host, an evaluation process starts to determine whether the host is suitable for the development of their offspring (Pak 1988). Host examination and attack may include different steps such as host encounter or contact (touching the egg), drumming (examining the egg with the antennae), drilling (boring the egg chorion with the ovipositor), feeding (on the egg exudates due to egg puncturing), oviposition and marking and different stimuli may be necessary to elicit this chain of behaviour (Fig 3-3). During the parasitization process a female wasp can reject the host egg either after

examination the host volume and curvature with the antenna (drumming) or after insertion and examination the internal contents with the ovipositor (van Dijken et al. 1986, Pak 1988). Frequency of host rejection differs from one species / strain to another. Some strains reject the egg mostly after drumming, while others reject the eggs after drilling (Pak 1988, Pak et al. 1986). Once the host has been located, final acceptance (oviposition) depends on host species and quality, expressed as age, size, or previous parasitism (Vinson 1976). Host location and recognition by the parasitoid depends on either olfactory cues, which elicit the parasitoid to search and locate the host, or visual cues, which triggers oviposition (Godfray 1994). Host selection is defined as selection by one parasitoid between hosts of different age (stages) or of different species. Host selection and acceptance are dependent on the parasitoid ability to discriminate between parasitized and non parasitized host, learning ability, and host age. Host age could be an important factor in host acceptance. Some *Trichogramma* spp. showed higher tendency to parasitize young over old eggs (Godin and Boivin 2000; Monje et al. 1999). This could be either due to poor nutrient quality or defensive mechanism in the old eggs (Mattiacci and Dicke 1995).

Non-preferred stages / species have an increasing probability of remaining unparasitized (Pak 1988). Therefore, candidate species / strains should have, whenever possible, no preference for a determined host age. In this chapter, assessment of several German strains of *T. aurosum* were tested towards their host age preference by offering choice conditions, in which a female wasps can chose and parasitized two age types of eggs of the target host, *C. pomonella*.

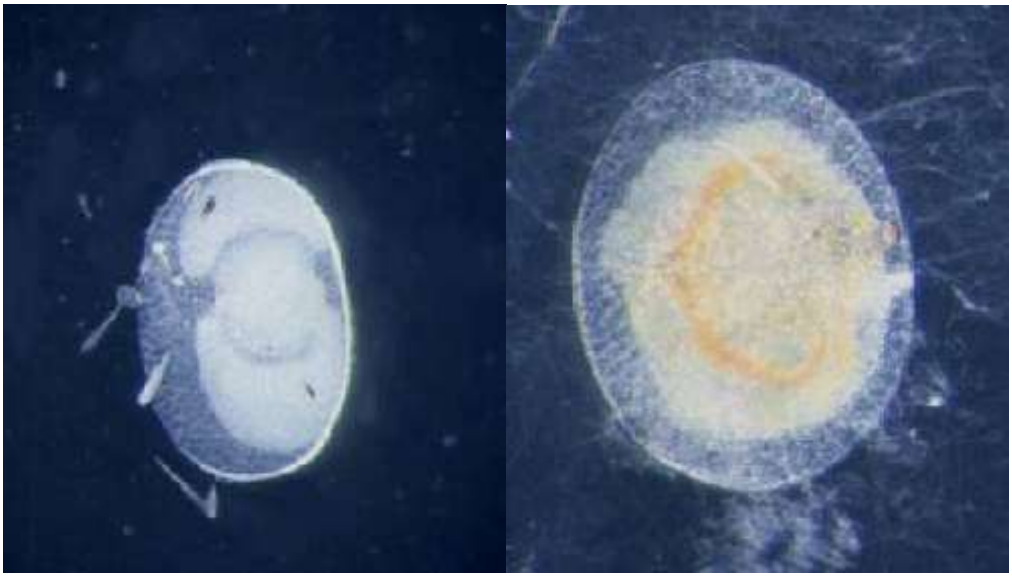
3.3 MATERIAL AND METHODS

Following strains were used for the experiments: Ta4, Ta10, Ta13, Ta19, and Ta20 (see Chapter 2 for details). For details on the rearing of *C. pomonella* see also Chapter 2.

Experimental design

Experiments were conducted at room temperature by exposing combinations of two host ages (0 vs. 4 and 1 vs. 5 old days; Fig. 3-1) to a single female wasp in a choice test. Each test was conducted in a plastic petri-dish (5.3 cm diameter) carrying a piece of graph-paper (2 x 2 cm). Eight eggs of each age were arranged alternatively in a grid, the hosts being 4 mm apart (Fig 3-2). Then a *Trichogramma* female was released and allowed to parasitize the hosts. Duration and frequency of every behavioural event was recorded using the Observer® software 3.0 (Noldus information Technology 1993) for 90 min after the first contact with one egg: Walking, cleaning, inactivity, contact, drumming (touching the host egg with the antennae), acceptance (by starting drilling), or rejection (by leaving the host and walking away), drilling, and oviposition (movement of the abdomen can be clearly seen) (Fig 3-3). Since parasitized hosts were not removed, recurrent visits to already attacked host eggs were also recorded. Each treatment was replicated 20 times. Host age preference was statistically

analysed by summing the number of acceptances and rejections of eggs at the first visit. Then, a contingency table for the distribution of contacts was constructed. The acceptance-contact–ratios (Pak 1988) were then evaluated by Chi-square test. Analysis of variance (ANOVA) of mean differences in recognition times and number of parasitizied eggs was conducted using the General Linear Models (PROC GLM) procedure (SAS Institute 1996). The Student Newman Keuls (SNK) procedure was used to separate the means.



a. **b.**

Figure 3-1. Different age stages of the Codling moth (a) fresh egg (14 degree hours old), (b) red-ring egg (70 degree hours old) (Richardson et al. 1982).

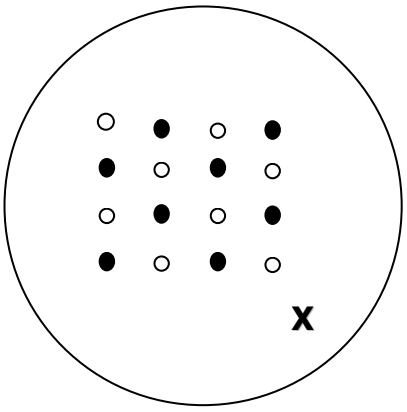


Figure 3-2. Eggs of different age arranged in a glass petri–dish in a rectangular grid. X is the female release point.

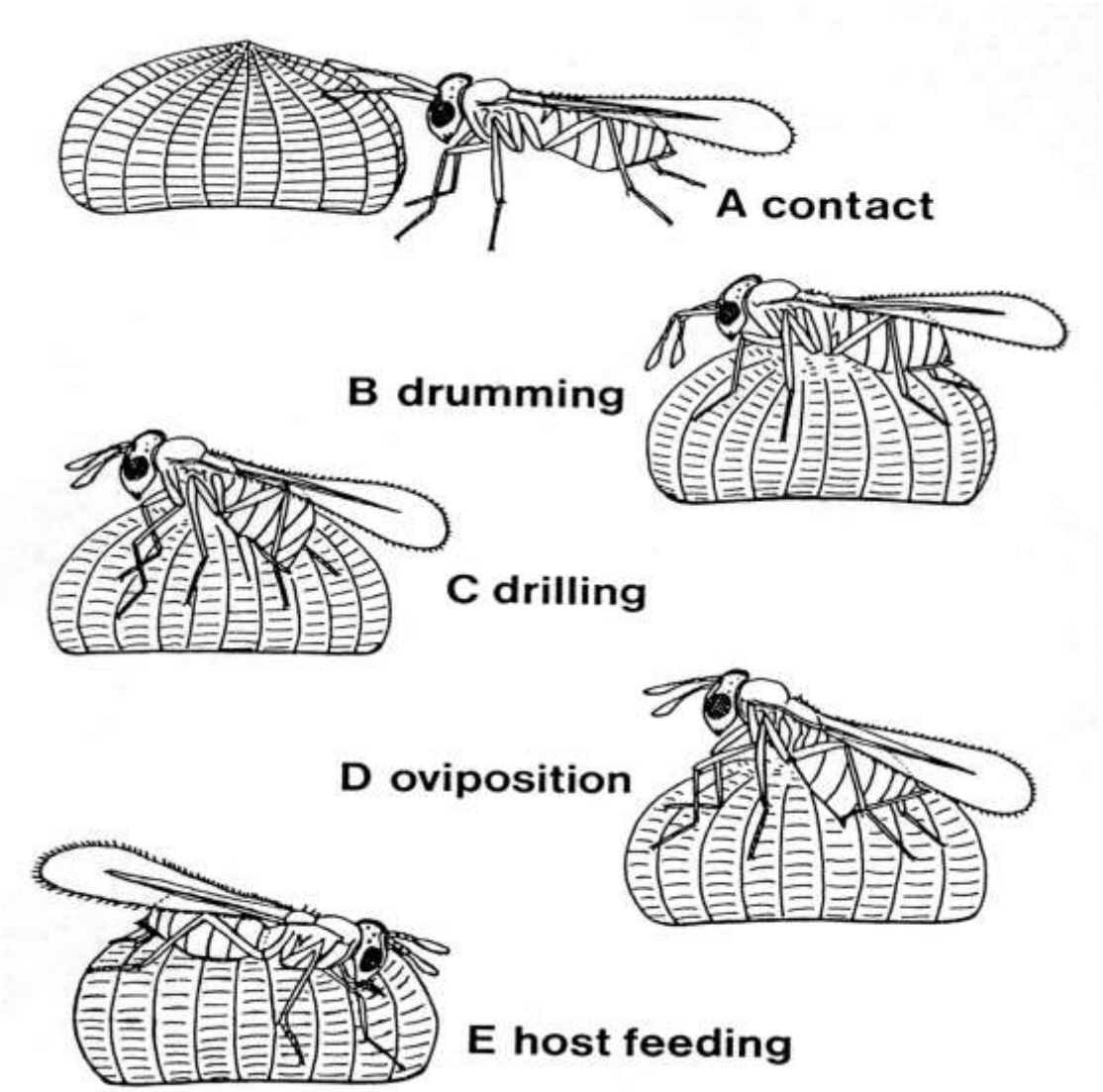


Figure 3-3. Behaviour of *Trichogramma* female wasps (Pak 1988). See explanations in the text.

3.4 RESULTS

Time consumed for drilling on old hosts (four or five days old) was longer than time consumed on fresh ones (Table 3-1). The strains Ta4 and Ta13 showed a significantly longer drilling time in old eggs (in the test with 0 vs. 4 day old eggs) compared with fresh ones, while no significant differences were detected in the strains Ta10, Ta19 and Ta20. By statistical analysis of the means, drilling time spent on old eggs was about 50-60% longer than on fresh eggs (Figure 3A-1 and 3A-2 in Appendix). However, these differences were not that pronounced when 1 d vs. 5 d old eggs were offered. Mean time consumed on drumming either fresh or old eggs did not show any significant differences (Table 3-2), although time consumed on drumming fresh eggs seems to last equal or longer than drumming old eggs (Figure 3A-1 to 3A-4; 3A-3 and 3A-4 Appendix).

The five strains of *T. aurosum* studied have no age preference, where the mean number of eggs attacked either old or fresh eggs did not differ significantly. Ta10, Ta13, and Ta20 showed a higher preference to attack fresh eggs at the age of 0 days and 1 day old but of no significant difference to older eggs. Meanwhile all strains showed a higher though not significant preference to parasitize fresh eggs than old ones (Figures 3-4 to 3-7). Mean number of eggs attacked was noticeably higher than the number of parasitized eggs. Egg attacking frequency for all strains studied were > 30% of the offered eggs (Figure 3-4 and 3-6) while the frequency of accepted eggs were > 25% of the offered eggs; either when eggs were old or fresh (Figure 3-5 and 3-7).

Table 3-3 shows that in most tests, the acceptance / contact (a/c) ratios were high (>0.7). This indicates that eggs of different ages tested were contacted and examined at similar rates. Chi square test to evaluate the a/c ratios per test revealed a statistical difference at the 5% level between 0 and 4 day old eggs for both Ta13 and Ta19; and between 1 and 5 day old eggs for the Ta20 strain. Here the females preferred the younger eggs. In all other tests, no preference was observed for any of the strains.

Host discrimination. Observation of females contacting host eggs previously parasitized by themselves evidently showed that each *Trichogramma* strain was able to discriminate between unparasitized and parasitized hosts of different ages. Females of all strains were able to discriminate parasitized eggs of each age of *C. pomonella*, (Chi square X^2, $P < 0.05$). Differences between a/c ratios of parasitized and unparasitized hosts were highly significant for all strains, except for Ta20, where difference with 5 day old eggs were visible but not that pronounced (Table 3-4).

There is no clear sign suggesting that learning takes place on the first host encountered, thus reducing handling time on the subsequent hosts (Figure 3-8 and 3-9), however, this point is worth to investigate separately. A more detailed analysis of the behaviour events (especially walking and resting) between the host encounters may help to explain the longer duration of drilling time on the last host attacked. The wasps spent about 75, 80, 85, and 95% of the handling time on drilling the 0, 1, 4 and 5 day old eggs, respectively, 5% for drumming and 10% for resting (Figure 3-10).

Table 3-1. Mean duration of drilling by five strains of *T. aurosum* on *C. pomonella* eggs of different age at room temperature (n = 20).

Age (d)	Ta4	Ta10	Ta13	Ta19	Ta20
0	251.51±130 [b]	414.25±359 [a]	263.87±109 [b]	227.71±182 [a]	259.44±228 [a]
4	344.64±177 [a]	535.09±404 [a]	322.60±143 [a]	253.72±121 [a]	287.07±237 [a]
	F = 6.34; P = 0.014; DF = 1: 68	F = 2.46; P = 0.12; DF = 1: 96	F = 6.17; P = 0.02; DF = 1: 112	F = 0.67; P = 0.42; DF = 1: 93	F = 0.42; P = 0.52; DF = 1: 118
1	341.02±237 [a]	366.77±213 [a]	319.00±161 [a]	364.47±316 [a]	378.45±271 [a]
5	386.75±271 [a]	479.45±294 [a]	371.64±249 [a]	353.90±158 [a]	344.98±209 [a]
	F = 0.92; P = 0.34; DF = 1: 112	F = 5.15; P = 0.03; DF = 1: 103	F = 1.72; P = 0.19; DF = 1:106	F = 0.04; P = 0.85; DF = 1: 8	F = 0.42; P = 0.52; DF = 1: 88

* Within a column, means followed by the same letter are not significantly different (P > 0.1: Student Newman Keuls (SNK) test)

(F = F-value; P = probability; DF = degree of freedom of the treatment and the error, respectively).

Table 3-2. Mean duration of drumming by five strains of *T. aurosum* on *C. pomonella* eggs of different age at room temperature (n = 20).

Age (d)	Ta4	Ta10	Ta13	Ta19	Ta20
0	31.59±11.4 [a]	30.94±15.0 [a]	35.43±27.2 [a]	30.09±20.5 [a]	32.62±17.7 [a]
4	31.43±12.4 [a]	27.26±15.6 [a]	33.88±20.9 [a]	29.55±17.2 [a]	28.27±13.8 [a]
	F = 0; P = 0.95; DF = 1: 107	F = 1:4; P = 0.23; DF = 1: 97	F = 0.11; P = 0.74; DF = 1: 109	F = 0; P = 0.97; DF = 1: 92	F = 2.2; P = 0.14; DF = 1: 115
1	37.84±15.3 [a]	30.26±16.6 [a]	30.09±20.5 [a]	31.63±15.4 [a]	31.27±17.0 [a]
5	37.32±16.7 [a]	29.35±12.9 [a]	29.55±17.2 [a]	27.93±9.9 [a]	33.00±19.3 [a]
	F = 0.03; P = 0.86; DF = 1: 115	F = 0.1; P = 0.75; DF = 1: 104	F = 0.02; P = 0.89; DF = 1: 105	F = 1.8; P = 0.18; DF = 1: 89	F = 0.19; P = 0.66; DF = 1: 83

* Within a column, means followed by the same letter are not significantly different (P > 0.1: Student Newman Keuls (SNK) test)

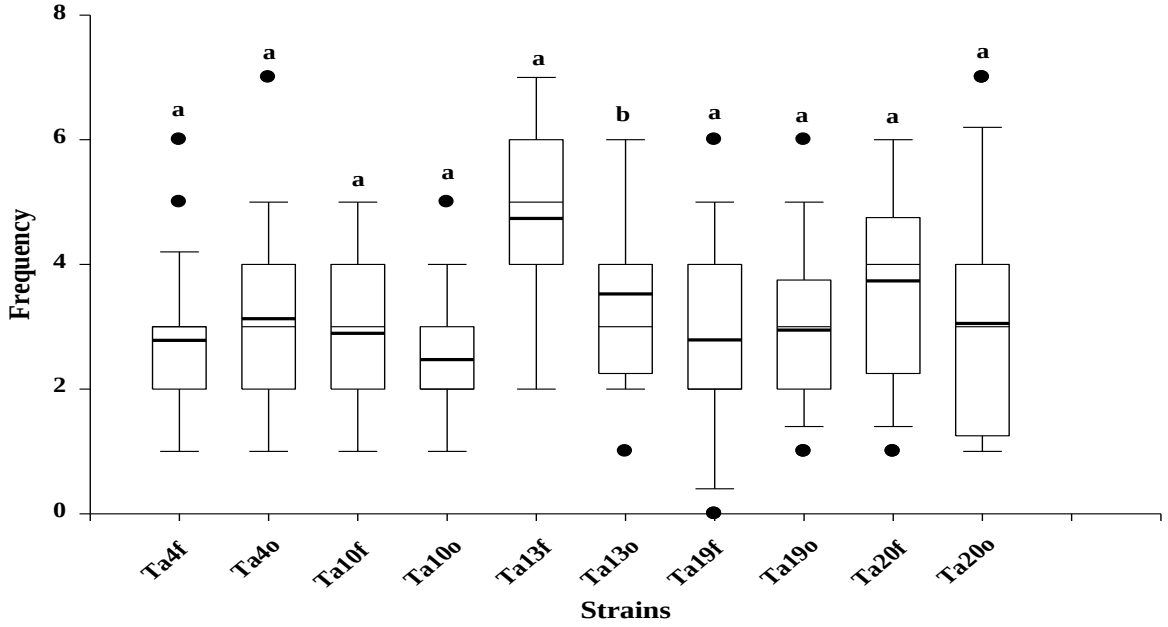


Figure 3-4. Mean attacking frequency on 0 and 4 days old eggs of *C. pomonella* for five strains of *T. aurosum* at room temperature. Ta10, Ta13, and Ta20 (F = fresh eggs, O = old eggs; F = 1.15; DF = 1: 36; P = 0.29; F = 5.8; P = 0.021; DF = 1: 36; F = 1.45; P = 0.24; DF = 1: 36; respectively. F/O-pairs with the same index do not differ significantly at the $p < 0.05$ level after Student Newman Keuls test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line)).

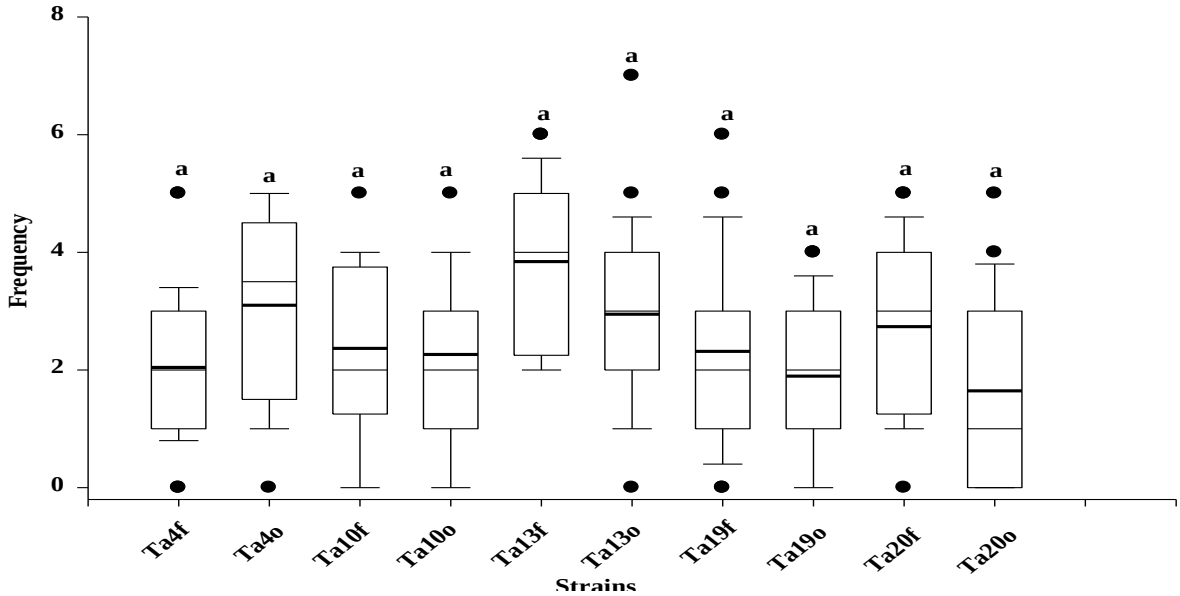


Figure 3-5. Mean parasitization frequency on 0 and 4 days old eggs of *C. pomonella* for five strains of *T. aurosum* at room temperature. Ta10, Ta13, and Ta20 ($F = 0.03$; $P = 0.85$; $DF = 1: 36$; $F = 0.64$; $P = 0.43$; $DF = 1: 36$; $F = 0.40$; $P = 0.53$; $DF = 1: 32$. F/O-pairs with the same index do not differ significantly at the $p < 0.05$ level after Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line)

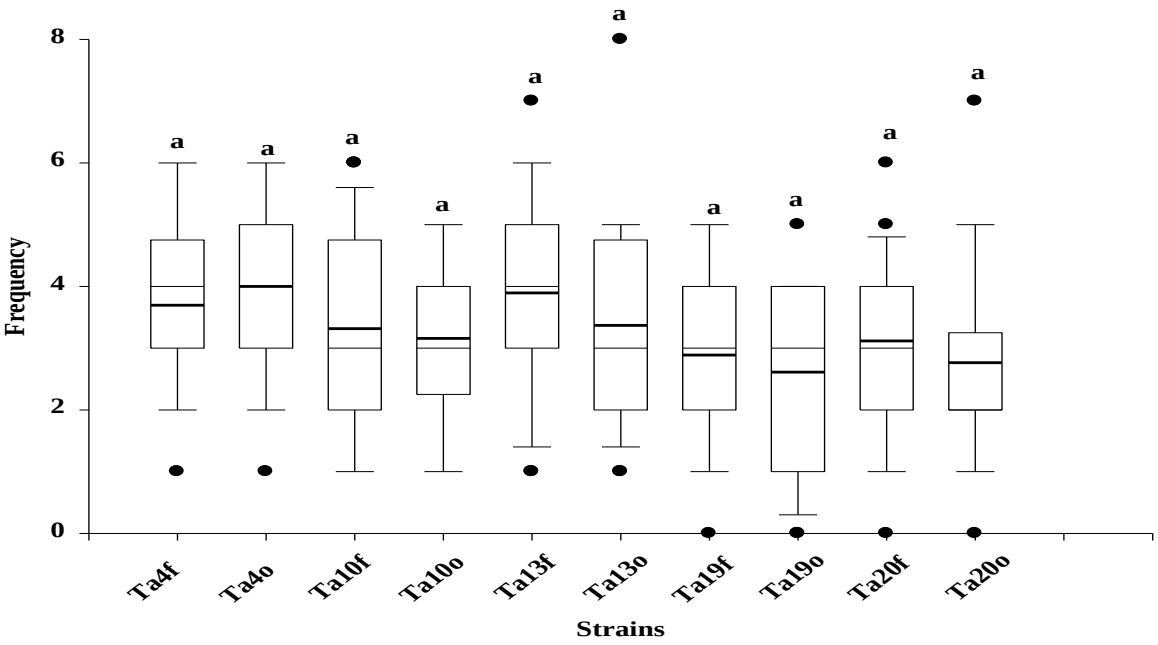


Figure 3-6. Mean attacking frequency on 1 and 5 days old eggs of *C. pomonella* for five strains of *T. aurosum* at room temperature. (F = fresh eggs, O = old eggs. F/O-pairs with the same index do not differ significantly at the $p < 0.05$ level after Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line)

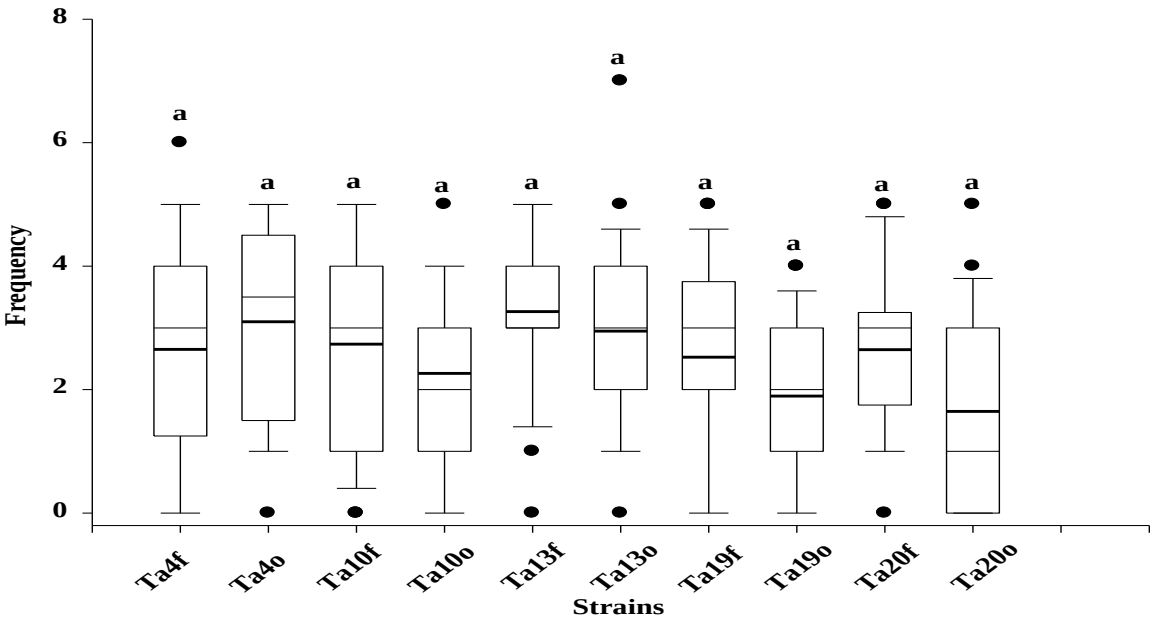


Figure 3-7. Mean parasitization frequency on 1 and 5 days old eggs of *C. pomonella* for five strains of *T. aurosum* at room temperature. (F/O-pairs with the same index do not differ significantly at the $p < 0.05$ level after Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

Table 3-3. Number of contacts and acceptances for *C. pomonella* eggs of different age by five strains of *T. aurosum* in paired–choice tests and statistical analysis of host age preference. (n = 20).

Strain name	Age (day)	No. Contact	No. Acceptance	a/c ration	Chi square	Df	P
Ta4	0	64	47	0.73	1.85	1	0.174
	4	72	45	0.63			
	1	76	58	0.76	0.03	1	0.862
	5	80	62	0.78			
Ta10	0	55	45	0.82	0.20	1	0.655
	4	47	40	0.85			
	1	63	52	0.83	2.07	1	0.150
	5	60	43	0.72			
Ta13	0	90	73	0.81	7.18	1	0.007
	4	67	64	0.96			
	1	74	62	0.84	0.38	1	0.538
	5	64	56	0.88			
Ta19	0	53	44	0.83	6.66	1	0.009
	4	56	34	0.61			
	1	56	48	0.86	3.62	1	0.057
	5	51	36	0.71			
Ta20	0	71	52	0.73	0.12	1	0.729
	4	58	44	0.76			
	1	53	45	0.85	8.11	1	0.004
	5	47	28	0.60			

Table 3-4. Discrimination between parasitized and unparasitized *C. pomonella* eggs of various ages by five *Trichogramma aurosum* strains (n = 20).

Strain name	Host age	parasitized hosts # cont	# acc.	a/c ratio	Unparasitized host a/c ratio*	Statistical comparison Chi-square	P
Ta4	0	37	1	0.03	0.73	47.04	0.001
	4	30	6	0.20	0.63	15.30	0.001
	1	51	5	0.10	0.76	54.01	0.001
	5	58	3	0.05	0.78	70.59	0.001
Ta10	0	24	4	0.17	0.82	30.11	0.001
	4	13	1	0.08	0.85	28.20	0.001
	1	18	1	0.06	0.83	36.68	0.001
	5	10	0	0.00	0.72	18.58	0.001
Ta13	0	15	1	0.07	0.81	34.25	0.001
	4	14	4	0.29	0.96	38.53	0.001
	1	15	4	0.27	0.84	21.23	0.001
	5	14	2	0.14	0.88	32.30	0.001
Ta19	0	9	3	0.33	0.83	10.36	0.001
	4	23	2	0.09	0.61	17.79	0.001
	1	20	0	0.00	0.86	46.53	0.001
	5	11	0	0.00	0.71	18.52	0.001
Ta20	0	12	4	0.33	0.73	7.45	0.006
	4	7	1	0.14	0.76	11.12	0.001
	1	8	3	0.38	0.85	9.32	0.002
	5	5	1	0.20	0.60	2.81	0.09

* a/c ratio for unparasitized are calculated in Table 3-3.

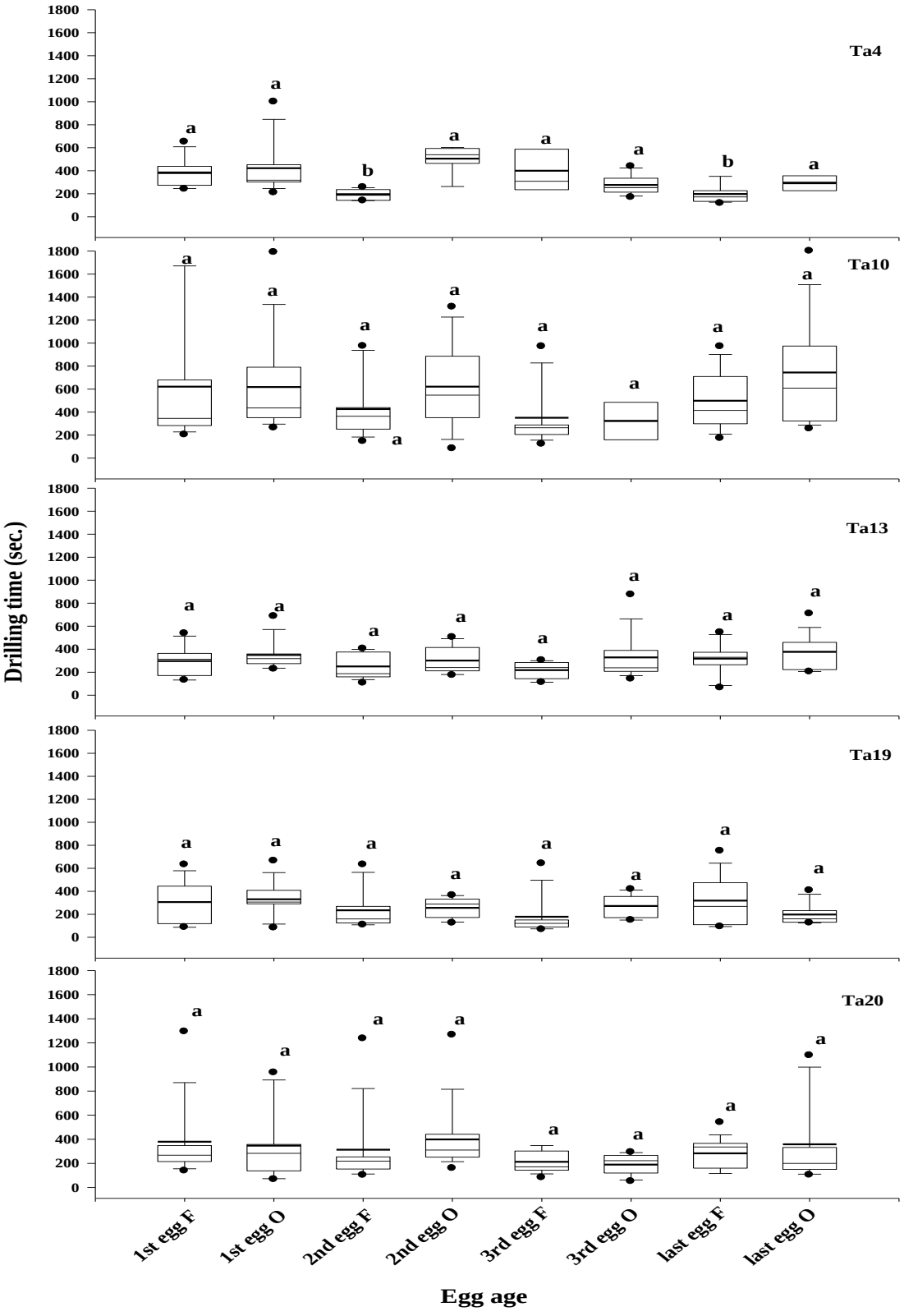


Figure 3-8. Mean drilling time of the first, second, third and last egg by five *T. aurosum* strains parasitizing 0 vs. 4 days old day eggs of *C. pomonella* at room temperature. (F = fresh eggs, O = old eggs. F/O-pairs with the same index do not differ significantly at the $p < 0.05$ level after Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

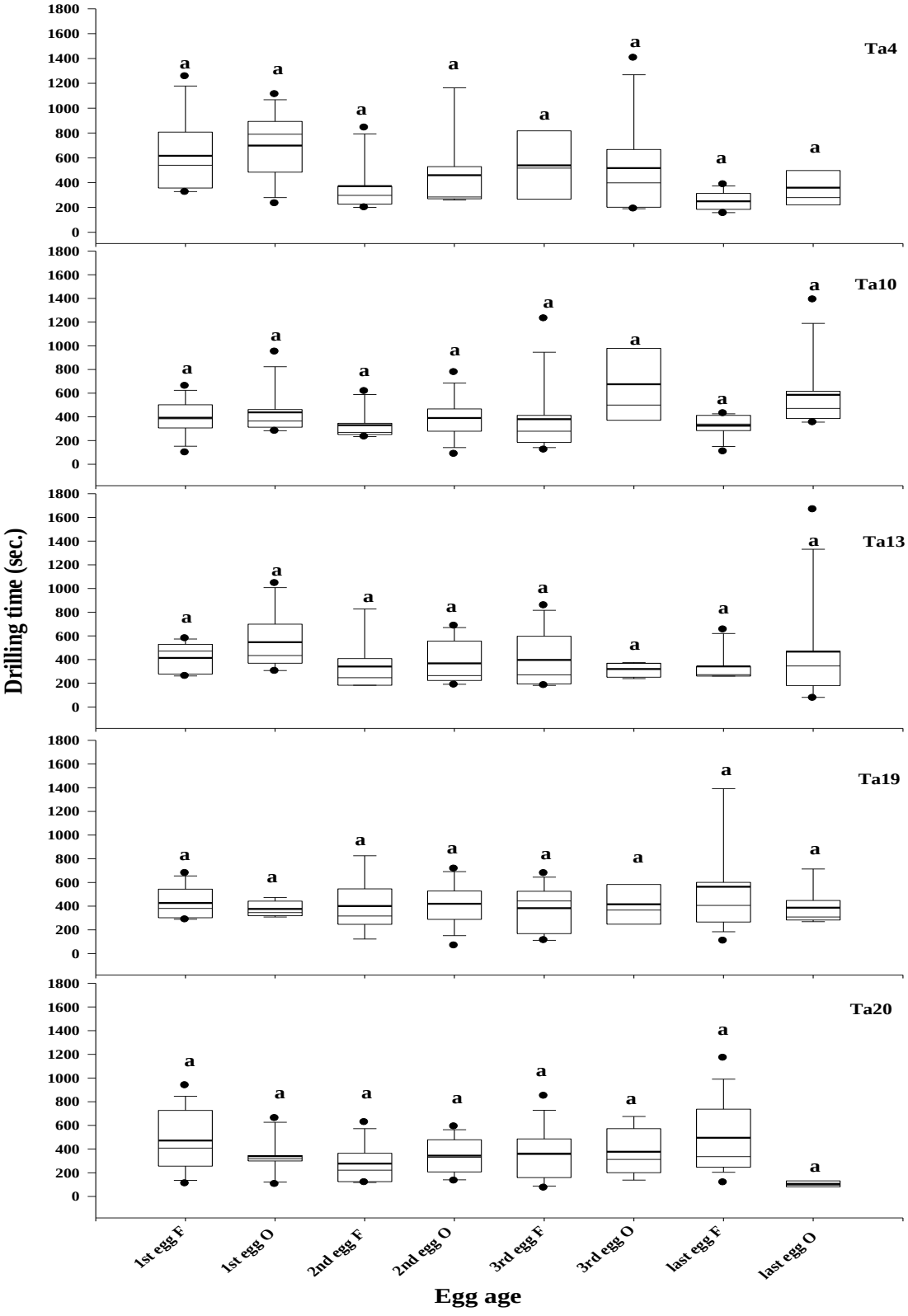


Figure 3-9. Mean drilling time of the first, second, third, and last egg by five *T. aurosum* strains parasitizing 1 vs. 5 days old day eggs of *C. pomonella* at room temperature. (F = fresh eggs, O = old eggs. F/O-pairs with the same index do not differ significantly at the $p < 0.05$ level after Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

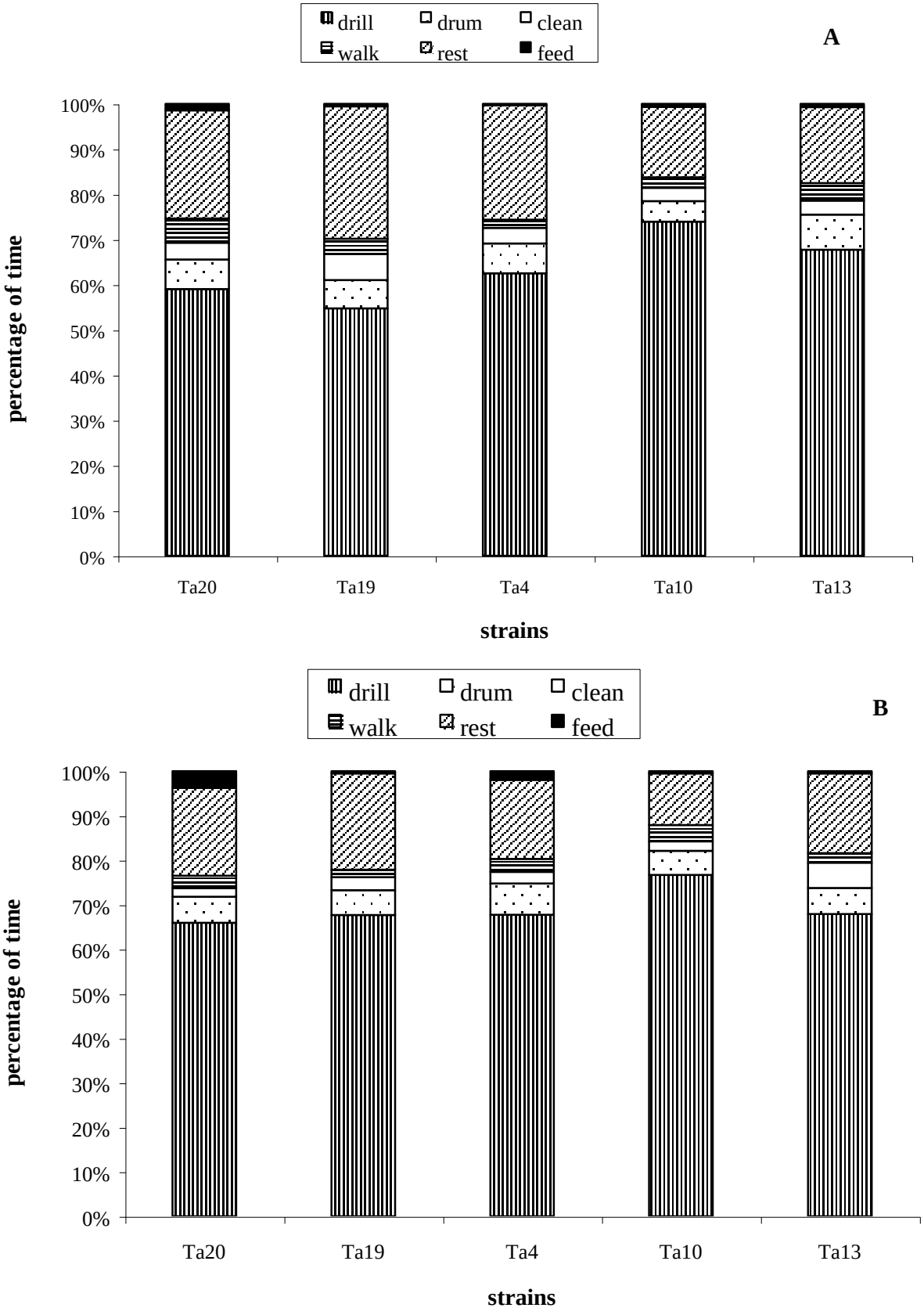


Figure 3-10. Percent time spent for behavioural steps of host searching and handling for five strains of *T. aurosum* parasitizing fresh (A) or old (B) eggs of *C. pomonella* at room temperature. (drill = drilling; drum = drumming; clean = cleaning; walk = walking; rest = resting; feed = feeding)

3.5 DISCUSSION

The reproductive ability of a female parasitoid is controlled by host factors such as host age, size and physical defences (Godfray 1994). Host age affects host preference and host suitability of parasitoids (Pak et al. 1986). Also, some species of parasitoids can control the sex ratio of their progeny in response to host age (Pak and Oatman 1982), while others do not (Nakamura and Noda 2001). For some parasitoids, host age was shown to have a significant effect on offspring size (Husni et al. 2001), and even a greater effect on sex allocation in other parasitoids (Nakamura and Noda 2001). So studying the effect of host age and size on parasitoid reproductive characters will not only allow a better understanding of their ecological strategies, but it will also be useful to screen candidates as biological control agents.

Many studies have shown that host age can affect the parasitoid host acceptance behaviour especially drumming and drilling behaviour (Marston and Ertle 1969, Pak et al. 1986). In the present study, ovipositor penetration and oviposition were not behaviourally distinguishable. After oviposition, parasitoids withdrew their ovipositor and some females started host feeding. Some females repeated ovipositor penetration followed by host feeding two or three times (data not shown). Two explanations are possible: 1) during the previous drilling only examination of the internal content of the egg was done without taking the decision of laying egg and 2) female wasps examine the possibility of laying another egg in the same host. After contacting and drumming the host, some females left the host immediately without trying to oviposit. The acceptance and suitability of various hosts is not a direct evidence that the wasps successfully parasitize these species under natural conditions, while preference studies in the laboratory can only give an indication for the field conditions (van Dijken et al. 1986).

Females of *T. aurosum* spent longer time drilling on old eggs (4 and 5 days old) compared with fresh ones (0 and 1 day old). This not necessarily means that they prefer fresh eggs over old ones, since both type of hosts were parasitized in the choice test. Similar results were obtained by Brand et al. (1984) and Godin and Boivin (1994) with *T. evanescens* and *T. pretiosum*, respectively. Host age generally does not appear to affect contact or acceptance of eggs of different host species, but duration of the oviposition behaviour can sometimes be influenced by host age (Pak et al. 1986). For instance, drilling period was prolonged on old eggs when compared with young ones in *T. brassicae* (Babendreier et al. 2003). Furthermore, Reznik et al. (1997) found out that host acceptance depends not only on current host age, but also on the age of the previously offered host. Possibly, an increased mechanical resistance of the chorion in response to development of the host embryo in the older eggs was responsible for the prolonged drilling time (Reznik et al. 1997). It was reported that cell masses of the host egg differentiate after 11 hr (Marston and Ertle 1969). However, this does not explain the possible alteration of any change in the mechanical resistance of the chorion. Nakamura and Noda (2001) reported that the duration of ovipositor insertion and oviposition of the larval

parasitoid *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) was significantly longer on fourth larval instar than on second- and third larval instar of the host. Pak et al. (1986) related elongation in handling time due to unsuitability of older host eggs, whereas wasps can recognize this only after internal examination of the host with their ovipositor. Pak and Oatman (1982) reported that drumming could serve as a tool for the wasps to measure the size of the host, in order to control number of eggs being oviposited. Our results show that the mean drumming time was independent of host age, where females spent about the same time drumming on fresh and old eggs.

In general, mean duration of drilling was highest in the first egg and decreased only slightly until the last attacked egg (Figure 3-8 and 3-9). By drilling the last egg, an obvious pattern was noticed: in the third observation period (after about 3,600 s) female tend to spend more time on cleaning and resting. We assume that the female was exhausted after she spent a lot of energy on host searching and prasitization and she gathered her strength. These results agree with the findings of Nakamura and Noda (2001). Behavioural differences may indicate a difference in chemical and/or visual conditions between young and old hosts and explain how the parasitoid recognizes host size.

The results showed that a/c ratios were high, i.e. eggs of different age were contacted and examined at similar rates. *T. minutum* was shown to deposit more eggs in younger hosts than in older ones of the same size, because old eggs provide fewer resources for the parasitoid larvae than young eggs (Nakamura and Noda 2001). But most *T. aurosum* strains observed had no preference for any host age, although two strains have shown some preference to younger eggs than two old ones. Marston and Ertle (1969) suggested that parasitoid mortality could be higher in old eggs because the parasitoid eggs collapsed when they were forced into the embryonic tissue.

Host discrimination is the ability of a female to distinguish parasitized from unparasitized hosts, probably detecting external and/or internal host-marking pheromones using the antennae and /or ovipositor (Salt 1937, Vinson 1976). Salt (1937) reported that *T. evanescens* could discriminate between parasitized and unparasitized hosts by marking them externally and internally. All the strains studied discriminated between parasitized and unparasitized host eggs during the drilling phase. This suggests that they use the ovipositor to detect internal stimuli or marks left previously. According to Miura et al. (1994), discrimination between parasitized and unparasitized hosts may depend on the parasitoid prior ovipositional experience. Conversely, Pak and Oatman (1982) reported that *T. brevicapillum* and *T. pretiosum* females accept host eggs that were parasitized by other species, which could lead to superparasitism.

Since in nature a mixture of eggs of different age (fresh and old) is common, lack of preference for any host age makes *T. aurosum* an interesting candidate for release purposes.

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CHAPTER 4

4. HOST PREFERENCE AND OVIPOSITION BEHAVIOUR

4.1 ABSTRACT

Oviposition behaviour and host selection of different German strains of the egg parasitoid *Trichogramma aurosum* were examined on eggs of five lepidopteran hosts (*Cydia pomonella* (L.), *Spodoptera littoralis* (Boisd.), *Helicoverpa armigera* (Hübner), *Agrotis segetum* (Schiff.), *Lobesia botrana* (Den. and Schiff.)). The parasitization behaviour of individual female wasps was examined in choice and non-choice tests. Single female wasps were observed for 90 min using a rectangular grid. Results from the choice test revealed that 75 – 90% of *C. pomonella* eggs attacked by *T. aurosum* strains were successfully parasitized. Values for *L. botrana* and for *A. segetum* ranged between 40 – 80% and 40 – 70%, respectively. No significant preference was found between *C. pomonella* eggs and host eggs of both *L. botrana* and *A. segetum*. Time needed by the female wasps to drill on eggs of *L. botrana* was shorter than the time needed for drilling on *C. pomonella* eggs. This could be due to differences in egg chorion thickness and, presumably, differences in chorion hardness. During direct observation of the parasitism behaviour it was noticed that all strains spent about 20% of the observation time for drilling on either *C. pomonella* or *L. botrana* eggs, 30 – 60% on resting, 4 – 15% on cleaning, < 4% on walking and < 1% on feeding. Results from the non-choice test showed that a significantly higher number of *C. pomonella* and *L. botrana* eggs were parasitized in comparison to the other hosts offered. The presence of hair-like structures deposited on eggs of *S. littoralis* and a thick egg chorion in *H. armigera* seems to represent a physical barrier that impedes successful parasitization.

Key words: *Trichogramma aurosum, Cydia pomonella, Spodoptera littoralis, Helicoverpa armigera, Agrotis segetum, Lobesia botrana*, host acceptance, host selection, parasitization behaviour.

4.2 INTRODUCTION

The Codling moth, *Cydia pomonella* (L.), is a key pest of apple trees distributed world-wide, with exception of both Japan and China (Madsen and Morgan 1970). Damage caused by this pest on apple trees could reach 95% when no control methods were applied (Anonymous 2002b). Insecticides are widely used to control this pest. Thus, the increase of resistance has prompted interest in alternative control methods such as inundative biological control. *Trichogramma* spp. are egg parasitoids used worldwide for this control strategy against lepidopterous pests in forestry, orchards, and row crops (Li 1994, Pak et al. 1990, Smith 1996). However, not only inoculative releases (classical biological control) but also inundative releases failed to reveal successful control of the target pest(s) (Stiling 1993,

Monje 1996). In more than 10% of the reported cases, failing biocontrol could be traced back to selection of either the wrong species or inappropriate or unsuitable strains (Stiling 1993). Thus, biological control programmes require pre-introductory basic research on the performance of potential candidates (Monje et al. 1999, Noldus 1989).

One of the most important aspects of the parasitoid-host relationship is research done on host selection process (van Dijken et al. 1986). Selection process is usually based on *Trichogramma* biological characteristics, which includes fecundity, emergence rate, sex ratio, longevity, host preference for the target species, host searching activity and tolerance to local climatic conditions. According to Flanders (1937), Gordh et al. (1999) and Vinson (1976), successful parasitism is divided into 5 steps: host habitat location, host location, host acceptance, host suitability and host regulation. The first three steps are referred to as aspects of the host selection process, which can be by selecting between hosts of different ages and or species (van Lenteren 1981). Host suitability is concerned with factors affecting the development of a parasitoid within potential hosts. Following the host finding and host selection process, host suitability is a final step in the host-parasitoid relationship toward successful parasitism (Pak et al. 1990), while host preference under controlled conditions is important in the risk assessment of biological control agents (Howarth 1991, Mansfield and Mills 2004).

Host selection can be affected by environmental conditions or by host factors such as host chemical or physical cues, shape, size and host age. For biological control programs host preference tests were carried out on *Trichogramma* species / strains between its target pest and its factious host eggs (Bourchier et al. 1994: Brand et al. 1984: van Dijken et al. 1986, Hassan 1994: Hassan 1989, Taylor and Stern 1971). This assessment of the host preference showed to be insufficient while various host eggs may not occur together in the field on the same crop and at the same time (Taylor and Stern 1971).

Host preference tests should include a native *Trichogramma* species / strain found in the field with the target pest. Theses wasps may have adapted to the field conditions and synchronized with the presence of the pest. Since host preference tests are difficult to be carried out under field conditions, this study aimed at examining the host preference of five strains of *T. aurosum* for eggs of different lepidopterous host species in the laboratory. Van Dijken et al. (1986) suggested that observation of the wasp behavioural performance using the rectangular grid is the most suitable method for determining host preference. Two experiments were designed for determining host preference: 1) choice tests, where female wasps have to choose between eggs of two host species, 2) non-choice tests, experiment was carried out to determine the wasps host range. These experiments were expected to provide more information of this *Trichogramma* species the parasitization behaviour and host preference.

4.3 MATERIALS AND METHODS

4.3.1 Insect rearing

Stock rearing of the Codling moth, *Cydia pomonella* L. (abbr. CP), the Cotton leaf worm, *Spodoptera littoralis* Boisd. (abbr. SL), the African bollworm, *Helicoverpa armigera* Hübner (abbr. HA), the Turnip moth, *Agrotis segetum* Schiff. (abbr. AS), the Grapevine moth, *Lobesia botrana* Den. and Schiff. (abbr. LB), and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (abbr. EK) were reared and maintained according to the methods described in Chapter 2. *Trichogramma aurosum* wasps collected from field were reared and maintained on eggs of *E. kuehniella* according to the methods described in Chapter 2.

4.3.2 Experimental design

4.3.2.1 Non-choice test

Non-choice tests are sufficient for an initial evaluation of both the physiological host range and host preference of *Trichogramma* spp. Therefore, 16 eggs of each host species (*C. pomonella, S. littoralis, H. armigera, A. segetum, L. botrana*) were arranged as described above. A single newly hatched female wasp was allowed to parasitize them for 90 min. Each treatment was replicated 20 times.

4.3.2.2 Choice test

Van Dijken et al. (1986) found that the best method for studying the parasitoid parasitism behaviour is by direct observation using a rectangular grid. According to them, the distance between the eggs offered should not exceed 2- 4 mm. Newly hatched *T. aurosum* test females were placed singly in small tubes with a droplet of honey for 10 min Subsequently they were transferred singly into a Petri-dish with eight host eggs of *L. botrana,* or *A. segetum,* in combination with eight eggs of *C. pomonella*. Eggs were arranged in a grid in alternative manner (Chapter 3 Fig 3-2). The distance between single eggs was ca. 4 mm to ensure that females could not perceive adjacent eggs. Every behavioural event was recorded for 90 min by direct observation with The Observer® software 3.0 (Noldus Information Technology 1993), i.e. walking, cleaning, resting (handling time), contact (touching the egg with part of the wasps body), drumming (touching the host egg with the antennae), acceptance (by starting drilling), or rejection (by leaving the host and walking away), drilling, and oviposition (movement of the abdomen can be clearly seen). Each treatment was replicated 20 times. The observation time started after the fist contact with an egg. The second part of the experiment was by indirect observation. Eight eggs of *C. pomonella* were arranged in a Petri-dish in combination with eight eggs of *S. littoralis*, or *H. armigera* in the same manner as described above and test females were allowed to parasitize them for 90 min.

4.4 RESULTS

In the choice test, no significant differences in the number of parasitized eggs in both host combinations were detected for all tested strains of *T. aurosum* (Table 4-1). The average number of parasitized eggs ranged from 3.2 - 4.6 CP eggs, and 2.7 - 3.9 LB eggs. The values in the combination CP vs. AS ranged from 1.1 - 2.7 eggs of CP and 0.8 - 1.8 eggs of AS. In contrast, all strains parasitized a significant higher number of LB and CP eggs in comparison to the other hosts in the non-choice tests (Table 4-2). The average number of parasitized eggs ranged from 4.4 - 7.5 in CP, and from 5.3 - 9.3 eggs in LB. The average number of parasitized eggs of SL, HA, and AS ranged from 0.3 – 1.4, 2.1 – 4.1 and 2,1 – 4.1 eggs, respectively.

Host acceptance contact ratio (a/c ratio) was higher for CP across all strains when two different hosts were offered in the choice test (Table 4-3). The a/c ratio for the strains Ta4, Ta10, Ta19, and Ta20 ranged from 0.76 – 0.93 on CP eggs and from 0.44 – 0.91 on LB eggs. In the second test, the a/c ratios for Ta4, Ta10, and Ta13 ranged from 0.77 – 1.00 for CP and from 0.60 – 0.82 on AS eggs. Significant differences in the a/c ratios were detected for both Ta4 and Ta10 in the combination CP vs. LB and for Ta10 in the combination CP vs. AS eggs. Here the females preferred eggs of CP over the other offered eggs in the choice test. In all other tests, no preference was observed for any of the strains to contact and to accept one of the hosts offered. The female wasps were able to parasitize 75 – 90% of the attacked eggs of CP compared with 40 – 98% of the attacked eggs of LB in the choice test (Figure 4-2). Also, they were able to parasitize 70 – 80% of the attacked eggs of the CP compared with 40- 77% of the attacked eggs of AS.

Table 4-1. Host selection expressed as number of parasitized eggs of *A. segetum, L. botrana,* and *C. pomonella* by female wasps of *T. aurosum* in choice tests at room temperature (n = 20).

	Ta4	Ta10	Ta19	Ta20
C. pomonella	3.22 ± 0.97 [a]	3.5 ± 1.76 [a]	3.41 ± 1.94 [a]	4.59 ± 2.6 [a]
L. botrana	2.78 ± 1.99 [a]	3.17 ± 0.98 [a]	2.71 ± 1.93 [a]	3.93 ± 2.41 [a]

	Ta4	Ta10	Ta13	Ta19	Ta20
C. pomonella	2.69 ± 1.60 [a]	1.13 ± 0.74 [a]	2.08 ± 1.12 [a]	1.27 ± 0.8 [a]	1.25 ± 0.87 [a]
A. segetum	1.77 ± 1.42 [a]	1.0 ± 1.0 [a]	1.62 ± 1.67 [a]	0.8 ± 0.77 [a]	1.08 ± 0.79 [a]

Within a column, means followed by the same letter are not significantly different (P > 0.05, Student Newman Keuls (SNK) test)

Table 4-2. Host selection expressed as number of parasitized eggs of *S. littoralis, H. armigera., A. segetum, C. pomonella,* and *L. botrana* by female wasps of *T. aurosum* non-choice tests at room temperature.

	Ta4	Ta10	Ta13	Ta19	Ta20
S. littoralis	0.65 ± 0.933 [c] (n = 20)	0.313 ± 0.48 [c] (n = 16)	1.4 ± 0.91 [c] (n = 16)	0.5 ± 0.22 [d] (n = 20)	0.75 ± 0.97 [d] (n = 20)
H. armigera	3.35 ± 3.28 [b] (n = 20)	2.13 ± 1.41 [b] (n = 15)	4.10 ± 3.29 [b] (n = 20)	2.06 ± 2.82 [c] (n = 18)	4.05 ± 3.26 [c] (n = 19)
A. segetum	--	4.13 ± 2.45 [a] (n = 16)	3.00 ± 2.08 [bc] (n = 13)	2.13 ± 1.41 [c] (n = 16)	2.92 ± 1.93 [c] (n = 15)
C. pomonella	5.32 ± 2.06 [a] (n = 20)	4.37 ± 2.34 [a] (n = 20)	7.53 ± 2.65 [a] (n = 20)	5.15 ± 2.96 [b] (n = 20)	5.93 ± 2.63 [b] (n = 20)
L. botrana	5.75 ± 4.49 [a] (n = 16)	5.25 ± 1.91 [a] (n = 15)	7.59 ± 3.97 [a] (n = 20)	6.77 ± 3.65 [a] (n = 15)	9.27 ± 3.41 [a] (n = 15)

* Within a column, means followed by the same letter are not significantly different (P > 0.05, Student Newman Keuls (SNK) test)

Table 4-3. Number of contacts and acceptances for *C. pomonella*, *L. botrana* and *A. segetum*, eggs by five *T. aurosum* strains in choice tests and statistical analysis of host preference (n = 20).

Strain		no. Contacts	No. Acceptances	a/c ratio	Chi square	Df	P
Ta4	*C. pomonella*	58	44	0.76	11.47	1	0.001
	L. botrana	50	22	0.44			
Ta10	*C. pomonella*	42	36	0.86	5.14	1	0.020
	L. botrana	38	24	0.63			
Ta19	*C. pomonella*	58	54	0.93	0.12	1	0.730
	L. botrana	46	42	0.91			
Ta20	*C. pomonella*	78	70	0.90	3.17	1	0.080
	L. botrana	67	53	0.79			

		no. Contact	no. Acceptance	a/c ration	Chi square	Df	P
Ta4	*C. pomonella*	40	35	0.88	0.377	1	0.540
	A. segetum	28	23	0.82			
Ta10	*C. pomonella*	9	9	1.00	4.8	1	0.028
	A. segetum	15	9	0.60			
Ta13	*C. pomonella*	26	20	0.77	0.004	1	0.947
	A. segetum	18	14	0.78			

P values lower than 0.05 indicate significant differences

Handling time was calculated in the choice test experiment between *C. pomonella* and *L. botrana*: Ta19 and Ta4 spent 23.7 and 25.4% of the observed time for drilling on eggs of CP and 16.3, 17.6% on LB eggs, respectively (Figure 4-1). In contrast, Ta20 and Ta10 spent 16.6, 18.1% of the observed time for drilling on eggs of CP and 24, 15.2% on LB eggs, respectively. Drumming time did not differ between the two hosts. Resting behaviour dominated the handling time in all strains. It was 29.5, 36.8, 40.0 and 60.0% for Ta20, Ta19, Ta4 and Ta10, respectively. It was followed by cleaning, walking and feeding, which always made out less than 1% of the total observation time (Figure 4-3).

Ta4 and Ta19 needed longer time for drilling and ovipositing when the first egg encountered was an egg of CP, while this was the case in Ta10 and Ta20 when the first egg encountered was an egg of LB (Figure 4-4).

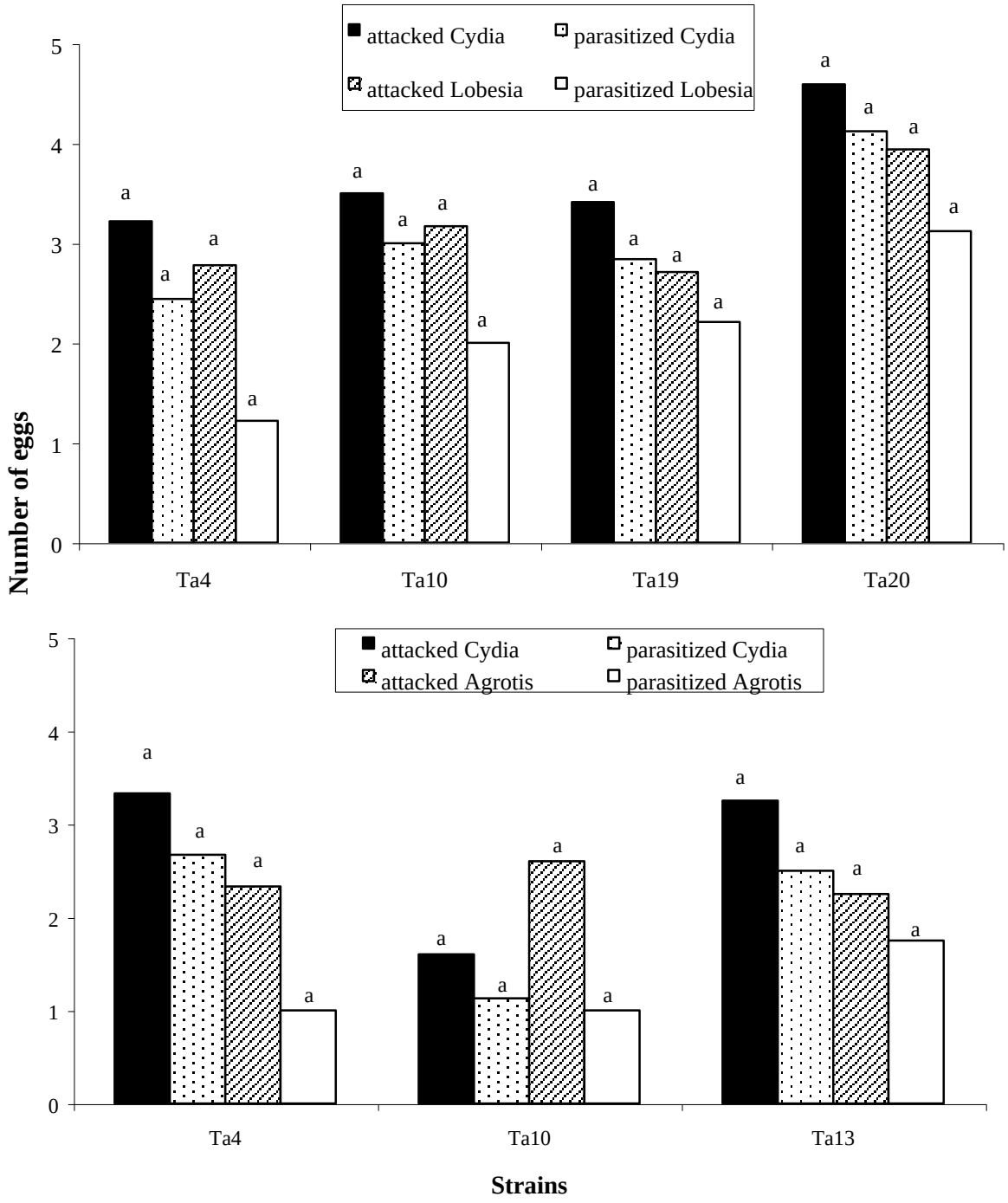

Figure 4-1. Host selection expressed as number of eggs of *A. segetum*, *L. botrana*, and *C. pomonella* attacked and parasitized by female wasps of *T. aurosum* in choice tests at room temperature (n = 20).

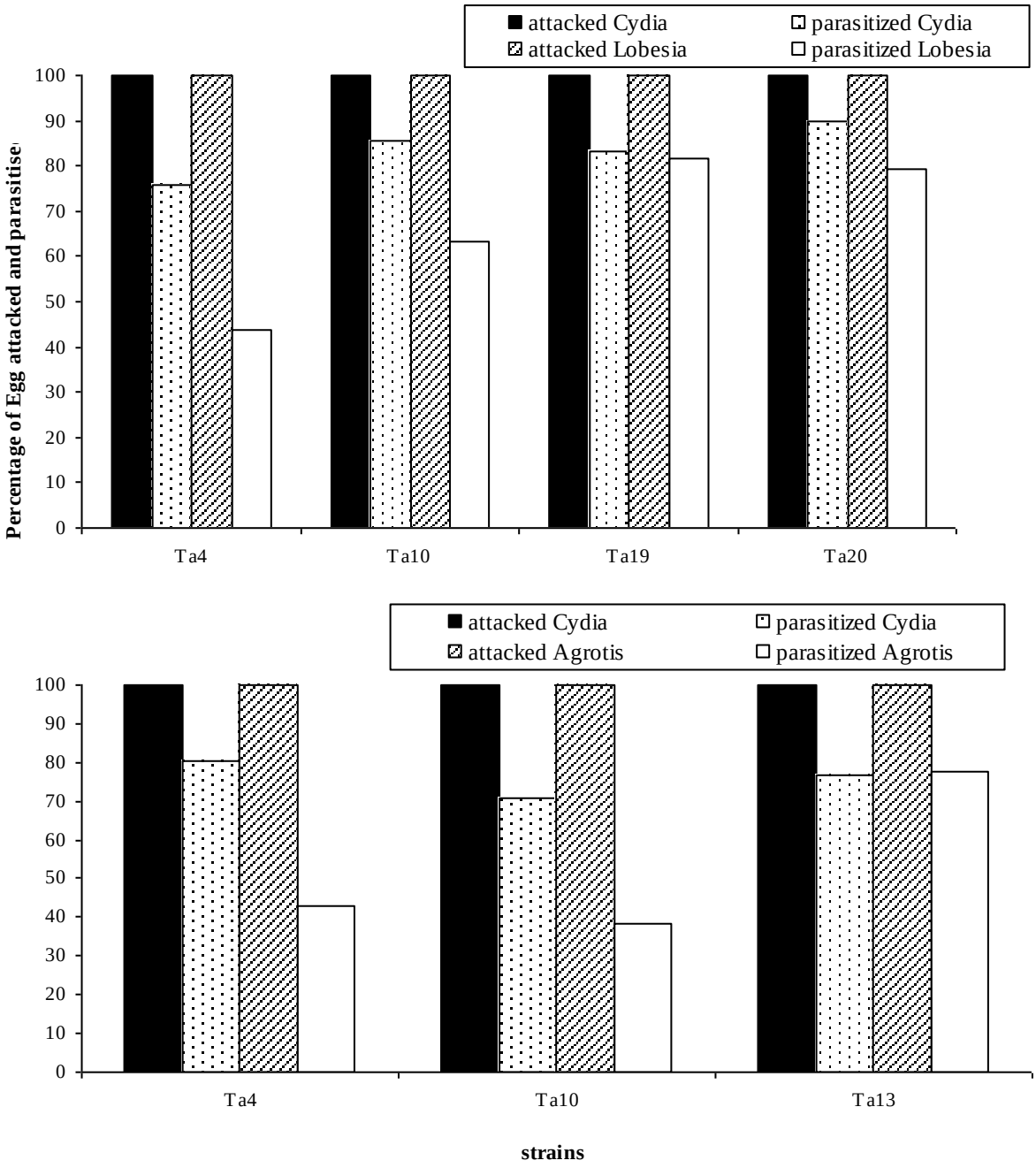

Figure 4-2. Host suitability expressed as percentage of eggs of *A. segetum*, *L. botrana*, and *C. pomonella* attacked and parasitized by female wasps of *T. aurosum* in choice tests at room temperature (n = 20).

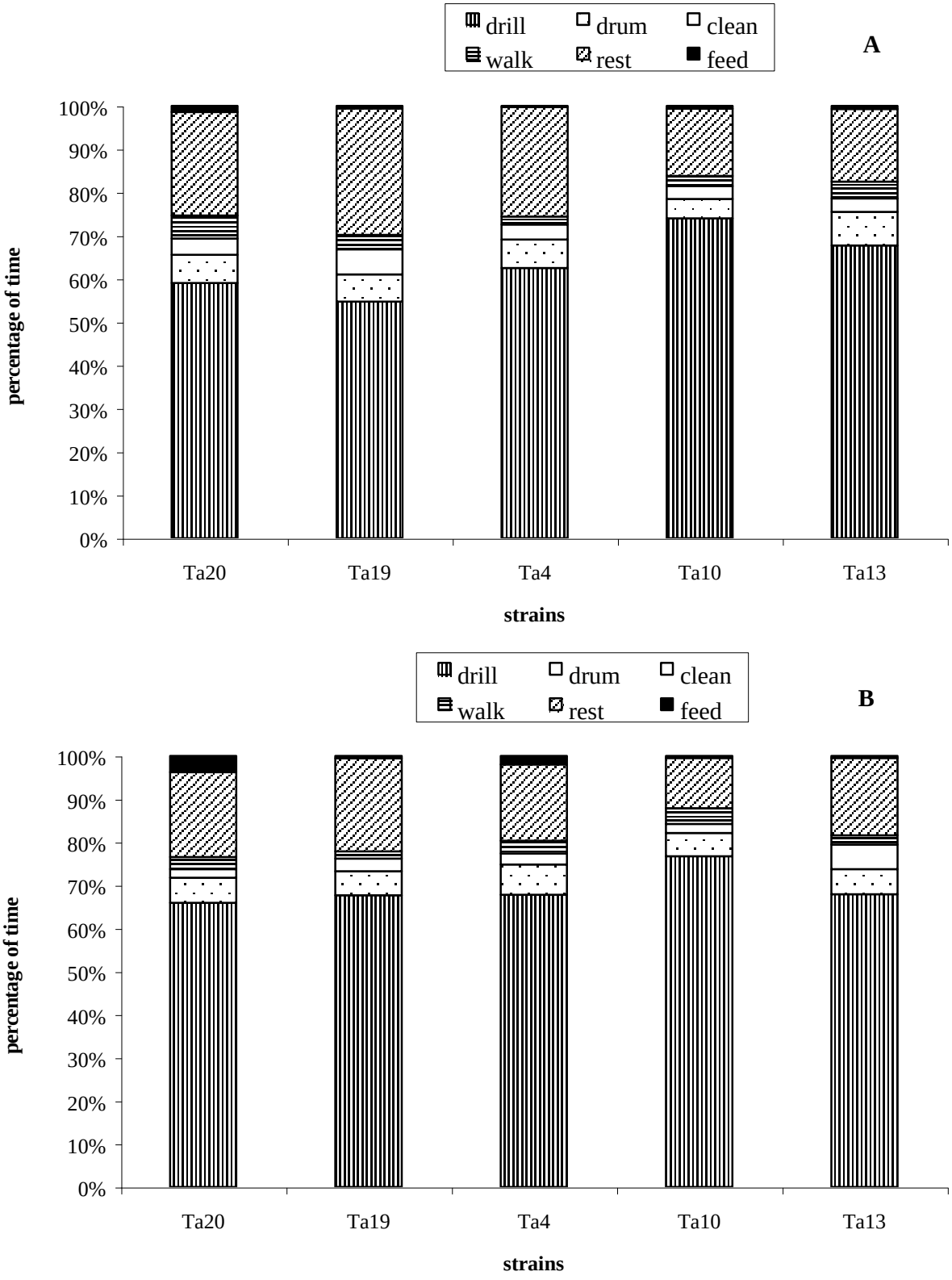

Figure 4-3. Percent time spent for behavioural steps of host searching and handling for four strains of *T. aurosum* parasitizing eggs of *C. pomonella* (A) and *L. botrana* (B) at room temperature in choice test (n = 20). (drill = drilling, drum = drumming, clean = cleaning, walk = walking, rest = resting, feed = host-feeding).

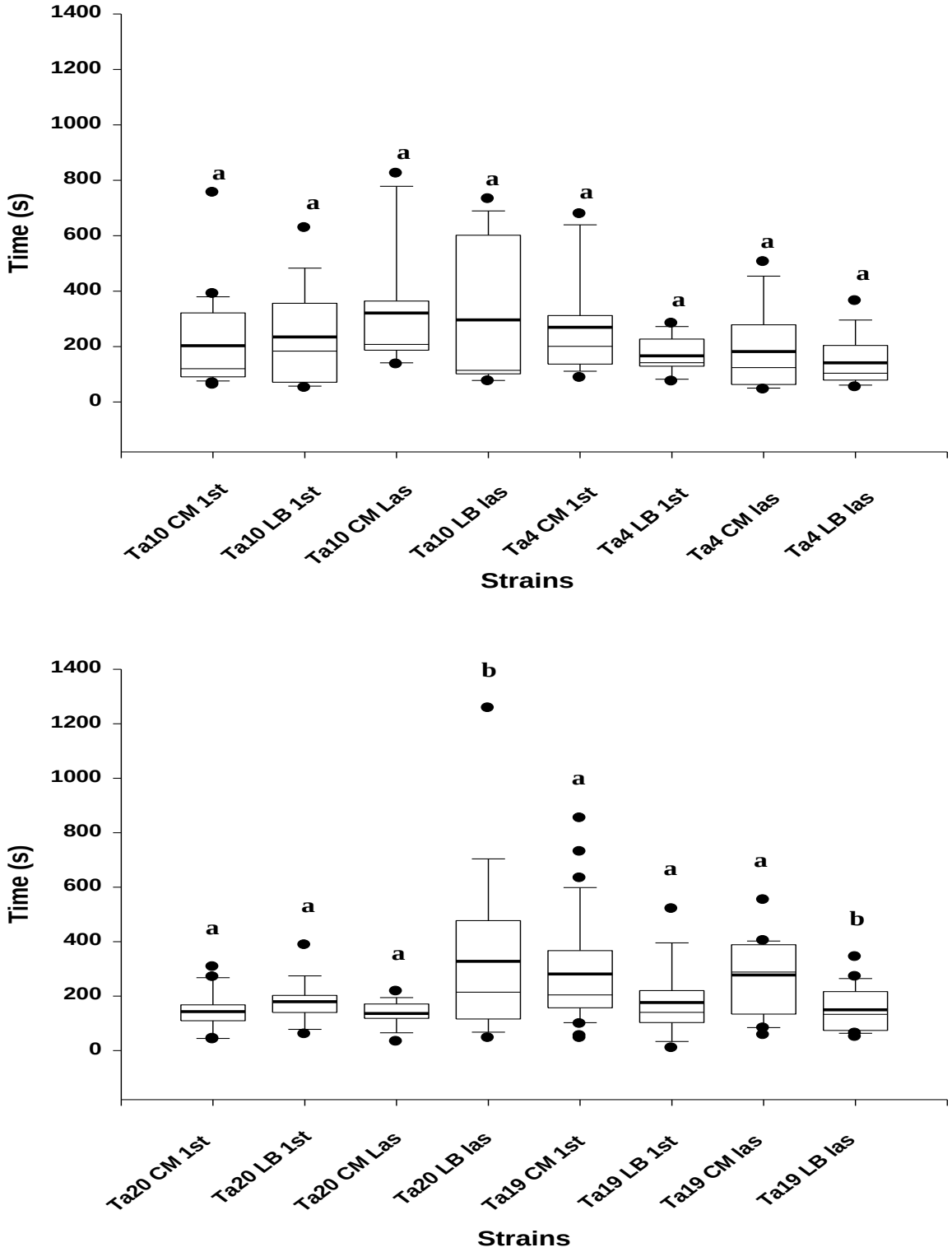

Figure 4-4. Box-Whisker-Plots of time consumed for drilling on the first and the last egg of *C. pomonella* (CM) and *L. botrana* (LB) by four strains of *T. aurosum* at room temperature in choice test (n = 20). (CM/LB-pairs with the same index do not differ significantly at the $p < 0.05$ level after Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

4.5 DISCUSSION

Preference for a certain host can be expressed as the total number of accepted hosts divided by the total number of contacts made with these hosts before the first acceptance occurs. A highly preferred host would have the a/c ratio near to 1. A host egg can be encountered several times, and it can be rejected after have being accepted in the first time. Hosts can be rejected by the parasitoids during different phases of the host inspection, e.g. after approaching at a distance of 1-2 mm, or after contact with the antennae, or after drumming the host surface, or after internal inspection with the ovipositor (van Dijken et al. 1986).

No significant differences in the number of parasitized eggs in both host combinations were detected for all tested strains of *T. aurosum* in the choice tests. This suggests that no preference for any host species was present (Table 4-1). Interestingly, the total number of parasitized eggs was consistently higher when CP and LB were offered simultaneously compared with the number of parasitized eggs in the combination CP and AS. This suggests that host acceptance may be influenced when hosts of similar shape and size (i.e. CP and LB) are available for parasitization. Offering hosts of different shape and size (i.e. CP and AS) apparently had a negative effect on host acceptance. Chorion thickness and hardness may also have played a role (Consoli et al. 1999). The number of contacts and acceptances of the hosts also provide additional indication (Table 4-3). In general, hosts in the combination CP and LB were more frequently contacted and accepted than in the combination CP and AS. It could be hypothesized that the presence of a less attractive host (i.e. AS) has a negative impact on overall parasitism activity, but additional work is necessary to verify this in detail. For instance, shape and texture of the host has been shown to play a major role in host acceptance (Vinson 1976).

In contrast to the choice tests, all strains parasitized a significant higher number of *L. botrana* and *C. pomonella* eggs in comparison to the other hosts in the non-choice tests (Table 4-2). For example, the average number of parasitized eggs of CP and LB by strain Ta4 was 8 times higher than the parasitized eggs of SL and 2 times higher than the parasitized eggs of HA (Table 4-3). The results suggest that *T. aurosum* has a higher affinity for members of the Tortricidae as for Noctuidae when offered alone (see also explanations below). Host size had a obvious effect on choice of the first host in *T. galloi* (Monje et al. 1999), while the probability of ovipositional probing after the antennal contact increases with increasing size of the host (Reznik et al. 1992). The shape and texture of the host is very important in host acceptance. It was found that odour played a key role while shape and texture have secondary effect on acceptance of a host (Vinson 1976).

Morphological characters reflect the adaptation of the species to their habitats (Consoli et al. 1999). *Trichogramma aurosum* was collected from eggs of the locust sawfly *Nematus tibialis* (Hymenoptera: Tenthredinidae), which lay its eggs on the leaves and stem of *Robinia pseudoacacia*. The eggs are oval in shape (ca. 1,000 – 1,500 µm in length and 500 – 700 µm

in width). *Cydia pomonella* and *L. botrana* belong to the family Tortricidae with different egg shapes. Eggs of *C. pomonella* belong to the lying flat type (1350 µm in length and 1050 µm in width). The upper face of the shell is convex, the surface finely structured (Fehrenbach et al. 1987). Eggs of *L. botrana* are circular, of a diameter of 600 to 700 µm, slightly convex, whitish green with a rainbow hue. The factitious host eggs of *E. kuehniella* have both ends rounded (520.7 µm long x 289.8 µm wide). Egg sculpture is homogenous, the surface has a granular texture and it is marked by a regular polygonal pattern.

Eggs of *H. armigera* are cylindrical in shape (551.8 µm long x 499.5 µm wide). The chorion shows a well-defined sculpturing with 9 – 10 conspicuous ridges crossing the egg longitudinally. The chorion has a rough surface due to the presence of many tiny holes. Egg of *S. littoralis* have a dense layer of hairy-like material that covers them when laid by young females and is less frequent on eggs of older females. Eggs are spherical in shape (454.9 µm long x 390.2 µm wide). The presence of hairy-like structures on egg clusters of *S. littoralis* reduces the parasitism rate by *Trichogramma* in field and laboratory conditions, thus representing a physical barrier (Consoli et al. 1999).

These findings suggest that shape of the host can affect the degree of acceptance by *T. aurosum,* where the natural host is oval in shape and large in size. This is also the case in CP and LB. Mansfield and Mills (2002) reported that *Trichogramma* spp. could not penetrate a chorion thicker than 20 µm. Drilling time spent on LB eggs was noticeably lower than drilling time on CP (25 – 130 s), independent on whether it was the first or the last egg. Consoli et al. (1999) found that the thickness of the exochorion might result in different drilling time by *T. galloi* and *T. pretiosum* when drilling on eggs of the same chorion thickness.

Additionally, the structural integrity of the chorion was an important factor limiting successful oviposition by *T. platneri* in larger host eggs (Mansfield and Mills 2002). The chorion of *C. pomonella* eggs have 4 layers and it varies in thickness (Fehrenbach et al. 1987), while the chorion of *E. kuehniella* eggs is 2.63 – 3.23 µm thick with four layers. In contrast, the chorion of *H. virescens* eggs, which belongs to the same genus as *H. armigera* has 5 layers with 4.00 - 13.15 µm thickness and the chorion of *S. frugiperda* eggs, which belongs to the same genus as *S. littoralis* is 2.50 – 4.44 µm thick. This may explain the wasp host preference for eggs of *E. kuehniella* (factitious host), *C. pomonella*, *L. botrana* over eggs of *S. littoralis* and *H. armigera.* As a result, many factors could have affected the selection process and *T. aurosum* strains host preference such as host shape, structure, texture, chorion thickness and structural integrity.

Both average mean drilling and oviposition time spend by Ta4, Ta10, Ta19 and Ta20 either did not change between the first and the last egg of CP and / or LB (Ta19), or increased (Ta10, Ta20) or decreased (Ta4). However, these changes were not significantly but noticeably. This might be an indication that the wasp test females learn to differentiate and to inspect the egg content so as to reach a faster decision whether to parasitise the hosts or not (Figure 4-4).

Godfray (1994) related learning behaviour with switching behaviour and pointed out that the first contact made by the wasp is very important. *Trichogramma evanescens* strains tended to parasitise the host species that was initially encountered although it was the less preferred host (van Dijken et al. 1986). Our results showed that host switching from CP to LB eggs for Ta4 and Ta20 was relatively high (> 55%) compared with host switching from LB to CP (34%). In contrast, host switching from CP to LB eggs for Ta10 and Ta19 was relatively low (30%) compared with switching from LB to CP (20 and 50%, respectively).

Host switching is a fundamental part of the parasitoid biology, allowing it to attack a variety of different hosts (Bourchier et al. 1994), and to regulate the pest population (van Dijken et al. 1986). The parasitoids tend to switch to the most abundant host type (type in the meaning of species and its age) when presented with a range of different species. It is believed that parasitoid tendency to switch is correlated with innate host preference (van Dijken et al. 1986). This behaviour can be explained as a type of learning or host preference. When a parasitoid prefers a host species over another, they tend to switch to the preferred host (Godfray 1994). Host switching is a fundamental component of mass rearing programmes for *Trichogramma* spp. Normally, parasitoids are mass-reared on a relatively small factitious host and then released to attack a larger target host (Bourchier et al. 1994).

Percentage of parasitized eggs from attacked eggs of CP was higher than those of LB and AS. This showed that *T. aurosum* examine all eggs it comes in contact with, but that does not mean that the females accept them for oviposition. Our results showed that host acceptance : contact ratio was higher for CP in some strains when offered together with eggs of LB or AS in the choice test. Although the number of the contacts made by a female to the latest host eggs was high, it seems that the females rejected many of the eggs they examined after drumming or drilling. This agrees with the finding of Godfray (1994), who reported that the parasitoids frequently insert their ovipositor into a host without laying eggs. Castañeda-Samayoa et al. (1993) reported that *T. dendrolimi* continue drilling and attempt to oviposit in eggs of *Eupoecilia ambiguella* Hb. and *L. botrana* before rejecting the eggs.

Anaphes iole Girault (Hymenoptera: Mymaridae) females use chemicals derived from host eggs or adults in host recognition (Takasu and Nordlund 2001). Godfray (1994) found that the wasps would attempt to oviposit on glass beads coated with the chemical if they were about the same size as a host egg, but they would refuse to oviposit either on uncoated glass beads or on flat surfaces smeared with accessory gland extract. Therefore, laboratory behavioural observations of the relative time spent handling different host species can provide at least an indication towards preference for any host, but it does not give obvious indication of their field efficiency (Castañeda-Samayoa et al. 1993). They may be useful to assess the host range by non-choice test before the choice test (Mansfield and Mills 2004).

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CHAPTER 5

5. FERTILITY LIFE-TABLE OF EUROPEAN STRAINS

5.1 ABSTRACT

Life table parameters were assessed for seven strains of *Trichogramma aurosum* Sugonjaev and Sorokina (Hymenoptera: Trichogrammatidae) collected in different European countries from 2001 to 2003, in order to compare their performance when reared on eggs *of Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as a potential factitious host for mass-rearing. The average number of progeny per female, cumulative fertility and emergence rate did not differ significantly, whereas female longevity and female proportion significantly differed between the seven parasitoid strains. The Ta34 strain survived the longest (6.05 days) and the Ta33 strain survived the shortest (2.75 days). Progeny was always female-biased with varying proportions (57.7 – 96.7%). Survival rates started to decrease rapidly after 3 days for some of the strains studied. The mean cohort generation duration (T_c) was 11.40, 10.15, 10.62, 9.28, 9.70 and 11.30 days for the Ta22, Ta27, Ta28, Ta30, Ta33, Ta34 and Ta4 strains, respectively. Population doubling time (D_t) was 4.50, 7.96, 3.56, 5.30, 5.23, 7.36 and 3.30 days, respectively. Daily intrinsic rate of increase (r_m) and finite rate of increase (exp. r_m) ranged between 0.087 – 0.210 and 1.091 – 1.233, respectively. Ta4 strain might be a potential candidate for mass rearing and releases purposes, due to its high net reproduction rate (R_0 = 10.65 female), a high intrinsic rate of natural increase (r_m = 0.210), a high finite rate of increase (exp. r_m = 1.23 days), and a short population doubling time (D_t = 3.3 days). The relevance of intra- and interstrain variability as well as the usefulness of fertility life tables for pre-introductory research is discussed.

Keywords: *Trichogramma aurosum*, egg parasitoids, longevity, cumulative fertility, realised fertility, life table, intrinsic rate of increase, *Ephestia kuehniella*.

5.2 INTRODUCTION

Fertility life tables are appropriate tools to study the population dynamics of insects (Southwood 1978), to enable the development of biological control programmes (Pratissoli and Parra 2000), and to evaluate natural enemies for their antagonistic potential (Zhang et al. 2001). The parameters usually calculated from fertility life tables are the net reproductive rate (R_0), the intrinsic rate of increase (r_m), which is a measure of the growth rate of a population per female (Pak and Oatman 1982); the mean cohort generation time (T_c), the doubling time (Dt), and the finite rate of increase (λ) (Southwood 1978; Maia et al. 2000; Nagarkatti and Nagaraja 1978). To assess the usefulness of natural enemies, intra- and/or interspecific biological studies are necessary depending on whether populations of a single species or those of at least two species are considered as potential candidates. Comparison of longevity,

fecundity, and sex ratio is regarded to have higher importance for intraspecific studies (Smith and Hubbes 1986b), while successful development on potential hosts, i.e. host suitability, and competition relationships need to be assessed for interspecific studies (Pak and Oatman 1982; Pratissoli and Parra 2000).

The Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is considered to be one of the key pests in apple, peach, plum, pear, and walnut worldwide (Blomefield 1989). A female can lay 25-60 eggs on the surface of the leaves or fruits (Almatni 2003). Emerging larvae from the late generations can produce crop losses up to 100% in apple, if not sufficiently controlled (Schwartz and Klassen 1981). Several strategies have been followed to control this pest, e.g. chemical control using insecticides (Madsen and Morgan 1970), mating disruption technique using pheromones and additives (Cardé and Minks 1995; Hapke et al. 2001), sterile male technique (Barnes 1959), and biological control using natural enemies.

Among the latter, several species of the gregarious egg parasitoid *Trichogramma* (Hymenoptera: Trichogrammatidae) have been employed for attempts at controlling this pest. They include *T. platneri* Nagarkatti (Mansfield and Mills 2002), *T. minutum* Riley, and *T. pretiosum* Riley (Yu et al. 1984) in the Nearctic region, *T. dendrolimi* Matsumura, and *T. cacoeciae* (Marchal) (also erroneously identified as *T. embryophagum* Hartig) in the Palearctic region (Hassan et al. 1993). In Germany, both *T. cacoeciae* and *T. dendrolimi* have been experimentally released with promising results where they were able to reduce 40-60% of pest damage (Hassan et al. 1993). Although the level of efficiency is usually calculated as corrected mortality or reduction of pest damage, the only true parameter to assess the efficiency is% damage in the treated plots at the end of the growing period. Particularly under strong competitive apple production conditions, any potential candidate species for effective biocontrol must meet these high expectancies. Thus, it is recognized that there is a need for testing additional candidate species.

Furthermore, a clear tendency towards exploiting the potential of indigenous species is evident because 1) they are believed to be better adapted to the local climatic conditions (Hassan 1994) and 2) safety of releasing exotic *Trichogramma* spp. has become an important topic (van Lenteren et al. 2003).

Trichogramma aurosum Sugonjaev and Sorokina is a holarctic species that occurs naturally in Middle Europe (see chapter 8), the former Soviet Union (Lopatina 1983; Livshits and Mitrofanov 1986), and in North America (Pinto et al. 2002). Though this species was described by Sugonjaev and Sorokina in 1975, only studies on its distribution (Lopatina 1983; Livshits and Mitrofanov 1986) and systematics (Pinto 1999; Pinto et al. 2002) have been carried out in the former Soviet Union and in the USA, respectively. Among other hosts, *T. aurosum* has been also retrieved from eggs of *C. pomonella* in both mentioned regions.

This *Trichogramma* species was collected in Germany for the first time in 2000 from eggs of *Nematus tibialis* Newman (Hymenoptera: Tenthredinidae) on *Robinia pseudoacacia* (L.). In preliminary host preference experiments it has been shown that it prefers eggs of *C. pomonella* than other hosts, and therefore, it may be a potential candidate for attempts at

controlling this insect pest. From 2001 to 2003, a wide collection of this species was conducted in the German Federal Republic and on selected sites in six European countries from eggs of *N. tibialis* on *R. pseudoacacia*, in order to obtain different strains for pre-introductory research. It is generally accepted that this is necessary before a species or a strain can be recommended for release. Pre-introductory research includes assessment of biological (i.e. life table characteristics, influence of abiotic factors) and behavioural parameters (i.e. host age and host preference) as well as testing promising strains under semi-field and field conditions.

Adaptability to adverse abiotic conditions and suitability for mass rearing are important selection criteria for use in inundative releases/augmentative biological control (Pak 1988). Additionally, species/strains with high parasitic potential under laboratory conditions may achieve better results in the field, though this has not been confirmed in all species tested (Thomson and Hoffmann 2002). Fertility life tables have received increasing attention in the last five years as a tool to evaluate the antagonistic potential of Trichogramma spp. (e.g. Haile et al. 2002), and also to compare the suitability of different species for mass rearing (Pratissoli et al. 2004). In this study we compare the life table characteristics of *T. aurosum* strains collected from seven European countries, their parasitization potential and the population growth parameters when reared on the factitious host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae).

5.3 MATERIALS AND METHODS

The experimental host species, the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae), was maintained as described by Cerutti et al. (1992) in chapter 2. The parasitoids were collected from seven European countries (Table 2-1 chapter 2) and maintained on eggs of *E. kuehniella*.

Cumulative fertility, realised fertility, longevity, mean number of progeny, development time, emergence rate, sex ratio, and survival rate of *T. aurosum* from sting to adult emergence was determined by allowing a 16 - 24 h old mated females to oviposit on *E. kuehniella* eggs. Females of each strain were placed singly (n = 20) in glass test tubes (75 x10 mm) with 50 *E. kuehniella* eggs and a small drop of honey. Egg cards were supplied daily in the morning until the death of the test females. Each group of test tubes was placed in a cabinet at 25 ± 0.5 °C, 18:6 h L:D photoperiod, and relative humidity of 80 ± 5%.

Development time from oviposition to adult emergence was determined by two visual controls every 12 h (07:00, 19:00) for *Trichogramma* emergence. Longevity (number of days) until death of the adults was also recorded. Due to the absence of superparasitism, we define cumulative fertility of a female wasp as the number of parasitized host eggs (as evidenced by blackening) over the full life span. Realised fertility is the number of eggs parasitized by individual females over the first 3 days of adult life (Kuhlmann and Mills 1999). The number of parasitized host eggs and the number of adults emerging from them were counted.

Parasitized eggs with unhatched parasitoids were dissected to assess the onset of mortality of the parasitoid. We refer to the number of living females born per female in each age interval as age-specific fertility (m_x) of the strain tested (Southwood 1978). Assessment of fertility life table parameters was conducted using the Jackknife technique (Maia et al. 2000).

DATA ANALYSIS

Data on female longevity and fertility were transformed to $\log_{10}(x+1)$, while the data on emergence rates and sex ratio were arcsine transformed. The transformed data were analysed by ANOVA test using the General Linear Models (PROC GLM) procedure (SAS Institute 1996). The Student Newman Keuls (SNK) procedure was used to separate the means.

5.4 RESULTS

Cumulative female fertility ranged from 6.8 to 14.5 parasitized eggs, but differences were not significant (Figure 5-1: F = 2.11: DF = 6, 133, P = 0.0565). A significant difference in female longevity was found between the Ta34 and the Ta33strain (Table 5-1: F = 2.33, DF = 6, 133; P = 0.0357). Noticeable female mortality (ca. 30-50%) was recorded on the second day of the experiment (Fig. 5-5). This parameter reached ca. 80% on the 4th day in the Ta30, Ta27, and Ta33 strains. Interestingly, more than 60% of the females from the Ta34 strain survived eight days, but 100% mortality was reached on the 9th day. The same value was reached after seven days in the Ta33 strain, whereas this was the case in the rest of the strains between 11 and 15 days (Fig. 5-5).

No significant differences in adult emergence rate (Figure 5-3, F = 1.93, DF = 6, 133, P = 0.080) and number of progeny produced per female could be detected (Figure 5-4: F = 2.48, DF = 6, 133; P = 0.026, respectively). However a high number of females did not produce any offspring in some strains. Dissection of blackened host eggs revealed pre-imaginal death, predominantly during prepupal stage or as pharate adults (Table 5-1). Results obtained for the pre-imaginal mortality ranged between 11 to 24% but did not differ significantly between the strains studied (F = 0.79, DF = 6, 30, P = 0.586).

Age-specific fertility was highest in the first three days for all the strains studied (Figure 5-6). It decreased gradually until the death of the females. The realised fertility in the first three days post-eclosion differed significantly (F = 2.70, DF = 6, 133; P = 0.0165). The Ta4 strain had the highest number of parasitized eggs, whereas the Ta22, Ta28, Ta33, and Ta34 strain did not differ significantly from each other (Figure 5-2). Post-oviposition period from 1-2 days was recorded for the Ta4, Ta27, Ta33, and Ta28 strains, while the rest of them continued to lay eggs until their natural death (Figure 5-6).

Sex ratio differed significantly (F = 8.33, DF = 6, 108; P = 0.0001). Progeny was extremely female-biased in the Ta30 strain, followed by the Ta4 and Ta28 strains (Table 5-1). The remaining strains had sex ratios below 80%. Development time differed significantly between the strains studied (F =6.25, DF = 6, 110; P = 0.0001). The Ta4 strain needed the longest time to develop from egg to adult, while the rest of the strains did not differ significantly from each other.

Table 5-1 Longevity, development time, sex ratio, and pre- imaginal mortality of the female wasps of the different strains (Means ± SE, n = 20)

Strain	Longevity (D)*	Development time (D)*	Sex ratio (%)**	Pre-imaginal mortality (%)**
Ta22	4.80 ± 3.85^{ab}	10.29 ± 0.77^{b}	66.56 ± 13.65^{bcd}	19 ± 14^{a}
Ta27	3.75 ± 2.86^{ab}	9.95 ± 0.76^{b}	58.48 ± 22.69^{d}	17 ± 8^{a}
Ta28	5.25 ± 3.46^{ab}	10.18 ± 0.81^{b}	77.14 ± 23.23^{bc}	12 ± 9^{a}
Ta30	3.60 ± 2.84^{ab}	10.21 ± 0.80^{b}	96.72 ± 9.27^{a}	24 ± 11^{a}
Ta33	2.75 ± 1.52^{b}	10.07 ± 0.83^{b}	61.70 ± 18.41^{d}	20 ± 10^{a}
Ta34	6.05 ± 3.07^{a}	10.00 ± 0.85^{b}	57.76 ± 16.84^{d}	14 ± 13^{a}
Ta4	3.85 ± 4.63^{ab}	11.35 ± 1.04^{a}	81.33 ± 23.00^{b}	11 ± 10^{a}

* Log transformed data were used for the mean values

**Arcsine transformed data were used for the mean proportion.

Within a column, means followed by the same letter are not significantly different (P > 0.05, Student Newman Keuls (SNK) test)

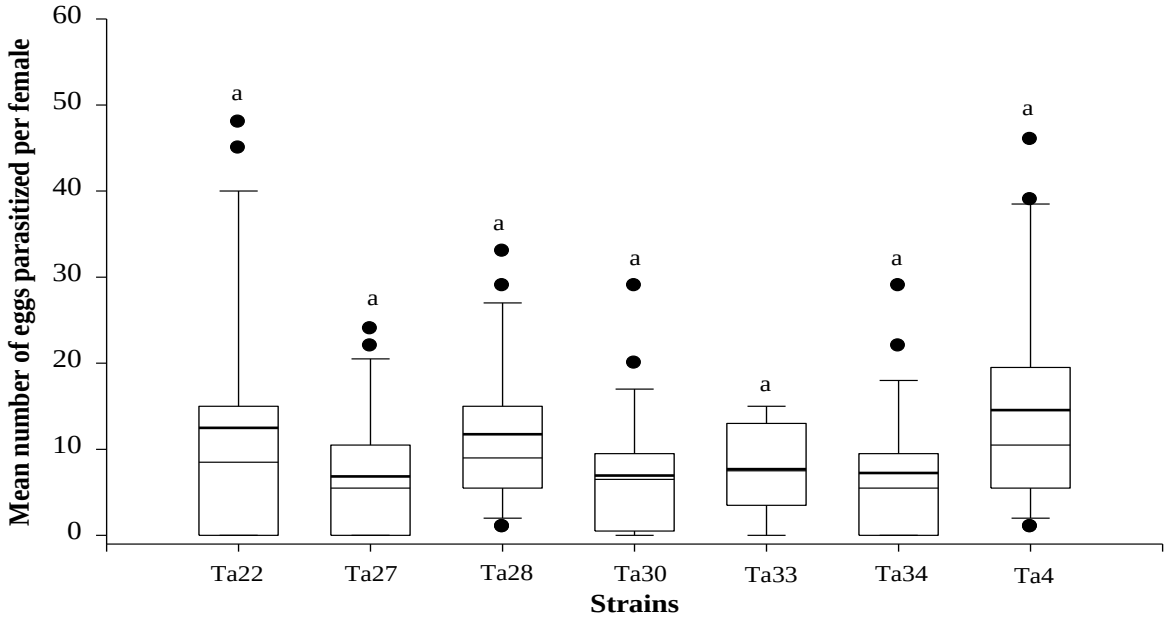

Figure 5-1. Mean accumulative female fertility of seven different strains of *T. aurosum* (n = 20). Log transformed data were used for the mean values; data sets followed by the same letter are not significantly different (P > 0.05, Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

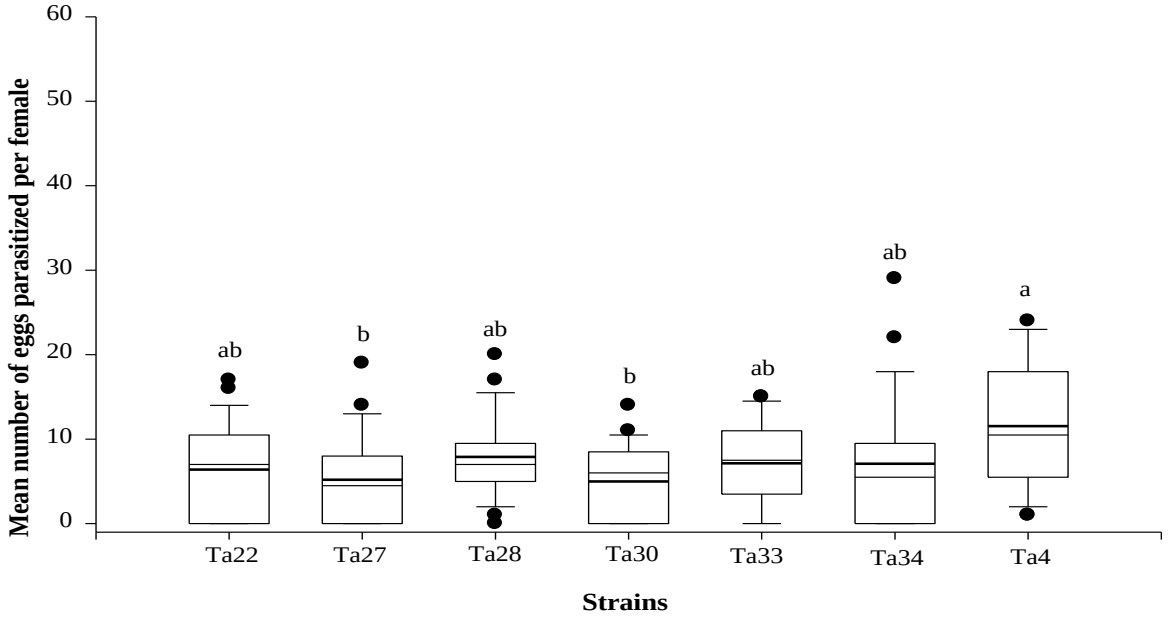

Figure 5-2. Mean realised female fertility of seven different strains of *T. aurosum* (n = 20). Log transformed data were used for the mean values; data sets followed by the same letter are not significantly different (P > 0.05, Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

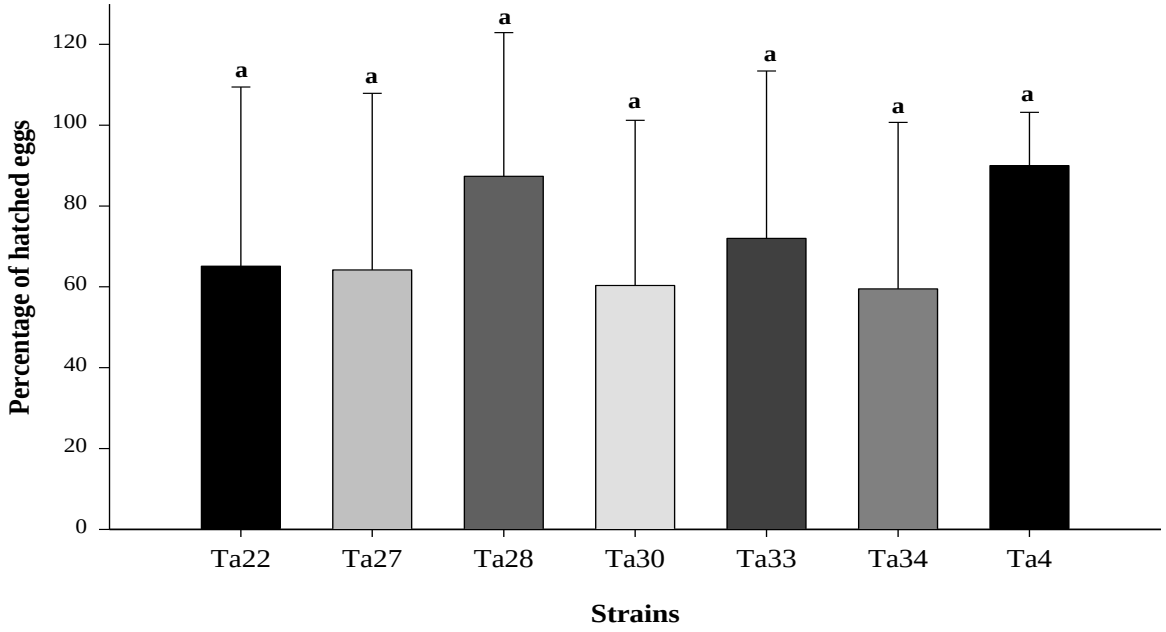

Figure 5-3. Emergence rate of seven different strains of *T. aurosum* (n = 20). Arcsine transformed data were used for the mean proportion; data sets followed by the same letter are not significantly different (P > 0.05, Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

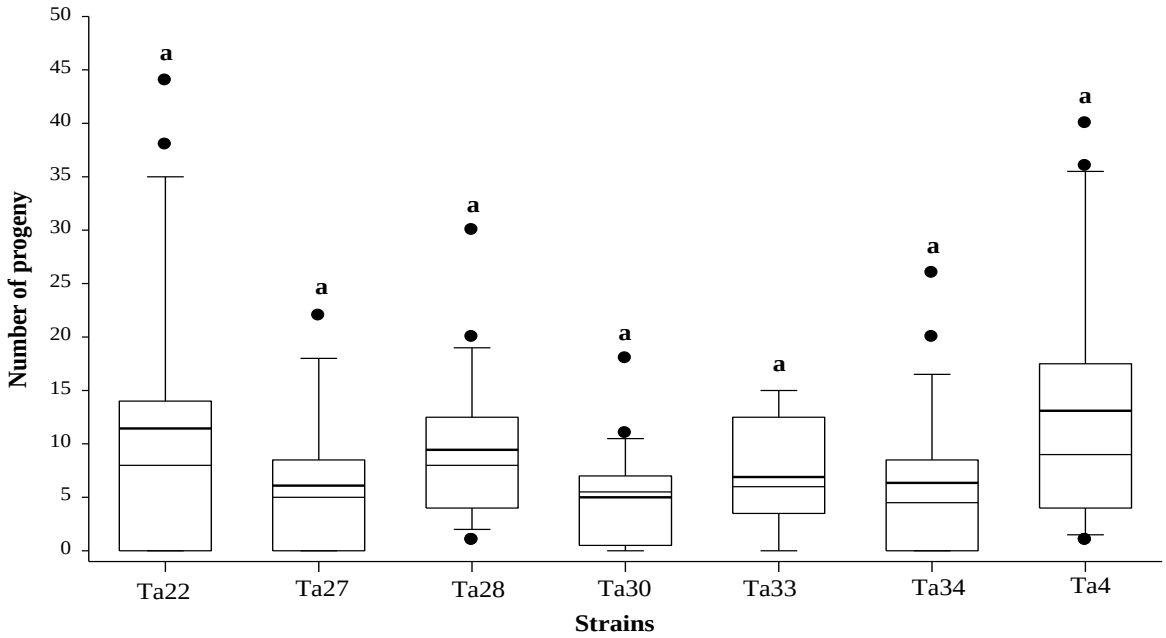

Figure 5-4. Mean number of progeny produced by one female wasp of seven different strains of *T. aurosum* (n = 20). Log transformed data were used for the mean values; data sets followed by the same letter are not significantly different (P > 0.05, Student Newman Keuls (SNK) test; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots

above and under the whisker cap; mean value = thick line; median = thin line).

The net reproduction rate (R_0) differed significantly between the Ta4 and the Ta34 strain, but no other significant differences were detected (Table 5-2). The intrinsic rate of increase and the finite rate of increase did not differ significantly between the strains studied. The mean cohort generation duration (T_c) was significantly different for the Ta4, Ta22, Ta28, and Ta27 strains. The Ta33 strain had the lowest mean cohort generation time. Population doubling time (D_t) was significantly higher for the Ta27 and Ta34 strains, while this parameter was significantly lower for the Ta4 and Ta28 strains (Table 5-2).

Table 5-2. Life table parameters of the seven *T. aurosum* strains. R_0 = net reproductive rate, T_c = mean cohort generation time, r_m = daily intrinsic rate of increase, λ = finite rate of increase, D_t = population doubling time.

Strain	R_0	T_c	r_m	D_t	λ
Ta22	5.70 ± 3.15^{abc}	11.4 ± 0.92^{a}	0.156 ± 0.04^{a}	4.366 ± 1.40^{ab}	1.165 ± 0.05^{a}
Ta27	2.42 ± 1.34^{bc}	10.15 ± 0.75^{a}	0.091 ± 0.05^{a}	6.853 ± 5.44^{a}	1.091 ± 0.06^{a}
Ta28	7.92 ± 2.87^{ab}	10.62 ± 0.76^{a}	0.196 ± 0.03^{a}	3.521 ± 0.55^{b}	1.215 ± 0.04^{a}
Ta30	4.03 ± 2.08^{bc}	10.63 ± 1.54^{ab}	0.133 ± 0.04^{a}	5.073 ± 1.76^{ab}	1.140 ± 0.05^{a}
Ta33	3.42 ± 1.14^{bc}	9.28 ± 0.51^{b}	0.134 ± 0.03^{a}	5.101 ± 1.36^{ab}	1.141 ± 0.04^{a}
Ta34	2.49 ± 1.24^{c}	9.69 ± 0.82^{ab}	0.097 ± 0.05^{a}	6.559 ± 4.50^{a}	1.099 ± 0.06^{a}
Ta4	10.65 ± 4.52^{a}	11.30 ± 2.02^{a}	0.210 ± 0.028^{a}	3.283 ± 0.45^{b}	1.233 ± 0.03^{a}

Within a column, means followed by the same letter are not significantly different ($P > 0.05$, Student Newman Keuls (SNK) test)

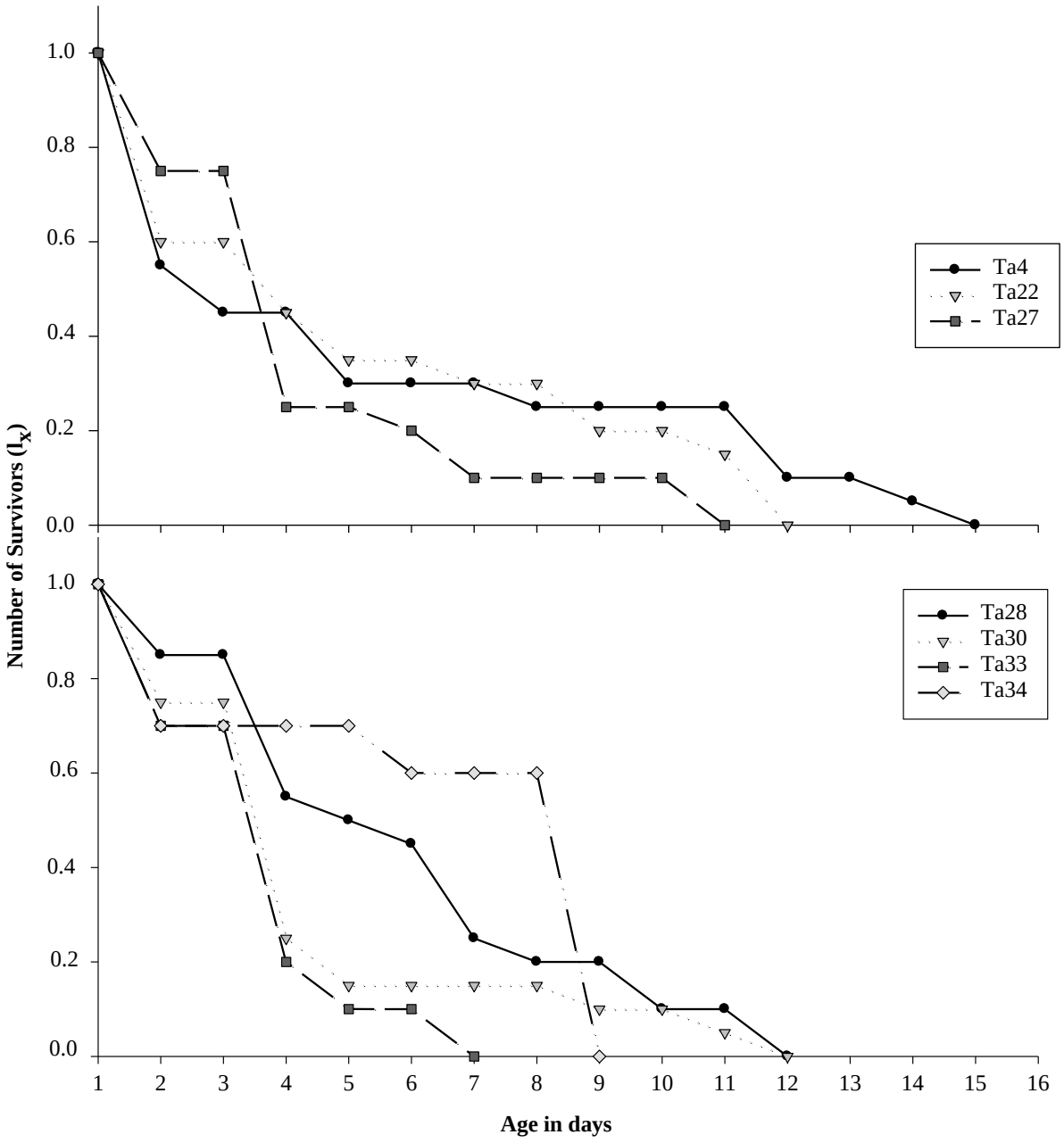

Figure 5-5. Age specific survival (l_x) of seven strains of *T. aurosum* at 25 °C , reared from eggs of *E. kuehniella.*

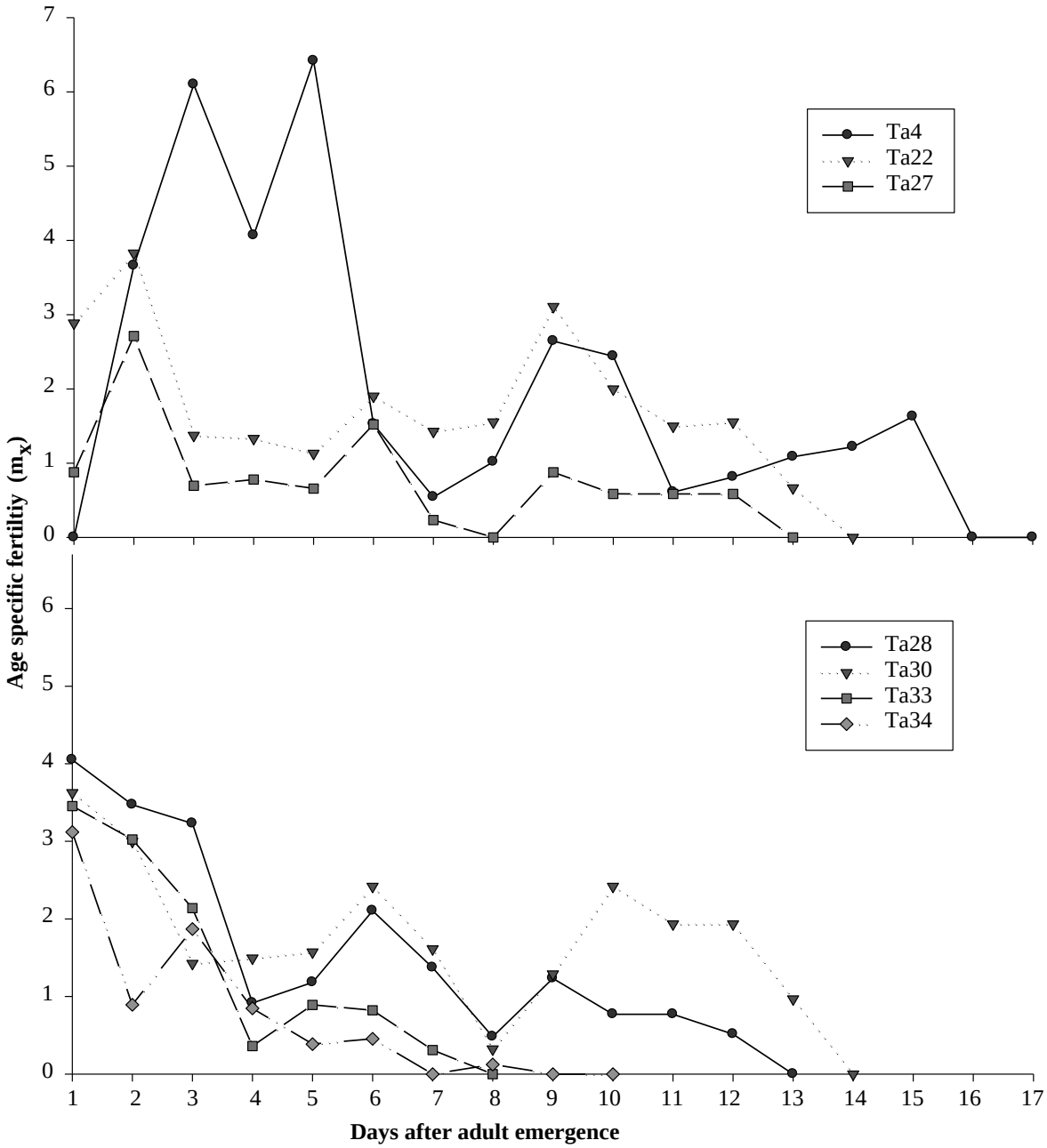

Figure 5-6. Age specific life time fertility (m_x) of seven strains of *T. aurosum* at 25 °C , reared from eggs of *E. kuehniella.*

5.5 DISCUSSION

Selection of the most appropriate species or strain of *Trichogramma* is considered to be one of the most critical factors affecting the success of biological control. In our opinion, a considerable part of the (recent) studies towards selecting promising candidates relied on assessing interspecific differences between single, or, in the best case, between two strains of different *Trichogramma* species (e.g. Schöller and Hassan 2001; Pak and Oatman 1982). With few exceptions, intraspecific differences of strains representing the range of distribution of a species have been assessed (Smith and Hubbes 1986a; Ram et al. 1995).

Our experiments have shown noticeable differences between strains of *T. aurosum* from seven European countries with respect to their longevity, realised fertility, and sex ratio. In contrast, cumulative fertility and emergence rate did not differ. The data on net reproductive rate and cohort generation time also indicate that the strains studied clearly differ from each other. Hence, we feel that interspecific differences may have been overestimated in the past, as the degree of variation between the strains we tested might be within the range reported for different species of *Trichogramma*.

We assume that the differences observed might be due to ecological pressure inferred from local climatic conditions and, consequently, to genetic variability. Intraspecific genetic variability has been reported in *T. brassicae* Bezdenko for traits including locomotion and activity rhythm (Pompanon et al. 1999), the area searched by a female (Wajnberg and Colazza 1998), the reactive distance (Bruins et al. 1994), and progeny allocation (Wajnberg et al. 1989). Unfortunately, only one population (probably the same) of *T. brassicae* was tested in the mentioned studies and hence, neither differences between populations/strains of a species nor between different species can be drawn. Nonetheless, our results revealed, additionally to inter-strain differences, considerable intra-strain variability for all seven strains tested. This suggests that genetic variability is present at the intra- and inter-strain level.

Survivorship curves obtained for the strains studied were of different type according to Southwood (1978). Survivorship curve for the Ta28 strain was of type II, i.e. a relatively constant number of individuals died per unit of time. The Ta4 and Ta22 strains had the type III (logarithmic curve), where the mortality rate is constant, while the Ta30, Ta33 and Ta27 strains showed the curve type IV, in which mortality does affect mostly young individuals. In contrast, the Ta34 strain had the curve type I, i.e. mortality was stronger in older individuals. This underlines our assumption that interspecific differences have been overestimated in the past. Mean values for female longevity ranged between 2.7 – 6.0 d in our experiments. In work conducted under comparable conditions, values ranged between 8.3 d for *T. brassicae* (Zhang et al. 2004) and 21.0 for *T. cacoeciae* Marchal (Quednau 1957). Hence, *T. aurosum* can tentatively be regarded as a “short-lived” species, at least for the experimental conditions we used. It is difficult to discern whether this is a disadvantage or not, if this species has to be considered as potential candidate for control of the Codling moth. Several authors (e.g. Zhang et al. 2001) have shown that *Trichogramma* and *Trichogrammatoidea* females deposit the

majority of their eggs within the first three days after emergence, and hence, the relatively short life span in *T. aurosum* may not impede acceptable levels of parasitization depending on availability of hosts and food sources.

Cumulative fertility ranged between 6.8 and 14.5 parasitized eggs in our study. In comparable studies with *E. kuehniella* as host, values ranged between 38.0 for *T. cordubensis* Vargas and Cabello (Garcia et al. 2001) and 112.0 for *T. ostriniae* Pang and Cheng (Pavlik 1993). At first glance, the low cumulative fertility of *T. aurosum* may represent a disadvantage for using this species in inundative releases/biocontrol/augmentative biological control. Under field conditions, however, *Trichogramma* adults do live for a maximum of three days (Mansfield and Mills 2002). Hence, realised fertility may be a more reliable parameter for estimating the parasitic potential of candidate species or strains. Realised fertility for *T. aurosum* ranged between 5 and 12 eggs/ female in our study (Figure 5-2). If the realised fertility is taken into consideration instead of the cumulative fertility, then the performance of the *T. aurosum* strains tested can be regarded as acceptable.

Two aspects are remarkable regarding the mean number of progeny produced: 1) the values were much closed to those of the realised fertility and 2) a high standard deviation was noticed. The later means, that a high variation was recoded between the individuals of the same strain. This again suggest that intraspecific genetic variations is a very important factor for the evaluation of the potential parasitism of this parasitoid. By comparing it with the pre-imaginal mortality rate, it is clear that mortality in the pupal stage was noticeable. This could be an indication that the factitious host *E. kuehniella* is not very suitable of the mass rearing of *T. aurosum*.

In general, development time at 25 °C has been found to be about 10 d for several species of *Trichogramma* (e.g. Harrison et al. 1985, Hansen 2000; Haile et al. 2002), which is in accordance with the results we obtained. Development time was significantly longer in the Ta4 strain (Tab. 2), while it did not differ in the rest of the strains. If the Ta4 strain is to be selected for mass-rearing, the longer development time should be taken into consideration.

The emergence rate is an essential parameter to measure the suitability of factitious hosts for mass-rearing (e.g. Hassan 1994). Emergence rates ranged from 60 - 90% in our study. Although differences were not significant, preference should still be given to strains with emergence rates above 80% (e.g. the Ta4 and Ta28 strain), since this is of economic importance for rearing facilities. Female proportion in the progeny differed significantly, but no relationship to lower or higher emergence rates was apparent (Table 5-1). Similarly, Ram et al. (1995) recorded a high variability of female proportion (30.6 – 74.2%) in strains of *T. evanescens* despite of very high emergence rates (89.6 – 91.4%). Additional studies are needed to clarify whether the differences observed in female proportion are of genetic origin or if symbionts are possibly involved.

The daily intrinsic rate of increase and the net reproductive rate appear to offer some potential as a biological method for differentiating strains or species (Orphanides and Gonzalez 1971). Interestingly, this approach has received increasing attention only in the last

four years. A number of species have been subject of study but unfortunately, only single strains of different species have been compared. In general, r_m values range between 0.170 – 0.380 (Zhang et al. 2001: Haile et al. 2002; Schöller and Hassan 2001). R_m values for the tested *T. aurosum* strains ranged between 0.087- 0.209, which is within the range reported for different species of *Trichogramma.* We are concerned that the results reported in the literature did not consider intraspecific variability and thus, potential candidates might be over-, or underestimated. As for *T. aurosum*, the increase of the r_m value could be the product of a low net reproductive rate (R_0) and a relatively long cohort generation time (T_c). This results in a short, but intensive reproduction period with the majority of the eggs being laid in the first 3 days post-eclosion. Still, one has to be cautious when using r_m values for selection purposes, as the host species seems to have a major influence on this parameter (Orphanides and Gonzalez 1971; Pak and Oatman 1982; Zhang et al. 2001). Parallel studies on the target host, *C. pomonella*, will provide additional information on this topic.

Our study has shown noticeable differences between strains of the same species towards biological and growth parameters. As stated by Smith and Hubbes (1986a), this has important consequences (high net reproduction rate, long cohort generation time and short doubling time) for mass production and release, as some strains might be better suited to the factitious host and hence, can be easier mass-produced than others. The Ta4 and the Ta28 strain had high values for R_0, r_m, T_c and λ and short D_t, whereas the Ta27 and the Ta34 strains had very low R_0, r_m, T_c and λ values and long D_t. From these results we can tentatively conclude that the Ta4 and perhaps the Ta28 strain have good potential for rearing and release purposes.

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CHAPTER 6

6. EFFECT OF CONSTANT AND ALTERNATING TEMPERATURES

6.1 ABSTRACT

The influence of constant and alternating temperatures on biological parameters of five German strains of *Trichogramma aurosum* Sugonjaev and Sorokina (Hymenoptera: Trichogrammatidae) was evaluated in the laboratory at four constant temperatures (15 °C, 20 °C, 25 °C, and 30 °C) and three alternating temperatures (20/10, 25/15 and 30/20 °C) on eggs of *Cydia pomonella* L. (Lepidoptera: Tortricidae). The development time and longevity of all strains were reduced as temperature increased. Developmental time of the strains differed significantly when exposed to 15 °C, 20 °C, 25 °C and 20/10 °C, but it did not differ significantly when maintained at 25/15 and 30/20 °C. Cumulative fertility and longevity differed significantly at 20/10 °C, 30/20 °C, 15 °C and 20 °C, while no significant effect was recorded on the other rearing temperatures. Realised fertility differed significantly at all constant and intermediate alternating temperatures. Emergence rates of all strains were less than 65% and were still reduced as temperature increased. Sex ratio ranged from 65-100% at constant temperatures and from 80-100% at alternating temperatures. The low temperature threshold for *T. aurosum* was 10 °C at constant temperatures and 5 °C at alternating temperatures. The mean number of degree-days (DD) required for the development of the five strains at 15 °C, 20 °C, 25 °C, and 30 °C was 175, 183, 173, and 185 DD, respectively. At 20/10, 25/15, and 30/20 °C it was 221, 212 and 218 °D, respectively. The Bavarian strain (Ta13) tolerated high temperatures and had the highest parasitization capability, while the Hessian strain (Ta10) had the lowest parasitization at all temperatures tested. Fertility life table analysis revealed a major effect of temperature on the population growth parameters. Net reproductive rate (R_0) was highest at medium temperatures (20 °C, 25 °C and 20/10 °C) in all strains studied. Net reproductive rate was the highest for the Bavarian strain (Ta13) and Baden-Wuerttemberg strain (Ta4) at all constant and alternating temperatures, respectively. The mean cohort generation time (T_c), and the population doubling time (D_t) decreased as temperature increased. The daily intrinsic rate of increase (r_m) and finite rate of increase (exp. r_m) were positively correlated with temperature. At 30 °C and 30/20 °C, all strains showed a high intrinsic rate of natural increase and finite capacity for increase, a short generation time, and a short doubling time. The short parasitization period at such high temperatures is considered as a specific survival strategy, where a faster oviposition at higher temperatures will allow the parasitoids to lay most of its available eggs in a short lifetime period. The relevance of our results is discussed in the context of climatic adaptation, intraspecific variability, and biological control.

Keywords: *Trichogramma aurosum*, egg parasitoids, longevity, cumulative fertility, life time fertility, sex ratio, development time, *Cydia pomonella*, *Ephestia kuehniella*.

6.2 INTRODUCTION

The Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), is a major pest of apple, pear, peach, plum, cherry, and walnut worldwide (Madsen and Morgan 1970). Infestation rates have been reported to reach 95% when no adequate control methods were applied (Ahmad and Abul-Hab 1977). Control of the Codling moth has been tried in commercial orchards by pheromone trapping, trunk banding, sanitation, application of pesticides, mating disruption, and biological control (Barnes 1957, Madsen and Morgan 1970). At present, the management of the Codling moth has shifted from a strictly chemical to integrated management that also incorporates horticultural practices such as pruning and thinning. Although these practices are primarily aimed at producing a healthy, productive fruit tree, they may also improve control of the pest through habitat management. Emphasis is placed on the minimum use of pesticides that have a disruptive impact on the beneficial arthropods present in apple orchards (Blomefield et al. 1997).

The gregarious egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are the most widely used natural enemies worldwide with several species being mass-produced and sold by a number of commercial companies (Smith 1996). Five species of *Trichogramma* have been reported to be potentially useful for control of the Codling moth. They include *T. platneri* Nagarkatti (Mills et al. 2000), *T. minutum* Riley, and *T. pretiosum* Riley (Yu et al. 1984) in the nearctic region, while *T. dendrolimi* Matsumura and *T. cacoeciae* (Marchal) (also erroneously identified as *T. embryophagum* Hartig) have been tested in the palearctic region (Hassan 1993). Mills et al. (2000) reported 60% reduction of damage in California walnut and apple orchards through releases of *T. platneri*, while Hassan (1993) regarded the results of releasing *T. dendrolimi* as promising.

Substantial reduction in pest damage does, however, not necessarily mean that% damage at the end of the growing period will be below the economic threshold. Hence, there is necessity to seek for additional candidate species. For instance, Pinto et al. (2002) reported eleven species of *Trichogramma* attacking tortricid eggs in apple and pear orchards in the USA. One of them, *Trichogramma aurosum* Sugonjaev and Sorokina, is a holarctic species that occurs naturally in Middle Europe (see chapter 8), the USSR (Lopatina 1983; Livshits and Mitrofanov 1986), and in the USA (Pinto et al. 2002). It was collected in Germany for the first time in 2000. In host preference experiments it was shown that this species prefers eggs of the Codling moth, *C. pomonella*, than other lepidopteran eggs. It may therefore be a potential candidate antagonist for attempts to control *C. pomonella* in apple orchards. From 2001 - 2003, a wide collection of this species was carried out in the German Federal Republic from eggs of *Nematus tibialis* Newman (Hymenoptera: Tenthredinidae) on *Robinia pseudoacacia* (L.), in order to obtain strains that could be used for pre-introductory research.

Temperature plays a major role in the activity and metabolic processes of poikilothermic organisms such as insects (Suverkropp et al. 2001). Suitability of *Trichogramma* spp. and their use as bioagents is dependent on their ability to tolerate and

adjustment to adverse abiotic conditions (Pak 1988). Adaptability of *Trichogramma* species and/or strains to high or low temperature extremes has been subject of several studies (e.g. Smith and Hubbes 1986; Garcia and Tavares 1994; Hansen 2000). In general, temperature is inversely correlated with developmental time, developmental rate, preimaginal mortality, and emergence rate in *Trichogramma* spp. In contrast, walking activity is positively correlated with temperature (Suverkropp et al. 2001).

According to Pak and Heiningen (1985), climatic adaptability is one of the criteria for the comparative selection of a strain through laboratory experiments. Field temperature alternate between day and night temperatures, but in the costal and tropical regions temperature is somehow constant. In contrast, in deserts and mountains the difference between day and night temperatures is extreme. Therefore, studying the parasitic potential and biological characteristics of *Trichogramma* species under constant temperatures is not accurate, while parasitic wasps would consume at night more energy than under natural field conditions.

Unfortunately, many studies were conducted at constant temperatures to assess the biological characteristic of many *Trichogramma* species (Garcia and Tavares 1994, Hansen 2000, Schöller and Hassan 2001, Sakr 2003, Maceda et al. 2003), and only few studies were conducted using alternating temperature in an attempt to resemble and imitate the natural conditions (Consoli and Parra 1995, Ram et al. 1995). The latter can provide more reliable results and information on the parasitoid performance.

In this paper we report the results of experiments carried out to investigate the effect of constant and alternating temperatures on the parasitization potential and the population growth parameters of different German strains of *T. aurosum*, in order to obtain suitable candidates that can possibly be used against *C. pomonella* in different parts of the country.

6.3 MATERIALS AND METHODS

A stock culture of the Codling moth, *C. pomonella*, was maintained in the laboratory according to Bathon et al. (1991) in Chapter 2. Strains of *T. aurosum* collected from the field were reared and maintained on eggs of the factitious host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Chapter 2.). Strains were placed in culture tubes (70 x 20 mm) plugged with a plastic lid, which had a small hole for aeration. The tubes were kept in a climatic cabinet at ca. 25 °C, 85% RH and 18:6 h L:D photoperiod during pupal development of the parasitoids. To feed the wasps, a droplet of honey was placed in the tube prior to or upon their emergence. Emerged parasitoids were provided with fresh host eggs on an ‘egg card’. Egg cards were prepared by sprinkling host eggs on a drop of gum arabic on a piece of paper index card (50 x 15 mm). Females used for the experiments were 24 hr old, mated, fed on honey and had no experience with hosts prior to the tests.

6.3.1 Effect on preimaginal development, emergence, and mortality

Temperature-dependent development of *T. aurosum* from sting to adult emergence was determined by allowing females to oviposit on 24 h old *C. pomonella* eggs for 24 hs. Females of each strain were placed singly (n = 20) in glass test tubes (70 x 20 mm) with 10-20 *C. pomonella* eggs, to follow individual development and emergence more closely. Development duration was calculated from the time when the test females were removed. Each group of test tubes was placed in separate cabinets at constant temperature regimes of 15, 20, 25, and 30 ºC and at alternating temperature regimes of 20/10 °C, 25/15 °C and 30/20 °C (all ± 0.5 C) at a 18:6 h L:D photoperiod, and a relative humidity of 80 ± 5%. Development time from oviposition to adult emergence was determined by two visual controls for each parasitized egg every 12 h (07:00, 19:00). Degree-days and mean developmental threshold were determined according to Smith and Hubbes (1986).

6.3.2 Fertility and longevity

Mean cumulative female fertility and relative cumulative parasitized eggs per day were determined under the same conditions as given in the previous paragraph. Test females were obtained from egg cards with parasitized *E. kuehniella* eggs in a small glass vial (40 mm long, 15 mm diameter), 3-6 h before the experiments. In these experiments, a single mated female, maximum 6 h old, was placed on a circular filter paper that covered the bottom of a glass petri dish (70 mm diameter, 10 mm deep). One minute drop of undiluted honey was provided in the middle of the petri dish to feed the adult during this test. Fresh Codling moth eggs (10 - 20 ± 1 eggs per dish per day) were supplied daily until the natural death of the female wasps. Twenty replications were performed for each wasp strain and for each temperature treatment. Longevity (number of days) until death of the adults was also recorded. Hatched *C. pomonella* larvae from unparasitized eggs were removed daily from the vials to avoid parasitized eggs being eaten in the vials.

Cumulative female fertility is defined as the total number of successfully parasitized eggs by a female over the full life span (here as evidenced by black coloration of the eggs). The number of parasitized host eggs as well as the numbers of adults emerging from the host eggs and the number of unhatched eggs were counted. We refer to the number of living females born per female in each age interval as age specific fertility (m_x) (Southwood 1978). Cumulative realised fertility is the number of eggs successfully parasitized by a female wasp over the first 3 days of adult life (Kuhlmann and Mills 1999), since most *Trichogramma* die within this period in nature if they are not able to find a food source. The parameters usually estimated from fertility life tables are the net reproductive rate (R_0); the intrinsic rate of increase (r_m), which is a measure of the growth rate of a population per female (Pak and Oatman 1982); the mean cohort generation time (T_c); the doubling time (D_t), and the finite

rate of increase (λ); all were estimated according to Southwood (1978), Maia et al. (2000), and Nagarkatti and Nagaraja (1978).

DATA ANALYSIS

Female longevity, cumulative fertility, realised fertility, and development time data were transformed to $\log_{10}(x + 1)$, while the data of emergence rate and sex ratio were arcsine transformed. The transformed data were then analysed by ANOVA test using the General Linear Models (PROC GLM) procedure (SAS Institute 1996). The Student Newman Keuls (SNK) procedure was used to separate the means.

6.4 RESULTS

6.4.1 Effect of constant temperatures

Significant differences in cumulative fertility were detected at 15 °C. Cumulative fertility was highest for Ta4 and Ta20, while Ta10 had the lowest values among all strains at 15 °C (Table 6-1). The highest cumulative fertility was measured in the strain Ta19 at the intermediate temperatures of 20 °C and 25 °C. In contrast no significant differences were recorded between the rest of the strains. At the extreme temperature (30 °C), cumulative fertility of Ta13 was significantly higher than the rest of the strains (Table 6-1), which did not differ significantly. Female longevity did not differ significantly at 25 °C, and 15 °C in all *T. aurosum* strains. At the highest temperature (30 °C), females of all strains died after max. 3 days. Females of Ta19 were able to live longest at all temperatures (Table 6-1). A significant difference was recorded at 30 °C and at 20 °C for female longevity.

Realised fertility for *T. aurosum* was calculated in response to temperature impact. Temperature had a significant impact on the female realised fertility for all strains studied. Ta20 had the highest realised fertility at 15 °C, and Ta4 had the highest significant realised fertility at 20 °C, meanwhile no significant differences were found between the rest of the strains. At 25 °C and 30 °C, Ta19 and Ta13 had the highest significant differences, respectively (Table 6-1). Parasitism percentages reached the highest values in the first three days after emergence of the female *T. aurosum*, 60-80, 70-100, 40-80 and 30-100% between the temperature 20-30 °C for Ta4, Ta10, Ta13 and Ta19, respectively. While at the low temperature (15 °C) the parasitization percentage ranged between 20- 40% for the above strains.

Mean development time at 30 °C ranged from 8.6 to 9.2 days, but the differences were not significant (Table 6-2). A significant difference was found between the strains at the rest temperatures studied, at 15 °C it ranged from 30.3 to 33.6 days, at 20 °C ranged from 15.8 to 19.1 days and at 25 °C ranged from 10.7 to 12 days. No development was recorded for all strains at 35 °C (preliminary experiments). The developmental rate from egg to adult of both female and male parasitoids increased with increasing temperature (Figure 6-1). The R^2 values

obtained by linear regression were 0.9985, 0.9916, 09864, 0.9667, and 0.9864 for Ta4, Ta10, Ta13, Ta19, and Ta20, respectively. The low temperature development threshold was 9.25, 9.33, 9.5, 10.55 and 9.07 °C, respectively.

Number of relative cumulative parasitized eggs per day was temperature dependent. Females reached 100% parasitization after 2-6 days at 30 °C, 6-11 days at 25 °C, 11-16 days at 20 °C and 26-30 days at 15 °C (Figure 6-2). Sex ratio was significantly affected by exposing the test females to different temperatures. Interestingly, progeny of Ta10 was extremely female-biased (Table 6-2), though the remaining strains had also a high sex ratio (> 67%). Emergence rate of Ta10 was affected by low temperature (15 °C). Meanwhile, Ta4 had the highest emergence rate at all temperatures studied (Table 6-2). The sum of degree-days (thermal constant) of Ta4, Ta10, Ta13, Ta19, and Ta20 was 180, 180, 185, 159 and 186 °D respectively. At 30 °C no progeny hatched from the parasitized eggs of the strain Ta19 even though black eggs were observed. This is an indication that the parasitoids reached the larval stage, but died during the prepupal stage. Therefore, the low temperature development threshold was higher and the sum of degree-days (DD) was shorter than in the other strains. An inverse relationship was found between rearing temperature and developmental time and a direct relationship between rearing temperature and developmental rate.

Fertility life table parameters differed significantly in response to the different temperatures treatments. The net reproduction rate (R_0) of Ta4, Ta10, and Ta19 varied from 3.22 to 8.12, 0.14 to 2.67, and 1.76 to 7.40 times, respectively, according to the temperature variation. The maximum increase in capacity was reached at 20 °C (Table 6-3). The values for Ta13 and Ta20 varied from 1.56 to 2.77 and from 0.71 to 4.59 times, respectively; the maximum was reached at 30 °C and 25 °C, respectively. The intrinsic rates of increase (r_m) for the different strains are shown in Table 6-3. Ta4 and Ta13 had the highest intrinsic rate of increase at 30 °C, Ta19 and Ta20 had the highest intrinsic rate of increase at 25 °C. Cohort generation time (T_c) for Ta4, Ta10, Ta13, Ta19 and Ta20 ranged from 8.26 – 32.58, 8.01 – 49.31, 9.34 – 33.69, 11.14 – 31.69 and 11.67 – 33.89 days, respectively. The finite capacity for increase (λ) of the parasitoids increased as temperature increased. Doubling time (D_t) decreases with the increase of temperature. The longest time was required at 15 °C, and the shortest was at 30 °C for the parasitoids to double its number.

Table 6-1. Effect of different constant temperatures on the female mean cumulative fertility, longevity, realised fertility of five strains of *T. aurosum.*

Temperature (°C)	Code	Cumulative fertility *	Longevity *	Realised fertility *
15	Ta4	17.11 ± 10.85 [a]	16.14 ± 11.82 [a]	3.60 ± 3.1 [b]
	Ta10	4.75 ± 3.25 [c]	11.7 ± 9.94 [a]	1.15 ± 2.1 [b]
	Ta13	14.81 ± 12.39 [ab]	11.85 ± 14.23 [a]	2.65 ± 4.2 [b]
	Ta19	9.21 ± 5.21 [bc]	18.10 ± 11.07 [a]	3.15 ± 2.8 [b]
	Ta20	18.7 ± 10.6 [a]	22.05 ± 15.63 [a]	7.85 ± 5.9 [a]
		F = 5.71; DF = 4, 77; P = 0.0004	F =2.38; DF = 4, 96; P = 0.057	F = 8.49; DF = 4, 94; P = 0.0001
20	Ta4	14.06 ± 10.47 [b]	8.45 ± 5.92 [b]	10.00 ± 6.9 [a]
	Ta10	6.35 ± 9.58 [b]	3.94 ± 4.23 [b]	4.35 ± 4.8 [b]
	Ta13	13.05 ± 8.59 [b]	7.20 ± 4.92 [b]	6.30 ± 3.6 [b]
	Ta19	22.05 ± 11.63 [a]	12.80 ± 6.07 [a]	5.60 ± 4.4 [b]
	Ta20	6.65 ± 6.48 [b]	6.60 ± 5.22 [b]	3.65 ± 3.4 [b]
		F = 8.7; DF = 4, 88; P = 0.0001	F = 7.11; DF = 4, 87; P = 0.0001	F = 4.86; DF = 4, 89; P = 0.0014
25	Ta4	14.05±11.59 [a]	4.14 ± 3.44 [a]	6.70 ± 6.1 [bc]
	Ta10	5.05 ± 3.677 [b]	4.45 ± 1.90 [a]	4.10 ± 3.0 [c]
	Ta13	6.90 ± 5.5 [b]	5.15 ± 3.12 [a]	5.65 ± 3.8 [bc]
	Ta19	18.10 ± 12.94 [a]	4.85 ± 3.39 [a]	12.40 ± 7.2 [a]
	Ta20	11.74 ± 9.97 [ab]	4.20 ± 3.12 [a]	8.30 ± 6.5 [b]
		F = 6.31; DF = 4, 95; P = 0.0002	F = 0.41; DF = 4, 95; P = 0.803	F = 6.51, DF = 4, 95; P = 0.0001
30	Ta4	6.74 ± 6.37 [b]	2.53 ± 2.45 [a]	6.82 ± 4.7 [b]
	Ta10	5.05 ± 3.15 [b]	2.95 ± 2.67 [a]	4.63 ± 3.1 [bc]
	Ta13	13.05 ± 10.27 [a]	2.60 ± 2.01 [a]	10.05 ± 6.5 [a]
	Ta19	7.40 ± 3.97 [b]	1.60 ± 1.1 [b]	6.85 ± 3.9 [b]
	Ta20	2.45 ± 4.03 [b]	2.40 ± 1.73 [a]	3.00 ± 4.3 [c]
		F = 7.99; DF = 4, 92; P = 0.0001	F = 1.21; DF = 4,92; P = 0.313	F = 6.52; DF = 4, 91; P = 0.0001

* Log transformed data were used for the mean values

**Arcsine transformed data were used for the mean proportion.

Data means ± □SE. The data in same column followed by the same letter means no significant difference (P < 0.05, ANOVA, Student Newman Keuls procedures (SNK)

Table 6-2. Effect of different constant temperatures on the development time, sex ratio, emergence rate and degree-days of five strains of *T. aurosum*

Temperature (°C)	Code	Development time*	Sex ratio**	Emergence rate**	Degree day
15	Ta4	30.3 ±1.8 [b]	69.22 ± □22.74 [b]	30.17 ± 32.16 [a]	174.23 ± 10.4 [c]
	Ta10	32.7 ± 1.2 [a]	100 ± 0.00 [a]	4.58 ± 8.61 [a]	185.41 ± 6.5 [b]
	Ta13	32.6 ± 1.5 [a]	80.56±25.73 [ab]	18.94 ± 19.85 [a]	179.3 ± 8.3 [bc]
	Ta19	32.0 ± 1.4 [a]	93.52 ± 15.27 [ab]	23.11 ± 45.91 [a]	142.4 ± 6.3 [d]
	Ta20	33.6 ± 0.9 [a]	67.57 ± 25.72 [b]	34.00 ± 26.13 [a]	199.25 ± 5.3 [a]
		F = 13.2; DF = 4, 54; P = 0.0001	F = 5.5; DF = 4, 104; P = 0.0005	F = 2.03; DF = 4, 80; P = 0.0994	F = 85; DF = 4, 54; P = 0.0001
20	Ta4	17.3 ± 1.7 [b]	86.73 ± 20.59 [a]	65.88 ± 32.58 [a]	185.98 ± 17.7 [b]
	Ta10	15.8 ± 0.4 [c]	100 ± 0.00 [a]	42.82 ± 39.66 [ab]	168.59 ± 4.7 [c]
	Ta13	18.9 ± 0.6 [a]	67.59 ± □31.86 [b]	15.85 ± 30.38 [b]	198.45 ± 5.9 [a]
	Ta19	19.1 ± 0.8 [a]	65.77 ± 30.7 [b]	52.25 ± 29.78 [a]	180.5 ± 7.9 [b]
	Ta20	16.8 ± 0.77 [b]	56.90 ± 25.59 [b]	42.60 ± 33.46 [ab]	183.62 ± 8.4 [b]
		F = 23.44; DF = 4, 72; P = 0.0001	F = 8.88; DF = 4, 97; P = 0.0001	F = 5.83; DF = 4, 82; P = 0.0004	F = 9.2; DF = 4, 72; P = 0.0001
25	Ta4	11.3 ± 1 [b]	87.77 ± 18.18 [a]	44.90 ± 33.26 [a]	177.98 ±16.1 [b]
	Ta10	12 ± 1 [a]	100 ± 0.00 [a]	18.25 ± 17.69 [b]	188.04 ± 15.7 [a]
	Ta13	11.2 ± 0.9 [b]	73.33 ± 26.26 [b]	34.40 ± 32.76 [ab]	173.6 ± 13.6 b
	Ta19	10.7 ± 0.5 [b]	70.80 ± 23.44 [b]	27.32 ± 25.59 [ab]	154.62 ± 6.8 [c]
	Ta20	10.7 ± 0.7 [b]	92.19±18.67 [a]	35.78 ± 28.95 [ab]	170.45 ± 10.5 [b]
		F = 6.53; DF = 4, 88; P = 0.0001	F = 10.06; DF = 4, 141; P = 0.0001	F = 2.2; DF = 4, 88; P = 0.0759	F = 15.3; DF = 4, 88; P = 0.0001
30	Ta4	8.7 ± 0.8 [a]	86.67 ± 20.62 [a]	56.61 □± 38.31 [a]	179.48 ± 16.9 [a]
	Ta10	8.6 ± 0.5 [a]	96.67± 20.69 [a]	22.72 ± 34.02 [b]	177.76 ± 10.7 [a]
	Ta13	9.2 ± 0.6 [a]	87.88±20.69 [a]	17.35 ± 28.61 [b]	188.6 ± 12.9 [a]
	Ta19	ND	ND	ND	ND
	Ta20	9.2 ± 0.79 [a]	66.68 ± 20.85 [b]	35.50 ± 47.74 [ab]	192.56 ± 16.5 [a]
		F = 2.46; DF = 3, 46; P = 0.0748	F = 7.75; DF = 3, 80; P = 0.0001	F = 3.69; DF = 4, 69; P = 0.0159	F = 2.6; DF = 4, 46; P = 0.067

* Log transformed data were used for the mean values

**Arcsine transformed data were used for the mean proportion.

Data means ± □SE. The data in same column followed by the same letter means no significant difference (P < 0.05, ANOVA, Student Newman Keuls procedures (SNK)

ND = no development was found, no hatching was recorded.

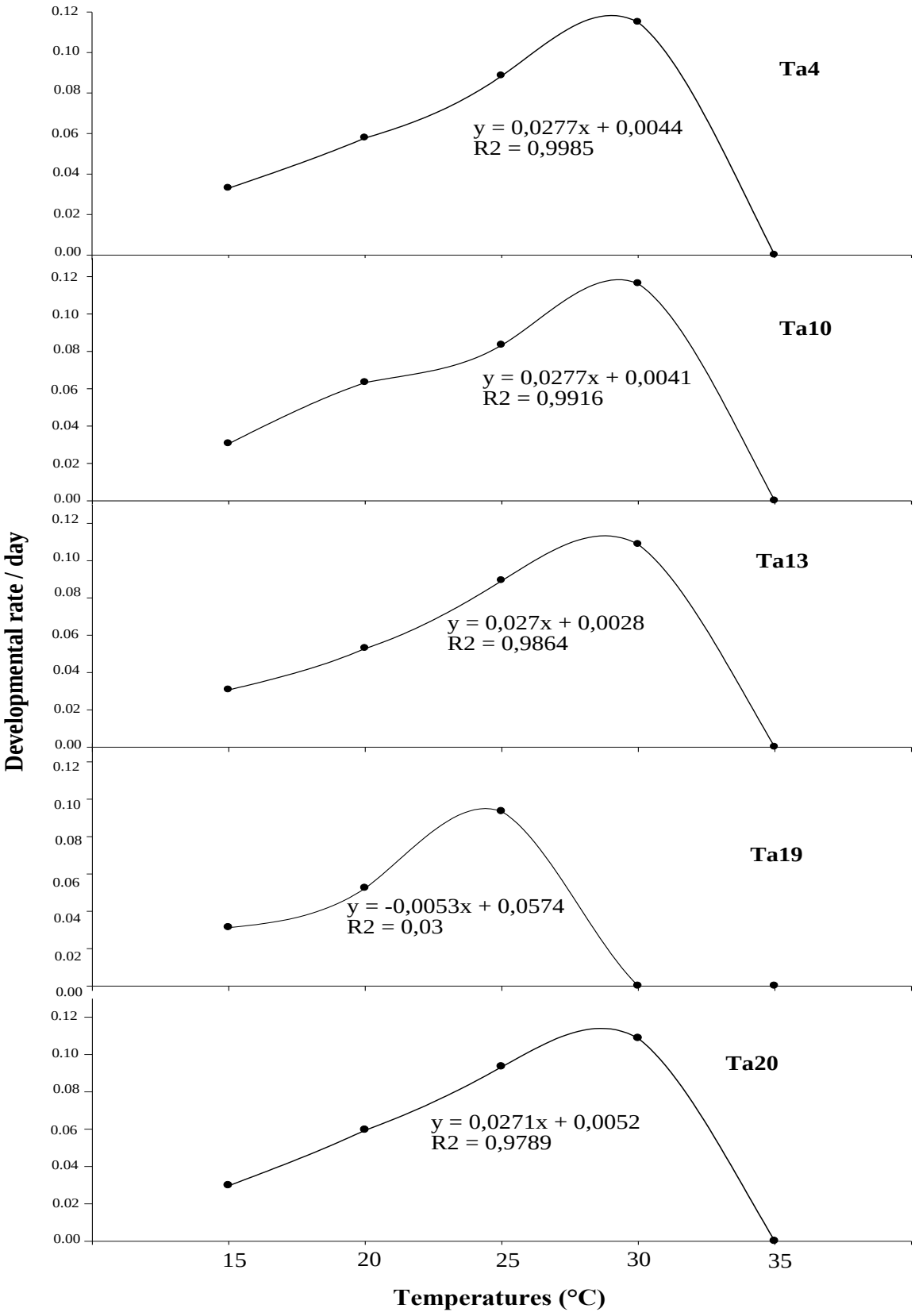

Figure 6-1. Effect of constant temperatures on the developmental rate per day of different strains of *T. aurosum.*

Table 6-3. Fertility life table parameters of *Trichogramma aurosum* on *Cydia pomonella* at 15, 20, 25 and 30 °C. R_0 = net reproductive rate, r_m = intrinsic rate of increase, T_c = cohort generation time (days), λ = finite capacity for increase, D_t = doubling time (days).

Strain	R_0	r_m	T_c	λ	D_t
			15 °C		
Ta4	3.22 ± 0.1 [a]	0.036 ± 0.012 [a]	32.58 ± 4.8 [b]	1.037 ± 0.011 [a]	19.32 ± 5 [ab]
Ta10	0.14 ± 0.9 [c]	-0.04 ± 0.015 [c]	49.31 ± 3.2 [a]	0.961 ± 0.015 [c]	-17.38 ± 36.8 [c]
Ta13	1.83 ± 0.5 [b]	0.018 ± 0.009 [b]	33.69 ± 1.2 [b]	1.018 ± 0.009 [b]	38.586 ± 19 [ab]
Ta19	1.76 ± 1.1 [b]	0.018 ± 0.008 [b]	31.69 ± 1.4 [c]	1.018 ± 0.008 [b]	38.78 ± 2.7 [a]
Ta20	4.56 ± 0.1 [a]	0.045 ± 0.012 [a]	33.89 ± 4.8 [b]	1.046 ± 0.011 [a]	15.49 ± 5 [b]
			20 °C		
Ta4	8.12 ± 3.01 [a]	0.132 ± 0.025 [a]	15.87 ± 0.63 [c]	1.141 ± 0.028 [a]	5.25 ± 0.99 [b]
Ta10	2.67 ± 2.05 [b]	0.06 ± 0.052 [bc]	15.20 ± 1.90 [bc]	1.067 ± 0.055 [b]	10.74 ± 11.09 [ab]
Ta13	1.56 ± 0.52 [b]	0.022 ± 0.016 [c]	19.71 ± 1.25 [a]	1.023 ± 0.017 [b]	30.86 ± 23.46 [ab]
Ta19	7.20 ± 1.99 [a]	0.096 ± 0.013 [b]	20.67 ± 0.65 [a]	1.100 ± 0.014 [c]	7.26 ± 0.97 [a]
Ta20	1.61 ± 0.74 [c]	0.028 ± 0.026 [c]	16.97 ± 1.52 [b]	1.029 ± 0.027 [b]	24.64 ± 25.48 [ab]
			25 °C		
Ta4	4.93 ± 2.4 [a]	0.135 ± 0.037 [a]	11.81 ± 1.2 [a]	1.145 ± 0.042 [a]	5.13 ± 1.4 [a]
Ta10	0.92 ± 0.3 [c]	-0.005± 0.021 [c]	16.55 ± 1.1 [b]	0.995 ± 0.021 [c]	-130.88 ± 769.1 [a]
Ta13	2.25 ± 0.8 [b]	0.072 ± 0.029 [b]	11.26 ± 1.1 [a]	1.075 ± 0.031 [b]	9.60 ± 4.1 [a]
Ta19	3.70 ± 1.1 [a]	0.117 ± 0.026 [a]	11.14 ± 0.7 [a]	1.125 ± 0.029 [a]	5.90 ± 1.4 [a]
Ta20	4.59 ± 2.3 [ab]	0.131 ± 0.038 [a]	11.67 ± 1.2 [a]	1.140 ± 0.043 [a]	5.31 ± 1.6 [a]
			30 °C		
Ta4	3.96 ± 1.83 [a]	0.167 ± 0.051 [a]	8.26 ± 0.63 [c]	1.181 ± 0.061 [a]	4.16 ± 1.35 [a]
Ta10	1.14 ± 0.29 [b]	0.017 ± 0.031 [b]	8.01 ± 0.41 [c]	1.017 ± 0.032 [b]	41.94 ± 102.50 [a]
Ta13	2.77 ± 1.06 [a]	0.109 ± 0.040 [a]	9.34 ± 0.44 [b]	1.115 ± 0.044 [a]	6.36 ± 2.37[a]
Ta19	…	…	….	…	…
Ta20	0.71 ± 0.48 [b]	-0.027 ± 0.056 [b]	12.73 ± 0.46 [a]	0.973 ± 0.054 [b]	-25.78 ± 42.98 [a]

Data means ± SE. The data in same column followed by the same letter means no significant difference ($P < 0.05$, ANOVA, Student Newman Keuls procedures (SNK)

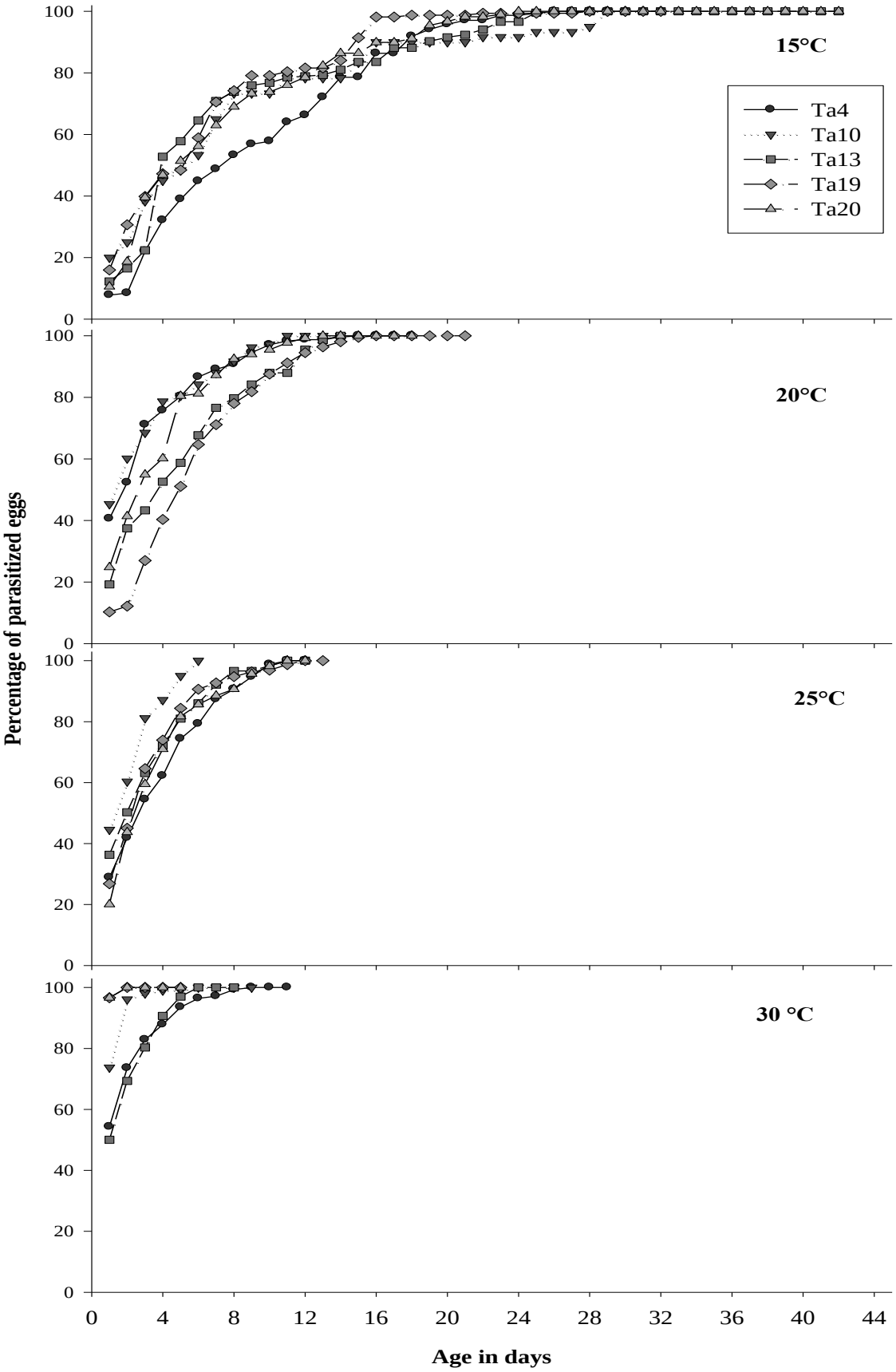

Figure 6-2. Relative cumulative parasitized eggs per day of five German strains at four constant temperatures

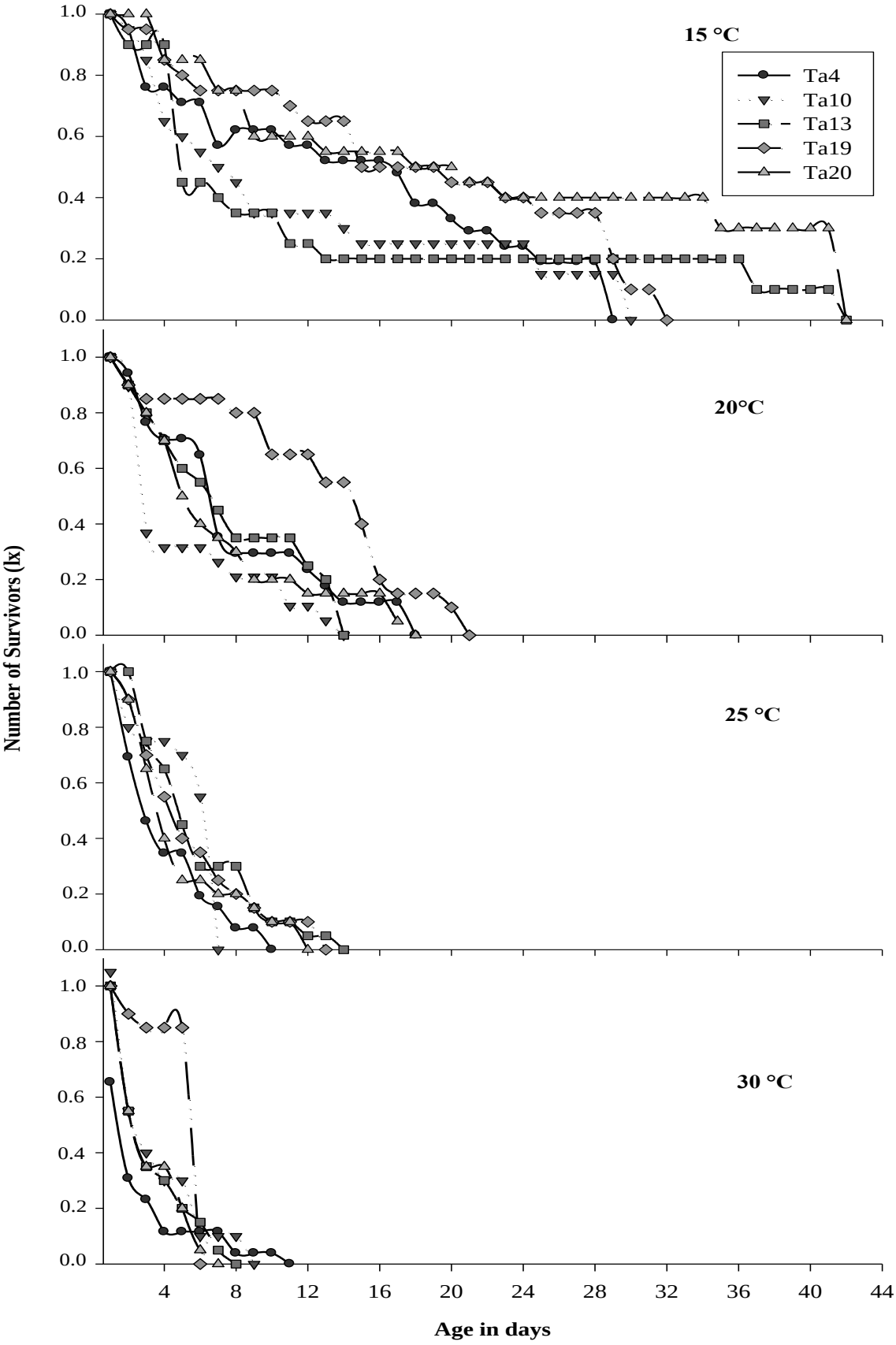

Figure 6-3. Survivorship in five strains of *T. aurosum* at four constant temperatures.

6.4.2 Effect of alternating temperatures

Significant differences in cumulative fertility were detected at the low temperature 20/10 °C and the high temperature 30/20 °C. It was highest for Ta4 and Ta13 at the low temperature and for Ta13 and Ta20 at the high temperature (Table 6-4). At the intermediate temperature 25/15 °C, no significant difference between the strains could be observed. Female longevity differed significantly at the low and the high temperature, too. Ta13 had the longest lifespan at all temperatures (Table 6-4). Longevity decreased as temperature increased. Realised fertility differed significantly between the strains at 25/15 °C, but it did not differ significantly at 20/10 °C and 30/20 °C. The strains Ta10, Ta20 and Ta13 had the highest realised fertility at the low, intermediate and high temperature, respectively (Table 6-4). Relative cumulative parasitized eggs per day was affected with alternating temperatures. The female parasitization reached 100% after 6-8 days at 30/20 °C, 8-12 days and at 25/15 °C. At low alternating temperatures rate (20/10 °C) some strains responded differently most strains have reached 100% parasitization after 16-22 days, and Ta4 reached that after 12 days (Figure 6-6). Parasitism percentages reached the highest values in the first three days after emergence of the female *T. aurosum,* 30-60%, 60-85% and 90-100% at 20/10, 25/15 and 30/20 °C, respectively, for Ta4, Ta10, Ta13 and Ta19, respectively.

Mean development time at 20/10 °C ranged from 19.7 – 22.5 days, at 25/15 °C ranged from 13.4-14.4 days and at 30/20 °C it ranged from 10.6 – 11 days, but the differences were not significant at the intermediate and high temperatures (Table 6-5). A significant difference was found between the strains at the low temperature only. Developmental rate from egg to adult of both female and male parasitoids increased with increasing temperature (Figure 6-4). The r^2 values obtained by regression analysis were very high and ranged from 0.9897 to 0.9995.

Sex ratio was not affected by exposing the test females to low and intermediate temperatures. The strains Ta4, Ta10, Ta13, and Ta19 did not differ significantly at 20/10 °C and 25/15 °C (Table 6-5). At 30/20 °C significant differences were recorded. The progeny of Ta10 was extremely female-biased (Table 6-5), although the remaining strains had also a high sex ratio (> 75%). Emergence rate of all strains was affected at high temperature (30/20 °C). It ranged from 8 – 47%. At low temperature it ranged from 30 – 80% and at intermediate temperature it ranged from 30 – 48% (Table 6-5). The degree-day (thermal constant) of Ta4, Ta10, Ta13, Ta19 and Ta20 was 257, 280, 285, 280 and 280 °D respectively.

The net reproduction rate (R_0), the intrinsic rate of increase (r_m), and the finite capacity for increase (λ) at alternating temperatures differed significantly between the strains (Table 6-6). At 20/10 °C Ta10 and Ta13 had the highest R_0 and r_m value. At 25/15 °C Ta20 and at 30/20 Ta13 and Ta20 had the highest R_0 and r_m value. The intrinsic rate of increase (r_m) for the different strains increased with increasing temperature (Table 6-6). Cohort generation time (T_c) at low and high temperature differed significantly but at intermediate temperature no significant differences were recorded. The finite capacity for increase (λ) of the parasitoids

differed significantly between all strains at temperatures studied. Ta10 and Ta20 had the highest T_c value at 20/10 and 25/15 °C, respectively. Doubling time did not differ significantly at low and intermediate temperatures, whilst at high temperature a significant difference was recorded between the strains studied.

Table 6-4. Effect of different alternating temperatures on the female cumulative fertility, longevity, life time fertility and released fertility of five strains of *T. aurosum.*

Temperature °C	Code	Cumulative fertility *	Longevity *	Released fertility *
20/10	Ta4	14.33 ± 6.5 [a]	3.55 ± 3.52 [b]	5.10 ± 6.15 [a]
	Ta10	10.10 ± 8.21 [ab]	7.95 ± 6.50 [b]	5.65 ± 5.60 [a]
	Ta13	13.76 ± 9.69 [a]	13.50 ± 8.21 [a]	4.35 ± 4.38 [a]
	Ta19	8.85 ± 11.59 [ab]	6.5 ± 6.10 [b]	4.25 ± 4.20 [a]
	Ta20	5.50 ± 6.53 [b]	5.70 ± 5.05 [b]	3.15 ± 3.69 [a]
		F = 2.99; DF = 4, 88; P = 0.023	F = 7.58; DF = 4, 95; P = 0.0001	F = 0.75; DF = 4, 95; P = 0.5599
25/15	Ta4	8.26 ± 9.50 [a]	4.4 ± 5.2 [a]	4.63 ± 4.78 [b]
	Ta10	8.20 ± 8.10 [a]	3.6 ± 3 [a]	6.50 ± 5.31 [b]
	Ta13	11.60 ± 12.08 [a]	4.3 ± 6.4 [a]	8.25 ± 6.65 [ab]
	Ta19	7.10 ± 7.54 [a]	3.6 ± 2.6 [a]	6.00 ± 4.74 [b]
	Ta20	12.58 ± 9.29 [a]	3.9 ± 2 [a]	10.70 ± 7.16 [a]
		F = 1.25; DF = 4, 93; P = 0.294	F = 0.26; DF = 4, 94; P = 0.0903	F = 3.18; DF = 4, 94; P = 0.017
30/20	Ta4	8.25 ± 6.08 [ab]	3.7 ± 2.5 [ab]	7.75 ± 4.78 [a]
	Ta10	4.10 ± 4.52 [b]	2.2 ± 1.7 [b]	4.58 ± 4.26 [a]
	Ta13	12.55 ± 9.13 [a]	4.3 ± 2.6 [a]	11.75 ± 6.73 [a]
	Ta19	6.05 ± 6.02 [b]	2.3 ± 1.7 [b]	5.60 ± 5.25 [a]
	Ta20	10.85 ± 9.02 [a]	2.8 ± 1.6 [ab]	10.95 ± 8.54 [a]
		F = 4.57; DF = 4, 95; P = 0.002	F = 3.81; DF = 4, 95; P = 0.0064	F = 5.27; DF = 4, 94; P = 0.0007

* Log transformed data were used for the mean values

** Arcsine transformed data were used for the mean proportion.

Data means ± □SE. Data in the same column followed by the same letter are not significantly different (P < 0.05 ANOVA; Student Newman Keuls procedures (SNK).

Table 6-5. Effect of different alternating temperatures on the development time, sex ratio, emergence rate and degree-days of five strains of *T. aurosum* (P < 0.05).

Temperature (°C)	Code	Development time*	Sex ratio **	Emergence rate**	Degree day
20/10	Ta4	19.7 ± 1 [b]	88.82 ± 19.14 [a]	30.03 ± 34.25 [b]	190.7 ± 10.1 [c]
	Ta10	22.1 ± 1.5 [a]	88.27 ± 21.55 [a]	80.66 ± 30.61 [a]	224.7 ± 15.6 [a]
	Ta13	22.5 ± 1[a]	78.11 ± 22.35 [a]	56.34 ± 33.12 [ab]	208.6 ± 10.1 [b]
	Ta19	22.2 ± 1 [a]	78.77 ± 24.02 [a]	68.00 ± 28.68 [a]	204.2 ± 9.7 [b]
	Ta20	22.3 ± 1.2 [a]	55.78 ± 20.51[b]	49.95 ± 24.89 [ab]	201.4 ± 11.2 [b]
		F = 18.77; DF = 4, 84; P = 0.0001	F = 10.61; DF = 4, 146; P = 0.0001	F = 5.4; DF = 4, 69; P = 0.0008	F = 21; DF = 4, 84; P = 0.0001
25/15	Ta4	13.5 ± 0.9 [a]	94.32 ± 15.3 [a]	40.50 ± 35.95 [a]	198.1 ± 13.9 [b]
	Ta10	14.1 ± 1.3 [a]	94.79 ± 14.55 [a]	28.34 ± 24.46 [a]	213.8 ± 18.9 [a]
	Ta13	14.4 ± 0.7 [a]	88.22 ± 19.48 [a]	35.48 ± 31.73 [a]	206.1 ± 10.4 [ab]
	Ta19	14.1 ± 1 [a]	87.53 ± 23.5 [a]	40.44 ± 25.90 [a]	199.5 ± 14.2 [b]
	Ta20	13.9 ± 1 [a]	80.76 ± 21.67 [a]	47.85 ± 22.76 [a]	194.9 ± 13.6 [b]
		F = 1.62; DF = 4, 81; P = 0.1768	F = 2.05; DF = 4, 123; P = 0.0913	F = 0.99; DF = 4, 68; P = 0.4175	F = 4.8; DF = 4, 81; P = 0.0015
30/20	Ta4	10.8 ± 0.4 [a]	73.19 ± 19.86 [b]	24.00 ± 26.29 [ab]	211.8 ± 8.8 [ab]
	Ta10	11.0 [a]	100 [a]	7.67 ± 21.29 [b]	221.8 ± 0.0 [a]
	Ta13	10.8 ± 0.4 [a]	70.32 ± 22.76 [b]	35.93 ± 38.04 [a]	208.1 ± 7.9 [ab]
	Ta19	10.6 ± 0.6 [a]	90 ± 27.33 [ab]	22.07 ± 21.37 [ab]	202.9 ± 12.4 [b]
	Ta20	11 ± 0.9 [a]	85.28 ± 20.70 [ab]	47.38 ± 34. 55 [a]	209.2 ± 17.5 [ab]
		F = 1.12; DF = 4, 73; P = 0.3552	F = 5.14; DF = 4, 79; P = 0.001	F = 4.07; DF = 4, 77; P = 0.0048	F = 2.3; DF = 4, 73; P = 0.063

* Log transformed data were used for the mean values

** Arcsine transformed data were used for the mean proportion.

Data means ± □SE. The data in the same column followed by the same letter are not significantly different (P < 0.05 ANOVA. Student Newman Keuls procedures (SNK)).

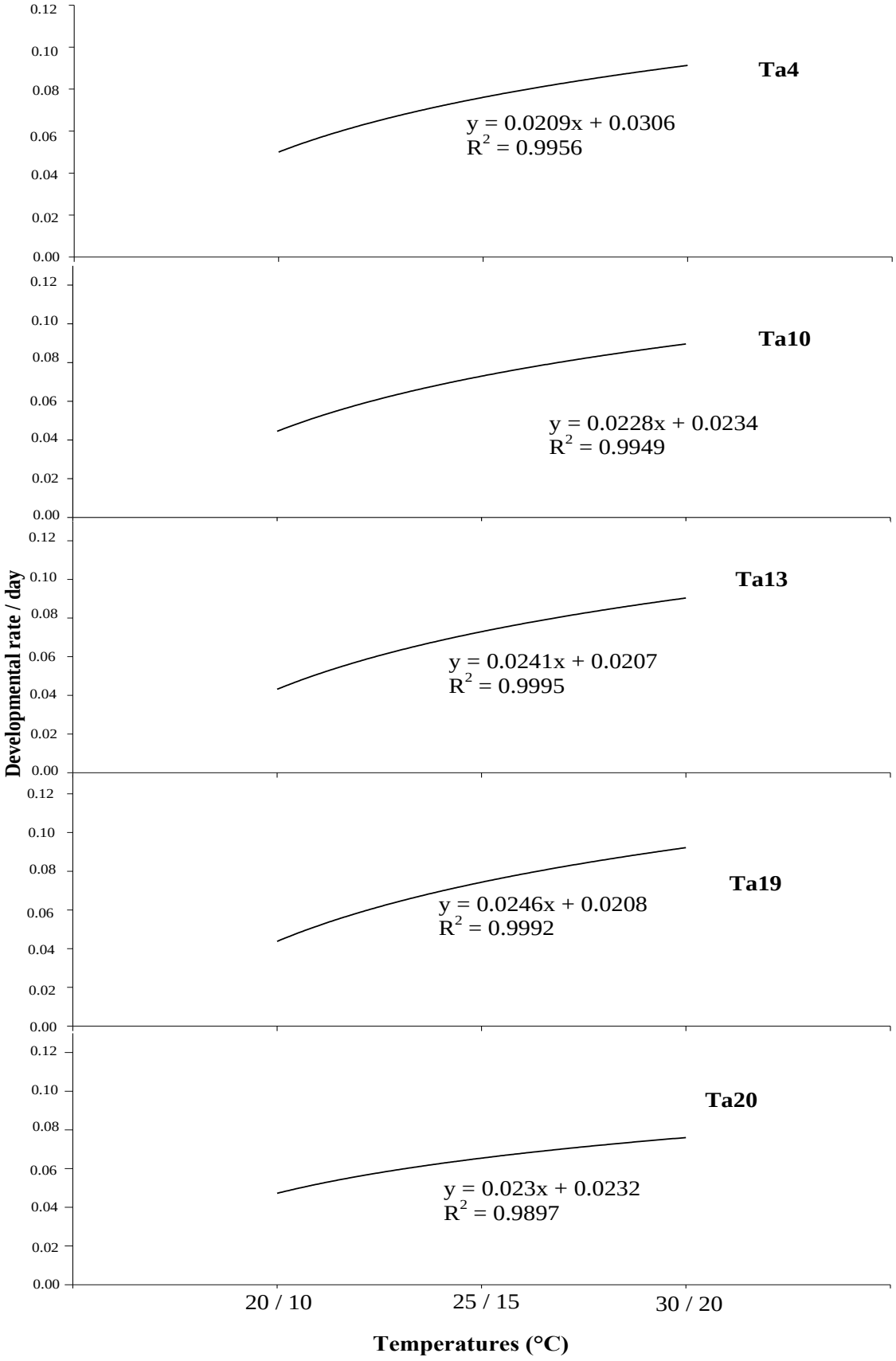

Figure 6-4. Effect of alternating temperature on the developmental rate per day of the different strains of *T. aurosum.*

Table 6-6. Life table parameters of *Trichogramma aurosum* on *Cydia pomonella* at 20/10, 25/15 and 30/20 °C. R_0 = net reproductive rate. r_m = intrinsic rate of increase. T_c = cohort generation time (days). λ = finite capacity for increase. D_t = doubling time (days).

Strain	R_0	r_m	T_c	λ	D_t
			20 / 10 °C		
Ta4	2.15 ± 1.1 ^{bc*}	0.040 ± 0.03 ^{bc}	19.24 ± 1.03 ^{c}	1.04 ± 0.03 ^{b}	17.45 ± 12.39 ^{a}
Ta10	6.84 ± 2.8 ^{a}	0.088 ±0.02 ^{a}	21.71 ± 2.26 ^{b}	1.09 ± 0.02 ^{a}	7.83 ± 1.59 ^{a}
Ta13	6.38 ± 1.9 ^{a}	0.075 ± 0.01 ^{a}	24.72 ± 1.44 ^{a}	1.08 ±0.01 ^{ab}	9.24 ± 1.55 ^{a}
Ta19	4.74 ± 2.9 ^{ab}	0.069 ± 0.03 ^{ab}	22.64 ± 2.72 ^{ab}	1.07 ± 0.03 ^{ab}	10.08 ± 3.75 ^{a}
Ta20	1.53 ± 0.9 ^{c}	0.020 ± 0.03 ^{c}	20.92 ± 1.19 ^{bc}	1.02 ± 0.03 ^{b}	33.97 ± 59.39 ^{a}
			25 / 15 °C		
Ta4	3.15 ± 1.8 ^{ab}	0.08 ± 0.04 ^{ab}	14.13 ± 1.48 ^{a}	1.08 ± 0.04 ^{ab}	8.53 ± 4.27 ^{a}
Ta10	2.37 ± 1.0 ^{b}	0.064 ± 0.03 ^{b}	13.42 ± 0.96 ^{a}	1.067± 0.04^{b}	10.79 ± 5.93 ^{a}
Ta13	3.91 ± 1.8 ^{ab}	0.095 ± 0.03 ^{ab}	14.42 ± 1.64 ^{a}	1.099 ± 0.03 ^{ab}	7.33 ± 2.21 ^{a}
Ta19	3.43 ± 1.4 ^{ab}	0.092 ± 0.03 ^{ab}	13.44 ± 0.88 ^{a}	1.096 ± 0.03 ^{ab}	7.55 ± 2.42 ^{a}
Ta20	5.02 ± 2.1 ^{a}	0.124 ± 0.03 ^{a}	13.07 ± 0.79 ^{a}	1.13 ± 0.03 ^{a}	5.611 ± 3.34 ^{a}
			30 / 20 °C		
Ta4	1.75 ± 0.6 ^{b}	0.05 ± 0.03 ^{b}	10.32 ± 0.32 ^{b}	1.056 ± 0.03 ^{b}	12.81 ± 7.49 ^{a}
Ta10	0.35 ± 0.2 ^{c}	-0.07 ± 0.10 ^{c}	14.95 ± 0.48 ^{a}	0.93 ± 0.03 ^{c}	-9.84 ± 4.41 ^{b}
Ta13	3.78 ± 1.1 ^{a}	0.122 ± 0.03 ^{a}	10.89 ± 0.60 ^{b}	1.13 ± 0.03 ^{a}	5.68 ± 1.18 ^{a}
Ta19	1.21 ± 0.6 ^{b}	0.02 ± 0.049 ^{b}	9.58 ± 0.29 ^{c}	1.020 ± 0.05 ^{b}	34.60 ± 16.58^{ab}
Ta20	4.99 ±2.0 ^{a}	0.15 ± 0.033 ^{a}	10.68 ± 1.50 ^{b}	1.16 ± 0.04 ^{a}	4.60 ± 1.03 ^{a}

* Data means ± □SE. The data in same column followed by the same letter means no significant difference ($P < 0.05$ ANOVA. Student Newman Keuls (SNK) test (ANOVA. SAS Institute. 1996)

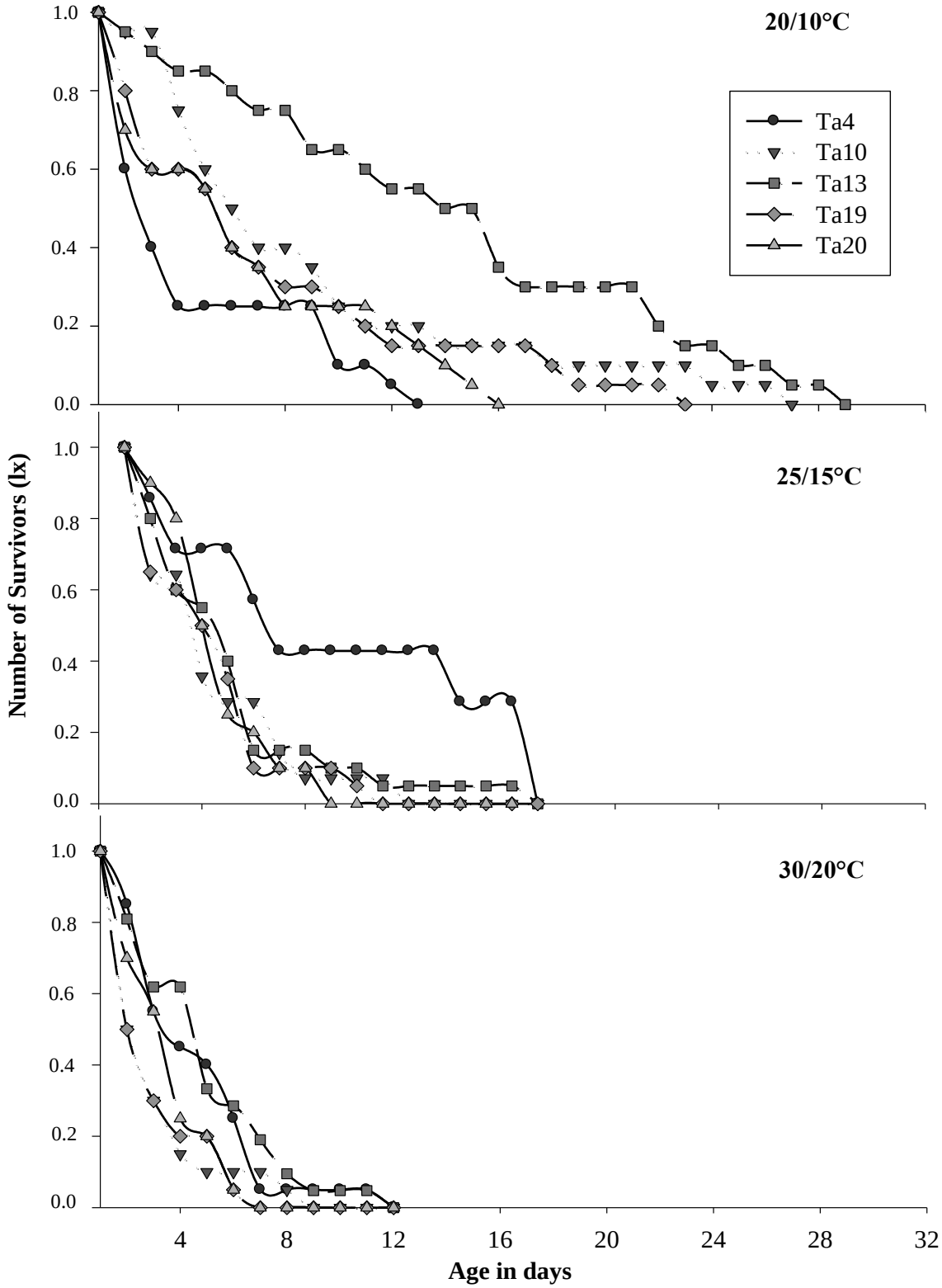

Figure 6-5. Survivorship in five strains of *T. aurosum* at three alternating temperatures

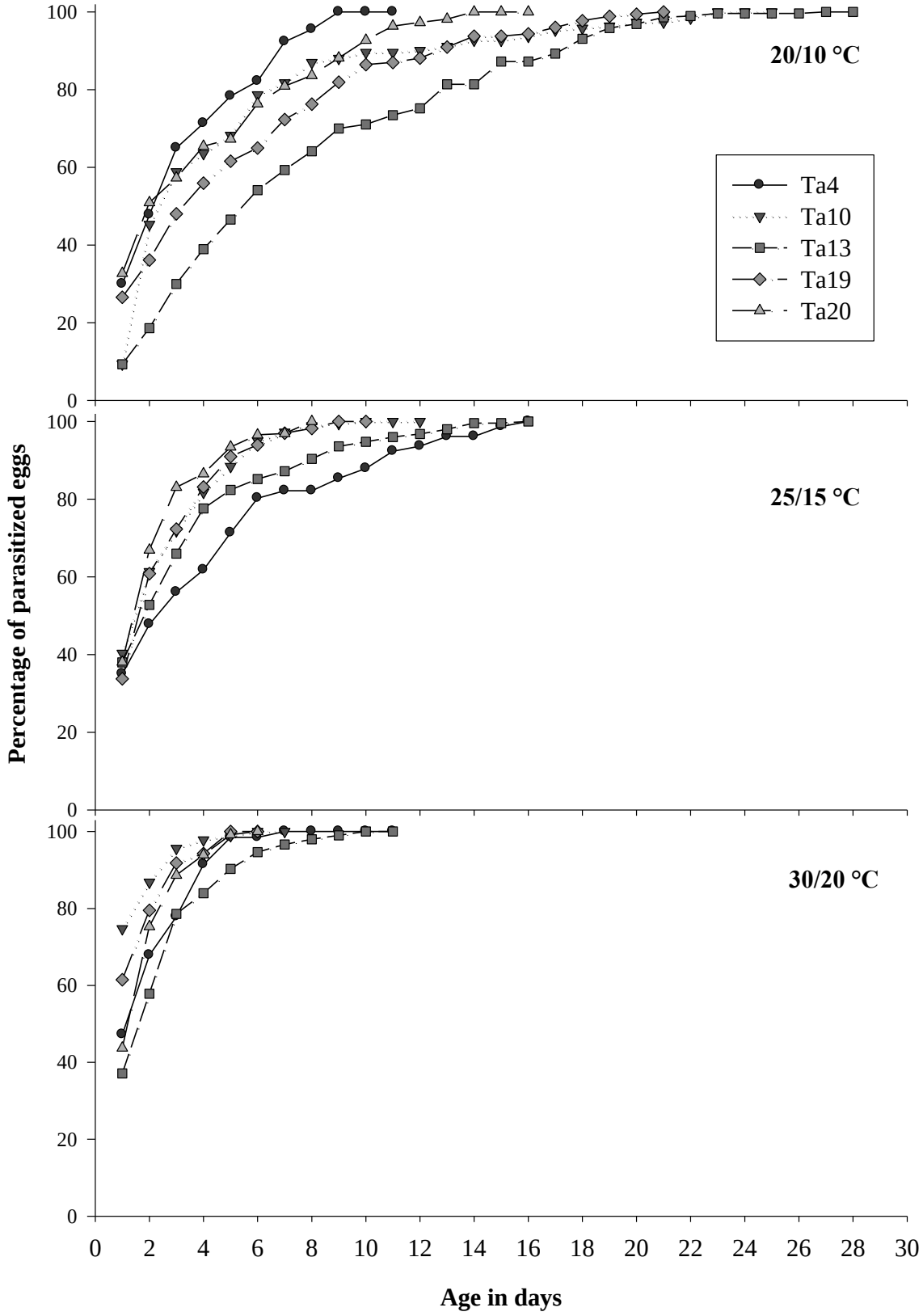

Figure 6-6. Relative cumulative parasitized eggs per day of five German strains at three alternating temperatures

6.5 DISCUSSION

Temperature has a major influence on both activity and metabolic processes in poikilotherm organisms such as insects (Suverkropp et al. 2001). As for *Trichogramma* spp. and their use for biological control, candidate species should be adapted to adverse abiotic conditions (Pak 1988). This is important for two reasons: Firstly, the parasitoids are normally distributed in the field as immature stages (Knutson 1998) and hence, they must be able to successfully develop even under unfavourable conditions. Secondly, it is necessary that emerged adults search for hosts and parasitize them independent of whether abiotic conditions are extreme or not. Therefore temperature is one of the most important abiotic factor affecting the developmental rate, cumulative fertility, longevity, sexual ratio and emergence rate of *Trichogramma* spp.

Tolerance of immature stages of *Trichogramma* species / strains to high or low temperature extremes has been subject of several studies, e.g. for *T. cordubensis* Vargas and Cabello (Garcia and Tavares 1994), *T. turkestanica* Meyer (Hansen 2000; also as *T. evanescens* Westwood by Schöller and Hassan 2001), *T. maidis* Pintureau and Voegelé (= *T. brassicae*), *T. pretiosum* Riley, and *T. semblidis* Aurivillius (Pak 1988), *T. cacoeciae* Marchal, *T. dendrolimi* Matsumura, and *T. principium* Sugonjaev and Sorokina (Sakr 2003), *T. confusum* Viggiani (= *T. chilonis* Ishii, Nagarkatti and Nagaraja 1978), and *T. minutum* Riley (Smith and Hubbes 1986). But most of the published work was conducted only on constant temperatures, which may be useful for laboratory rearing and commercial mass production. However, we have found that the response of the *T. aurosum* strains differed, when they were reared under alternating or constant temperatures. These behavioural variations could be related to the reaction of the wasps to the lower and upper temperatures involved in the thermic cycle (Beck 1983). Barfield et al. (1977) found that alternating temperature increases the fecundity of *Bracon mellitor*. Therefore, the assessment of insect biology at constant laboratory temperatures could be unrealistic.

No significant differences were observed in longevity and development time at some temperatures tested, where as the duration of development and longevity decreased as temperature increased. The presence of honey increased longevity at all temperatures (Hawkins and Smith 1986). *Trichogramma aurosum* is able to live only for 1-2 days at 25 °C without a food source (unpublished data). Cumulative fertility and longevity at alternating temperatures was reduced when compared with constant temperatures. This reduction could be due to the long rearing of the wasps under constant temperature (the wasps used in these experiments were reared for at least 40 generation under laboratory conditions). It is not well known whether the long laboratory rearing affect the wasps activity and vigour. According to Nagarkatti and Nagaraja (1978) female fertility of *T. confusum* wasps reared for long time under laboratory conditions was significantly lower than the wild females. However, female longevity is affected by many factors such as temperature (Pak and Oatman 1982), humidity (Stinner et al. 1974), host size (Stinner et al. 1974), and food (Yu et al 1984). Almatni (2003)

found that the high temperature 30/20 °C could have caused sterilization of European strains *T. cacoeciae,* because they stopped laying eggs but lived for few days more without laying new eggs. Also a reduction of female longevity of *T. platneri* was recorded from 53 day at 10 °C and 3 days at 35 °C (McDougall and Mills 1997).

The highest parasitization rate for the strains studied was at intermediate temperatures (20 and 25 °C). These results agree with Pak (1988), who found that number of parasitized hosts increased with increasing temperature to a maximum at 20-25 °C and declined at 30 °C. Some strains examined in this study showed better tolerance to high temperatures and others were able to tolerate low temperature. The strains Ta4, Ta19 and Ta20 were able to live up to 6-11 days longer than the rest of the strains at 15 °C, while Ta13 were able to live up to 1-2 days longer than the rest of the strains at 30 °C.

Relative cumulative parasitized eggs per day was temperature dependent, females reached 100% parasitization at the high temperature regimes in short time after hatching. On the contrary, females held under low temperatures reached 100% parasitization after longer period of time. The short period of parasitism can be considered as a specific survival strategy, because a faster oviposition at higher temperatures will allow this pro-ovogenic parasitoid to lay most of its available eggs in a short lifetime period (Garcia and Tavares 1994).

Garcia and Tavares (1994) found significant differences between all temperatures for *T. cordubensis* longevity, which increased with the decrease of temperature. These results are similar to our results. However, it was noticed that the decrease in temperature increased the pre-oviposition period for all the studied strains. At 15 °C the average pre-oviposition period was 3, 5, 2, 1.5 and 2 days for Ta4, Ta10, Ta13, Ta19 and Ta20, respectively. Mean while the pre-oviposition period on the higher temperature was 0 day, where the wasps activity at low temperature was reduced. According to Al-Ahmed and Kheir (2003), temperature is considered an important factor affecting the duration of pre-oviposition, were high temperature increases the metabolic rate, which in turn increases the rate of physiological processes involved in egg production resulting in a decrease of the pre-oviposition period.

Realised cumulative fertility was calculated according to Mills and Kuhlmann (2000). The realised cumulative fertility was the highest at the high temperature regime. This parameter was reduced at the lower temperature regime. The total cumulative fertility values did not differ from the values of the realised cumulative fertility at high constant and alternating temperatures. But both values were found to differ when *T. aurosum* strains were reared at 15 °C and 20/10 °C. Similar results were reported for *T. minutum* (Smith and Hubbes 1986), and *Trichogramma* spp. (Pak and van Heiningen 1985). According to Jervis and Copland (1996), there is an optimal range of temperature for insect development. Beyond this range they would be unable to continue oogenesis and laying eggs or unable to function appropriately for long period of time. This could be due to the increase of respiration rate, i.e. the insects would be unable to produce fertile eggs due to the high consumption of energy (Mills 1981).

Data recorded for sex ratio from the present experiments agree with some data from the literature but disagree with other. Pintureau and Bolland (2001) found that the percentage of males in the offspring of virgin females increased faster according to temperature in *T. cordubensis* than in *T. pretiosum*. Sex ratio at low and intermediate alternating temperatures was higher than at constant temperatures, while at high alternating temperatures it was reduced. These results agree with Bowen and Stern (1966). In general, sex ratio was not affected with increase of temperature, it ranged from 65 – 100%. Our finding agrees with Haile et al. (2002), who found that sex ratio was biased to female production at all temperatures. It seems that rearing the wasps at lower temperature have a significant effect on the biological characteristics. Crozier (1977) suggested that lower temperatures could promote fusion of nuclei and increase the proportion of diploid offspring, this can explain the high sex ratio at 15 °C in all strains studies.

Developmental time needed by the *T. aurosum* strains was shorter at alternating temperatures compared with constant temperatures. These results agree with Consoli and Parra (1995), who assumed that this might be due to more appropriate metabolic process of the immature stages at alternating temperatures. The optimal temperature for development and survival was 25 °C (Figure 6-1 and 6-3) and 25/15 °C (Figure 6-4 and 6-5), where 35 °C was assumed as upper vital threshold (McDougall and Mills 1997, Pak 1988). Total developmental time was four times faster at 30 °C than at 15 °C, and two times faster at 30/20 °C than at 20/10 °C. But the total mortality was greater at the higher temperature; these results are similar to the findings of Hawkins and Smith (1986). Mean development time at constant temperature ranged between 32.2 and 8.8 days at temperatures between 15 °C and 30 °C. Although development occurred at 10.8 °C (noticed from the parasitized eggs turning black and development takes place up to the third larval stage), no emergence was observed during six months incubation (unpublished data). For all the temperatures tested, a linear regression model was calculated for egg-adult development of all *Trichogramma* strains. As temperature increased, the duration of development decreased. Mean development time of females *T. turkestanica* on *E. kuehniella* were 32.9, 18.2, 9.1, and 7.0 days at 15, 20, 25, and 30 °C, respectively (Pintureau and Bolland 2001; Hansen 2000).

Degree-days at alternating temperature were 1.5 times higher than at constant temperatures. The results imply that rearing parasitoids at constant temperatures may overestimate their efficiency in biological control under field conditions, as the results showed that parasitism was higher at constant temperatures compared to alternating temperatures. It can not be excluded that an adaptation has occurred, as the *T. aurosum* strains were reared in the laboratory under constant temperature.

Survivorship curves according to Southwood (1978) obtained from our results at different constant and alternating temperatures for the strains studied differed remarkably. At low constant temperature (15 °C) all strains had the type I, where the mortality acts most heavily on the old females. At low alternating temperature regime (20/10 °C), Ta13 had the type II (a straight line when the l_x is arithmetic), i.e. a constant number of individuals died per

unit of time. Ta10, Ta19 and Ta20 had the type III (the logarithmic curve), where the mortality rate is constant, while Ta4 had the type IV, in which the mortality is stronger in young individuals. At 20 °C, all strains had the curve type II (a straight line when the l_x is arithmetic) a constant number of individual die per unit of time. At the intermediate alternating temperature (25/15 °C), Ta10, Ta13, Ta19 and Ta20 the curve type III, whereas Ta4 had type II. At 25 °C strains have the curve type III; the survivor rate is logarithmic where the mortality rate is constant. Finally at high alternating temperature regime (30/20 °C) and at 30 °C, all strains had the curve type IV (Figure 6-3 and 6-5).

The Ta13 strain showed a better adaptation to all temperatures studied, Ta4 and Ta20 also showed promising results in response to rearing temperature. The relationship between the geographical origins of the strains and their climatic adaptability was evaluated. We found that Ta13 which was collected form southeast Germany; where the average temperature form June-September is 15-19 °C, were able to tolerate the high constant temperature (30 °C) and all alternating temperature regimes studied. While Ta4, Ta19 and Ta20 were able to tolerate intermediate and low temperature; where the average temperature in the original locations ranged between 13-16, 14-17 and 14-18 °C, respectively (Table 6-7).

Table 6-7. Mean climatic temperatures at the collected origins during the growing season between the year 2002 and 2003.

Strain/location	May	June	July	August	September
Ta4 / Baden-Wuerttemberg	10	15	17	15	10
Ta10 / Hessian	15	18	20	19	15
Ta13 / Bavaria	13	17	19	18	15
Ta19 / Lower Saxony	13	16	17	17	14
Ta20 / Berlin	14	17	18	18	14

* Source: http://www.klimadiagramme.de/ (last update date was September 2004)

Emergence rate reached the highest values at intermediate temperatures in all strains studied. It was found to be more than 89% for *T. annulata* and *T. pretiosum* (Maceda et al. 2003). The reduction in the emergence rate and the long time required for the immature stages to develop from egg to adult was recorded at the low temperature 15 °C for all strains studied. This can be due to the high mortality in the immature stages. Smith and Hubbes (1986) noticed that the emergence rate was reduced when the parasitized eggs were reared for 24 days at 15 °C.

Fertility life table studies provide a powerful technique for evaluation of population dynamics because they provide a detailed description of age specific mortality of individuals in the population. Pratissoli and Parra (2000) found out that the net reproduction rate varied according to the temperature variation for *T. pretiosum*. It was the highest at 20 °C and 20/10 °C for all the *T. aurosum* strains studied. The net reproductive rate recorded for *T. mwanzai* at the constant temperatures 20, 25 and 30 °C, was 7.3, 35.9, and 31.9 (Lu 1992), and for *T. cacoeciae* at the same temperatures 48.69, 45.83 and 24.02, respectively (Uzun and Akten 1992). The finite increase rate was proportionally related to temperature. For *T. pretiosum* the relation between λ and temperature increase occurred for the range from 18 to 30 °C (Pratissoli and Parra 2000), and for *Trichogrammatoidea* sp. it was the highest at 27 °C (Baitha and Ram 1998) and in our results it was at 30 °C.

The mean cohort generation time (T_c) values show a decreasing trend from 18 to 30 °C (Baitha and Ram 1998). This agrees with our findings, where the generation time values decreased as the temperature increased. Pak and van Heiningen (1985) assumed that the higher temperatures might lead to an increased flight activity with a resulting faster dispersion. According to the differences between the strains among each other was very obvious, some strains showed good adaptability to high temperature (Ta13), where others showed good adaptability to intermediate temperatures (Ta4, Ta10 and Ta19), where others showed adaptability on low temperature (Ta20).

Cabello and Vargas (1988) related the reduction of the net reproductive rate at high temperatures to the production of both males and females at these temperatures. This can explain our results, where the R_0 was reduced at 30 °C and at 30/20 °C. Accordingly, values of daily intrinsic rate of increase (r_m) and finite rate of increase (exp. r_m) were affected. Development of each insect stage is dependent on alternating temperatures. Insect activity such as locomotion, searching behaviour is dependent on the day temperature, but night temperature has no effect on these activities at alternating temperature. On the other hand, high temperature at night increase the metabolic activity of the immature stages. Insect reared on alternating temperatures was due to higher food utilization and assimilation has a reduction in developmental time as a result of a suitable metabolic process (Consoli and Parra 1995). Field temperature always alternates between day and night, except in the coastal and temperate regions. In contrast in deserts and mountains the difference between the day and nigh temperature is very high. Therefore, studying *Trichogramma* parasitic potential and biological characteristic under constant temperatures is not accurate, because parasitic wasps would consume more energy at night than under natural field conditions. However, rearing the wasps under constant temperatures could be useful for laboratory rearing and commercial mass production.

This study can provide us with information to select one or several strains as a suitable candidate for biological control of the Codling moth according to their climatic adaptability. It is possible to select either those strains that showed high parasitization rate, higher longevity and higher tolerance to high temperatures, or those strains that showed high parasitization

activity at the low temperature conditions. However, further studies on host location and searching, parasitic behaviour and female dispersal are also required before the field release experiments.

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CHAPTER 7

7. PHYLOGENETIC STUDIES

7.1 ABSTRACT

Taxonomy and phylogeny of *Trichogramma* is often critical due to 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. The ITS2 (internally transcribed spacer 2) region of the rDNA was used to assess the identity strains of *Trichogramma aurosum* Sugonjaev & Sorokina collected on eggs of *Nematus tibialis* Newman (Hymenoptera: Tenthredinidae) from different locations in Middle Europe. Amplified products were identical in length (ca. 500 bp), sequences showed a high percent similarity (> 96%), and no cryptic species could be detected in the samples. Parsimony analysis of ITS2 sequences rendered *T. aurosum* together with *T. alpha* Pinto and *T. sibericum* Sorokina. All three species belong to the *exiguum*-section and have been recovered from eggs of hymenopteran hosts. This suggests that they might have evolved from a single ancestor. Relationships with other species of the *exiguum*-section are presented and discussed. In addition, AFLP (Amplified Fragment Length Polymorphisms) analysis was conducted with 230 female wasps of *T. aurosum* strains collected from 23 different locations. 180 AFLP fragments could be detected of which 170 (98.3%) were polymorphic in more than one individual, with a mean number of fragments per individual of 37. Fragment size ranged from 43 to 398 bp with an average of 337.12 ± 122.55 bp. An analysis of genetic relatedness based on the obtained AFLP markers revealed the existence of very low heterozygosity and very low gene flow between populations of *T. aurosum*.

KEY WORDS: *Trichogramma aurosum*, ITS2 region, biological control, rDNA, parasitoids, AFLP.

7.2 INTRODUCTION

Trichogramma spp. are minute parasitoid wasps (< 1 mm in length) that parasitize eggs of several insect orders, especially members of the Lepidoptera. Correct identification of *Trichogramma* spp. is crucial for successful biological control programmes (Stouthamer et al. 1999, Smith and Hubbes 1986a). 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. However, a large number of species show remarkable morphological homogeneity and, on the top of it, thelytokous populations/species present difficulties for taxonomy based on male characters (Querino and Zucchi 2002, Stouthamer et al. 1999).

Electrophoretic markers are powerful molecular tools that facilitate the study of diverse areas of biology, taxonomy, answering questions about phenology, evaluation ecology and population dynamics (Loxdale and Lushai 1998). Few studies have so far investigated the molecular genetics of *Trichogramma* spp. (Laurent et al. 1998, Stouthamer et al. 1999, Smith and Hubbes 1986a). Since *Trichogramma* individuals are minute in size compared with other insects, little amount of genomic DNA is obtained, which make investigations with common molecular techniques difficult, e.g. RFLP. 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 strains for mass production and subsequent field release.

The Ribosomal DNA (rDNA). Ribosomes consist of ribosomal DNA (rDNA) plus proteins. They are divided into two subunits, a large subunit (23-28S), a small subunit (16-18S) and the 5.8S nuclear rDNA (Fig 7-1). In eukaryotes, there are 100 to 500 copies of rDNA in the nuclear genome in repeated transcription units. These repeated transcription unit are composed of a leader promoter region known as the **E**xternal **T**ranscribed **S**pacer (ETS), an 18S rDNA coding region, an **I**nternal noncoding **T**ranscribed **S**pacer region (ITS), a 28S rRNA coding region, and an **I**nter **G**enic nontranscribed **S**pacer segment (IGS). The gene 5.8S rDNA is embedded in the internal transcribed spacer (ITS) region. ITS2 sequences have gained increasing importance in identification of cryptic *Trichogramma* species, in studying differentiation within populations as well as for the reconstruction of phylogenetic relationships between closely related species (Ciociola et al. 2001, Pinto et al. 2002, Stouthamer et al. 2000). The DNA sequence of the internal transcribed spacer regions (ITS1 and ITS2) have been used at species and intraspecific levels in many organisms groups (Hoy 1994). ITS2 sequences have been shown to be a suitable tool to separate closely related species, such as *T. deion, T. kaykai, T. sathon, T. pratti, T. pretiosum, T. interius, T. oleae* (Stouthamer et al. 1999).

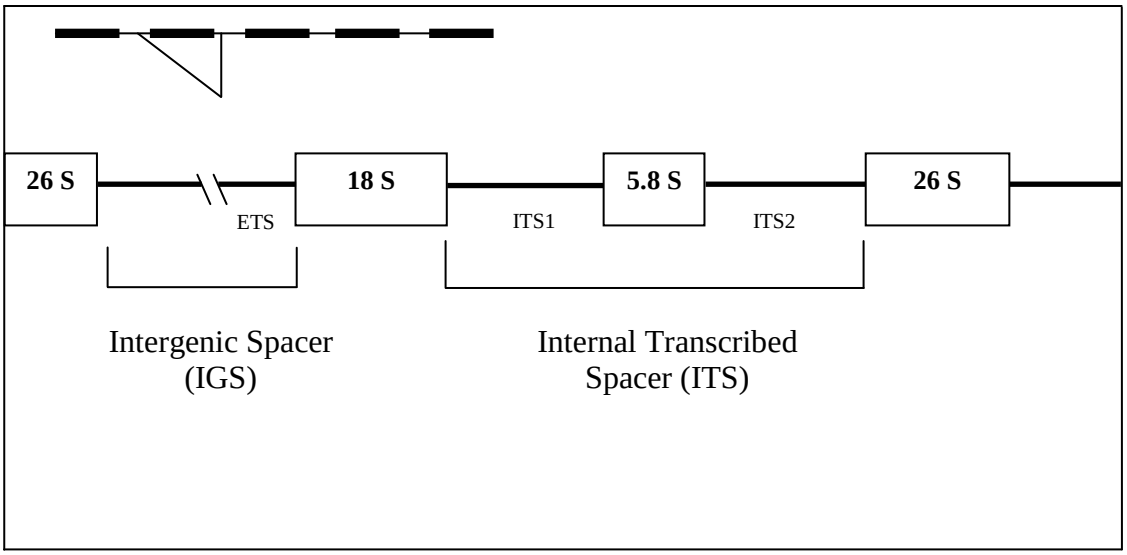

Figure 7-1. Structure of a repeat unit within the nuclear rDNA region. Each repeat consists of External Transcribed Spacer (ETS), followed by the small ribosomal subunit 18S, then the first Internal Transcribed Spacer (ITS1), 5.8S, the second Internal Transcribed Spacer (ITS2), then the large subunit 26S (Hoy 1994)

Differences within populations

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 frequent 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. A fingerprinting technique called AFLP (**A**mplified **F**ragment **L**ength **P**olymorphisms) 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, it requires only low amount of genomic DNA (Reineke et al. 1998), can be used with stored, dry or old samples (e.g. museum samples) (Loxdale and Lushai 1998), and it has a good reproducibility. However, it requires a complete digestion of intact, high-molecular weight DNA. AFLP technology is a random amplification technique, which in contrast to most other random amplification techniques makes use of stringent PCR conditions. The amplification primers are generally 17 - 21 nucleotides in length and anneal perfectly to their target sequences.

The ITS2 region has been shown to be useful in the identification of closely related species, subspecies or populations. Hence, this work aimed at differentiating the collected *Trichogramma* strains by amplifying and sequencing the ITS2 region to measure the homology between the populations from different geographical origin. On the other hand, the AFLP technique was applied to measure the intraspecific variability among 30 strains of *T.*

aurosum collected from 30 localities in Germany and in other six countries from Europe. Analysis of the ITS2 allows distinguishing closely related species or subspecies that may be difficult or impossible to differentiate on the basis of morphological or phenotypic characteristics. This study should contribute to provide additional information about the genetic variation between local and regional populations.

7.3 MATERIALS AND METHODS

Field trips and collection from the field were done during summer 2002 and 2003 by collecting parasitized (blackened) eggs of *Nematus tibialis* on leaves of Robinia trees in several locations in the German Federal Republic and its neighbouring countries (Table 2-1 chapter 2). The collected strains of *T. aurosum* were maintained for the laboratory experiments on eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), in a climatic cabinet at ca 25 °C, 85% RH and 18:6 h L:D photoperiod. Then the wasps were collected and stored in 100% ethanol for the genetic studies.

DNA extraction and ITS2

DNA was extracted from single males of each location following a modified cetyltrimethyl-ammonium bromide (CTAB) protocol with an additional polyethylene glycol precipitation (Reineke et al. 1998). Briefly, males were placed in 100 µl of TES lysis buffer (100 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2% SDS) and allowed to sink. The samples were kept for 1 h at room temperature and then manually crushed in a 1.5 ml microfuge tube using a Teflon-coated steel rod. The rods were washed and sterilized between each use. Proteinase K (20 mg/ml) was added and the solution was incubated for 1 h at 58 ºC with occasional gentle mixing. The salt concentration was adjusted to 1.4 *M* with 5 *M* NaCl, 1/10 volume of 10% CTAB (Sigma, St. Louis, MO) was added, and then the samples were incubated for 10 minutes at 65 ºC. After adding an equal volume of chloroform to isoamylalchol ratio of 24:1, the tubes were gently mixed, incubated for 30 minutes on ice and centrifuged for 20 minutes at 4 ºC and 12,000 rpm. The supernatant was then transferred to a new tube and 45 µl of 5 *M* ammonium acetate was added. The tubes were then mixed gently and placed on ice for 30 minutes. After centrifugation for 20 minutes at 4 ºC and 12,000 rpm, the upper phase was transferred to a new tube and 0.25 volume of 30% polyethylene glycol 6000 (Sigma, St. Louis, MO) was added. The samples were incubated on ice for 1 h and centrifuged for 20 min at 4 ºC and 12,000 rpm to precipitate the DNA. The supernatant was discarded and the DNA pellet was washed twice with cold (4 ºC) 70% ethanol, dried and re-suspended in 100 µl TE (100 mM Tris-HCl, 1 mM EDTA, pH 8.0) over night. PCR of the internal transcribed spacer 2 region (ITS2) of the ribosomal DNA (rDNA) was performed in 25 µl reaction mixtures containing 1 µl DNA template, 2.5 µl dNTP's (each in a 10 mM concentration), 2 µl $MgCl_2$ (in a 25 mM concentration), 0.5 µl BSA, 0.21 µl Taq DNA polymerase (0.625 U) (MBI

Fermentas, Vilnius, Lithuania), 1 µl of each primer (0.2 mM) in 2.5 µl (10x) PCR-buffer and 16 µl sterile water.

The specific forward 5'TGTGAACTGCAGGACACATG-3' and reverse 5' GTCTTGCCTGCTCTGAG-3' primers (Stouthamer et al. 1999) were used to amplify the ITS-2 region of rDNA. A PTC-100 thermocycler (Watertown, MA) was programmed for PCR as follows: initial denaturation at 94 ºC for 2 minutes followed by 35 cycles of 1 min at 94 ºC, 1 min at 53 ºC and 2 min at 72 ºC, with 1 min at 72 ºC after the last cycle. After amplification, 2 µl of a loading dye was added and 5 µl of the total mixture was applied to a 1.2% agarose gel in TBE-buffer. A 100 bp (base pairs) DNA ladder was used as a size marker. After the electrophoresis run at 50 V for 120 minutes, the gel was stained with ethidium bromide (0.5 µg/ml) and DNA was visualised in ultraviolet (UV) light. Negative controls lacking template DNA were included in all experiments.

PCR products were purified with QIAquick® purification kit (QIAGENE) following the recommendations of the manufacturer, and sent for sequencing (MWG The genomic Company, Ebersberg). Sequences were aligned with MEGALIGN (DNAStar Inc.) and phylogenetic parsimony analysis employed PAUP 4.0B10 (Swofford. 2002). *Trichogramma* (*Trichogrammaza*) carverae (GenBank accession number AY162998) was used as outgroup. Parsimony analysis used the branch and bound algorithm, with gaps treated as missing data. Initial parsimony analysis on unweighted characters was followed by successive approximations character weighting (Farris 1989).

AFLP

AFLP analysis was used to analyse the population genetic structure, genetic diversity and gene flow of *T. aurosum* strains. AFLP has been demonstrated to be a powerful method for the characterization of intraspecific polymorphisms among populations because of its high reproducibility (Vos et al. 1995). AFLP fingerprinting of complex genomes generally involve amplification in two steps. The first step is named pre-amplification, where all fragments are amplified at moderate stringency for 20 cycles, and the second step is the actual amplification reaction.

AFLP analysis was performed using a modified protocol of Vos et al. (1995). Adaptor and primer sequence used in this study are shown in Table 7-1. 200 ng of genomic DNA was digested with 1 U *Tru*1I and double stranded adaptors were ligated to the restriction fragments. This was followed by non-selective amplification of DNA fragments using primers with zero base pair extensions. Selective amplifications were performed on the pre-amplified fragments with primers each having two or three selective nucleotides (Reineke et al. 1999). For the selective amplification, pre-amplified products were used as a template: They were amplified with Cy5- labelled primers (Reineke and Karlovsky 2000). Amplification products were loaded on 5% denaturing polyacrylamide gel and separated for 1.5 h at 42 V/cm. As a size marker, a sequencing reaction of a 200 bp fragment cloned into pUC18 was run at regular

intervals on the same gel. After electrophoresis, the gel was dried and exposed to a phospho-imaging screen for approximately 18h, which was subsequently scanned using a bio – image analyser (BAS- 1000, Fuji photo Film, Kanaagua, Japan).

Table 7-1. List of AFLP primers and their sequences used in the present study:

Primer	**Code**	**Sequence 5' – 3'**
EcoRI adaptor		CTCGTAGACTGCGTACC
		CATCTGACGCATGGTTAA
MseI adaptor		GACGATGAGTCCTGAG
		TACTCAGGACTCAT
Eco RI primer		
Eco 14	E14	GACTGCGTACCAATTC**AAG***
Eco 15	E15	GACTGCGTACCAATTC**ACA***
Eco 16	E16	GACTGCGTACCAATTC**ACC***
Mse - primer		
Mse10	M10	GATGAGTCCTGAGTAA**CA**
Mse22	M22	GATGAGTCCTGAGTAA**T**
Mse23	M23	GATGAGTCCTGAGTAA**TGT**
Mse28	M28	GATGAGTCCTGAGTAA**GCT**
Mse33	M33	GATGAGTCCTGAGTAA**TAG**
Mse34	M34	GATGAGTCCTGAGTAA**GGA**

* Cy-5 fluorescently labelled primer

Briefly to 200 ng of genomic DNA, 10 U of *Eco*RI and 4 U of *Tru*1l (*Mse*I) (MBI Fermentas, St.Leon-Rot, Germany) were added and incubated at 37 °C for 90 min followed by incubation at 65 °C for 90 min. For adaptor ligation, *Eco*RI and *Mse*I double-stranded adaptors were prepared by mixing 2 pmol each of the oligonucleotides 5' CTCGTAGACTGCGTACC 3', 5' AATTGGTACGCAGTCTAC 3' (for *Eco*RI adaptor), 5' GACGATGAGTCCTGAG 3' and 5' TACTCAGGACTCAT 3' (for *Mse*I adaptor). The double-stranded adaptors were then ligated to the restriction fragments of the genomic DNA by incubating them with 4 U of T4 Ligase (MBI Fermentas) at 20 °C for 2 h. The ligase was inactivated after incubation by heating at 65 °C for 10 min.

Ligation of adaptors was followed by non-selective amplification (pre-amplification) of DNA fragments using primers with zero base pair extensions (*Eco*RI primer 5' GACTGCGTACCAATT 3', *Mse*I primer 5' GATGAGTCCTGAGTAA 3'). The pre-amplification reaction was conducted in a thermal cycler with 20 cycles of 30 s at 94 °C, 60 s at 56 °C and 60 s at 72 °C.

Selective amplifications were performed on the pre-amplified fragments with two different *Eco*RI primers each having three selective nucleotides at its 3'-end and labelled with

Cy5 (*Eco*RI-AAG, *Eco*RI-ACC), and with different *Mse*I primers each having one to two selective nucleotides at its 3'-end (*Mse*I-GAG, *Mse*I-CTC). The amplification reaction was conducted in a thermal cycler with the following thermal program: a first cycle of 30 s at 94 °C, 30 s at 65 °C and 60 s at 72 °C, repeated for 12 cycles with a stepwise decrease of the annealing temperature in each subsequent cycle by 0.7 °C, followed by 23 cycles of 30 s at 94 °C, 60 s at 56 °C and 60 s at 72 °C.

The amplified samples were diluted with 10 µl formamide dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylenecyanol FF, pH 8.0). Before electrophoresis, the samples were denatured for 3-5 min at 80 °C and 4 µl aliquots of the PCR products were loaded on a ReproGel Long Read (Amersham Biosciences, Freiburg, Germany) and analyzed on an ALFexpress II DNA Analysis System (Amersham Biosciences). As a size marker, ALFexpress sizer 50-500 (Amersham Biosciences) was run in the first and last lanes in the gels.

DATA ANALYSIS

AFLP digital images patterns were analysed using Gel Compare 4.0 software (Applied Maths, Belgium). A binary matrix of the AFLP-band presence (1) or absence (0) was created. Genetic similarities between two individuals were estimated according to the formula of Dice (1945), first applied to molecular data by Nei and Li (1979). 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%. Based on genetic similarities, a dendrogram was constructed using the unweighted pair group methods of arithmetric averages.

Since AFLP loci segregate as dominant markers, the following assumptions were made to estimate population heterozygosity: First, AFLP fragments segregate according to Mendelian expectations. Second, amplified fragments of the same size are identical in state among and between populations. Third, unamplified fragments of a locus are identical in state among and between populations. Finally, it is also assumed that genotypes at all AFLP loci are in Hardy-Weinberg-equilibrium. Using the program AFLP-surv version 1.0, genetic diversity within populations was estimated on the basis of Nei's (1978) average heterozygosities and percentages of polymorphic loci. In addition, Nei's (1973) gene diversity was calculated. To compare genetic distances between and within populations, genetic distances were calculated as total pairwise differences between several individuals from thirty two populations and compared by a one tailed Mann-Whitney test. In the same locality, pairwise comparisons were done by using the program MEGA 3.0 (Reineke et al. 1999).

7.4 RESULTS AND DISCUSSION

7.4.1 ITS2

Discovering the usefulness of male genitalia for distinguishing species allowed unambiguous identification of many species. Unfortunately, many important species share similar genitalic structure and this has forced workers to continue relying on less dependable characters that often are intraspecifically variable and subject to phenotypic plasticity (Pinto et al. 1990). The use of male genitalic characters was indeed a large improvement, but it still requires specialized skills and is time consuming. An especially severe limitation is that female *Trichogramma* are virtually unidentifiable unless associated with males. This creates a particular problem for completely parthenogenetic forms in which males are not present at all with the consequence that these forms cannot be determined by classical, i.e. morphology-based methods.

Other methods have been proposed to help in identification of *Trichogramma* species. The use of biochemical methods is one of these methods (Sappal et al. 1995, Smith and Hubbes 1986a, Pinto et al. 1993, 2002). More recently, DNA methods have been proposed using restriction length polymorphisms of the complete mitochondrial genome or RAPD PCR (Laurent et al. 1998; Vanlerberghe Masutti 1994; Sappal et al. 1995), and the DNA sequence of the internally transcribed spacer (ITS) of the ribosomal gene complex (Pinto et al. 2002; Orrego and Agudelo-Silva 1993; Stouthamer et al. 2000 The ITS2 sequences showed little variation) within species. Most of the variation was restricted to the number of microsatellite repeats. The molecular technique used for identifying *Trichogramma* based on ITS2 sequence was proved to be a reliable method (Stouthamer et al. 1999), and solved the limitation of the morphological identification basing on male features only.

In the present study, the amplified products of the ITS2 region of *Trichogramma* spp. showed all the same size (ca. 530 bp, Figure 7-2 and 7-3), and the sequences (411 nucleotides) possessed a high degree of homology (>96%) (Diagram 7-1 and Table 7-1 in appendix). However, when sequences were compared with the sequence of strains from the USA, homology ranged from 86 to 90%. Thus, further studies are needed to address the relationship between European and North American populations (Table 7-2). Based on the length of the amplified ITS2 region detected, one was able to distinguish between closely related species or strains such as *T. minutum* Riley, *T. brassicae* Bezdenko and *T.* near *sibericum* Sorokina, where the length of the ITS2 region was about 620, 600 and 660 bp, respectively (Sappal et al. 1995), 453 and 526 bp for *T. lasallei* Pinto and *T. rojasi* Nagaraja & Nagarkatti (Ciociola et al. 2001), and 580 and 510–520 bp for *T. kaykai* Pinto and Stouthamer and *T. deion* Pinto and Oatman, respectively (Stouthamer et al. 1999).

Parsimony analysis with unweighted characters resulted in 792 equally parsimonious trees each with a length of 938 (CI = 0.664: RI = 0.429). Successive approximations weighting produced 26 trees (Figure 7-4). Known relationships between *T. pretiosum* Riley,

T. kaykai, *T. deion* and *T. sathon* Pinto were confirmed (Stouthamer et al. 1999), as well as between *T. minutum* and *T. platneri* Nagarkatti (Stouthamer et al. 2000). Relationships between other species of this section were not previously known and deserve further study. As for *T. aurosum*, strains from Denmark, Austria and Nederland were grouped together, while strains from France, Germany, Belgium and Switzerland were clustered in another group.

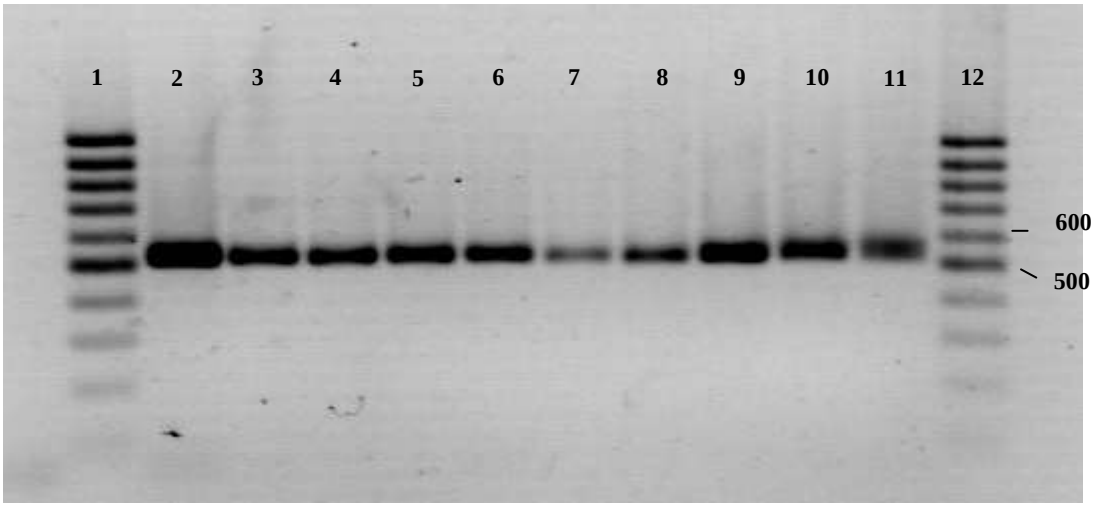

Figure 7-2: Absolute size of the amplified ITS2 region of *T. aurosum* strains collected from different locations. Lane 1 and 12: 100 bp size marker; 2: Ta11a; 3: Ta14; 4: Ta15; 5: Ta16; 6: Ta17; 7: Ta18a; 8: Ta18c; 9: Ta19; 10: Ta20; 11: Ta21.

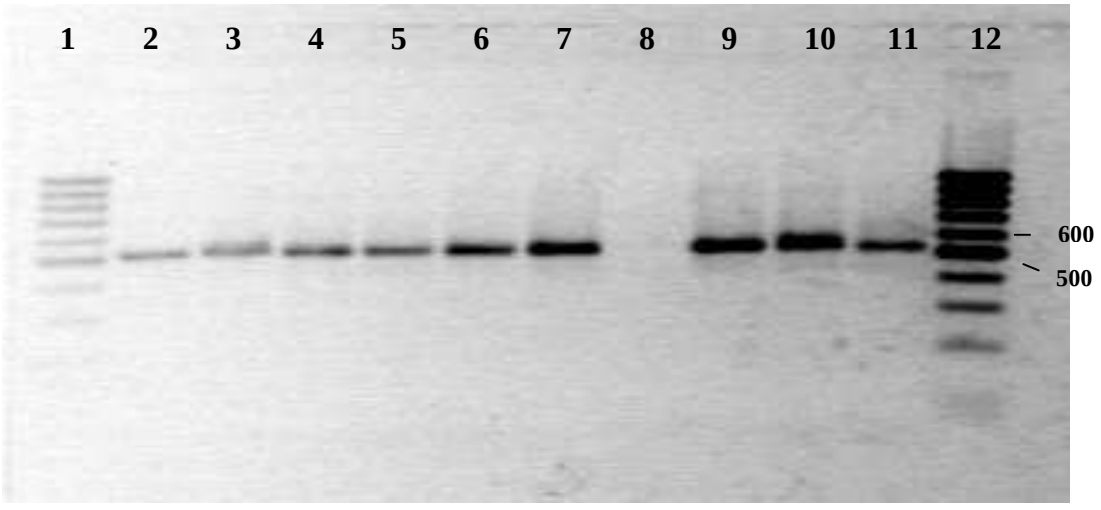

Figure 7-3: Absolute size of the amplified ITS2 region of *T. aurosum* strains collected from different locations. Lane 1 and 12: 100 bp size marker; 2: Ta4aa; 3: Ta6; 4: Ta22; 5: Ta24; 6: Ta28; 7: Ta29; 8: Ta30; 9: Ta31; 10: Ta33; 11: Ta34.

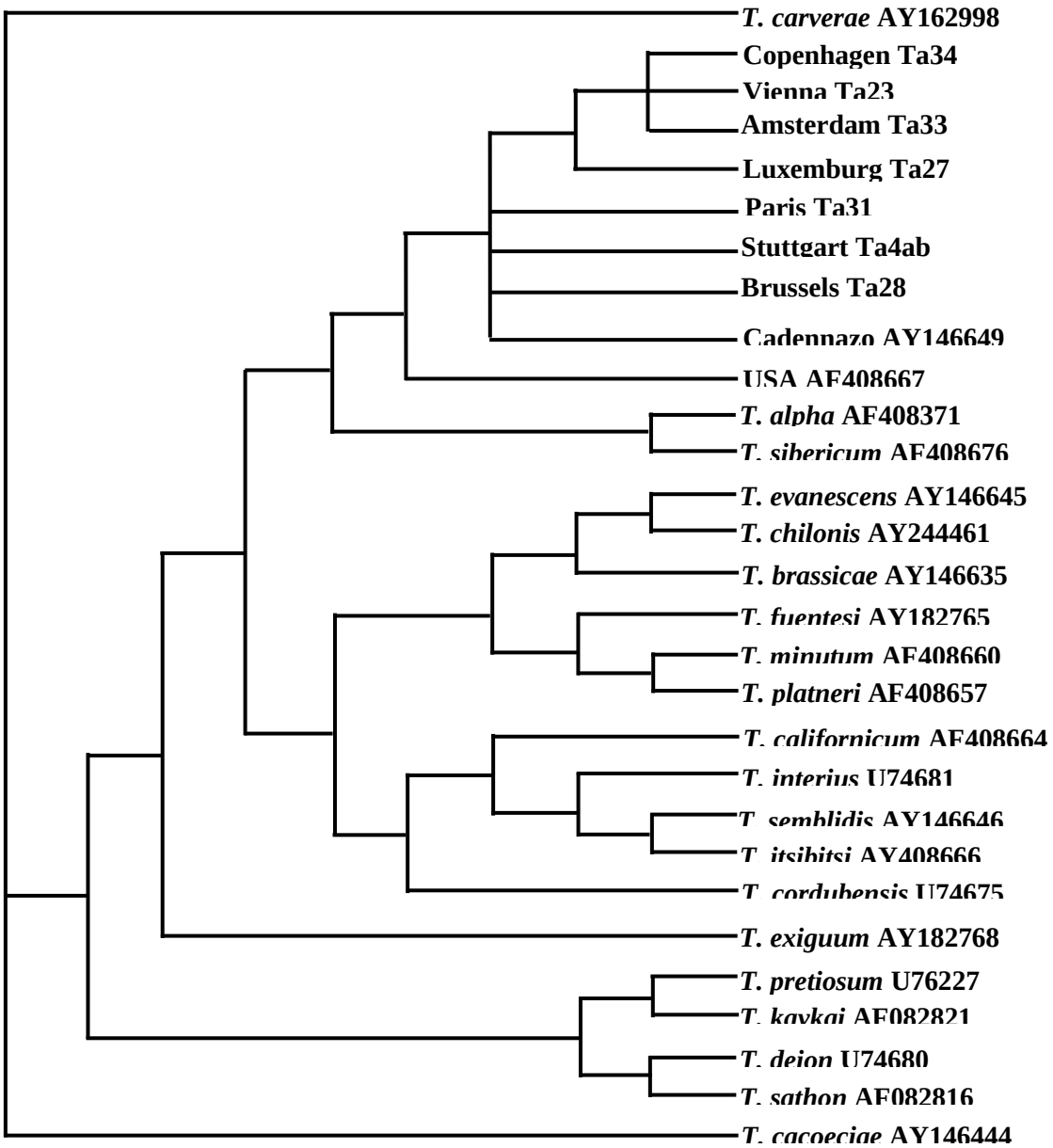

Figure 7-4. Strict consensus tree with successive approximations weighting obtained for different strains of *T. aurosum* and other species of the *exiguum*-section (Tree length = 471.5, CI = 0.8395, RI = 0.7931).

Phylogenetic tree obtained by applying PAUP* (maximum-likelihood methods using the p-distance model) applied to complete ITS2 sequences of the different strains of *T. aurosum* and other *Trichogramma* spp.

Table 7-2. Pair distances of *T. aurosum* alignment of the ITS2 region obtained by Clustal V (Weighted) (Percent Similarity in upper triangle, percent Divergence in lower triangle)

	28	11	16	19	8	6	15	34	26	27	12	9	13	14	7	31	30	4	4	17	23	24	10	33	T.ca	5	T.ex	T.ex	T.mi	T.pl	USII	USII	Swis
28	***	98.8	100	98.8	100	99.8	100	99.5	99.8	97.1	97.6	100	100	99.5	96.1	97.6	100	100	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
11	0	***	98.8	97.3	98.8	98.5	98.8	98.3	98.5	97.1	98.8	98.8	98.8	98.3	96.1	98.5	98.8	98.8	98.8	98.1	98	98.8	97	96.8	32.1	98.8	68.1	68	70.3	70.6	85.9	89.1	97.5
16	0	0	***	98.8	100	99.8	100	99.5	99.8	97.1	97.6	100	100	99.5	96.1	97.6	100	100	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
19	0	0	0	***	98.8	98.5	98.8	98.3	98.5	96.8	97.6	98.8	98.8	98.3	97.8	97.6	98.8	98.8	98.8	96.8	98	98.8	97.3	98	28	98.8	69.5	68.8	71.2	70.5	89.8	87	97.5
8	0	0	0	0	***	99.8	100	99.5	99.8	97.1	97.6	100	100	99.5	96.1	97.6	100	100	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
6	0.2	0.2	0.2	0.2	0.2	***	99.8	99.3	99.5	96.8	97.3	99.8	99.8	99.3	95.8	97.3	99.8	99.8	99.8	96.6	99	99.8	96.8	96.6	34.5	99.8	68.1	68	71.4	70.7	89.3	86	98.5
15	0	0	0	0	0	0.2	***	99.5	99.8	97.1	97.6	100	100	99.5	96.1	97.6	100	100	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
34	0.5	0.5	0.5	0.5	0.5	0.7	0.5	***	99.8	97.1	97.1	99.5	99.5	100	95.6	97.1	99.5	99.5	99.5	97.3	99.8	99.5	96.6	97.3	34.2	99.5	67.9	67.7	71.1	70.4	89.5	85.5	98.3
26	0.2	0.2	0.2	0.2	0.2	0.5	0.2	0.2	***	97.3	97.3	99.8	99.8	99.8	95.8	97.3	99.8	99.8	99.8	97.1	99.5	99.8	96.8	97.1	34.5	99.8	68.1	68	71.4	70.7	89.8	85.7	98.5
27	0.2	0.2	0.2	0.2	0.2	0.5	0.2	0.2	0	***	96.8	97.1	97.1	97.1	96.3	96.6	97.1	97.1	97.1	96.6	96.8	97.1	98	98.3	29.1	97.1	70	69.3	69.4	69.4	89.8	86	95.8
12	0	0	0	0	0	0.2	0	0.5	0.2	0.2	***	97.6	97.6	97.1	96.6	99.8	97.6	97.6	97.6	99.3	96.8	97.6	97	96.6	31.7	97.6	68.4	68.2	70	70.2	89.8	85.7	96.3
9	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	***	100	99.5	96.1	97.6	100	100	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
13	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	0	***	99.5	96.1	97.6	100	100	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
14	0.5	0.5	0.5	0.5	0.5	0.7	0.5	0	0.2	0.2	0.5	0.5	0.5	***	95.6	97.1	99.5	99.5	99.5	97.3	99.8	99.5	96.6	97.3	34.2	99.5	67.9	67.7	71.1	70.4	89.5	85.5	98.3
7	0.7	0.7	0.7	0.7	0.7	1	0.7	1.2	1	0.7	0.7	0.7	0.7	1.2	***	96.6	96.1	96.1	96.1	95.8	95.3	96.1	98.3	97.1	33.6	96.1	69.2	68.5	70.6	70.8	89.8	87	95.8
31	0	0.2	0	0	0	0.2	0	0.5	0.2	0.5	0.2	0	0	0.5	0.7	***	97.6	97.6	97.6	99	96.8	97.6	97	96.4	32.2	97.6	68.4	68.2	69.2	69.5	89.5	85.7	96.3
30	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	0	0	0.5	0.7	0	***	100	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
4	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	0	0	0.5	0.7	0	0	***	100	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
4	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	0	0	0.5	0.7	0	0	0	***	96.8	99.3	100	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
17	0.7	0.7	0.7	0.7	0.7	1	0.7	0.2	0.5	0.5	0.7	0.7	0.7	0.2	1.5	1	0.7	0.7	0.7	***	97.1	96.8	96.3	96.9	30.8	96.8	67.9	67.7	69.5	69.2	89.3	84.9	95.6
23	0.7	0.7	0.7	0.7	0.7	1	0.7	0.2	0.5	0.5	0.7	0.7	0.7	0.2	1.5	0.7	0.7	0.7	0.7	0.5	***	99.3	96.3	97.1	34	99.3	71.3	67.5	70.9	70.2	89.3	85.2	98
24	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	0	0	0.5	0.7	0	0	0	0	0.7	0.7	***	97	96.8	34.7	100	68.1	68	71.4	70.7	89.8	86	98.8
10	0.5	0.5	0.5	0.5	0.5	0.7	0.5	1	0.7	0.7	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	1.2	1.3	0.5	***	96.6	31	97	69.7	69	71.2	71.4	90.3	86.8	96.1
33	0.7	0.7	0.7	0.7	0.7	1	0.7	0.2	0.5	0.5	0.7	0.7	0.7	0.2	1.5	1	0.7	0.7	0.7	0.5	0.5	0.7	1.2	***	31.4	96.8	69.7	69	68.8	68.8	89.5	86.2	95.6
T.ca	65.2	65.8	65.2	65	65.2	64.5	65.2	66.4	65.8	65.4	65.9	65.2	65.2	66.4	65.4	65.9	65.2	65.2	65.2	67.6	67	65.2	64.9	65.6	***	34.7	30.8	32.5	33.8	35.3	35.1	30.9	32.2
5	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	0	0	0.5	0.7	0	0	0	0	0.7	0.7	0	0.5	0.7	65.2	***	68.1	68	71.4	70.7	89.8	86	98.8
T.ex	6.7	7	6.7	6.7	6.7	6.7	6.7	7	6.7	7	7	6.7	6.7	7	7.1	6.6	6.7	6.7	6.7	7.6	7.3	6.7	6.4	7.6	65.5	6.7	***	97.9	70.2	69.7	68.1	70.8	68.1
T.ex	7.4	7.7	7.4	7.4	7.4	7.4	7.4	7.7	7.4	7.7	7.6	7.4	7.4	7.7	7.7	7.3	7.4	7.4	7.4	8.3	8	7.4	7.1	8.2	66.6	7.4	0.8	***	69.6	69	68	69.6	68
T.mi	8.9	8.8	8.9	8.9	8.9	9.2	8.9	9.5	9.2	9.1	8.8	8.9	8.9	9.5	9.2	9.1	8.9	8.9	8.9	9.7	9.8	8.9	8.3	9.7	61	8.9	13.7	14	***	95.2	72	73.2	71.7
T.pl	8.1	8	8.1	8	8.1	8.4	8.1	8.7	8.4	8.3	8	8.1	8.1	8.7	8.3	8.3	8.1	8.1	8.1	8.8	8.9	8.1	7.5	8.8	60.1	8.1	13.3	13.7	0.7	***	73.3	73	71
USII	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.9	1.6	1.6	1.6	1.6	1.6	1.9	1.9	1.6	1.6	1.6	1.6	2.1	2.1	1.6	1.3	1.9	60.2	1.6	6.7	7.4	8.8	7.9	***	94.5	90.1
USII	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.6	1.3	1.3	1.3	1.3	1.3	1.6	1.6	1.3	1.3	1.3	1.3	1.9	1.9	1.3	1.1	1.9	59.1	1.3	6	6.6	8.1	7.2	1.3	***	85.7
Swis	0	0	0	0	0	0.2	0	0.5	0.2	0.2	0	0	0	0.5	0.7	0	0	0	0	0.7	0.7	0	0.5	0.7	64.5	0	6.4	6.8	8.9	8.1	1.6	1.3	***

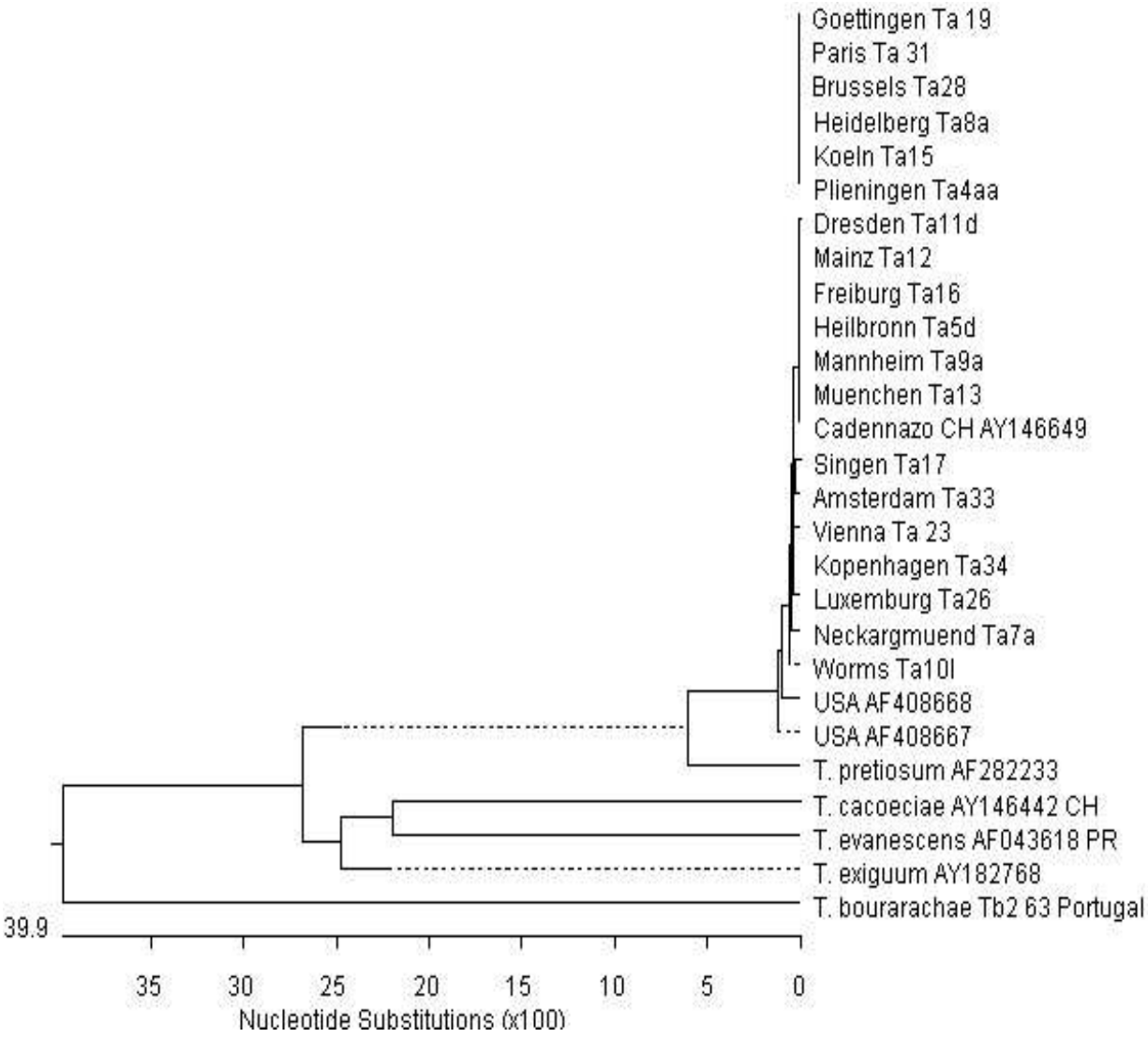

Diagram 7-1. Phylogenetic tree of *T. aurosum* alignment of the ITS2 region. Clustal V (Weighted)

In general, *T. aurosum* strains collected from Germany and other European countries were grouped in a third group together with the strains from both Switzerland and the USA. Moreover, ITS2 sequences of *T. aurosum* were rendered together with *T. alpha* Pinto and *T. sibericum* Sorokina. All three species have been recovered from eggs of hymenopteran hosts, thus suggesting that they might have evolved from a single ancestor.

7.4.2 AFLP

Since 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 primer combinations either with 1, 2 or 3 selective nucleotides at their 3'end. Accordingly, five AFLP primer combinations were chosen which showed a clear and reproducible banding pattern and were used for generating AFLP fingerprints in all *T. aurosum* strains collected (Figure 7-5). The total number of fragments recovered from the wasp populations by the presence of markers was 170 (about 98.3%) and mean number of fragments per individual was 36.5. AFLP fragment size ranged from 43 to 398 bp with an average of 337.12 ± 122.55 bp. The correlation coefficient between fragment sizes and frequencies is 0.2819 (P = 0.00017).

The AFLP genotypic data were used to calculate a similarity matrix according to the formula of Nei and Li (1979), and cluster analysis was performed using the unweighted pair group method of arithmetric averages (UPGMA). The expected heterozygosity (Hw) under Hardy Weinberg genotypic proportions (Nei's gene diversity) ranged from 0.482 to 0.484. To account for the dominant nature of AFLP markers, allele frequencies were estimated in each colony using both the Bayesian method with uniform prior distribution and square root method. Total gene diversity (Ht) was calculated for each population. Results revealed that gene diversity was higher when allele frequencies were estimated using the Bayesian method than the square root method. Results presented in Table 7-3, showed that the mean gene diversity within population was higher than between populations.

It has been suggested that values of Fst (Wright's fixation index) in the range of 0.05 to 0.15 indicate moderate genetic differentiation, values in the range 0.15 to 0.25 indicate high genetic differentiation and values above 0.25 indicate very high genetic differentiation. Using the Bayesian analysis, the Fst value obtained in the present study (-0.1143) suggests that there is very low gene flow between the populations of *T. aurosum*. This would indicate very low genetic differentiation, although the Fst value estimated by the square root method pointed to a very high genetic differentiation (Table 7-4).

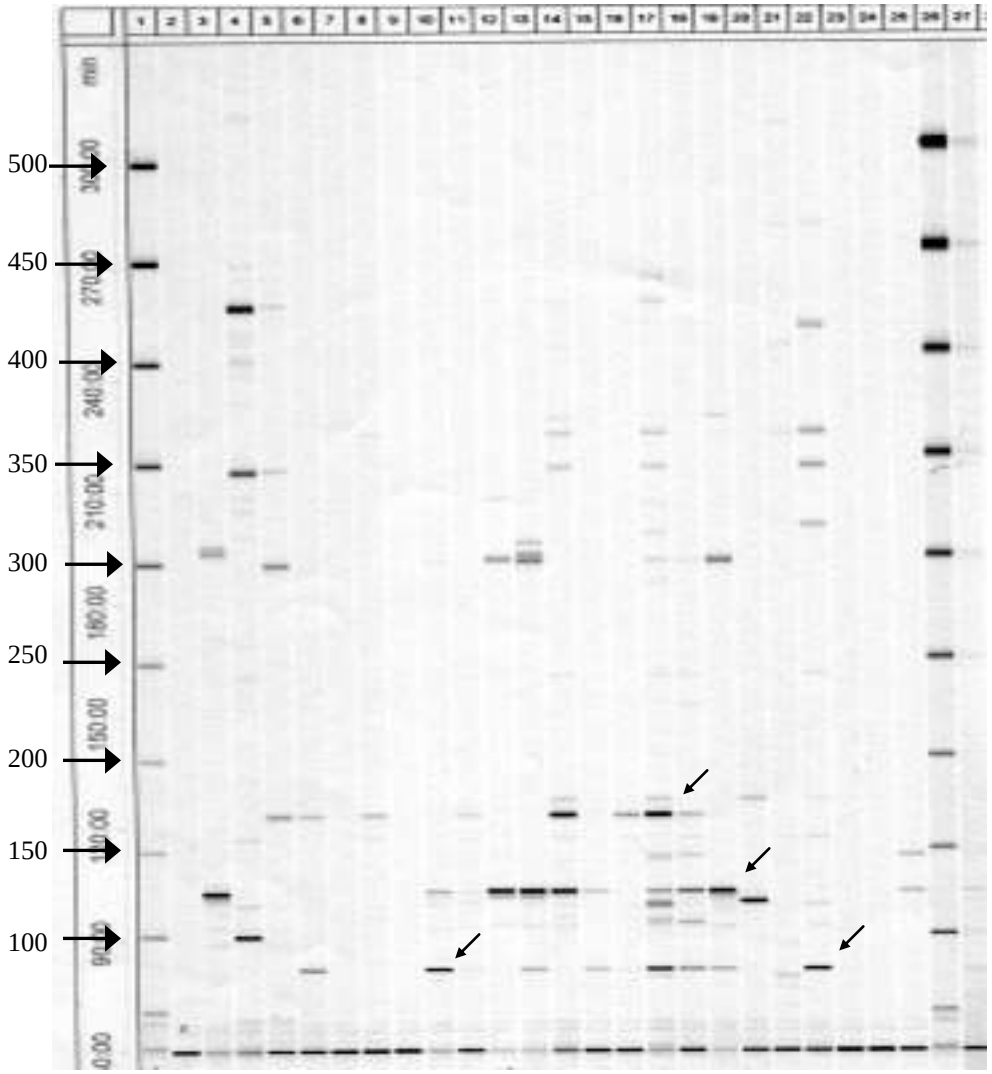

Figure 7-5 AFLP fingerprints generated from genomic DNA of 31 *T. aurosum* strains from seven different geographic origins using primer combination E16/M34 (E+ACC / M+GGA). Lane 2-7 *T. aurosum* from Austria; lanes 8-11 from Luxembourg; lanes 12-15 from Belgium; lanes 16-21 from France; lanes 23 from The Netherlands and lanes 24-25 from Denmark. Lanes 1 and 26 size marker, fragment sizes are indicated on the left.

Table 7-3. Genetic variation within *Trichogramma aurosum* populations based on results obtained by AFLP analysis [Lynch and Milligan method].

Population	Mean no. of fragments per wasp	No. of polymorphic loci	Proportion of polymorphic loci (%)	Hj [1]	S.E.(Hj) [2]
Ta10	173	173	100	0.48497	0.00457
Ta11	132	132	100	0.48125	0.00503
Ta12	173	173	100	0.47782	0.00421
Ta13	173	173	100	0.47941	0.00430
Ta15	173	173	100	0.47782	0.00421
Ta16	132	132	100	0.48125	0.00503
Ta17	132	132	100	0.48542	0.00525
Ta18	173	173	100	0.48577	0.00460
Ta19	173	173	100	0.47623	0.00412
Ta20	173	173	100	0.47861	0.00426
Ta21	173	173	100	0.47543	0.00407
Ta22	173	173	100	0.48020	0.00434
Ta26	141	141	100	0.48121	0.00487
Ta28	100	100	100	0.48438	0.00598
Ta30	100	100	100	0.48988	0.00627
Ta33	141	141	100	0.48511	0.00507
Ta34	173	173	100	0.48974	0.00475
Ta4	173	173	100	0.49212	0.00483
Ta5	141	141	100	0.49778	0.00553
Ta6	173	173	100	0.48815	0.00469
Ta7	100	100	100	0.49400	0.00645
Ta8	141	141	100	0.48511	0.00507
Ta9	173	173	100	0.48100	0.00438

[1] Gene diversity

[2ss] Standard error of gene diversity over individuals and loci

Table 7-4. Total gene diversity (Ht), average gene diversity within populations (Hw) and between (Hb) populations, and Wright's fixation index (Fst), according to Lynch and Milligan (1994) obtained by using two different methods (Bayesian and square root). Values are based on results obtained by AFLP analysis.

	Ht	Hw	Hb	Fst
Bayesian	0.4331	0.4826	-0.0495	-0.1143
Square root	0.2559	0.1185	0.1374	0.5377

Very little genetic variability was found among the 31 strains of *T. aurosum* collected from different locations in Germany and six other European countries. The similarity between the strains was very high, ranging from 75 to 95% (Table A7-1). Smith and Hubbes (1986b) found no variation among strains of *T. minutum*, and the authors attributed this to the long period of rearing the strains in the laboratory. In the present work, no clear clustering related to geographical origin was formed in the UPGMA dendrogram tree. Exceptions were the strains from Munich (Ta13-Ta14), Austria (Ta22-Ta23), Freiburg, and Singen (Ta16-Ta17), which clustered together according to their geographical origin (Figure 7-6). In contrast, other strains were clustered together indicating a close relationship, although they were collected from widely separated regions. This was the case for the strains collected from Stuttgart and Denmark (Ta4-Ta34), France and Netherlands (Ta31-Ta33), Berlin, Ulm, and Göttingen (Ta20-Ta18- Ta19).

Trichogramma aurosum is a holarctic species belonging to the *exiguum*-section (Pinto 1999). Sugonjaev and Sorokina described it for the first time in 1975. In Eastern Europe it was found to be distributed in Russia (central Russia, Altay region, western Siberia and Zabaykalie), Moldavia and Bulgaria (Sorokina 1993), and in Western Europe it was collected from Germany, France, Austria, Netherlands, Belgium, Luxembourg and Denmark (this Chapter), and Switzerland (Kuske et al. 2003). In North America it was found in northern California, central Illinois, Maryland, and southern Arizona. No evidence was found in the literature about an introduction of *T. aurosum* to Western Europe or to Germany or even to North America. This species is widely distributed in Western Europe, so we assume that it is indigenous to this region on Robinia trees.

In Western Europe it was only found and collected from eggs of *Nematus tibialis*, while in Russia it was collected from egg of *Cimbex femorata* L., *Cydia pomonella, Grapholita inopunata* Heinrich, *Acronycta* sp., *Pygaera curtula* (L.). On the other hand in North America it was collected from *Nematus tibialis* and *Cydia pomonella* (Sorokina 1993, Pinto 1999). These insects belong either to the order Hymenoptera or Lepidoptera, which indicate that *T. aurosum* could be a strong potential candidate for controlling insect belonging to both orders.

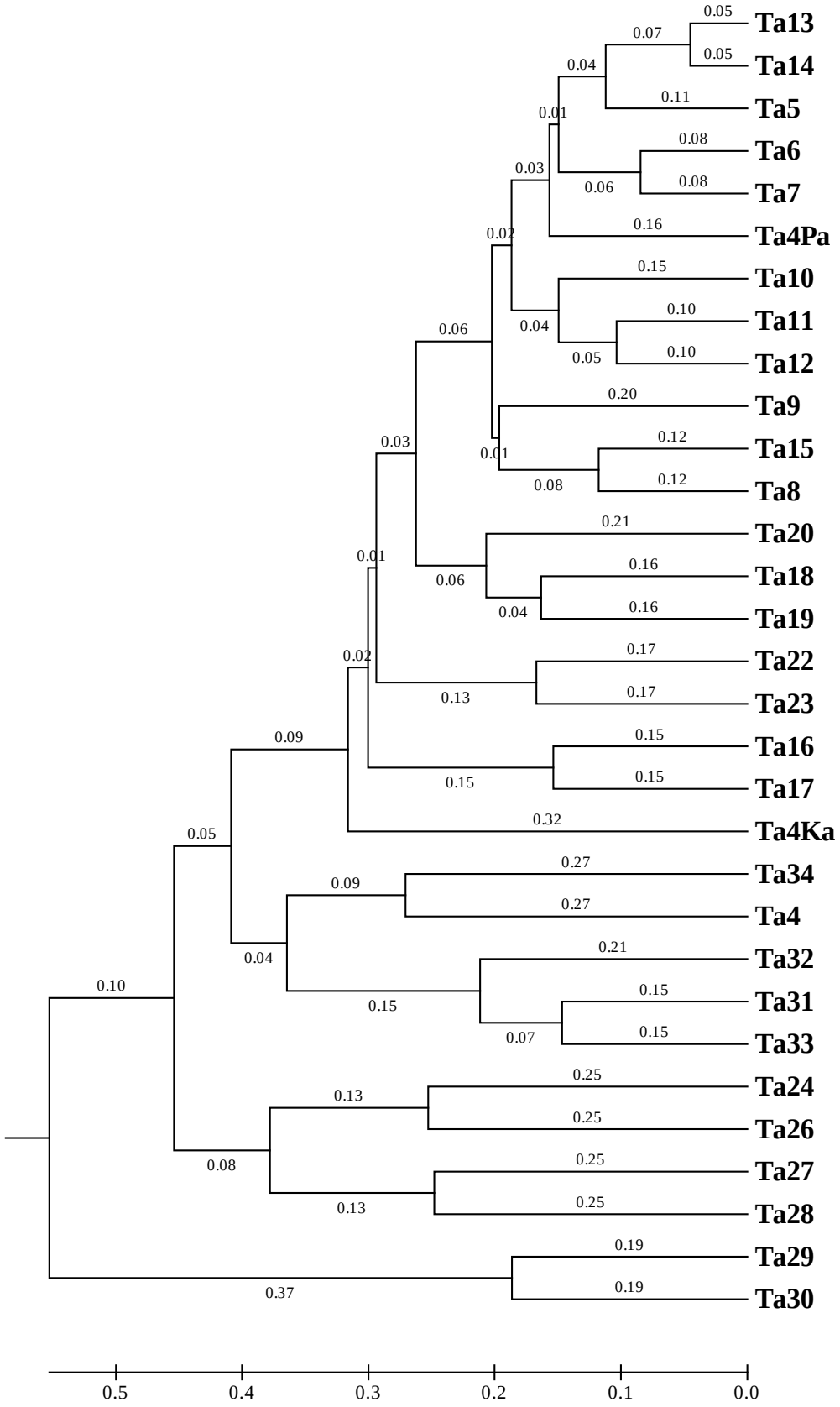

Figure 7-6 UPGMA dendrogram of 31 *T. aurosum* strains from seven different geographic origins showing genetic similarities based on AFLP data of 170 polymorphic markers obtained from five AFLP primer combinations.

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CHAPTER 8

8. GENERAL DISCUSSION

8.1 General information about biological control methods

Biological control is one component of the integrated pest management strategy. It is defined as using bioagents to reduce the pest populations under the economic threshold. These bioagents include predators, parasitoids, and pathogens (Croft and Hoyt 1983). Biological control is applied by three general approaches: importation, augmentation and conservation of natural enemies (Gordh et al. 1999). One of the major problems occurs when an insect pest is accidentally introduced into a new geographic area without its associated natural enemies (Barnett et al. 1991).

Importing natural enemies from their site of origin and releasing them in field can reduce the exotic but native pest insect populations too. Augmentative release of natural enemies have been known by inoculative releases, were small numbers of beneficial are released in order to colonize the crop for long term pest reduction (Debach and Rosen 1991). In contrast, the inundative approach involves releasing a large number of biological agents to cause an immediate and direct mortality in the pest population. Yet, long term regulation is not expected. Conservation of natural enemies is the most important biological control practice. It can be achieved either by using wild plants or backyard gardens to overwinter (Debach and Rosen 1991, Barnett et al. 1991, Falcon and Huber 1991).

8.2 Biological control using Trichogramma (historical)

Experiments with *Trichogramma* started in the beginning of 1920s in the USA, USSR and China. But soon, the interest in this egg parasitoid declined in the West due to the rise of organic pesticides (Smith 1996). Meanwhile the work in USSR and China continued, where the treated areas almost reached 20 million ha (Li 1994). In the 1960s, the research on *Trichogramma* was resumed in both Europe and in the USA (Stinner 1977).

Most biological control programmes involve pre-introductory research to select potential candidates. Studying the interspecific and intraspecific variation in biology and behaviour is very important in this process (Smith 1996). Many authors have listed desirable traits of natural enemies. For inundative and seasonal inoculative biological control they should include: adaptability to climatic extremes and various habitats, searching efficiency, host specificity, host discrimination, host utilization, reproductive capacity and lack of negative side effects (van Dijken et al. 1986, Smith and Hubbes 1986a, Pak and Oatman 1982, Pak et al. 1986, Hohmann et al. 1988, Schmidt and Smith 1987, Stein and Parra 1987, Pavlik 1992). Studying the genetic variability between and within the population is essential too (Bruins et al. 1994, Hoy 1994, Pompanon et al. 1999, Stouthamer et al. 1999, Wajnberg and Colazza 1998, Wajnberg et al. 1989, Wajnberg 1994).

8.3 Parasitization behaviour

Successful parasitism is divided into five steps: host habitat location, host location, host acceptance, host suitability and host regulation. The host selection process involves the first three steps (Flanders 1937, Vinson 1976, Gordh et al. 1999). It has been suggested that host location starts with random searching, but once the host area is located, searching is converted into oriented search with the assistance of physical (Consoli et al. 1999), and chemical cues (Takasu and Nordlund 2001, Noldus et al. 1988, Nordlund et al. 1985). Host location involves host finding within the habitat and recognition of the host. This could be achieved with the assessment of signals emitted from the host or from the host plants (Vinson 1976). Host recognition and acceptance in egg parasitoids can be influenced by the shape, size or by physical and chemical cues from the egg surface.

Host age plays a major role on parasitoid host preference and host suitability (Pak et al. 1986), sex selection of the progeny (Pak and Oatman 1982), and size of the offspring (Husni et al. 2001). Vinson and Iwantsch (1980) found that the suitability of the host for the development of the parasitoid might depend on factors such as nutritional adequacy of the host, host immune system, and toxins. According to Godfray (1994), it is easier for the egg parasitoid to metabolise yolk rather than tissue of the embryonic larvae. Thus, older eggs are unsuitable for the development of the parasitoid (Pak et al. 1986). Host age can affect the duration of major parasitic behaviour such as drumming and drilling time (Marston and Ertle 1969, Pak et al. 1986, Babendreier et al. 2003). Some authors attributed this to the increase of mechanical resistance of the chorion in response to development of the host embryo in older eggs (Reznik et al. 1997), and consequently to host egg differentiation in cell masses after 11 hr (Marston and Ertle 1969).

A second important element in the parasitoid selection process is the preference for certain host species. Host selection in *Trichogramma* spp. was found to be dependent on environmental and host factors (Vinson 1976, Gordh et al. 1999, Takasu and Nordlund 2001). Host factors, which include physical and chemical characteristics, such as host size, shape, texture and odour were found to have an obvious effect on host choice of *Trichogramma* (Monje et al. 1999, Vinson 1976, Reznik et al. 1992, Consoli et al. 1999). The ability of *Trichogramma* spp. to discriminate between parasitized and non parasitized host egg is another important criterion. The wasps can examine the host eggs either externally (using the antennae) or internally (using the ovipositor), in order to search for some marks or pheromones, which indicate previous parasitism (Salt 1937, Vinson 1976, Miura et al. 1994).

8.4 Biological characteristics

Fertility life tables can be used to compare biological traits of insect strains and or species exposed to different biotic and abiotic factors such as temperature, adaptability to different hosts, and reproductive potential of species (Maceda et al. 1994, Zhang et al. 2001), as well as comparing the biological performance of the parasitoid in different diets or hosts

(Pratissoli and Parra 2000). The net reproduction rate varies according to temperature, where its value decreases as temperature increases (Pratissoli and Parra 2000). Also the values of cohort generation time decreases as the temperature increases (Baitha et al. 1998, Pratissoli et al. 2004). According to Cabello and Vargas (1988), the decrease of the net reproduction values could be due to the production of both males and females at high temperatures. Thus, the number of living females born per female in each age interval (m_x) is reduced. Consequently, the value of the net reproductive rate declines. On the other hand, the intrinsic rate of increase and the finite capacity for increase in *Trichogramma* spp. were reported to increase as temperature increase (Pratissoli and Parra 2000, Pratissoli et al. 2004).

The ability of natural enemies to tolerate extreme abiotic conditions is a determining factor for their survival and reproductive capability. Temperature is one of the main factors affecting the adaptability of *Trichogramma* spp. to their habitat, either for the inoculative and / or inundative release. Since parasitoids are normally released into the field as immature stages, they must be able to develop successfully even under unfavourable conditions. On the other hand, the emerged adults must search for hosts and parasitize them regardless of whether abiotic conditions are extreme or not. Tolerance of immature stages of *Trichogramma* species / strains to high or low temperature extremes has been reviewed by many authors (Garcia and Tavares 1994, Hansen 2000, Schöller and Hassan 2001, Pak 1988, Nagarkatti and Nagaraja 1978, Smith and Hubbes 1986a).

Temperature can affect the biological characteristics of *Trichogramma* in different manner. For instance, longevity and development time decrease when temperature increases (McDougall and Mills 1997, Schöller and Hassan 2001). On the other hand fertility increases as temperature increases (Kuhlmann and Mills 1999, Hansen and Jensen 2002, Garcia et al. 2001, Pavlik 1993). It was found that females of *Trichogramma* and *Trichogrammatoidea* deposit the majority of their eggs within the first three days after emergence at high and intermediate temperatures (Mills and Kuhlmann 2000, Bai and Smith 1993, Naranjo 1993). This short life span of some *Trichogramma* species cannot be considered as an adverse character. The velocity of parasitism can be considered as a specific survival strategy, because a faster oviposition at higher temperatures will allow this pro-ovogenic parasitoid to lay most of its available eggs in a short lifetime period (Garcia and Tavares 1994). Pre-oviposition period was found to be long at low temperatures and almost zero at extreme high temperatures. High temperatures increase the metabolic rate, which in turn increases the rate of physiological processes involved in egg production resulting in a decrease of the pre-oviposition period (Al-Ahmed and Kheir 2003).

Effect of temperature on sex ratio differs between *Trichogramma* species: Some species produce more males as temperature increases (Pintureau and Bolland 2001), other species are female-biased at all temperatures (Haile et al. 2002). It has been suggested that lower temperature increases the fusion of nuclei, therefore increasing the proportion of diploid offspring (Crozier 1977). Emergence rate is considered an important parameter to measure the suitability of factitious hosts for mass rearing (Hassan 1994). High emergence rates (89.6 –

91.4%) were recorded in many *Trichogramma* species at intermediate temperatures (Ram et al. 1995, Smith and Hubbes 1986a, Maceda et al. 2003). Mean while emergence of the adult parasitoids was reduced when reared at low temperature. This could be due to the higher mortality in the immature stages (Smith and Hubbes 1986a).

Selection of the most appropriate species or strain of *Trichogramma* is considered to be one of the most critical factors affecting the success of mass releases. Selection of promising candidates has relied so far on the assessment of interspecific differences between different *Trichogramma* species (Almatni 2003, Schöller and Hassan 2001, Pak and Oatman 1982), rather than on assessing intraspecific differences of strains representing the range of distribution of a species (Smith and Hubbes 1986b, Pak 1988, Ram et al. 1995). Therefore, interspecific differences could have been overestimated in the past, if the variation within a strain is at least equal to the variation between strains. However, the reasons for the variation between strains deserve further study.

8.5 Phylogenetical characteristics

During the last decade, and due to the tremendous development in PCR-based techniques, there was an important shift toward studying insect ecology, taxonomy and genetics using a variety of molecular methods, such as RFLP, RAPD, SCAR, and AFLP. These methods differ with respect to key features, such as genomic abundance, level of detected polymorphism, locus specificity, reproducibility, technical and financial requirements. The appropriate choice of the genetic marker depends on the specific application, the expected level of polymorphism, the presence of sufficient technical facilities, time and financial restrictions. Genetic markers can be used in biodiversity studies while the polymorphism of genetic markers is related with heredity. In this way, genetic relationships among organisms can be established and phylogenetic trees can be constructed.

The internal transcribed spacer region 2 (ITS2 of ribosomal DNA) has proven to be a useful source for phylogenetic studies in many insect families (Pinto et al. 2002, Stouthamer et al. 2000), for identification of cryptic *Trichogramma* species and to distinguish between closely related species, subspecies, and populations (Ciociola et al. 2001). These methods depend on amplification of this region and then sequencing it using universal primers. The size of the amplified region of *T. aurosum* strains was identical in size (ca. 530 bp). Further, the sequences showed a high degree of homology (> 96%) between the strains collected from Europe and Germany, while 86-90% homology was recorded with the sequences of the strains from the USA.

Many of the known relationships between *Trichogramma* species were found in our results, such as the relationship between *T. pretiosum*, *T. kaykai*, *T. deion* and *T. sathon*, as well as between *T. minutum* and *T. platneri*. In addition, relationships between other species were found, which were not previously known. As for *T. aurosum*, strains from Copenhagen (Ta34), Vienna (Ta22) and Amsterdam (Ta33) were grouped together, while strains from

Paris (Ta30), Germany (Ta4), Brussels (Ta28) and Switzerland were clustered in another group. In general, *T. aurosum* strains collected from Germany and other European countries were grouped in a third group together with the strains from both Switzerland and the USA. Moreover, ITS2 sequences of *T. aurosum* were rendered together with *T. alpha* Pinto and *T. sibericum* Sorokina. All three species have been recovered from eggs of hymenopteran hosts, thus suggesting that they might have evolved from a single ancestor.

The AFLP technique is one major fingerprinting technique, which has drawing large attention especially in studying the molecular genetics of minute insects, because it needs only little amount of genomic DNA. Consequently, a high amount of polymorphisms can be achieved via PCR and hence more information. Our study resulted in 170 fragments obtained from 23 strains of *T. aurosum* with a mean number of 36.5 fragments per individual. The heterozygosity ranged from 0.482 to 0.484, confirming that the variation between the strains is very low. This suggests that the strains studied might have developed from a single origin. Gene diversity analysis revealed that the mean gene diversity within strains was higher than between strains.

The Bayesian analysis of Fst values suggests that there was very low gene flow between the strains of *T. aurosum*: As a result, very low genetic differentiation and very little genetic variability was found. The homogeneity between the strains was very high; it ranged from 75 to 95% similarity. Only two strains (Ta29 and Ta30) showed polymorphic fingerprint pattern. In this study, no clear correlation between genetic and geographical distance could be detected in the UPGMA dendrogram tree, with exception of some strains (Ta13-Ta14, Ta22-Ta23, and Ta16- Ta17). In contrast, other strains were grouped together, although they were collected form widely separated regions (Ta4-Ta34, Ta31-Ta33, and Ta20-Ta18- Ta19).

The present study showed that intraspecific variation between biological, behavioural and genetical traits of *Trichogramma* species are important in evaluating their efficiency and in selecting suitable candidates for biological control. Further studies on field releases and dispersal are still necessary.

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9. SUMMARY

Biological control of Lepidopterous pests with egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) failed to provide successful control of the target pests in several cases because of selection of either the wrong species or inappropriate strains. Thus, biological control programmes of the Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), with *Trichogramma* spp. require pre-introductory basic research on the performance of potential candidates. In the present study, 32 strains of *Trichogramma aurosum* Sugonjaev and Sorokina collected from different locations in the Federal Republic of Germany and from six countries in Europe were reared in the laboratory, in order to select a suitable strain and/or strains for attempts at controlling the Codling moth. The candidate strains were examined to obtain detailed information about their biology, behaviour, and genetic diversity.

Experiments on host-age preference and host preference of five strains were carried out in the laboratory at room temperature. In the choice tests for host preference, the wasps did not show a significant preference for eggs of *C. pomonella* compared with the eggs of both *Lobesia botrana* Den. and Schiff, and *Agrotis segetum* Schiff. In contrast, in the non-choice tests all strains parasitized a significantly higher number of eggs of *C. pomonella* and *L. botrana* compared with the other hosts tested. Experiments on host age preference were conducted on eggs of *C. pomonella* by directly observing the behaviour of the parasitoids. None of the strains tested showed a significant preference for either fresh or old hosts. Drilling time on old eggs (four or five days old) was longer than the time consumed on fresh ones. This could be due to the mechanical resistance of the host chorion in old eggs or to cell differentiation due to aging. Females of all strains were able to discriminate between parasitized and unparasitized eggs. The acceptance/contact ratio for unparasitized host eggs was significantly higher. Resting made out 30-60% of the total handling time, followed by cleaning and walking.

Fertility life tables have received increasing attention as a tool to evaluate the antagonistic potential of *Trichogramma* spp., and also to compare the suitability of different factitious hosts for mass-rearing. Hence, fertility life tables were constructed at 25 °C for seven strains of *T. aurosum* collected from seven European countries. The mean accumulative fertility ranged from 6.9 – 14.6 eggs / female, while the realised fertility (i.e. the accumulative fertility during the first three days) ranged from 5.2 – 11.6 eggs / female. Development time from egg to adult ranged from 9.9 – 11.35 days, while the adults were only able to live from 2.8 – 6.1 days. Adult emergence rate was higher than 60%, and the female proportion ranged from 58 – 96%. The mean number of progeny emerging from the eggs ranged from 5 – 13 wasps, whereas mortality in the prepupal stage ranged from 11 – 24%. Adaptability of *Trichogramma* spp. to adverse abiotic factors such as high or low temperature is one of the major selection criteria in biological control. Accordingly, the impact of constant and alternating temperature regimes on the parasitization potential and the population growth

parameters of five German strains of *T. aurosum* was studied. Accumulative fertility was positively correlated with temperature, while development time from egg to adult and female longevity were negatively correlated with temperature. The lowest development threshold temperature was 9 – 10 °C. Extreme high temperatures had a significant adverse effect on the sex ratio and the emergence rate. The values of cumulative fertility, longevity and developmental time were reduced at alternating temperatures compared with constant temperatures. This could be due to the better metabolic process of the immature stages at alternating temperatures. We conclude that rearing parasitoids at constant temperatures may overestimate their efficiency in biological control under field conditions, because the values of the biological parameters were higher at constant temperatures than at alternating temperatures. This might be due to an adaptation of *Trichogramma* to the constant laboratory rearing conditions. The strain from Munich (Ta13) showed the best biological properties at all alternating temperatures studied.

Molecular techniques have gained increasing interest in the past ten years to assist assessing biological diversity, phenology, and population dynamics of insects. This is also the case in *Trichogramma* spp., especially what is concerned with the identification of morphologically close related species or those that cannot be assessed morphologically (e.g. parthenogenetically reproducing forms). In this part of the work, the ITS2 region (rDNA) of the collected strains was assessed and compared with those of other *Trichogramma* spp. The amplified products of the *T. aurosum* strains tested had all the same size (ca. 530 bp), and the sequences (411 nucleotides) possessed a high degree of homology (> 96%). However, when sequences were compared with those of strains from the USA, homology ranged from 86 to 90%. Thus, it is not clear yet, whether the collected strains and those from the USA belong to the same species. Here further work is needed. Parsimony analysis with unweighted characters resulted in 792 equally parsimonious trees each with a length of 938 (CI = 0.664, RI = 0.429). In general, strains collected from Germany and other European countries were grouped together with the strains from both Switzerland and the USA. Moreover, ITS2 sequences of *T. aurosum* were rendered together with *T. alpha* Pinto and *T. sibericum* Sorokina. All three species have been recovered from eggs of hymenopteran hosts, thus suggesting that they might have evolved from a single ancestor. Analysis of Amplified Fragment Length Polymorphisms (AFLPs) has been confirmed as a powerful method for the characterization of intraspecific genetic variability among populations of insects because of its high reproducibility. Therefore, this technique was used to analyse the genetic structure, genetic diversity and gene flow between strains of *T. aurosum*. The analysis of five AFLP primer combinations among the 31 strains of *T. aurosum* showed that the mean gene diversity within strains was higher than between strains. Using the Bayesian analysis the Wright's fixation index value (Fst) it could be shown that there was very low gene flow between the populations of *T. aurosum* (i.e. very low genetic differentiation), as well as very little genetic variability. The homogeneity between the strains was very high; it ranged from 75 to 95% similarity. Only two strains (Ta29 and Ta30) showed a polymorphic fingerprint pattern.

Finally, no clear clustering in relation to geographical origin was formed in the UPGMA dendrogram, with few exceptions. Conversely, other strains were grouped together, although they were collected from widely separated regions.

10. ZUSAMMENFASSUNG

Die biologische Bekämpfung von Schadlepidopteren mit Eiparasitoiden der Gattung *Trichogramma* (Hymenoptera: Trichogrammatidae) schlug häufig allein deshalb fehl, weil eine falsche Art oder ein falscher Stamm des Nützlings eingesetzt wurde. Daher erfordern Versuche, den Apfelwickler, *Cydia pomonella* L. (Lepidoptera: Tortricidae), mit Hilfe dieser wichtigen Parasitoide zu bekämpfen, eine Überprüfung der Eignung potentieller Kandidaten. Während der vorliegenden Studie wurden 32 Stämme von *Trichogramma aurosum* Sugonjaev and Sorokina (Hymenoptera: Trichogrammatidae) an unterschiedlichen Standorten in der Bundesrepublik Deutschland und in sechs anderen Ländern Europas gesammelt und im Labor gezüchtet. Aus Stämmen dieser Art, die bereits als Parasitoid des Apfelwicklers bekannt war, sollte ein geeigneter Stamm bzw. mehrere Stämme selektiert werden, der/die hohe Effizienz gegen den Apfelwickler aufweisen. Die Untersuchungen dienten dazu, detaillierte Informationen über die Biologie, Verhalten und genetische Diversität dieser Art zu erhalten.

Fünf näher untersuchte Stämme von *T. aurosum* zeigten im zweiseitigen Wahlversuch zwar eine erhöhte, aber nicht signifikante Bevorzugung von Eiern des Apfelwicklers im Vergleich zu Eiern von *Lobesia botrana* oder *Agrotis segetum*. Wurden die Wirtseier ohne Wahlmöglichkeit angeboten, so wurden die Eier von *C. pomonella* oder *L. botrana* deutlich bevorzugt. Laborversuche zur Wirtsalterspräferenz derselben fünf Stämme für Eier von *C. pomonella*, ergaben keinerlei Präferenz zwischen frischen und/oder alten Wirtseiern, die kurz vor dem Schlupf der Erstlarve waren. Lediglich die benötigte Zeit, das Chorion des Wirtseis zu durchbohren (drilling time), war auf alten Eiern (4 oder 5 Tage alt) gegenüber frischen Eiern etwas erhöht. Dies könnte am zunehmenden mechanischen Widerstand des Chorions alter Eier oder an der Zelldifferenzierung im alternden Wirt liegen. Weibchen aller Stämme waren in der Lage, parasitisierte von nicht parasitisierten Eiern zu diskriminieren. Die Kontakt-Annahme-Rate war signifikant höher für nicht-parasitisierte Wirte. Die inaktive Zeit (Ruhephasen) machte 30 - 60% der Beobachtungszeit aus, gefolgt von Putzen und Suchlauf.
Die Erstellung von Lebenstafeln ist ein unverzichtbares Werkzeug zur Bewertung der Qualität und Leistungsfähigkeit einer Art. Dies gilt besonders für Nützlinge der Gattung *Trichogramma*, um auf diesem Wege auch die Eignung von Ersatzwirten für die Massenzucht zu ermitteln. Folglich wurden Lebenstafeln für sieben Stämme von *T. aurosum* bei 25 °C erstellt. Die mittlere Gesamtfertilität reichte von 6.9 bis 14.6 Eier/Weibchen, wobei die Fertilität an den ersten drei Lebenstagen der Weibchen (realised fertility) mit 5.2 - 11.6 Eier/Weibchen den größten Anteil hatte. Die Entwicklungsdauer vom Ei bis zur Imago reichte von 9.9 bis 11.35 Tagen. Die anschließende Lebensdauer als Imago reichte von 2.8 bis 6.1 Tagen. Die Schlupfrate der Parasitoide lag in allen Fällen über 60%, das Weibchenanteil reichte von 58 – 96%. Die mittlere Anzahl Nachkommen schwankte zwischen 5 und 13 Wespen bei einer durchschnittlichen präimaginalen Mortalität von 11 – 24%. Die Anpassungsfähigkeit von *Trichogramma*-Arten an abiotische Faktoren, besonders hohe oder niedrige Temperaturen, ist eines der Hauptkriterien für qualitativ gut geeignete Nützlinge.

Daher wurde das Parasitisierungspotential und die Populations-Wachstumparameter von fünf Stämmen von *T. aurosum* unter verschiedenen konstanten oder wechselnden Temperaturen untersucht. Stämme aus Regionen mit höherer Jahresmitteltemperatur aus dem Süden oder Osten Deutschlands wiesen eine breitere Temperaturadaptation auf als der Stamm aus Norddeutschland, der eher als stenotherme Art angesprochen werden kann. Allerdings fiel auf, daß die Gesamtfertilität nicht mit der Temperaturabhängigkeit der Entwicklungsgeschwindigkeit übereinstimmte. Als unterer Entwicklungsnullpunkt wurde für alle Stämme 9 – 10 °C angenommen. Lebensdauer und Entwicklung waren, wie zu erwarten, negativ mit der Temperatur korreliert. Hohe Temperaturen führten zum Rückgang der Schlupfrate und zu einer Verschiebung des Geschlechterverhältnisse zugunsten der Weibchen. Vergleicht man die erhobenen Parameter im Bezug auf konstante oder wechselnde Temperatur, so fällt auf, daß Wechseltemperatur eine deutliche Reduktion der Entwicklungsdauer, der Lebensdauer und der Nachkommenzahl zur Folge hat. Obwohl die Erstellung von Lebenstafeln unter konstanten Temperaturen als Standard gilt, sollte aus Gründen der Übertragbarkeit der Daten auf Freilandverhältnisse auf Wechseltemperaturen umgestellt werden. Erstaunlicherweise weichen auch die unter konstanter Temperatur ermittelten Temperatursummen der Entwicklungsdauer von denen unter Wechseltemperaturen errechnete Werte ab. Stellt man alle erhobenen Parameter in Rechnung, so erwies sich der in München gesammelte Stamm von *T. aurosum* als der unter Wechseltemperaturen am besten geeignete Stamm.

Molekulare Methoden haben in den letzten 10 Jahren sehr an Bedeutung gewonnen, um die biologische Diversität, die Phänologie und die Populationsdynamik von Insekten zu erfassen. Dies ist auch der Fall bei *Trichogramma* spp., insbesondere was die Bestimmung morphologisch nah verwandter Arten oder derjenigen Formen betrifft, die nicht morphologisch untersucht werden können (z.B. sich parthenogenetisch vermehrende Arten). In diesem Teil der Studie wurde die ITS2-Region (rDNA) der gesammelten Stämme untersucht und mit derjenigen anderer Arten verglichen. Die amplifizierten Produkte der getesteten Stämme hatten identische Länge (ca. 530 bp) und die Sequenzen (411 Nukleotide) wiesen eine hohe Homologie auf (> 96%). Wurden jedoch die Sequenzen mit Stämmen aus den USA verglichen, so erreichten die Werte 86 bis 90%. Daher steht es noch nicht fest, ob die untersuchten Stämme und die Stämme aus den USA der gleichen Art angehören. Hier sind weitere Studien notwendig. Die Parsimonieanalyse mit ungewichteten Merkmalen ergab 792 parsimonisch gleichwertige Bäume mit einer Länge von 938 (CI = 0.664, RI = 0.429). Im allgemeinen wurden *T. aurosum*-Stämme, die in Deutschland und in anderen europäischen Ländern gesammelt wurden, in eine dritte Gruppe mit den Stämmen von der Schweiz und von den USA zusammengefasst. Außerdem wurden die ITS2-Sequenzen von *T. aurosum* mit denjenigen von *T. alpha* Pinto und *T. sibericum* Sorokina zusammen gruppiert. Alle drei Arten wurden in Eiern von Hymenopteren gefunden und dies deutet darauf hin, dass sie aus dem gleichen Vorfahren hervorgegangen sein könnten.

Die AFLP–Methode (Amplified Fragment Length Polymorphism) hat sich als ein starkes Werkzeug etabliert, um die intraspezifische Variabilität innerhalb von Insektenpopulationen zu erfassen, vor allem aufgrund der guten Reproduzierbarkeit der Ergebnisse. Daher wurde diese Methode angewandt, um die genetische Struktur, die genetische Diversität und den Genfluss zwischen Unterpopulationen von *T. aurosum* zu erfassen. Die Analyse mit fünf verschiedenen Primerkombinationen an 31 Stämmen ergab, dass die mittlere Gendiversität innerhalb der Stämme höher als zwischen den Stämmen war. Durch die bayesische Analyse des Fixierungsindexwertes (fixation index value, Fst) konnte gezeigt werden, dass der Genfluß zwischen den untersuchten Stämmen von *T. aurosum* sehr niedrig war (d.h. sehr niedrige genetische Differenzierung) und dass die, Die Homogenität zwischen den Stämmen war sehr hoch. Sie schwankte zwischen 75 bis 95% Ähnlichkeit. Nur zwei Stämme (Ta29 und Ta30) hatten ein polymorphes Bandenmuster. Das UPGMA-Dendrogram zeigte, mit wenigen Ausnahmen, keine eindeutige Gruppierung in Abhängigkeit vom geographischen Ursprung. Es wurden sogar Stämme zusammen gruppiert, die aus weit entfernten Regionen stammten.

APPENDIX

Preparing of Codling moth larval media

Ingredients:

1,600 ml water

40 g Agar Agar

100 g Semolina

100 g Brewers yeast (Bierhefe)

100 g Wheat germ

3.6 g Nipagin (p-Hydroxy-benzoic-acid-methylester (Methyl proban)

3.6 g Benzoic acid dissolved in 40 ml absolute ethanol

10 g Ascorbic acid dissolved in 40 ml water

Preparation:

1. Heat the water until boiling
2. Add agar and mix well
3. at 70 °C add Semolina
4. at 60 °C add wheat germ
5. at 50 °C add Brewers yeast
6. at 40 °C the rest of the materials, but add ascorbic acid when the temperature is below 40 °C pour the mixture in clean and sterilized plastic boxes and let the stand to cool at room temperature, then store it in - 20 °C up to 6 month.

Preparation of Arabic Gum

Arabic Gum was obtained as a wettable powder (MERCK), 10 g of the powder is dissolved in 100 ml distilled water and placed on a heatable magnetic stirrer at 50 °C for half an h or until the solution is well dissolved. This solution was kept in a suitable flask at room temperature for further use.

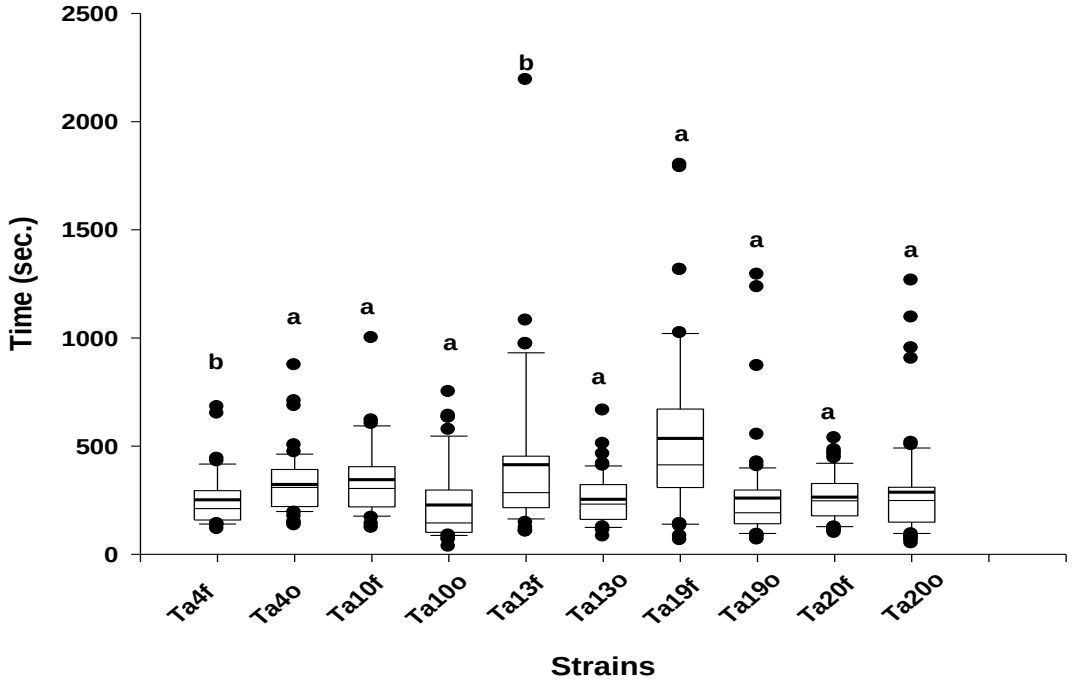

Figure A3-1 Mean duration of drilling by five *T. aurosum* strains parasitizing 0 vs. 4 days old eggs of *C. pomonella* at room temperature. (F = fresh eggs, O = old eggs; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

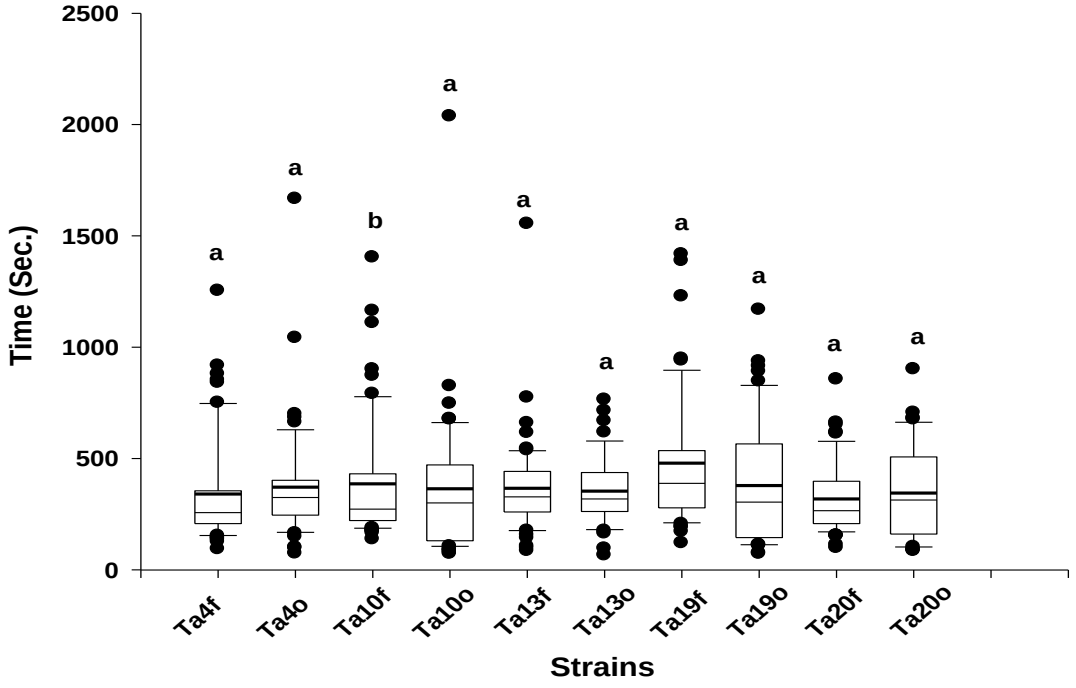

Figure A3-2 Mean duration of drilling by five *T. aurosum* strains parasitizing 1 vs. 5 days old eggs of *C. pomonella* at room temperature.

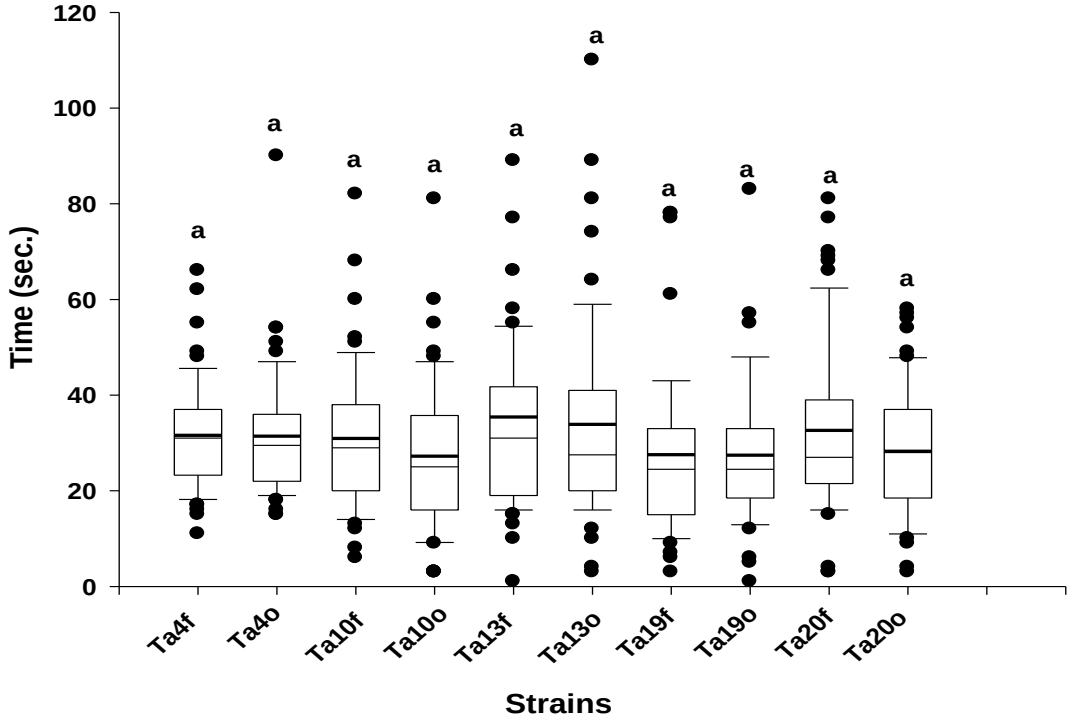

Figure A3-3 Mean duration of drumming by five *T. aurosum* strains parasitizing 0 vs. 4 days old eggs of *C. pomonella* at room temperature. (F = fresh eggs and O = old eggs; Box = 25 - 75 percentile; whiskers = 10 and 90 percentile; extreme values = dots above and under the whisker cap; mean value = thick line; median = thin line).

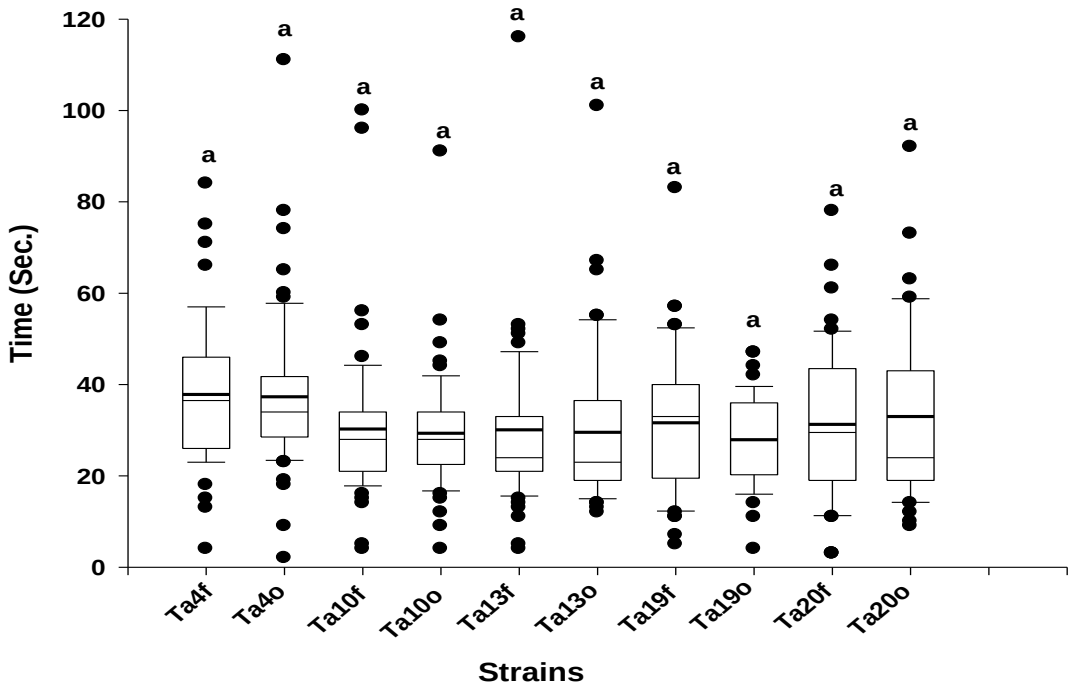

Figure A3-4 Mean duration of drumming by five *T. aurosum* strains parasitizing 1 vs. 5 days old eggs of *C. pomonella* at room temperature.

Table 6-1. Life and fertility table for five strains of *T. aurosum* at four constant temperatures.

15 °C	AGE	SURV	LX	MX	LXMX	MXLXX
	29.5	0.30	0.17	1.00	0.17	4.89
	30.5	0.30	0.17	0.13	0.02	0.63
	31.5	0.30	0.18	0.58	0.10	3.26
	32.5	0.30	0.24	1.25	0.30	9.75
	33.5	0.30	0.24	0.86	0.21	6.93
	34.5	0.30	0.24	1.51	0.36	12.50
	35.5	0.30	0.24	0.69	0.17	5.88
	36.5	0.30	0.24	0.95	0.23	8.31
	37.5	0.30	0.24	0.56	0.13	5.05
	38.5	0.30	0.24	0.69	0.17	6.38
Ta4	39.5	0.30	0.21	0.44	0.09	3.68
	40.5	0.30	0.17	0.75	0.12	5.03
	41.5	0.30	0.17	0.25	0.04	1.72
	42.5	0.30	0.17	0.88	0.14	6.16
	43.5	0.30	0.17	0.50	0.08	3.60
	44.5	0.30	0.17	0.94	0.16	6.91
	45.5	0.30	0.15	1.45	0.22	9.89
	46.5	0.30	0.15	0.41	0.06	2.89
	47.5	0.30	0.14	0.77	0.10	4.92
	48.5	0.30	0.12	0.43	0.05	2.51
	49.5	0.30	0.12	0.60	0.07	3.59
	50.5	0.30	0.12	0.52	0.06	3.14
	51.5	0.30	0.12	0.43	0.05	2.67
	52.5	0.30	0.11	0.39	0.04	2.17
	53.5	0.30	0.09	0.23	0.02	1.11
	54.5	0.30	0.09	0.23	0.02	1.13
	55.5	0.30	0.08	0	0	0
	56.5	0.30	0.08	0	0	0
	57.5	0.30	0.06	0.35	0.02	1.19
	58.5	0.30	0.06	0	0	0
	59.5	0.30	0.06	0	0	0
	60.5	0.30	0.06	0	0	0
	61.5	0.30	0.06	0	0	0
	62.5	0.30	0.06	0	0	0
	63.5	0.30	0.05	0	0	0
	64.5	0.30	0.05	0	0	0
	65.5	0.30	0.05	0	0	0
	66.5	0.30	0.05	0	0	0
	32.5	0.05	0.03	0.23	0.01	0.24
	33.5	0.05	0.05	0.42	0.02	0.67
	34.0	0.05	0.01	0	0	0
	34.3	0.05	0.01	0	0	0
	34.5	0.05	0.04	0.63	0.03	0.86
	34.6	0.05	0.01	0.50	0	0.09
	34.9	0.05	0	0	0	0
	35.2	0.05	0	0	0	0
	35.5	0.05	0.04	0.33	0.01	0.44
Ta10	35.8	0.05	0	0	0	0
	36.1	0.05	0	0	0	0
	36.4	0.05	0	0	0	0
	36.5	0.05	0.03	0.25	0.01	0.27
	36.7	0.05	0	0	0	0
	37.0	0.05	0	0	0	0
	37.3	0.05	0	0	0	0
	37.5	0.05	0.03	0.17	0.01	0.19
	37.6	0.05	0	0	0	0
	37.9	0.05	0	0	0	0

	38.2	0.05	0	0	0	0
	38.5	0.05	0.03	0.50	0.02	0.58
	38.8	0.05	0	0	0	0
	39.1	0.05	0	0	0	0
	39.4	0.05	0	0	0	0
	39.5	0.05	0.03	0.20	0.01	0.20
	39.7	0.05	0	0	0	0
	40.0	0.05	0	0	0	0
	40.5	0.05	0.02	0	0	0
	41.5	0.05	0.02	0.29	0.01	0.21
	42.5	0.05	0.02	0.33	0.01	0.21
	43.5	0.05	0.01	0	0	0
	44.5	0.05	0.01	0	0	0
	45.5	0.05	0.01	0.60	0.01	0.34
	46.5	0.05	0.01	0	0	0
	47.5	0.05	0.01	1.00	0.01	0.48
	48.5	0.05	0.01	0	0	0
	49.5	0.05	0.01	0	0	0
	50.5	0.05	0.01	0	0	0
	32.5	0.81	0.69	0.17	0.12	3.75
	33.5	0.81	0.61	0.09	0.05	1.80
	34.5	0.81	0.61	0.16	0.10	3.45
	35.5	0.81	0.57	0.77	0.44	15.57
	36.5	0.81	0.41	0.32	0.13	4.77
	37.5	0.81	0.41	0.30	0.12	4.62
	38.5	0.81	0.41	0.21	0.08	3.26
	39.5	0.81	0.36	0.44	0.16	6.38
	40.5	0.81	0.32	0.26	0.08	3.43
	41.5	0.81	0.24	0.16	0.04	1.60
	42.5	0.81	0.20	0.19	0.04	1.64
	43.5	0.81	0.20	0.08	0.02	0.67
	44.5	0.81	0.16	0.19	0.03	1.37
	45.5	0.81	0.16	0.19	0.03	1.40
	46.5	0.81	0.16	0.33	0.05	2.50
Ta13	47.5	0.81	0.16	0.10	0.02	0.73
	48.5	0.81	0.16	0.24	0.04	1.87
	49.5	0.81	0.16	0.05	0.01	0.38
	50.5	0.81	0.16	0.24	0.04	1.94
	51.5	0.81	0.16	0.19	0.03	1.59
	52.5	0.81	0.16	0.19	0.03	1.62
	53.5	0.81	0.16	0.14	0.02	1.24
	54.5	0.81	0.16	0.10	0.02	0.84
	55.5	0.81	0.16	0.14	0.02	1.28
	56.5	0.81	0.16	0.05	0.01	0.43
	57.5	0.81	0.16	0.29	0.05	2.65
	58.5	0.81	0.16	0.05	0.01	0.45
	59.5	0.81	0.16	0.14	0.02	1.37
	60.5	0.81	0.16	0.05	0.01	0.47
	61.5	0.81	0.16	0.05	0.01	0.47
	62.5	0.81	0.16	0.05	0.01	0.48
	63.5	0.81	0.16	0	0	0
	64.5	0.81	0.16	0	0	0
	65.5	0.81	0.16	0	0	0
	66.5	0.81	0.16	0	0	0
	67.5	0.81	0.16	0	0	0
	68.5	0.81	0.12	0	0	0
	69.5	0.81	0.12	0	0	0
	70.5	0.81	0.12	0	0	0
	71.5	0.81	0.12	0	0	0
	72.5	0.81	0.08	0	0	0
	73.5	0.81	0.08	0	0	0

	29.5	0.23	0.02	1.88	0.04	1.28
	30.5	0.23	0.02	0.47	0.01	0.33
	31.5	0.23	0.05	0.71	0.03	1.02
	32.5	0.23	0.18	0.65	0.12	3.86
	33.5	0.23	0.17	1.00	0.17	5.79
	34.5	0.23	0.22	1.43	0.31	10.82
	35.5	0.23	0.20	0.94	0.18	6.52
	36.5	0.23	0.18	0.35	0.06	2.37
	37.5	0.23	0.17	0.94	0.16	6.08
	38.5	0.23	0.17	0.56	0.10	3.75
	39.5	0.23	0.17	0.50	0.09	3.42
	40.5	0.23	0.17	0.63	0.11	4.38
	41.5	0.23	0.17	0	0	0
	42.5	0.23	0.17	0.13	0.02	0.92
	43.5	0.23	0.15	0.22	0.03	1.41
	44.5	0.23	0.14	0	0	0
Ta19	45.5	0.23	0.14	0.47	0.06	2.95
	46.5	0.23	0.14	0.47	0.06	3.02
	47.5	0.23	0.13	0.60	0.08	3.59
	48.5	0.23	0.13	0.17	0.02	1.05
	49.5	0.23	0.12	0.56	0.06	3.21
	50.5	0.23	0.12	0	0	0
	51.5	0.23	0.12	0	0	0
	52.5	0.23	0.12	0	0	0
	53.5	0.23	0.12	0.09	0.01	0.58
	54.5	0.23	0.10	0	0	0
	55.5	0.23	0.10	0	0	0
	56.5	0.23	0.08	0	0	0
	57.5	0.23	0.08	0	0	0
	58.5	0.23	0.08	0	0	0
	59.5	0.23	0.08	0	0	0
	60.5	0.23	0.08	0	0	0
	61.5	0.23	0.02	0.47	0.01	0.66
	62.5	0.23	0.01	0	0	0
	63.5	0.23	0.01	0	0	0
	32.5	0.34	0.07	0	0	0
	33.5	0.34	0.14	0.85	0.12	3.87
	34.5	0.34	0.31	1.52	0.47	16.09
	35.5	0.34	0.31	1.85	0.57	20.11
	36.5	0.34	0.29	2.28	0.66	24.05
	37.5	0.34	0.29	1.24	0.36	13.44
	38.5	0.34	0.29	1.00	0.29	11.13
	39.5	0.34	0.29	0.68	0.20	7.76
	40.5	0.34	0.29	0.76	0.22	8.90
	41.5	0.34	0.26	1.13	0.29	11.99
	42.5	0.34	0.22	0.94	0.21	8.84
Ta20	43.5	0.34	0.20	0.06	0.01	0.50
	44.5	0.34	0.20	0.45	0.09	4.12
	45.5	0.34	0.20	1.08	0.22	9.99
	46.5	0.34	0.20	0.51	0.10	4.84
	47.5	0.34	0.19	0.93	0.17	8.24
	48.5	0.34	0.19	0.19	0.03	1.68
	49.5	0.34	0.19	0.37	0.07	3.43
	50.5	0.34	0.19	0.80	0.15	7.59
	51.5	0.34	0.19	0.31	0.06	2.98
	52.5	0.34	0.19	0.62	0.12	6.07
	53.5	0.34	0.17	0.14	0.02	1.24
	54.5	0.34	0.17	0.41	0.07	3.78
	55.5	0.34	0.17	0	0	0
	56.5	0.34	0.14	0.17	0.02	1.31

57.5	0.34	0.14	0	0	0
58.5	0.34	0.14	0	0	0
59.5	0.34	0.14	0	0	0
60.5	0.34	0.14	0	0	0
61.5	0.34	0.14	0	0	0
62.5	0.34	0.14	0	0	0
63.5	0.34	0.14	0	0	0
64.5	0.34	0.14	0	0	0
65.5	0.34	0.14	0	0	0
66.5	0.34	0.14	0	0	0
67.5	0.34	0.12	0	0	0
68.5	0.34	0.12	0	0	0
69.5	0.34	0.10	0	0	0
70.5	0.34	0.10	0	0	0
71.5	0.34	0.10	0	0	0
72.5	0.34	0.10	0	0	0
73.5	0.34	0.09	0	0	0
74.5	0.34	0.09	0.54	0.05	3.44

20 °C	AGE	SURV	LX	MX	LXMX	MXLXX
	14.5	0.66	0.04	3.47	0.13	1.95
	15.5	0.66	0.12	5.78	0.67	10.40
	16.5	0.66	0.23	3.76	0.87	14.39
	17.5	0.66	0.31	5.20	1.61	28.18
	18.5	0.66	0.39	3.47	1.34	24.82
Ta4	19.5	0.66	0.66	2.50	1.64	32.05
	20.5	0.66	0.62	0.33	0.20	4.13
	21.5	0.66	0.50	0.60	0.30	6.49
	22.5	0.66	0.35	1.35	0.47	10.57
	23.5	0.66	0.31	1.19	0.37	8.67
	24.5	0.66	0.27	0.99	0.27	6.57
	25.5	0.66	0.19	0.17	0.03	0.86
	26.5	0.66	0.15	0.65	0.10	2.67
	27.5	0.66	0.12	0	0	0
	28.5	0.66	0.08	0.87	0.07	1.91
	29.5	0.66	0.08	0.43	0.03	0.99
	15.5	0.43	0.15	4.33	0.65	10.14
	16.5	0.43	0.28	2.09	0.58	9.55
	17.5	0.43	0.18	2.29	0.40	7.04
Ta10	18.5	0.43	0.15	1.50	0.23	4.19
	19.5	0.43	0.15	1.83	0.28	5.40
	20.5	0.43	0.15	0.33	0.05	1.03
	21.5	0.43	0.15	0.67	0.10	2.16
	22.5	0.43	0.13	0.80	0.10	2.26
	23.5	0.43	0.10	0.75	0.08	1.77
	24.5	0.43	0.10	1.25	0.13	3.08
	25.5	0.43	0.05	0	0	0
	26.5	0.43	0.03	3.00	0.08	2.00
	27.5	0.43	0.03	0	0	0
	28.5	0.43	0.03	0	0	0
	18.5	0.16	0.03	1.01	0.03	0.59
	19.5	0.16	0.14	2.40	0.34	6.68
	20.5	0.16	0.13	1.39	0.19	3.84
	21.5	0.16	0.12	0.72	0.09	1.84
	22.5	0.16	0.11	1.30	0.14	3.25
	23.5	0.16	0.09	1.52	0.14	3.39
	24.5	0.16	0.09	1.29	0.11	2.75
	25.5	0.16	0.06	2.28	0.14	3.68
	26.5	0.16	0.06	0.48	0.03	0.71

	27.5	0.16	0.06	1.45	0.08	2.21
Ta13	28.5	0.16	0.06	0.97	0.05	1.52
	29.5	0.16	0.06	0.29	0.02	0.47
	30.5	0.16	0.05	1.58	0.07	2.28
	31.5	0.16	0.04	1.62	0.06	2.02
	32.5	0.16	0.03	1.52	0.05	1.56
	33.5	0.16	0.02	0	0	0
	18.5	0.52	0.18	1.41	0.26	4.77
	19.5	0.52	0.29	1.26	0.36	7.04
	20.5	0.52	0.47	0.66	0.31	6.34
	21.5	0.52	0.44	1.20	0.53	11.46
Ta19	22.5	0.52	0.44	2.21	0.98	22.04
	23.5	0.52	0.44	2.40	1.07	25.04
	24.5	0.52	0.44	2.01	0.89	21.90
	25.5	0.52	0.44	0.85	0.38	9.64
	26.5	0.52	0.42	1.36	0.57	15.03
	27.5	0.52	0.42	1.15	0.48	13.24
	28.5	0.52	0.39	0.66	0.26	7.35
	29.5	0.52	0.37	1.08	0.40	11.66
	30.5	0.52	0.34	0.71	0.24	7.34
	31.5	0.52	0.34	0.46	0.15	4.87
	32.5	0.52	0.31	0.44	0.14	4.47
	33.5	0.52	0.26	0.46	0.12	4.03
	34.5	0.52	0.21	0.16	0.03	1.19
	35.5	0.52	0.16	0.22	0.03	1.22
	36.5	0.52	0.10	0	0	0
	37.5	0.52	0.08	0	0	0
	38.5	0.52	0.08	0	0	0
	39.5	0.52	0.05	0	0	0
	40.5	0.52	0.05	0	0	0
	15.5	0.43	0.02	1.14	0.02	0.38
	16.5	0.43	0.13	1.71	0.22	3.60
	17.5	0.43	0.34	0.82	0.28	4.88
	18.5	0.43	0.38	0.44	0.17	3.14
Ta20	19.5	0.43	0.34	0.50	0.17	3.31
	20.5	0.43	0.26	0.47	0.12	2.48
	21.5	0.43	0.19	0.70	0.13	2.87
	22.5	0.43	0.17	1.14	0.19	4.36
	23.5	0.43	0.13	0.38	0.05	1.14
	24.5	0.43	0.11	0.68	0.07	1.78
	25.5	0.43	0.09	1.00	0.08	2.16
	26.5	0.43	0.09	0.28	0.02	0.64
	27.5	0.43	0.09	0.14	0.01	0.33
	28.5	0.43	0.06	0.57	0.04	1.04
	29.5	0.43	0.06	0.38	0.02	0.72
	30.5	0.43	0.06	0	0	0
	31.5	0.43	0.06	0	0	0
	32.5	0.43	0.06	0	0	0
	33.5	0.43	0.06	0	0	0
	34.5	0.43	0.04	0	0	0
	35.5	0.43	0.02	0	0	0

25 °C	AGE	SURV	LX	MX	LXMX	MXLXX
	10.5	0.45	0.18	3.40	0.61	6.42
	11.5	0.45	0.27	3.07	0.83	9.53
	12.5	0.45	0.36	2.30	0.83	10.36
	13.5	0.45	0.29	1.69	0.49	6.66
	14.5	0.45	0.20	1.76	0.36	5.15
	15.5	0.45	0.13	4.10	0.55	8.57
Ta4	16.5	0.45	0.11	2.28	0.26	4.23
	17.5	0.45	0.11	3.51	0.39	6.91
	18.5	0.45	0.07	2.34	0.16	2.92
	19.5	0.45	0.07	2.93	0.20	3.85
	20.5	0.45	0.07	2.93	0.20	4.05
	21.5	0.45	0.02	2.63	0.06	1.27
	22.5	0.45	0.02	0	0	0
	23.5	0.45	0.02	0	0	0
	10.5	0.18	0.03	3.33	0.09	0.95
	11.5	0.18	0.08	1.67	0.14	1.56
	12.5	0.18	0.12	2.54	0.30	3.74
	13.5	0.18	0.15	0.82	0.13	1.71
	14.5	0.18	0.15	0.50	0.07	1.05
Ta10	15.5	0.18	0.11	0.92	0.10	1.55
	16.5	0.18	0.07	0.75	0.05	0.90
	17.5	0.18	0.05	0.20	0.01	0.16
	18.5	0.18	0.03	1.00	0.03	0.50
	10.5	0.34	0.07	3.30	0.23	2.38
	11.5	0.34	0.34	2.02	0.69	7.97
	12.5	0.34	0.34	0.99	0.34	4.25
	13.5	0.34	0.24	0.79	0.19	2.55
	14.5	0.34	0.21	1.10	0.23	3.29
	15.5	0.34	0.14	1.10	0.15	2.34
	16.5	0.34	0.10	1.47	0.15	2.49
	17.5	0.34	0.10	1.10	0.11	1.98
Ta13	18.5	0.34	0.10	0.86	0.09	1.63
	19.5	0.34	0.05	0	0	0
	20.5	0.34	0.03	1.47	0.05	1.03
	21.5	0.34	0.02	1.47	0.03	0.54
	22.5	0.34	0.02	0	0	0
	10.5	0.27	0.11	2.92	0.32	3.34
	11.5	0.27	0.27	3.15	0.86	9.86
	12.5	0.27	0.23	3.42	0.79	9.87
	13.5	0.27	0.16	3.89	0.64	8.58
	14.5	0.27	0.15	2.70	0.40	5.86
	15.5	0.27	0.11	2.92	0.32	4.93
Ta19	16.5	0.27	0.08	1.30	0.11	1.75
	17.5	0.27	0.05	1.06	0.06	1.01
	18.5	0.27	0.05	1.42	0.08	1.43
	19.5	0.27	0.03	0.71	0.02	0.38
	20.5	0.27	0.03	1.77	0.05	0.99
	21.5	0.27	0.03	2.48	0.07	1.45
	22.5	0.27	0.01	0	0	0
	10.5	0.36	0.14	2.30	0.33	3.45
	11.5	0.36	0.32	2.46	0.79	9.08
	12.5	0.36	0.29	3.28	0.94	11.72
	13.5	0.36	0.20	3.94	0.77	10.44
	14.5	0.36	0.12	4.61	0.58	8.35
	15.5	0.36	0.09	3.32	0.30	4.59
	16.5	0.36	0.09	2.58	0.23	3.80

	17.5	0.36	0.07	2.07	0.15	2.59
Ta20	18.5	0.36	0.05	3.07	0.16	3.04
	19.5	0.36	0.05	1.54	0.08	1.60
	20.5	0.36	0.04	4.15	0.15	3.04
	21.5	0.36	0.02	1.84	0.03	0.71
	22.5	0.36	0.02	4.61	0.08	1.85

30 °C	**AGE**	**SURV**	**LX**	**MX**	**LXMX**	**MXLXX**
	8.5	0.57	0.37	3.70	1.36	11.54
	9.5	0.57	0.40	3.25	1.30	12.34
	10.5	0.57	0.27	2.49	0.66	6.97
	11.5	0.57	0.10	1.44	0.14	1.66
Ta4	12.5	0.57	0.10	2.31	0.23	2.89
	13.5	0.57	0.10	1.16	0.12	1.56
	14.5	0.57	0.03	0.87	0.03	0.42
	15.5	0.57	0.03	2.60	0.09	1.34
	16.5	0.57	0.03	0.87	0.03	0.48
	8.5	0.23	0.09	4.11	0.37	3.17
	9.5	0.23	0.17	3.22	0.55	5.21
	10.5	0.23	0.09	1.45	0.13	1.38
Ta10	11.5	0.23	0.09	0.24	0.02	0.25
	12.5	0.23	0.05	0.24	0.01	0.14
	13.5	0.23	0.05	0.00	0.00	0.00
	14.5	0.23	0.03	1.29	0.04	0.64
	15.5	0.23	0.02	0.00	0.00	0.00
	16.5	0.23	0.06	0.19	0.01	0.18
	17.5	0.23	0.02	0.00	0.00	0.00
	8.5	0.17	0.03	5.86	0.15	1.30
	9.5	0.17	0.10	6.15	0.59	5.58
Ta13	10.5	0.17	0.12	6.36	0.77	8.12
	11.5	0.17	0.09	4.98	0.43	4.97
	12.5	0.17	0.08	4.98	0.39	4.86
	13.5	0.17	0.05	4.39	0.23	3.09
	14.5	0.17	0.03	3.08	0.11	1.55
	15.5	0.17	0.03	2.64	0.07	1.06
	16.5	0.17	0.02	1.76	0.03	0.50
	8.5	0.36	0.05	0.00	0.00	0.00
	9.5	0.36	0.14	1.58	0.22	2.14
Ta20	10.5	0.36	0.28	1.71	0.49	5.10
	11.5	0.36	0.18	0.00	0.00	0.00
	12.5	0.36	0.09	0.00	0.00	0.00
	13.5	0.36	0.09	0.00	0.00	0.00
	14.5	0.36	0.05	0.00	0.00	0.00

Table 6-2 Life table data of five strains of *T. aurosum* reared at three alternating temperatures

20/10 °C	AGE	SURV	LX	MX	LXMX	MXLXX
	18.5	0.30	0.05	0	0	0
	19.5	0.30	0.09	2.22	0.20	3.90
	20.5	0.30	0.15	2.04	0.31	6.29
	21.5	0.30	0.20	1.98	0.39	8.32
	22.5	0.30	0.12	3.89	0.47	10.50
	23.5	0.30	0.08	1.78	0.13	3.13
	24.5	0.30	0.08	1.95	0.15	3.59
Ta4	25.5	0.30	0.08	1.78	0.13	3.40
	26.5	0.30	0.08	1.07	0.08	2.12
	27.5	0.30	0.08	1.78	0.13	3.67
	28.5	0.30	0.03	2.22	0.07	1.90
	29.5	0.30	0.03	3.11	0.09	2.75
	30.5	0.30	0.02	0	0	0
	31.5	0.30	0.02	0	0	0
	20.5	0.81	0.08	0	0	0
	21.5	0.81	0.44	3.13	1.39	29.85
	22.5	0.81	0.52	1.49	0.78	17.62
	23.5	0.81	0.65	1.66	1.07	25.10
	24.5	0.81	0.65	0.83	0.53	13.08
	25.5	0.81	0.60	0.71	0.43	10.89
	26.5	0.81	0.48	1.25	0.61	16.04
	27.5	0.81	0.40	0.79	0.32	8.81
Ta10	28.5	0.81	0.32	0.99	0.32	9.13
	29.5	0.81	0.32	1.32	0.43	12.60
	30.5	0.81	0.28	0.38	0.11	3.26
	31.5	0.81	0.28	0.50	0.14	4.49
	32.5	0.81	0.24	0.15	0.04	1.16
	33.5	0.81	0.12	0.29	0.04	1.19
	34.5	0.81	0.12	0.88	0.11	3.68
	35.5	0.81	0.12	0.29	0.04	1.26
	36.5	0.81	0.12	0.59	0.07	2.60
	37.5	0.81	0.12	0.88	0.11	4.00
	38.5	0.81	0.08	0.44	0.04	1.37
	39.5	0.81	0.08	0.44	0.04	1.41
	40.5	0.81	0.08	0.88	0.07	2.88
	41.5	0.81	0.08	0	0	0
	42.5	0.81	0.08	0.88	0.07	3.03
	43.5	0.81	0.04	2.65	0.11	4.65
	44.5	0.81	0.04	0	0	0
	45.5	0.81	0.04	0	0	0

	21.5	0.56	0.17	1.30	0.22	4.73
	22.5	0.56	0.28	0.70	0.20	4.46
	23.5	0.56	0.37	0.54	0.20	4.65
	24.5	0.56	0.54	1.56	0.84	20.49
	25.5	0.56	0.54	0.90	0.48	12.34
	26.5	0.56	0.51	1.78	0.90	23.91
	27.5	0.56	0.48	0.78	0.37	10.29
	28.5	0.56	0.48	0.74	0.35	10.03
	29.5	0.56	0.39	0.95	0.37	11.03
	30.5	0.56	0.39	0.84	0.33	10.07
	31.5	0.56	0.37	0.42	0.15	4.85
	32.5	0.56	0.37	0.78	0.29	9.30
	33.5	0.56	0.34	0.91	0.31	10.32
	34.5	0.56	0.31	0.07	0.02	0.76
	35.5	0.56	0.28	0.47	0.13	4.69
Ta13	36.5	0.56	0.23	1.46	0.33	12.05
	37.5	0.56	0.23	0.39	0.09	3.30
	38.5	0.56	0.23	1.07	0.24	9.32
	39.5	0.56	0.20	0.78	0.15	6.08
	40.5	0.56	0.17	0.91	0.15	6.24
	41.5	0.56	0.17	0.52	0.09	3.65
	42.5	0.56	0.17	0.52	0.09	3.74
	43.5	0.56	0.14	0.31	0.04	1.91
	44.5	0.56	0.11	0	0	0
	45.5	0.56	0.06	0	0	0
	46.5	0.56	0.06	0	0	0
	47.5	0.56	0.03	0.78	0.02	1.05
	48.5	0.56	0.03	0	0	0
	21.5	0.68	0.17	0.95	0.16	3.45
	22.5	0.68	0.48	1.58	0.75	16.87
	23.5	0.68	0.48	2.14	1.02	23.92
	24.5	0.68	0.44	0.61	0.27	6.56
	25.5	0.68	0.41	0.72	0.29	7.51
Ta19	26.5	0.68	0.34	0.87	0.29	7.81
	27.5	0.68	0.34	0.95	0.32	8.84
	28.5	0.68	0.27	0.89	0.24	6.87
	29.5	0.68	0.20	1.31	0.27	7.90
	30.5	0.68	0.20	1.31	0.27	8.17
	31.5	0.68	0.14	0.98	0.13	4.22
	32.5	0.68	0.14	0.79	0.11	3.48

	33.5	0.68	0.14	0.79	0.11	3.59
	34.5	0.68	0.10	0.79	0.08	2.77
	35.5	0.68	0.10	0.53	0.05	1.90
	36.5	0.68	0.10	0.79	0.08	2.93
	37.5	0.68	0.10	0	0	0
	38.5	0.68	0.07	0.39	0.03	1.03
	39.5	0.68	0.07	1.18	0.08	3.17
	40.5	0.68	0.03	2.36	0.08	3.25
	41.5	0.68	0.03	1.58	0.05	2.22
	42.5	0.68	0.03	0.79	0.03	1.14
	43.5	0.68	0.03	0.79	0.03	1.17
	21.5	0.50	0.12	0.56	0.07	1.50
	22.5	0.50	0.60	0.95	0.57	12.85
	23.5	0.50	0.47	0.18	0.08	1.96
	24.5	0.50	0.25	0.78	0.20	4.78
	25.5	0.50	0.25	0.73	0.18	4.62
	26.5	0.50	0.15	0.28	0.04	1.11
	27.5	0.50	0.12	0.78	0.10	2.68
	28.4	0.50	0.02	2.23	0.06	1.58
Ta20	28.5	0.50	0.12	0.45	0.06	1.59
	29.1	0.50	0.02	0	0	0
	29.5	0.50	0.10	0.14	0.01	0.41
	29.8	0.50	0.02	0	0	0
	30.5	0.50	0.10	0.70	0.07	2.12
	31.5	0.50	0.10	0.28	0.03	0.88
	32.5	0.50	0.07	0.19	0.01	0.45
	33.5	0.50	0.07	0.19	0.01	0.47
	34.5	0.50	0.07	0.19	0.01	0.48
	35.5	0.50	0.07	0.37	0.03	0.99
	36.5	0.50	0.07	0	0	0
	37.5	0.50	0.05	0	0	0

25/15 °C	AGE	SURV	LX	MX	LXMX	MXLXX
Ta4	12.5	0.41	0.04	5.18	0.22	2.76
	13.5	0.41	0.19	1.78	0.34	4.61
	14.5	0.41	0.26	2.75	0.70	10.19
	15.5	0.41	0.21	2.36	0.50	7.78
	16.5	0.41	0.13	1.41	0.18	2.98
	17.5	0.41	0.13	2.36	0.30	5.27
	18.5	0.41	0.11	3.01	0.32	5.94
	19.5	0.41	0.09	0.24	0.02	0.39
	20.5	0.41	0.09	0	0	0
	21.5	0.41	0.09	1.65	0.14	3.02
	22.5	0.41	0.06	1.88	0.12	2.71
	23.5	0.41	0.06	0.94	0.06	1.42
	24.5	0.41	0.04	0.94	0.04	0.98
	25.5	0.41	0.04	1.88	0.08	2.05
	26.5	0.41	0.04	0	0	0
	27.5	0.41	0.04	1.88	0.08	2.21
	28.5	0.41	0.04	0.94	0.04	1.14
Ta10	12.5	0.28	0.06	4.50	0.26	3.19
	13.5	0.28	0.09	2.53	0.21	2.90
	14.5	0.28	0.07	2.09	0.15	2.14
	15.5	0.28	0.23	2.86	0.65	10.05
	16.5	0.28	0.14	2.75	0.39	6.43
	17.5	0.28	0.13	2.11	0.27	4.70
	18.5	0.28	0.13	1.47	0.19	3.48
	19.5	0.28	0.06	2.84	0.16	3.14
	20.5	0.28	0.03	0.47	0.01	0.28
	21.5	0.28	0.01	1.90	0.03	0.58
	22.5	0.28	0.01	1.90	0.03	0.60
	23.5	0.28	0.01	0.95	0.01	0.32
	24.5	0.28	0.01	0.95	0.01	0.33
	25.5	0.28	0.01	0	0	0
	26.5	0.28	0.01	0	0	0
	13.5	0.35	0.04	5.29	0.19	2.54
	14.5	0.35	0.16	2.35	0.38	5.45
	15.5	0.35	0.34	3.95	1.33	20.62
	16.5	0.35	0.25	1.89	0.47	7.75
	17.5	0.35	0.21	2.13	0.45	7.94
	18.5	0.35	0.16	2.45	0.39	7.24
	19.5	0.35	0.09	1.41	0.13	2.44
	20.5	0.35	0.05	0.88	0.05	0.96
	21.5	0.35	0.04	2.65	0.09	2.02

Ta13	22.5	0.35	0.04	3.53	0.13	2.82
	23.5	0.35	0.04	3.09	0.11	2.57
	24.5	0.35	0.04	1.32	0.05	1.15
	25.5	0.35	0.02	1.76	0.03	0.80
	26.5	0.35	0.02	2.65	0.05	1.24
	27.5	0.35	0.02	3.53	0.06	1.72
	28.5	0.35	0.02	0	0	0
	29.5	0.35	0.02	0.88	0.02	0.46
Ta19	12.5	0.40	0.04	0	0	0
	13.5	0.40	0.12	4.81	0.58	7.88
	14.5	0.40	0.24	3.36	0.81	11.80
	15.5	0.40	0.34	1.65	0.57	8.78
	16.5	0.40	0.28	1.56	0.44	7.30
	17.5	0.40	0.18	1.36	0.25	4.34
	18.5	0.40	0.12	3.06	0.37	6.88
	19.5	0.40	0.08	1.75	0.14	2.76
	20.5	0.40	0.06	2.04	0.12	2.54
	21.5	0.40	0.06	1.46	0.09	1.90
	22.5	0.40	0.06	0.88	0.05	1.19
	23.5	0.40	0.02	0	0	0
Ta20	12.5	0.48	0.05	9.29	0.44	5.56
	13.5	0.48	0.14	2.15	0.31	4.17
	14.5	0.48	0.31	4.04	1.26	18.21
	15.5	0.48	0.41	2.23	0.91	14.08
	16.5	0.48	0.36	3.23	1.16	19.13
	17.5	0.48	0.26	1.91	0.50	8.79
	18.5	0.48	0.17	0.46	0.08	1.43
	19.5	0.48	0.07	2.96	0.21	4.14
	20.5	0.48	0.02	4.04	0.10	1.98
	21.5	0.48	0.02	0.81	0.02	0.42
	22.5	0.48	0.02	1.62	0.04	0.87

30/20 °C	AGE	SURV	LX	MX	LXMX	MXLXX
Ta4	10.5	0.24	0.06	3.81	0.23	2.40
	11.5	0.24	0.23	3.00	0.69	7.88
	12.5	0.24	0.20	1.68	0.34	4.28
	13.5	0.24	0.12	1.54	0.18	2.49
	14.5	0.24	0.11	2.11	0.23	3.31
	15.5	0.24	0.10	0.55	0.05	0.82
	16.5	0.24	0.02	0	0	0
	17.5	0.24	0.01	2.20	0.03	0.46
	18.5	0.24	0.01	0	0	0
	19.5	0.24	0.01	0	0	0
	20.5	0.24	0.01	0	0	0
	21.5	0.24	0.01	0	0	0
Ta10	11.5	0.08	0.08	3.40	0.26	3.00
	12.5	0.08	0.04	1.10	0.04	0.53
	13.5	0.08	0.02	1.33	0.03	0.41
	14.5	0.08	0.01	0.67	0.01	0.11
	15.5	0.08	0.01	0.50	0	0.06
	16.5	0.08	0.01	0.50	0	0.06
	17.5	0.08	0	0	0	0
Ta13	10.5	0.36	0.05	4.22	0.23	2.39
	11.5	0.36	0.34	3.81	1.30	14.96
	12.5	0.36	0.29	2.33	0.67	8.37
	13.5	0.36	0.23	3.14	0.73	9.89
	14.5	0.36	0.18	1.20	0.21	3.11
	15.5	0.36	0.14	1.67	0.24	3.72
	16.5	0.36	0.13	1.31	0.16	2.71
	17.5	0.36	0.07	1.23	0.09	1.55
	18.5	0.36	0.05	1.17	0.06	1.17
	19.5	0.36	0.02	2.11	0.04	0.74
	20.5	0.36	0.02	2.11	0.04	0.78
	21.5	0.36	0.02	0	0	0
Ta19	9.5	0.22	0.01	5.40	0.06	0.57
	10.5	0.22	0.08	3.73	0.29	3.02
	11.5	0.22	0.20	3.20	0.64	7.31
	12.5	0.22	0.09	1.01	0.09	1.12
	13.5	0.22	0.06	2.16	0.12	1.61
	14.5	0.22	0.03	0	0	0
	15.5	0.22	0.02	0.90	0.02	0.31
	16.5	0.22	0.01	0	0	0

	10.5	0.47	0.12	3.07	0.36	3.82
	11.5	0.47	0.36	4.78	1.70	19.52
	12.5	0.47	0.28	4.26	1.21	15.15
Ta20	13.5	0.47	0.26	2.71	0.71	9.55
	14.5	0.47	0.12	3.41	0.40	5.86
	15.5	0.47	0.09	3.20	0.30	4.70
	16.5	0.47	0.02	0	0	0
	17.5	0.47	0.02	11.09	0.26	4.60
	18.5	0.47	0.02	1.71	0.04	0.75

Table A7-1 Pair distances of *T. aurosum* alignment of the ITS2 region obtained by Clustal V (Weighted) (Percent Similarity in upper triangle, percent Divergence in lower triangle)

	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	***

Curriculum Vita

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Education

1979 – 1990	Primary and Secondary School. Kuwait-Kuwait
1990 – 1991	Secondary School. Jordan-Amman
1991 – 1995	B.Sc. in Plant Protection, Faculty of Agriculture, University of Jordan, Amman
1995 – 1998	M.Sc. in Entomology, Faculty of Graduate Studies, University of Jordan, Amman. Thesis title "Reproduction potential, susceptibility to the infestation of some vegetables and broad bean cultivars *Vicia faba* and the flight activity of black bean aphid *Aphis fabae* Scopli (Homoptera: Aphididae)"
04/2001 – 09/2005	PhD in biological control, Department of Plant Protection, Institute of Phytomedicine, University of Hohenheim, Germany.

Experiences

06/1998 - 08/2000	Agricultural engineering in the Department of Pesticide Researches, Studies and Registration (VAPCO). Amman, Jordan.
07/1995 - 10/1995	Training in Al-Daqaq Nursery for out and indoor ornamentals Amman, Jordan.
09/1995 - 06/1997	Teaching the practical part of entomological courses in the University of Jordan, Amman
01/ 1996 - 06/1996	Research assistant University of Jordan, Amman
06/2001 - 10/2004	Training and supervising of BSc., MSc. and TA students. University of Hohenheim, Germany.
04/2001 - 09/2005	PhD at the Department of Plant Protection, University of Hohenheim, Germany.

Conference Contributions

1. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie. Band 15. Entomologen Tagung. Dresden, Germany.
2. 7th Annual meeting of the GfBS. Society of Systematics Biology. Stuttgart, Germany.
3. 4th Meeting on Biological Control; *Trichogramma* knowledge and future prospects. BBA, Darmstadt, Germany.
4. 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit Growing and Viticulture. Weinsberg , Germany.
5. 6th Arab Congress of Plant Protection. 1997. Beirut, Lebanon.