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Is the parasitization capacity of *Trichogramma cordubensis* influenced by the age of the females?

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Abstract

We investigated the parasitization capacity of *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera: Trichogrammatidae) females aged 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h, using *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as hosts. Wasps were held without hosts during the period of ageing, therefore being increasingly time-limited with respect to parasitization as they got older. The total number of parasitized hosts decreased as the age of the parasitoid increased. However, the proportion of lifetime parasitism carried out on the first day increased with wasp age, up to 120-h old females. These results show that the parasitization capacity of ageing *T. cordubensis* females changes as they become time-limited. The consequences of such changes for biological control programs are discussed.

Introduction

Trichogramma cordubensis Vargas & Cabello (Hymenoptera: Trichogrammatidae) is a native thelytokous species of São Miguel island (Azores) (Pintureau et al., 1991). The effectiveness of *T. cordubensis* as a biological control agent for agricultural pests existing in the Azores islands has been investigated in our laboratory by studying parasitoid biology (Pinto & Tavares, 1991; Garcia & Tavares 1995, 1997; Garcia et al., 1995a), population dynamics (Garcia et al., 1995b), and rearing techniques (Tavares & Vieira, 1992).

Trichogramma can be active in the field several days after being released, although life expectancy depends on factors such as temperature (Pak & van Heiningen, 1985). During their lives, egg parasitoids may not immediately find suitable hosts, being increasingly time-limited as they get older. The influence of ageing egg parasitoids on parasitization capacity is not well-documented despite its importance in biological control programs. However, some work has been performed on parasitoid age and changes in the offspring sex ratio. Honda & Trjapitzin (1995), working with *Telenomus hugi* Honda & S. Trjapitzin (Hymenoptera: Scelionidae), observed a change from a female- to a male-biased sex ratio as female wasps became older. Some authors also observed a gradual shift in the offspring sex ratio from female to male with the ageing of *Trichogramma* females (Houseweart et al., 1983; Bai & Smith, 1993; Leatemia et al., 1995). Other authors, studying the effects of host deprivation on parasitism, worked with ageing wasps. Fleury & Boulétreau (1993) and Leatemia et al. (1995) observed that *Trichogramma* lifetime offspring production decreased as the period of the withholding of host eggs increased. However, all previous studies were done with arrhenotokous species, while *T. cordubensis* is one of the two strictly thelytokous species in the genus *Trichogramma* (Silva & Stouthamer, 1996). The work of Stouthamer & Luck (1993), Wang & Smith (1996), Horjus & Stouthamer (1995), and Hoogenboom et al. (1998) demonstrates that some life-history traits, such as fecundity, could differ between thelytokous and arrhenotokous wasps. Therefore, such traits should be investigated in thelytokous species in order to evaluate their quality as biological control agents.

This work deals with how strictly thelytokous *T. cordubensis* females allocate offspring to the hosts they encounter, as their life expectancy decreases. The purposes of this research are: (i) to evaluate the influence of ageing wasps on the number of parasitized hosts; (ii) to consider the implications of changes in the parasitization capacity of increasingly time-limited wasps for biological control programs.

Materials and methods

Biological material. A *T. cordubensis* colony was established from parasitized eggs of *Autographa gamma* L. (Lepidoptera: Noctuidae) collected at Ribeira do Guilherme (São Miguel island, Azores) in August 1995. The colony was reared in the laboratory, at 20 ± 1 °C, 75 ± 5% r.h., and L16:D8, for approximately 60 generations on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, according to the methods of Tavares & Vieira (1992).

Experimental design. Six treatment groups of female wasps were assembled according to their age: 24 h ($n = 36$), 48 h ($n = 38$), 72 h ($n = 34$), 96 h ($n = 38$), 120 h ($n = 40$), and 144 h ($n = 40$). Each female was isolated in a glass tube (7 × 1 cm). Wasps were deprived of hosts during the period of ageing. A drop of honey solution (10%) was poured in each tube to provide the parasitoid with a carbohydrate source.

The experiments were run in an environmental chamber at 20 ± 0.5 °C, 75 ± 5% r.h., and L16:D8. Eggs of *E. kuehniella* were presented to wasps on egg cards. These were prepared by spraying a fixed area (0.4 × 0.8 cm) of an index card with a water solution of non-toxic glue, and then spreading host eggs (200 ± 10 eggs) on this surface. The host eggs were less than 24 h old and had previously been irradiated with ultra-violet light for 20 min. Egg cards were replaced daily with fresh ones during seven consecutive days. According to Garcia & Tavares (1995) females of *T. cordubensis* oviposit more than 65% of their eggs during the first seven days of parasitism at 20 °C. Cards with parasitized eggs were maintained under the same conditions for offspring development. The number of parasitized hosts was determined by counting the host eggs that turned black, using a dissecting microscope at 25×.

Egg chorions of insects infested by *Trichogramma* turn black when parasitoids are in the prepupal stage. This is caused by black deposits that forms a cocoon-shaped cuticle around the body of the larva and inner surface of the chorion of the host egg (Saakian-Baranova, 1991). The black deposits are secreted through the labial glands by *Trichogramma* larvae when larvae have consumed all the ooplasm, thus host-eggshell blackening indicates the onset of prepupal stage (Dahlan & Gordh, 1997). Therefore, when determining parasitization capacity by counting the number of host eggs that turned black, the number of parasitized hosts with parasitoids that died before the onset of pupation is not known. However, this is only relevant when parasitoids are dealing with competition within the host due to superparasitism (Corrigan et al., 1995). This is not the case in our study, since the number of host eggs available per female wasp was high enough to avoid superparasitism (Wajnberg et al., 1989).

Longevity was determined by checking the number of dead parental females once a day, starting from the day of their adult emergence. Wasps who drowned in the honey were eliminated from the statistical analysis.

Statistical analysis. Samples were first described with regular average and standard errors. Then, analyses of variance (ANOVA) were conducted on data regarding the number of parasitized eggs and parental longevity. Where statistical differences existed between data sets ($P<0.05$), Fisher's Protected Least Significant Difference tests (PLSD) were used to separate differing means (Zar, 1996). To reduce variance differences, data concerning the number of parasitized eggs were transformed by $\sqrt{(x + 0.5)}$, and data concerning the percentages of parasitism were transformed by arcsine $\sqrt{(x)}$, before ANOVA was performed (Zar, 1996). An analysis of Covariance (ANACOV) was used to study the relationship between longevity and the number of parasitized eggs per female for wasps of different age (Zar, 1996).

Results

The number of parasitized eggs decreased significantly ($F_{5,220} = 20.94$, $P = 0.0001$) as the age of the parasitoid increased (Figure 1). Furthermore, wasps parasitized the highest number of hosts on the first day, sharply decreasing thereafter, regardless of the age of the females (Figure 2). Wasp age had a signifi-

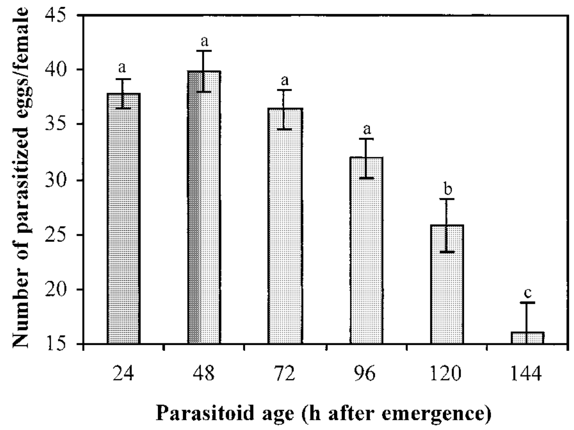

Figure 1. Age-specific number (detransformed mean ± s.e.) of parasitized eggs during seven days. Bars with different letters are significantly different at P<0.05 (PLSD procedure within ANOVA).

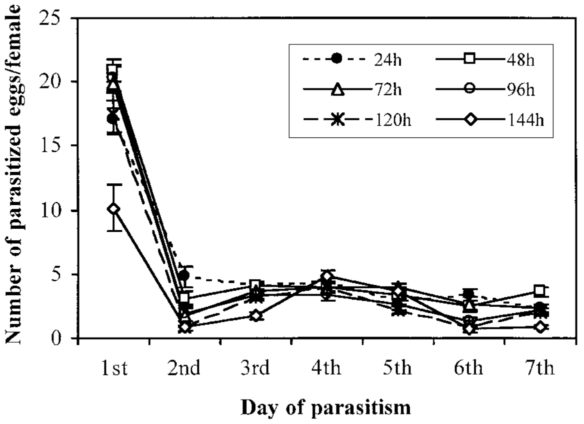

Figure 2. Age-specific number (detransformed mean ± s.e.) of parasitized eggs per wasp, for each day of parasitism.

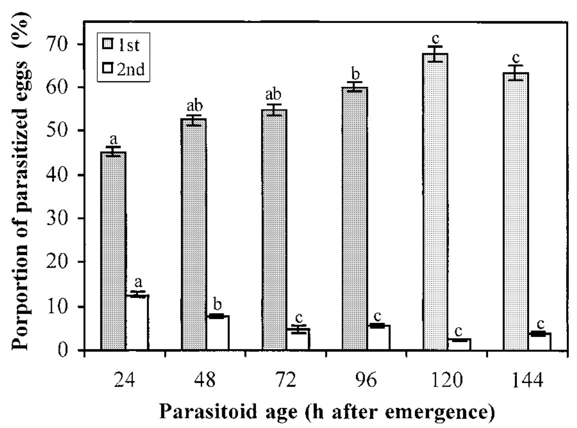

Figure 3. Age-specific percentages of the total lifetime parasitism (detransformed mean ± s.e.), that ocurred on the first and second day. Bars, within the same day of parasitism, with different letters are significantly different at P<0.05 (PLSD procedure within ANOVA).

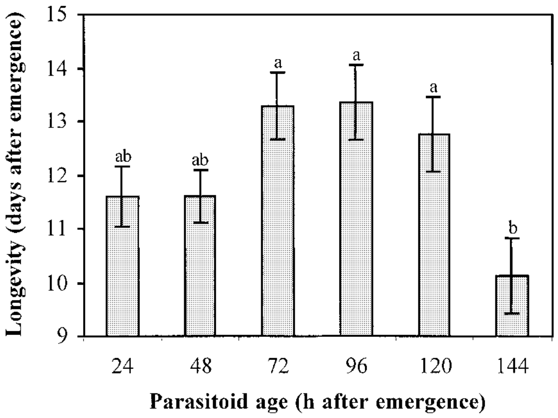

Figure 4. Longevity (mean ± s.e.) observed for *T. cordubensis* females of different age. Bars with different letters are significantly different at P<0.05 (PLSD procedure within ANOVA).

cant influence on the number of parasitized eggs, both for the first ($F_{5,220}$ = 9.45, P<0.0001) and for the second day of parasitism ($F_{5,220}$ = 9.45, P<0.0001; Figure 2). On the first day of parasitism, no significant differences were found for the number of parasitized eggs among females up to 120-h old (all PLSD tests at P>0.05). 144-h old females parasitized a significantly lower number of hosts on this day than did younger ones (P<0.0001, PLSD tests). On the second day, 24-h old wasps parasitized a significantly higher number of hosts than the older ones (P<0.05, PLSD tests). Finally, the mean percentage of the total lifetime parasitism that occurred on the first day increased with wasp age up to 120-h old females ($F_{5,191}$ = 4.28, P = 0.001; Figure 3). On the contrary, the mean percentage of the total lifetime parasitism that occurred on the second day decreased with wasp age up to 72-h old females ($F_{5,191}$ = 3.72, P = 0.0031; Figure 3).

The shortest life span was observed for the group of wasps at 144 h, differing significantly (P<0.05, PLSD tests) from the longevity of 72-h, 96-h, and 120-h old parasitoids (Figure 4). Although older females (144 h) show both the lowest longevity and parasitization capacity (Figures 1 and 4), survivorship does not seem to be the only mechanism to explain the decrease in the number of parasitized eggs as age increases. According to the analysis of covariance the wasp's longevity only explains 49% of the variation in the number of parasitized eggs ($F_{1,214}$ = 95.48, P<0.0001). Wasp age did not affect the number of parasitized eggs ($F_{5,214}$ = 1.55, P = 0.1769), and the interaction between longevity and wasp age was not significant, so the regression lines appeared to be

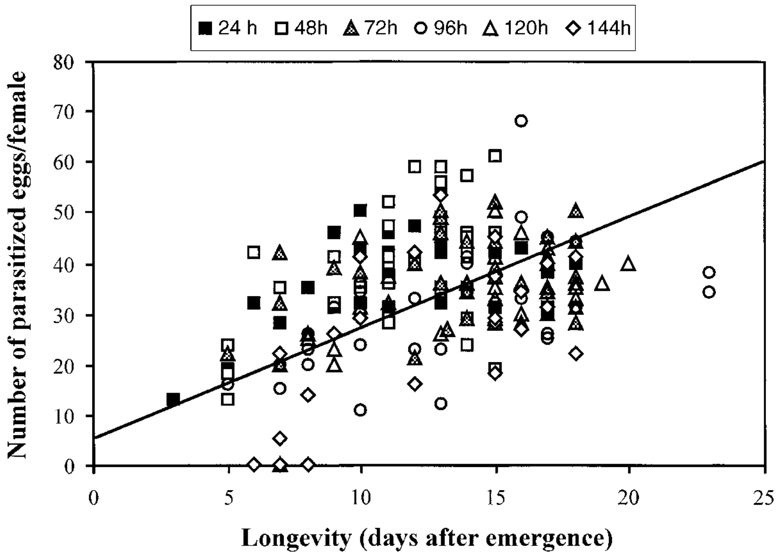

Figure 5. Relationship between the number of parasitized eggs per female of different age and longevity; $y = 4.94 + 2.15x$, $r^2 = 0.33$, P<0.0001.

statistically equivalent ($F_{5,214} = 0.79$, P = 0.5605; Figure 5). Since no significant difference was observed between the regressions computed for each individual wasp age, only the global regression ($y = 4.94 + 2.15x$, $r^2 = 0.33$, P<0.0001) was plotted in Figure 5.

Discussion

Our results show that *T. cordubensis* females that are becoming time-limited through ageing have different parasitization strategies. The total number of parasitized eggs decreased significantly as the age of the wasp increased. Although females with the lowest longevity are also those with the lowest parasitization capacity, survivorship does not appear to completely explain the decrease in the number of parasitized eggs as age increases. Fleury & Boulétreau (1993) also found that the longevity of *T. brassicae* females did not appear to explain the decrease in the number of parasitized eggs observed for older wasps.

According to the 'static optimization model' of host acceptance (Godfray, 1994), older wasps tend to maximize their progeny production by ovipositing a higher number of eggs as soon as suitable hosts are discovered. This model is directly applicable to parasitoids who are time-limited (both for host location and oviposition), as is the case in our experiments. In this model, parasitoids maximize their rate of gain of a quantity, such as the number of offspring produced (Godfray, 1994). Like all pro-ovigenic wasps, *T. cordubensis* parasitized the highest number of hosts on the first day, regardless of the age of the females. On the first day, age-specific percentages of the total lifetime parasitism increased up to 120-h old females, showing that as wasps approach the end of their lives, they maximize their progeny production. On the contrary, on the second day, age-specific percentages of the total lifetime parasitism decreased with age increase (up to 72-h old females). However, for wasps aged 144 h the number of parasitized eggs decreased sharply. Our results seem to indicate changes in host attack strategies adopted by ageing females of *T. cordubensis*, which are in accordance with the predictions of the 'static optimization model' for time-limited parasitoids. Furthermore, our results show no differences between *T. cordubensis* reproductive strategies and those observed by Fleury & Boulétreau (1993) and Leatemia et al. (1995) for arrhenotokous species, at least for the traits analysed in this study.

Rosenheim (1996) suggested that species which rarely become egg-depleted will often benefit by reallocating resources from 'excess' oocytes to other processes that make contributions to fitness, such as somatic maintenance. Our results show that wasps with longer life spans were those that were held without hosts for 72 h, 96 h, and 120 h. Longevity increase in these wasps may have occurred because oocytes were reallocated for somatic maintenance since females were held without hosts for more than three days. However, females with 72 h, 96 h, and 120 h were able to parasitize a large number of hosts, showing that they still had a high number of eggs in the ovaries. These results show that wasps approaching the end of their lives probably optimized their fitness, when equalizing between the risks of being egg depleted (due to egg resorption), but with a higher longevity, or dying with a large number of oocytes (due to the high probability of short life expectancy).

Under field conditions, host availability varies temporally and spatially. In both cases, a female wasp may not immediately find suitable hosts after being released. In such situations, the survival potential of *T. cordubensis* will likely be higher, since the parasitization capacity of this species was not negatively affected for wasps up to 96 h old. Furthermore, our results seem to indicate that ageing wasps allocated on the first day of parasitism a higher proportion of their lifetime production than on subsequent days, which also contributes to increase the survival potential of *T. cordubensis* when hosts are not immediately found after the parasitoid release.

Although *Trichogramma* releases are usually made with pupae, parasitoids can be released as adults in some biological control programs, to ensure a precise number of adult wasps in a given area (Smith, 1994). Thus, our results may have a potential application in such biological control programs, since the control potential of *T. cordubensis* might not be negatively affected by storage of adults for up to 96 h, allowing a greater flexibility in the timing of adult release. Experiments are now being performed in order to study the effects of wasp ageing on other important traits, such as parasitoid dispersal, host finding behavior, and host acceptance, since these traits might also be affected by prolonged unavailability of hosts.

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References

Bai, B. & S. M. Smith, 1993. Effect of host availability on reproduction and survival of the parasitoid wasp *Trichogramma minutum*. Ecological Entomology 18: 279–286.

Corrigan, J. E., J. E. Laing & J. S. Zubricky, 1995. Effects of parasitoid to host ratio and time of day of parasitism on development and emergence of *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) parasitizing eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). Annals of the Entomological Society of America 88: 773–780.

Dahlan, A. N. & G. Gordh, 1997. Development of *Trichogramma australicum* (Hymenoptera: Trichogrammatidae) at low and high population density in artificial diet. Entomophaga 42: 525–536.

Fleury, F. & M. Boulétreau, 1993. Effects of temporary host deprivation on the reproductive potential of *Trichogramma brassicae*. Entomologia Experimentalis et Applicata 68: 203–210.

Garcia, P., L. Oliveira & J. Tavares, 1995a. Comparative biology of three *Trichogramma* sp. populations captured in Azores. Boletim do Museu Municipal do Funchal 4: 311–318 (suppl.).

Garcia, P., L. Oliveira & J. Tavares, 1995b. *Trichogramma cordubensis* Vargas & Cabello (Hym., Trichogrammatidae): a dynamics study of an Azorean population. Les Colloques de l'INRA 73: 189–192.

Garcia, P. & J. Tavares, 1995. Parasitic capacity, longevity and development of *Trichogramma cordubensis* (Hym., Trichogrammatidae) at three temperature regimes. Les Colloques de l'INRA 73: 71–74.

Garcia, P. & J. Tavares, 1997. Biology of *Trichogramma cordubensis* (Hym., Trichogrammatidae) under different photoperiods. Boletin de la Associacion Española de Entomologia 21: 17–21 (suppl.).

Godfray, H. C. J., 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, NJ.

Honda, J. Y. & S. V. Trjapitzin, 1995. A species description and biological comparison between a new species of *Telenomus* Haliday (Hymenoptera: Scelionidae) and *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae): Two egg parasitoids of *Sabulodes aegrotata* (Guenée) (Lepidoptera: Geometridae). Pan-Pacific Entomologist 71: 227–236.

Hoogenboom, A., I. M. M. S. Silva, M. M. M. van Meer, M. M. Roskam & R. Stouthamer, 1998. Quality assessments of *Wolbachia* infected versus non infected lines of *Trichogramma deion*. Proceedings of Experimental & Applied Entomology of the Netherlands Entomological Society 9: 99–104.

Horjus, M. & R. Stouthamer, 1995. Does infection with thelytoky-causing *Wolbachia* in the pre-adult and adult life stages influence the adult fecundity of *Trichogramma deion* and *Muscidifurax uniraptor*? Proceedings of Experimental & Applied Entomology of the Netherlands Entomological Society 6: 35–40.

Houseweart, M. W., D. T. Jennings, C. Welty & S. G. Southard, 1983. Progeny production by *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) utilizing eggs of *Choristoneura fumiferana* (Lepidoptera: Tortricidae) and *Sitotroga cerealella* (Lepidoptera: Gelechiidae). Canadian Entomologist 115: 1245–1252.

Leatemia, J. A., J. E. Laing & J. E. Corrigan, 1995. Effects of adult nutrition on longevity, fecundity and offspring sex ratio of *Trichogramma minutum* Riley (Hym.: Trichogrammatidae). Canadian Entomologist 127: 245–254.

Pak, G. A. & T. G. van Heiningen, 1985. Behavioural variations among strains of *Trichogramma* spp.: Adaptability to field-temperature conditions. Entomologia Experimentalis et Applicata 38: 3–13.

Pinto, F. P. & J. Tavares, 1991. Longevity and parasitic capacity of the Azorean type of *Trichogramma cordubensis* (Hym., Trichogrammatidae). Les Colloques de l'INRA 56: 89–91.

Pintureau, B., L. Oliveira & L. Anunciada, 1991. Contribution to the study of the egg parasitic Hymenoptera of the Azores islands. Les Colloques de l'INRA 56: 115–118.

Rosenheim, J. A., 1996. An evolutionary argument for egg limitation. Evolution 50: 2089–2094.

Saakian-Baranova, A. A., 1991. Morphological study of preimaginal stages of six species of *Trichogramma* Westwood (Hymenoptera, Trichogrammatidae). Entomological Review 17–23.

Silva, I. M. M. S. & R. Stouthamer, 1996. Can the parthenogenesis-*Wolbachia* lead to unusual courtship behaviour in *Trichogramma*? Proceedings of Experimental & Applied Entomology of the Netherlands Entomological Society 7: 27–31.

Smith, S. M., 1994. Release of *Trichogramma* to control Lepidoptera. In: E. Wajnberg & S. A. Hassan (eds.), Biological Control with Egg Parasitoids. CAB International, Wallingford, UK, pp. 113–144.

Stouthamer, R. & R. F. Luck, 1993. Influence of microbe-associated parthenogenesis on the fecundity of *Trichogramma deion* and *T. pretiosum*. Entomologia Experimentalis et Applicata 67: 183–192.

Tavares, J. & V. Vieira, 1992. Produção em massa de *Ephestia kuehniella* Zeller (Lep., Pyralidae). IV- Técnicas de recolha dos adultos e ovos. Açoreana 7: 461–470.

Wajnberg, E., J. Pizzol & M. Babault, 1989. Genetic variation in progeny allocation in *Trichogramma maidis*. Entomologia Experimentalis et Applicata 53: 177–187.

Wang, Z. & S. M. Smith, 1996. Phenotipic differences between thelytokous and arrhenotokous *Trichogramma minutum* from *Zeiraphera canadensis*. Entomologia Experimentalis et Applicata 78: 315–323.

Zar, J. H., 1996. Biostatistical Analysis, 3rd ed. Prentice-Hall, Upper Saddle River, NJ.