BIOLOGICAL CONTROL

Host Deprivation Effect on Reproduction and Survival of *Wolbachia*-Infected and Uninfected *Trichogramma kaykai* Pinto & Stouthamer (Hymenoptera: Trichogrammatidae)

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Efeito da Suspensão de Hospedeiros sobre a Reprodução e Sobrevivência de *Trichogramma kaykai* Pinto & Stouthamer (Hymenoptera: Trichogrammatidae) Infectados e Não Infectados com *Wolbachia*

RESUMO – Comparou-se o efeito da suspensão temporária do fornecimento de hospedeiros sobre a reprodução e sobrevivência de *Trichogramma kaykai* Pinto & Stouthamer infectado e não infectado com *Wolbachia.* Ovos de *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) foram utilizados como hospedeiro. A progênie de fêmeas arrenótocas e telítocas foi reduzida com o aumento do período de ausência de hospedeiros, contudo fêmeas arrenótocas produziram significativamente mais progênie independentemente do período de ausência de hospedeiros (0 a 10 dias). Essas fêmeas produziram também um número maior de filhas. A ausência de hospedeiros não afetou o número de indivíduos emergentes por hospedeiro quando se consideraram as duas formas reprodutivas isoladamente. Por outro lado, menor número de parasitóides emergiu em ovos parasitados por fêmeas telítocas. Uma relação positiva existiu entre a duração da ausência de hospedeiros e a longevidade. Fêmeas arrenótocas viveram significativamente mais que fêmeas telítocas. Os resultados revelaram que as duas formas reprodutivas responderam de forma similar quando sujeitas a períodos de ausência de hospedeiro (de até 10 dias). Fêmeas arrenótocas, contudo foram mais fecundas e viveram mais que fêmeas telítocas em todos os tratamentos avaliados.

PALAVRAS-CHAVE: Insecta, arrenotoquia, disponibilidade de hospedeiros, partenogêneses, telitoquia.

ABSTRACT – The effect of temporary host deprivation on the reproduction and survival of *Trichogramma kaykai* Pinto & Stouthamer was determined by comparing a *Wolbachia*-infected (thelytokous) and an arrhenotokous line of *T. kaykai* when reared on *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) eggs. The progeny produced by both arrhenotokous and thelytokous females decreased with increasing periods of host deprivation, but arrhenotokous females produced significantly more progeny than thelytokous females regardless of the period of host deprivation (0 to 10 days). They also produced more daughters. Host deprivation did not affect brood size within arrhenotokous or thelytokous lines. However, fewer wasps emerged from hosts parasitized by *Wolbachia*-infected than by uninfected *T. kaykai.* A direct relationship existed between longevity and the length of time an arrhenotokous or a thelytokous wasp was deprived of hosts. The longer the deprivation period, the longer they lived. However, arrhenotokous females lived longer than their thelytokous counterparts. These findings showed that temporary host deprivation (up to 10 days) affected both reproductive forms similarly. However, in all conditions studied arrhenotokous wasps produced more progeny and lived longer than their thelytokous counterparts.

KEY WORDS: Insecta, arrhenotokous, host availability, parthenogenesis, thelytokous.

Trichogramma kaykai Pinto & Stouthamer (Hymenoptera: Trichogrammatidae) is a common egg parasitoid of *Apodemia mormo deserti* (C. & R. Felder) (Lepidoptera: Riodinidae), which lays eggs on *Eriogonium inflatum* (Torr. & Felder) (Polygonaceae) (Pinto *et al*. 1997). This parasitoid species consists of sympatric, interbreeding *Wolbachia*-infected parthenogenetic and uninfected arrhenotokous females that occur in the Mojave Desert of southern California (Stouthamer and Kazmer 1994, Pinto *et al.* 1997). As *A. mormo deserti* eggs are likely to be scarce during summer and fall most years and during years of low rainfall, the physiological state of the *T. kaykai* female (i.e., the appropriate balance of resources to be allocated to reproduction versus survival) will be a key factor for her reproductive success in the harsh climatic conditions of the desert.

Wolbachia infections have been associated with many different effects in their hosts, mating incompatibility, parthenogenesis, feminization and male killing (Werren 1997, Stouthamer *et al.* 1999). Parthenogenesis inducing infections are particularly common in the genus *Trichogramma* (Pinto & Stouthamer 1994) causing virgin females to produce only daughters (Stouthamer *et al.* 1990).

Studies have shown that carrying the bacterium comes at a cost for *Trichogramma* spp. *Wolbachia* infected females are less fecund (Stouthamer & Luck 1993, van Meer 1999, Hohmann *et al*. 2001) and suffer higher immature mortality (van Meer 1999, Hohmann & Luck 2000) than their uninfected counterparts. These differences, coupled with the observation that infected females mature eggs more slowly than uninfected females (Hohmann *et al.* 2001a), suggests that uninfected females have an advantage when they compete with the *Wolbachia* infected females for hosts in the Mojave desert. Moreover, Pak *et al.* (1985) found that *Trichogramma* spp., with a full complement of mature eggs, walk faster when foraging for hosts, and search longer than do females with fewer eggs. When *T. kaykai* females emerge, their ability to find hosts and to manipulate their egg load and life span, in response to host availability, have important implications for their reproductive success.

To determine whether host availability differentially affects progeny production and survival, the female offspring of field collected *Wolbachia*-infected and uninfected *T. kaykai* were deprived of hosts for different periods and then exposed to unlimited hosts for the rest of their lives. This study is an additional step of a larger project that aims to explain thelytokous and arrhenotokous coexistence in the desert of southern California.

Material and Methods

Parasitoid Culture. Two lines of *T. kaykai* were established. The first using the arrhenotokous offspring from two field-collected *A. mormo deserti* eggs, and the second line using the *Wolbachia*-infected (thelytokous) offspring from five different field-collected *A. mormo deserti* eggs. The hosts were collected on *E. inflatum* in Panamint Valley, Inyo County, California, spring 1998. Wasps after emerging from their natural hosts were individually exposed to *T. ni* eggs as described in Hohmann & Luck (2000). Few generations before the experiment started the offspring from the two arrhenotokous, and from the five thelytokous cultures were mixed resulting in two distinct reproductive form lines. To ensure that the thelytokous line was *Wolbachia* infected, a Polymerase Chain Reaction (PCR) assay with a specific *Wolbachia* primer *wsp*, was performed.

Experimental Procedures. To determine the effect of withholding hosts on the longevity and fecundity of arrhenotokous and thelytokous *T. kaykai* females (< 6h old), individual females from each line were randomly selected at emergence, isolated in an oviposition unit (9.5 x 2.5 cm glass shell vial), fed honey, and assigned to one of the five treatment groups, based on the number of days they were to be deprived of hosts: 0, 3, 6, 10, or their entire lifespan (for longevity only). After the host deprivation period was completed, each female was supplied daily with paper strips containing more than 50 eggs. The previous day's strips containing the parasitized eggs were transferred each to an empty vial, labeled, and held for parasitoid emergence. Total progeny (no. of F1 daughters + no. of F1 sons), total female progeny (no. of F1 daughters), % females [no. of females/(no. of females + no. of males) x 100], and brood size (no. of parasitoids emerging per host) produced by each female, parental female life span (days), and hind tibia length (HTL) (as described by Hohmann *et al.* 1988) were determined. The parasitoid cultures as well as the experimental units were maintained at 28 ± 1°C, RH 50 ± 10%, and a photophase of 16h.

Statistical Analysis. We compared treatment effects on life span, egg number, and lifetime progeny production by arrhenotokous and thelytokous females using ANCOVA (GLM procedure, SAS Institute Inc. 1994). The fecundity data were square root transformed, sqrt (x + 0.5), and the HTL was transformed using natural logarithms, ln (x). Data on sex ratio and clutch size were also analyzed using ANOVA (GLM procedure, SAS Institute Inc. 1994). Sex ratio data were arcsin transformed before analysis (Zar 1984). Comparisons between specific infected and uninfected treatment means were made using a Tukey Multiple Comparisons Test (Zar 1984).

Results

Temporary host deprivation had a similar effect on progeny production by both arrhenotokous (Table 1) and thelytokous *T. kaykai* females (Table 2), i.e., as the period of host deprivation increased, significantly fewer offspring were produced (ANCOVA with wasp strain, longevity, and HTL as covariates, $F_{[3, 82]} = 29.11$, $P<0.001$). Arrhenotokous wasps, however, consistently produced more progeny than thelytokous wasps (ANCOVA with host deprivation period and HTL as covariates, $F_{[1, 82]} = 187.88$, $P<0.001$). Arrhenotokous and thelytokous females deprived of hosts for three days following emergence produced a similar number of progeny as those provided of hosts since emergence. However, progeny production decreased by

Table 1. Lifetime progeny production and longevity (days) of field-collected arrhenotokous *T. kaykai* from Panamint Valley, California, deprived of hosts (*T. ni* eggs) for different periods of time.

Days without hosts	Sample size	Total progeny (mean±s.e.)	Sex ratio (% Females)	Brood size (mean±s.e.)	Longevity (mean±s.e.)
0	11	76.9 ± 4.85 a**	64 a*	1.56 ± 0.10 a**	16.1 ± 1.24 a**
3	11	74.5 ± 7.76 a	72 a	1.48 ± 0.06 a	19.5 ± 0.90 b
6	10	54.4 ± 5.40 b	73 a	1.55 ± 0.06 a	18.3 ± 1.20 a
10	11	36.9 ± 3.85 c	67 a	1.53 ± 0.05 a	21.2 ± 0.79 b
Life	10				20.9 ± 1.12 b

Means followed by the same letter within a column do not differ significantly at the 0.05 (*) and the 0.001 (**) level (Duncan's Multiple Range Test).

Table 2. Lifetime progeny production and longevity (days) of field-collected thelytokous *T. kaykai* from Panamint Valley, California, deprived of hosts (*T. ni* eggs) for different periods of time.

Days without hosts	Sample size	Total progeny (mean±s.e.)	Sex ratio (% Females)	Brood size (mean±s.e.)	Longevity (mean±s.e.)
0	11	26.8 ± 3.02 ab**	94 a*	1.29 ± 0.04 a**	9.0 ± 1.68 a**
3	12	31.0 ± 4.88 a	92 a	1.34 ± 0.03 a	14.7 ± 0.79 b
6	12	16.9 ± 2.01 bc	98 b	1.39 ± 1.20 a	16.1 ± 0.73 bc
10	11	13.5 ± 2.08 c	100 b	1.33 ± 1.10 a	19.2 ± 0.73 cd
Life	10				21.4 ± 1.22 d

Means followed by the same letter within a column do not differ significantly at the 0.05 (*) and the 0.001 (**) level (Duncan's Multiple Range Test).

30% and 37%, respectively, when wasps were deprived of hosts for six days, and approximately by 50% when they were deprived of hosts for ten days (Tables 1 and 2).

Wolbachia-infected *T. kaykai* produced a significantly higher percentage of female progeny (92-100%) than arrhenotokous females (64-73%), irrespective of the host deprivation period ($F_{[1,82]} = 166.17$, $P<0.001$). The sex ratio (% females) of the arrhenotokous offspring was independent of the host deprivation period ($F_{[3,41]} = 0.76$, $P>0.05$) (Table 1). In contrast, the sex ratio of thelytokous wasps increased with the host deprivation period ($F_{[3,42]} = 3.65$, $P<0.05$) (Table 2). This occurred because a thelytokous female ovipositing since emergence laid an increasing percentage of sons as she aged (Hohmann *et al.* 2001a). Thus, a thelytokous female lays more eggs when she is deprived of hosts for six or more days because she has a shorter reproductive lifespan. She produces a few sons towards the end of her reproductive lifespan when she is not deprived of hosts or deprived of hosts for three days. Even though thelytokous wasps produced a high percentage of females, arrhenotokous wasps produced a greater number of daughters than thelytokous wasps, regardless of the host deprivation period ($F_{[2,43]} = 4.66$, $P<0.05$) (Fig. 1). This occurred because arrhenotokous wasps produced substantially more progeny than thelytokous wasps (Table 1). However, fewer daughters were produced with increasing host deprivation period by both reproductive forms (ANCOVA with wasp strain and HTL as covariates, $F_{[3,83]} = 19.43$, $P<0.001$) (Fig. 1).

Host deprivation did not affect brood size emerging from a host in either the arrhenotokous (Table 1) or thelytokous (Table 2) wasps. However, significantly fewer offspring

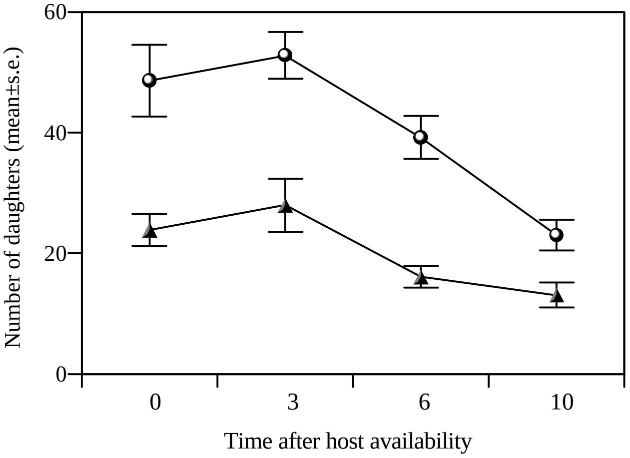

Figure 1. Effect of host availability on lifetime progeny produced by arrhenotokous (○) or thelytokous (△) *T. kaykai* females from Panamint Valley, Inyo Co., California, on *T. ni* eggs.

emerged per egg from hosts parasitized by thelytokous than by arrhenotokous females ($F_{[1, 85]} = 20.16$, $P<0.001$).

A direct and positive relationship existed between the life span of a parental female and the length of time she was withheld from hosts. As the host deprivation period increased, a female's life span increased in both arrhenotokous (Table 1) and thelytokous (Table 2) lines (ANCOVA with wasp strain and HTL as covariates, $F_{[3, 83]} = 15.97$, $P<0.001$). However, arrhenotokous females lived significantly longer than their thelytokous counterparts (ANCOVA, $F_{[1, 83]} = 24{,}15$, $P<0.001$), but only if they were offered hosts immediately after emergence (Tukey, $q= 6.56$, $P<0.001$, $n = 22$) or within three days of emergence (Tukey, $q = 4.89$, $P<0.05$, $n = 24$). Arrhenotokous and thelytokous females deprived of hosts for six or ten days lived for similar periods of time. Those withheld from hosts for their entire lives (ca. 21 days) lived significantly longer than their counterparts exposed to hosts since emergence (Table 1 and 2). Thus, wasps from both lines laid fewer offspring and lived longer with increasing periods of host deprivation.

Discussion

Temporary host deprivation had similar effects on reproduction parameters and survival of *Wolbachia*-infected and uninfected *T. kaykai*. An increase in the length of time during which a female was withheld from hosts decreased the total number of progeny she produced. High reproductive efforts early in life in both arrhenotokous and thelytokous females compromised their longevity. The longer the period of host deprivation the longer the wasps lived. Studies with different taxa have shown that organisms that invest more in reproductive effort may reduce their life expectancy because of a somatic-gametic trade-off (Reznick 1985, Bell & Kofoupanou 1986, Stearns 1992). Increasing longevity may occur at the expense of egg production. This does not appear to be the case with *Trichogramma* spp. since these parasitoids emerge with a full or almost full complement of eggs (Pak & Oatman 1982), and, if hosts are abundant, nearly all of their eggs are laid during the first few days of life (Pak *et al.* 1985, Hohmann *et al.* 1988). Increasing longevity, however, presumably increases the chance of a female encountering hosts.

Temporary host deprivation of up to 10 days followed by access to unlimited hosts for the remainder of her life did not affect the sex ratio of arrhenotokous wasps. This finding agrees with Fleury & Bouletreau (1993) who report no influence of host deprivation on the sex ratio of *T. brassicae* Bezdenko. In contrast, infected *T. kaykai* females, deprived of hosts for six or 10 days, produced significantly more females than those provided with unlimited hosts from emergence, or deprived of hosts for three days. Because the majority of the *Trichogramma*'s eggs are laid in the first 24h of their reproductive life (Pak & Oatman 1982, Hohmann *et al*. 1988), only daughters are laid during the first day of oviposition by a thelytokous female (Hohmann *et al.* 2001). A few male eggs begin to appear later in the reproductive life span of this reproductive form, thus the number of males that can potentially be produced by thelytokous females later in their reproductive life is reduced.

This study shows that temporary host deprivation (up to 10 days) affected both reproductive forms similarly. When females were deprived of hosts they lived longer, but they also produced fewer offspring. This suggests that a phenotypic trade-off exist between longevity and egg production. In all conditions studied arrhenotokous wasps produced more progeny and lived longer than their thelytokous counterparts.

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