

Phenotypic differences between thelytokous and arrhenotokous *Trichogramma minutum* from *Zeiraphera canadensis*

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Abstract

Both thelytokous and arrhenotokous *Trichogramma minutum* were collected from eggs of the spruce budmoth, *Zeiraphera canadensis* in New Brunswick, Canada and their phenotypic traits compared for use in biological control. The lower threshold temperature for development of thelytokous and arrhenotokous parasitoids was 9.7 and 10.1 °C, respectively; the former required significantly higher degree-days for development from egg to adult ($\bar{x} \pm SE = 165.1 \pm 5.8$ °D) than the latter (128.1 ± 4.9 °D). Thelytokous and arrhenotokous parasitoids had similar forewing length (0.49 ± 0.01 vs. 0.49 ± 0.01 mm) and adult lifespan (13.3 ± 0.7 vs. 14.0 ± 1.1 days), but significantly different fecundity and sex ratios. Thelytokous females produced fewer offspring (89.5 ± 6.6 vs. 173.9 ± 6.4) and fewer female progeny (77.2 ± 5.4 vs. 109.8 ± 3.3) despite an overall higher proportion of females ($91.6 \pm 1.1\%$ vs. $65.4 \pm 2.8\%$) than their arrhenotokous counterparts. Ovarian dissections showed that the number of eggs increased with parasitoid age in arrhenotokous parasitoids but remained steady in thelytokous parasitoids. The variation in ovarian development of the two forms was the major factor contributing to the differences in fecundity. Thelytokous parasitoids were more host-specific than arrhenotokous ones; when offered eight host species, the former rejected three whereas the latter rejected only one. Thelytokous parasitoids survived better than arrhenotokous ones when stored from 30 to 150 days at 4 °C. Thelytokous females were slower at initiating flight after emergence than arrhenotokous females but maintained flight activity longer (6 h). These results indicate that thelytokous *T. minutum* are different from their arrhenotokous counterparts physiologically, biologically and ecologically and that they may play different roles in the field. The potential for using thelytokous parasitoids in biological control programs is discussed.

Introduction

Parasitoids from the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) have been widely used as biological control agents in inundative release programs throughout the world. In Canada, the use of *T. minutum* Riley, an indigenous species in North American forests, has been developed as an effective alternative to chemicals against the destructive forest defoliator, spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). Experiments showed that inundative releases could result in egg parasitism rates of over 80% in release plots (Smith *et al.*, 1990). Because of these results and restrictions

on the use of chemical insecticides in the forest environment, the use of *Trichogramma* is now being examined for other forest insect pests, such as the spruce budmoth, *Zeiraphera canadensis* Mutuura & Freeman (Lepidoptera: Olethreutidae).

Most *Trichogramma* spp. used in inundative release programs are arrhenotokous, however, thelytokous forms have been found in 17 species of *Trichogramma* (Pinto & Stouthamer, 1994). Arrhenotoky is the common mode of reproduction in hymenopteran parasitoids, in which virgin females produce male offspring. By contrast thelytoky (virgin females almost always producing female offspring; Slobodchikoff & Daly, 1971; Stouthamer *et al.*, 1990a) is relatively

rare, but its distribution in the animal kingdom has led scientists to reexamine the traditional view of it as an 'evolutionary dead end' (Rössler & DeBach, 1973; White, 1984).

A thelytokous form of *T. minutum* was recently identified from eggs of *Z. canadensis* (J. D. Pinto & G. R. Platner, Univ. of California, Riverside, USA). A preliminary field survey showed that thelytokous individuals (>85%) dominated these natural *Trichogramma* populations from the spruce budmoth in New Brunswick (Wang, 1994).

From a practical viewpoint, thelytokous *Trichogramma* may be more efficient in biological control programs due to their higher female production and colonization rates (Aeschlimann, 1990; Stouthamer, 1993). Stouthamer *et al.* (1990a, b), Chen *et al.* (1992) and Stouthamer & Luck (1993) have all studied the origin of thelytoky, but a sound understanding of the biological and ecological attributes of thelytokous *Trichogramma* is necessary for their successful use as biocontrol agents.

Thelytokous and arrhenotokous *T. minutum* collected from the same host, *Z. canadensis*, were compared in terms of developmental time, size, longevity, fecundity, sex ratio, ovarian development, host range, cold tolerance and flight activity. These are important characteristics to analyze the potential of thelytokous *T. minutum* in biocontrol programs and to understand why thelytoky predominates in some natural populations.

Materials and methods

Parasitoid and host material. Both thelytokous and arrhenotokous *Trichogramma* used in the experiments emerged from overwintering eggs of the spruce budmoth. The parasitized eggs were collected from branch samples taken randomly from white spruce trees near Pokiok (46.5 ° W, 67.3 ° N), New Brunswick, Canada in 1992. Both lines were identified as *T. minutum* by J. D. Pinto and G. Platner (UC, Riverside, Calif). The two parasitoid lines were started from individual females ('isofemales') maintained separately (*c.* 5000 individuals/line) on irradiated eggs of the Mediterranean flour moth (MFM), *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae). The flour moth eggs were supplied weekly by the Biological Control Laboratory, Department of Environmental Biology, University of Guelph.

The host range experiments included eight species: black army cutworm, *Actebia fennica* Tauscher (Lepidoptera: Noctuidae), hemlock looper, *Lambdina fiscellaria* Guenee (Lepidoptera: Geometridae), eastern spruce budworm, *C. fumiferana*, western spruce budworm, *C. occidentalis* Freeman (Lepidoptera: Tortricidae), and gypsy moth, *Lymantria dispar* L (Lepidoptera: Lymantriidae) were obtained from the Forest Pest Management Institute, Canadian Forest Service, Sault Ste. Marie, Ontario. The spruce budmoth was collected from the field in New Brunswick. The wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) was reared in the Forest Pathology Laboratory, University of Toronto, and MFM eggs were provided by the University of Guelph. All experiments were conducted under laboratory conditions at 25 ± 1 °C, L16:D8 and 60 ± 10% r.h.

Developmental time. The developmental time of female parasitoids was determined by rearing the two lines on MFM eggs. After exposure to the parasitoids for 4 h at 25 °C, 100 eggs were kept in glass vials (25 × 95 mm) at each of six constant temperatures: 13, 15, 18, 20, 25, and 30 °C (±0.5 °C). Observations were made daily until the first adult emerged, after which the vials were checked every 8 h until all adults had emerged. The developmental time was considered to be that point where 50% of the females had emerged from each treatment. Linear regression was used to determine the developmental threshold (Wilson & Barnett, 1983). Degree-day development of the two lines was compared using a t-test (Wilkinson, 1990).

Body size, longevity, lifetime fecundity, sex ratio and ovarian development. Body size of females (n=30 females/line) was assessed by measuring the forewing length under a Wild M8 stereomicroscope as described by Bouchier *et al.* (1993). Longevity and lifetime fecundity were measured by providing individual females (n=30 females/line) with a 50% honey solution and *c.* 100 fresh MFM eggs daily until their death. Fecundity was determined by counting the number of parasitized (blackened) eggs. The sex ratio of the progeny was recorded after adult emergence. Differences between the two lines in these four traits were analyzed using t-tests (Wilkinson, 1990).

Ovarian development was assessed by taking newly emerged parasitoids from each line and dividing them into two groups (140 females/group). One group from each line was provided daily with *c.* 100 MFM eggs while the other group had no host eggs supplied.

Females from all four groups were kept individually in glass vials (35 × 10 mm) and fed a 50% honey solution. Ovarian development during the first seven days of adult life was measured for 20 females from each group by dissecting and counting the number of eggs in the ovaries each day after the start of the experiment. For those parasitoids supplied with host eggs, the number of eggs parasitized each day was also counted. The number of eggs in the ovaries upon emergence, eggs parasitized and eggs retained in the ovaries were transformed with reciprocal transformation and natural logarithms to normalize the data, and the differences were assessed using analysis of variance (Wilkinson, 1990).

Host range. The host range of the two lines of parasitoids was assessed by comparing their host recognition and ability to survive on the eight different lepidopteran species. To determine host recognition, one-day-old females from each line (n=30 females/line/host species) were placed individually in gelatin capsules (size 00) containing 1–5 host eggs. The time taken to initiate egg drilling was recorded during the first hour of observation. Only those parasitoids that had egg drilling during this period were counted. To determine host suitability, individual females from each line (n=30 females) were placed in 35 × 10 mm glass vials, provided with a 50% honey solution and offered *c. viable* 30–50 host eggs (>2 days old) for 24 h. The total number of offspring was counted after emergence. Differences between the two lines in the mean time taken to initiate drilling and the number of progeny were compared using t-tests (Wilkinson, 1990).

Cold tolerance. Tolerance to cold temperature in the two lines was determined by measuring adult emergence (survival) after long term cold storage. Thelytokous and arrhenotokous parasitoids were each provided with 6 egg cards of approximately 3000 fresh MFM eggs (5 replicates/line) for 8 h at 25 °C. The parasitized eggs were then placed at fluctuating temperatures of 20 °C for 16 h and 10 °C for 8 h under a photoperiod of L16:D8. They were kept under this regime until the prepupal stage, after which they were placed at 4 °C for either 30, 60, 90, 120 or 150 days (n=400 parasitized eggs/treatment, replicated 5 times). Emergence (counting the egg holes) and sex ratio were recorded for the two lines following their return to 25 °C. Two-way-analysis of variance (storage time and reproductive form as factors) and t-tests were used to compare

the effects of cold storage on adult emergence and sex ratio (Wilkinson, 1990).

Flight activity. Flight activity of the two lines was compared using the method described by Forsse *et al.* (1992). About 150 newly emerged females were placed in the bottom of a flight chamber for 12 h and the percentage of wasps flying to the top of the cylinder (fliers) recorded every hour. Five cylinders were used for each line. Differences between the two lines were examined using t-tests (Wilkinson, 1990).

Results

Developmental time. The thelytokous line took longer to develop than the arrhenotokous line, regardless of the temperature (Fig. 1). The developmental rate of the two lines increased linearly with temperature, the mean developmental rate (1/day) being 0.064 for thelytokous females and 0.080 for arrhenotokous females (Fig. 1). The lower threshold temperature for development of thelytokous and arrhenotokous parasitoids was 9.7 and 10.1 °C, respectively. The mean degree-days for development from egg to 50% female adult emergence was significantly greater (df=10, t=5.03, P<0.001) in thelytokous ($\bar{x} \pm \text{SE} = 165. \pm 5.8$ °D) than arrhenotokous females (128.2 ± 4.9 °D).

Body size, longevity, lifetime fecundity, sex ratio and ovarian development. Thelytokous and arrhenotokous females had similar forewing length and longevity, but had significantly different fecundity and offspring sex ratio (Table 1). Arrhenotokous females parasitized 1.9 times more host eggs than thelytokous ones, with a significantly greater proportion of parasitism during the first day of adult life (30.1 vs. 17.5%) (Table 1 and Fig. 2a). Thelytokous *T. minutum* produced only a few males one week after emergence (Fig. 2c). In contrast, the number of daughters produced by arrhenotokous females decreased with the age of the mother, and after the 11th day, all progeny were male (Fig. 2b+c). The total production of female offspring remained higher for arrhenotokous (109.8 ± 3.3) than for thelytokous females (77.2 ± 5.4) (Table 1).

Thelytokous females had significantly fewer mature eggs (df=38, t=10.02, P<0.001) in their ovaries (17.5 ± 0.5) upon emergence (day 0) than arrhenotokous ones (31.9 ± 1.4) (Fig. 3a). In parasitoids deprived of host eggs, the number of mature eggs in the ovaries fluctuated in the thelytokous line

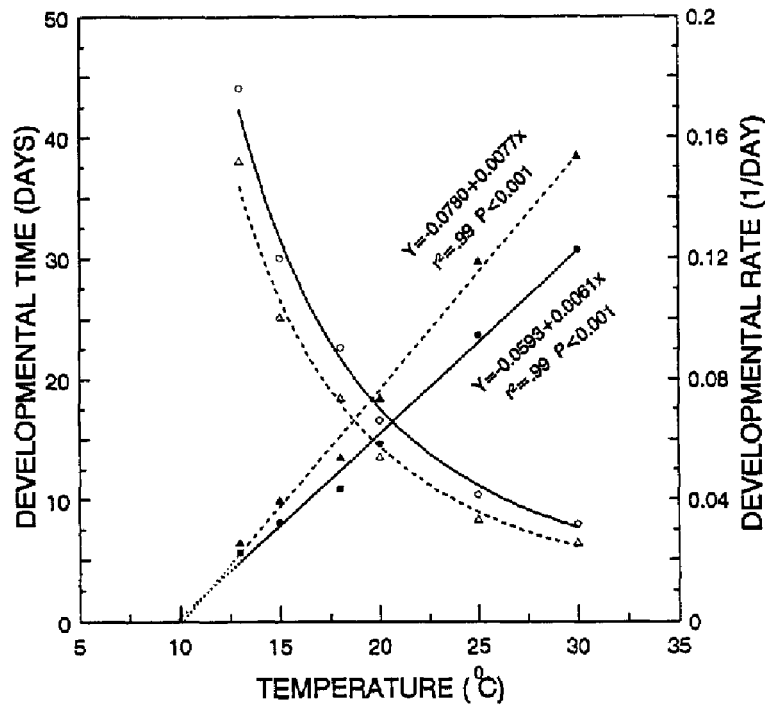


Fig. 1. Effect of temperature on the developmental time (clear symbols) and developmental rate (solid symbols) of thelytokous and arrhenotokous *Trichogramma minutum* females. Solid lines with circles represent thelytokous, dashed lines with triangles arrhenotokous parasitoids. The dotted lines represent extrapolations of the developmental rate to derive the lower threshold for development.

Table 1. Size, longevity, lifetime fecundity and progeny sex ratio for thelytokous and arrhenotokous *Trichogramma minutum* reared in *Ephestiu kuehniella* eggs (n=30, $\bar{x} \pm SE$)

Mode of reproduction	Wing length (mm)	Longevity (days)	No. eggs parasitized		Total production of progeny/		
			First day	Total	No. progeny emerged	No.	Sex ratio (%)
Thelytokous	0.49 \pm 0.01	13.3 \pm 0.7	15.7 \pm 0.9	89.5 \pm 6.6	84.3 \pm 6.1	77.2 \pm 5.4	91.6 \pm 1.1
Arrhenotokous	0.49 \pm 0.01	14.0 \pm 1.1	52.3 \pm 1.6	173.9 \pm 6.4	167.8 \pm 6.3	109.8 \pm 3.3	65.4 \pm 2.8
df	58	58	58	58	58	58	58
t	0.66	0.48	22.18	9.17	9.53	5.17	8.95
P	0.51	0.63	<0.001	<0.001	<0.001	<0.001	<0.001

(between 16.7 and 21.6 eggs/; ANOVA; $df=7,152$; $F=10.74$; $P<0.001$) and increased continuously in the arrhenotokous line (from 31.9 to 56.1 eggs/; ANOVA; $df=7,152$; $F=99.76$; $P<0.001$) (Fig. 3a). When host eggs were supplied, thelytokous females laid only part of their ovarian complement each day (i.e. 17.3, 3.1, 7.8, 2.0, 1.4, 4.0, and 2.8 eggs on each successive day after adult emergence) with an average of 6 eggs retained in the ovaries on any given day (Fig. 3b). In comparison, arrhenotokous females laid on average

46.1, 14.5, 14.8, 8.9, 5.1, 9.3 and 10.1 eggs on each successive day, retaining less than 3 eggs per day in their ovaries (Fig. 3b). The two lines differed significantly in the total number of eggs oviposited (ANOVA; $df=1,266$; $F=3279.09$; $P<0.001$) and the number of eggs retained ($df=1,266$; $F=148.12$; $P<0.001$) over the first seven days of adult life.

Host range. Thelytokous *T. minutum* took longer to initiate drilling into host eggs than arrhenotokous females

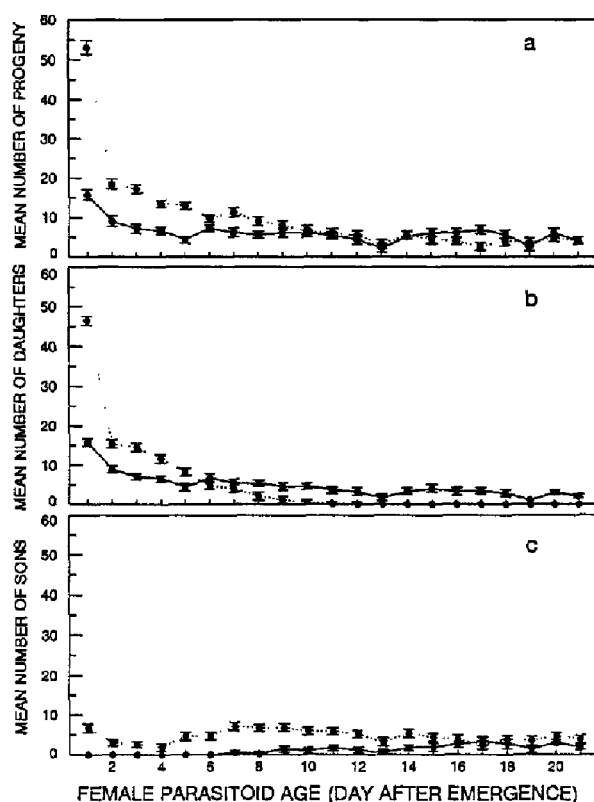


Fig. 2. Comparison of mean daily total progeny (a), daughters (b) and sons (c) produced by thelytokous and arrhenotokous *Trichogramma minutum*. Solid lines represent thelytokous parasitoids, and dashed lines represent arrhenotokous ones. Error bars are standard errors.

regardless of host species, the differences being significant for eggs of *A. fennica* ($df=48$, $t=2.91$, $P<0.05$), *G. mellonella* ($df=36$, $t=2.48$, $P<0.05$), and *L. dispar* ($df=34$, $t=2.18$, $P<0.05$) (Fig. 4a). Thelytokous parasitoids never contacted *C. fumiferana* eggs. Arrhenotokous parasitoids developed and produced adult progeny in seven of the eight host species, thelytokous in five only (Fig. 4b). Thelytokous parasitoids did not parasitize or develop in *C. fumiferana* and *C. occidentalis* but drilled into *C. occidentalis* (Fig. 4a and b). Both lines failed to develop in eggs of gypsy moth (Fig. 4b) although drilling behaviour was observed (Fig. 4a). Thelytokous females produced fewer progeny than arrhenotokous females, differences being significant for eggs of *A. fennica* ($df=58$, $t=2.28$, $P=0.03$), *E. kuehniella* ($df=58$, $t=10.9$, $P<0.01$), *G. mellonella* ($df=58$, $t=2.75$, $P<0.01$), and *Z. canadensis* ($df=58$, $t=2.97$, $P<0.01$) (Fig. 4b).

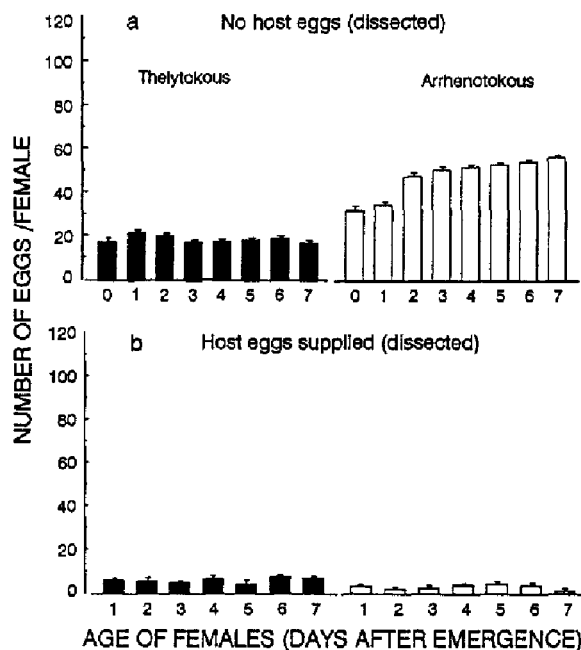


Fig. 3. Mean number of mature eggs in ovaries of thelytokous (solid bars) and arrhenotokous (clear bars) *Trichogramma minutum* during first seven days of adult life; (a) ovarian eggs dissected from females without host eggs. (b) ovarian eggs dissected from females with host eggs. Error bars are standard errors.

Cold tolerance. Adult emergence was reduced in both lines of *T. minutum* when parasitized eggs were held at 4 °C for varying periods (Fig. 5). There was a significant effect due to the storage time (ANOVA; $df=5,48$; $F=1209.71$; $P<0.001$), the reproductive mode ($df=1,48$; $F=84.68$; $P<0.001$) and the interaction between storage time and reproductive mode ($df=5,48$; $F=17.21$; $P<0.001$) with storage time being the most important in reducing adult emergence. Further analysis on the effect of storage time indicated that there were no significant differences between the two lines when parasitoids were not held (0 day) ($df=8$, $t=0.56$, $P=0.59$) or held for 90 days ($df=8$, $t=1.65$, $P=0.15$), while significantly ($P<0.05$) more thelytokous than arrhenotokous parasitoids emerged for all other storage times (Fig. 5). Storage time affected the sex ratio (female %) of the arrhenotokous line (i.e. 58.4, 51.5, 41.8, 47.9, 56.9 and 93.8% on each successive storage period) (ANOVA; $df=5,23$; $F=21.98$, $P<0.001$) but not of the thelytokous line (i.e. 100% female adults emerged from 0 to 150 days).

Flight activity. Thelytokous females flew significantly less than arrhenotokous females during the first ($df=8$, $t=6.37$, $P<0.001$) and second ($df=8$, $t=3.74$,

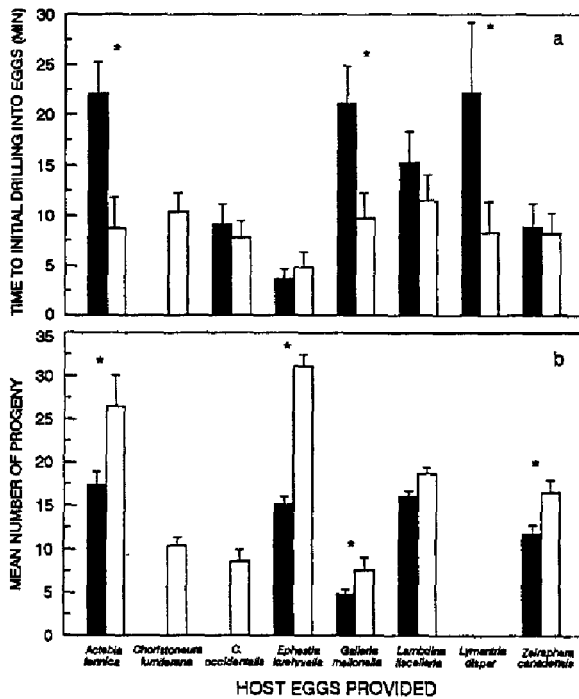


Fig. 4. (a) Time to initial drilling with ovipositor and (b) average number of adult progeny produced by thelytokous (solid bars) and arrhenotokous (clear bars) *Trichogramma minutum*. Error bars are standard errors. An asterisk indicates a significant difference between the two lines (t-test, $P < 0.05$) within a host species.

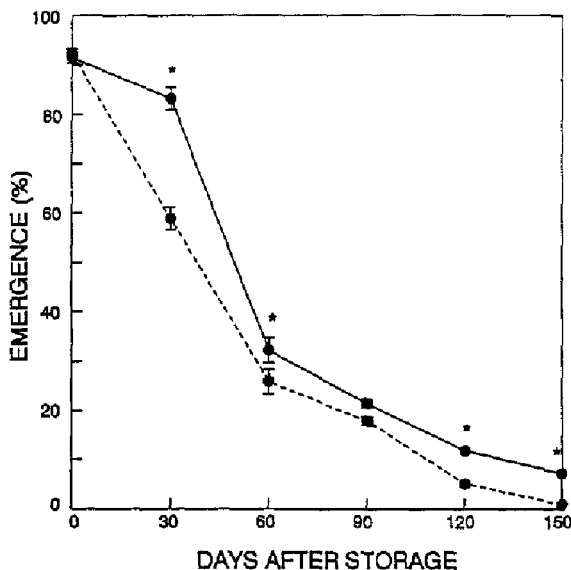


Fig. 5. Comparison of percent emergence of thelytokous (solid line) and arrhenotokous (dashed line) *Trichogramma minutum* after storage for varying lengths of time at 4 °C. Error bars are standard errors. An asterisk indicates a significant difference between the two lines at each hour (t-test, $P < 0.05$).

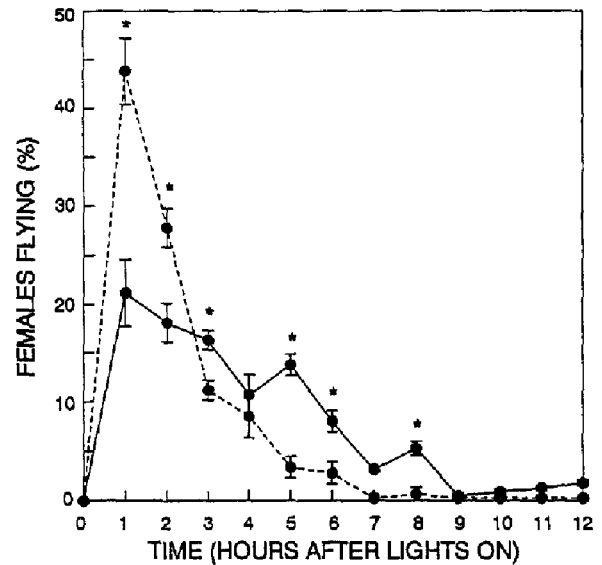


Fig. 6. Comparison of average flight activity in thelytokous (solid line) and arrhenotokous (dashed line) *Trichogramma minutum*. Error bars are standard errors. An asterisk indicates a significant difference between the two lines at each hour (t-test, $P < 0.05$).

$P = 0.006$) hours of flight (Fig. 6). For the next 6 h after emergence, however, flight propensity in thelytokous females was higher than that of arrhenotokous females; significant differences were observed in the third ($df = 8$, $t = 2.59$, $P = 0.03$), fifth ($df = 8$, $t = 4.17$, $P = 0.003$), sixth ($df = 8$, $t = 3.96$, $P = 0.004$), and eighth ($df = 8$, $t = 2.87$, $P = 0.02$) hours of flight (Fig. 6).

Discussion

Variation in developmental rates has been found among strains or species of *Trichogramma* reared on single or different host species over a range of temperatures (Butler & Lopez, 1980; Yu *et al.*, 1984; Smith & Hubbes, 1986). Thelytokous *T. minutum* required more time to develop than arrhenotokous females regardless of temperature and this was accompanied by a lower developmental threshold temperature and a higher number of degree-days. These characteristics might help to explain, physiologically and/or ecologically, why thelytokous parasitoids are the dominant form in the *T. minutum* complex from *Z. canadensis* in New Brunswick. Other reports have indicated that thelytokous insects have a lower thermal limit of activity and hence have a wider distribution in cold regions

than their sexually reproductive counterparts (Slobodchikoff & Daly, 1971; Suomalainen *et al.*, 1976).

Wasp size and fecundity are positively correlated (Bai *et al.*, 1992; Bouchier *et al.*, 1993). Despite their similar size and adult lifespan, thelytokous parasitoids produced significantly fewer offspring than arrhenotokous ones. This suggests that reproductive mode influences fecundity (Stouthamer & Luck, 1993). The reduction in the number of daughters produced by older arrhenotokous females may be due to a depletion of sperm in the spermatheca (Houseweart *et al.*, 1983; Chassain & Boulétreau, 1991). Thelytokous females produced only a few males toward the end of their adult life as was observed for other thelytokous parasitoids (Slobodchikoff, 1983; Stouthamer *et al.*, 1990a, b). Thelytokous parasitoids may have a distinct advantage over arrhenotokous ones in inundative release programs because they do not waste eggs producing males and this reduces the cost of mass production (Aeschlimann, 1990; Stouthamer, 1993). However, arrhenotokous females produced more female offspring and had higher parasitism levels than thelytokous females and this suggests that the production of males is not the only factor to be considered when evaluating parasitoid lines for release.

Trichogramma females are known to produce a large proportion of their progeny on the first day of adult life (Houseweart *et al.*, 1983; Yu *et al.*, 1984; Smith & Hubbes, 1986; Chassain & Boulétreau, 1991). This suggests that they are closer to pro-ovigenic (i.e. females having a large number of mature eggs in their ovaries upon emergence) reproduction than other parasitoids (Pak & Oatman, 1982; Pak *et al.*, 1985). Thelytokous parasitoids, however, are perhaps more similar to synovigenic parasitoids because they carry relatively few mature eggs in their ovaries at emergence and display a steady pattern of ovarian development. Pro-ovigenic parasitoids are able to exploit spatially abundant but temporally limited hosts because they can lay a large number of eggs in a relatively short period of time. On the other hand, synovigenic parasitoids are more suited to exploiting scarce hosts available over relatively long periods of time because they conserve reproductive material (Flanders, 1950). *Zeiraphera canadensis* is probably closer to the latter group of hosts than the former because its eggs are laid individually in hidden locations under the bud scales of the host tree and are acceptable for parasitism for several weeks (Ostaf & Quiring, 1994). Thelytokous parasitoids may be more adapted to this type of cryptic host because their slow pattern of ovarian development

allows them more energy for search than arrhenotokous parasitoids, and this may account for their predominance in natural populations on *Z. canadensis* (Wang, 1994). This also supports the view that thelytokous parasitoids are more effective against sparse hosts than arrhenotokous parasitoids (Slobodchikoff & Daly, 1971; Gerritsen, 1980; Suomalainen *et al.*, 1987; Aeschlimann, 1990; Stouthamer, 1993), and also partially explains why thelytokous females are less fecund than arrhenotokous ones.

Thelytokous females took longer to initiate drilling than arrhenotokous females, perhaps due to differences in ovarian development. Neither line of parasitoids accepted gypsy moth eggs, but thelytokous *T. minutum* were more host restricted like the thelytokous *T. nr. sibiricum* (Bai *et al.*, 1995).

Thelytokous parasitoids appear to be more tolerant of cold temperatures than arrhenotokous ones and their relatively lower threshold temperature and higher degree-days for development may be linked to this trait. Moreover, the field surveys showed a higher proportion of thelytokous individuals than arrhenotokous ones in overwintering populations (Wang, 1994). Cold tolerance in parasitoids is an important advantage for mass rearing and handling (Stinner *et al.*, 1974; Zhu & Zhang, 1987).

Differences between the two lines in flight activity may be related to differences in ovarian development. Smits (1982) found that newly emerged *Trichogramma* females had more mature eggs in the ovaries and hence, had a stronger flight response than older females. Pak *et al.* (1985) reported that *Trichogramma* females walked more frequently and faster when the ovarian egg supply was high and Flanders (1950) suggested that synovigenic parasitoids were less mobile than pro-ovigenic parasitoids because they had fewer mature eggs in their ovaries. As a trade-off in metabolic costs, thelytokous parasitoids may spend more time and energy in locating host eggs than arrhenotokous parasitoids and hence, be more efficient at searching for cryptic hosts.

Thelytokous parasitoids have been regarded as candidates to improve the success of biocontrol operations (Aeschlimann, 1990; Stouthamer, 1993), but all aspects of thelytoky should be considered. Thelytokous *Trichogramma* spp. have a lower fecundity but a higher cold tolerance. Due to their synovigenic characteristics, they may be better suited overall for use against *Z. canadensis* in Canadian forests.

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