Long-Term Mass Rearing Impacts Performance of the Egg Parasitoid *Telenomus remus* (Hymenoptera: Platygastridae)[1]

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Abstract The effects of long-term laboratory rearing on the performance of *Telenomus remus* Nixon (Hymenoptera: Platygastridae), an egg parasitoid of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), were investigated by comparing a laboratory-reared strain with a wild strain of the parasitoid. Restriction fragment length polymorphism revealed no genetic variation between the two strains. The wild strain exhibited a higher level of egg parasitism, a longer reproductive period, and a higher level of fecundity than did the laboratory-reared strain while developmental time and female longevity did not differ between the strains. Furthermore, host search efficiency and flight ability were superior in the wild strain compared to the laboratory strain, but oviposition behavior did not differ. Our results indicate that, over time, laboratory rearing may select for individuals that are less fertile, less aggressive, and have lower flight activity than their wild counterparts, which also exhibit a higher parasitism performance and superior potential for population increase in a shorter period of time. Implications for biological control using *T. remus* and strategies to avoid losses of parasitism efficiency are discussed.

Key Words mass rearing, parasitoid performance, *Telenomus remus*, *Spodoptera frugiperda*

The success of biological control using parasitoid releases depends on the efficiency of mass rearing of the parasitoids. Performance of insects subjected to prolonged mass rearing can be altered over generations due to adaptation to laboratory conditions, founder effect, processes of inbreeding, and genetic selection (Baitha 2005; Canale and Benelli 2012; Dutton and Bigler 1995; Nagarkatti and Nagaraja 1978; Oliveira et al. 2005; van Lenteren 2003,). Long-term mass rearing in the laboratory may decrease genetic variability and fitness in the population, thereby leading to suboptimal field performance of released individuals (van Lenteren 2003). Indeed, alterations in reproductive and behavioral traits have been reported in mass-reared hymenopteran egg and larval parasitoids (Baitha 2005; Canale and Benelli 2012; Nagarkatti and Nagaraja 1978, van Hezewijk et al. 2000).

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Periodic reinvigoration of laboratory colonies with introductions of individuals collected from the wild may be required to avoid such declines in fitness and performance (Joyce et al. 2010).

The egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae), native to New Guinea, has been successfully used in classical biological control programs targeting armyworms (*Spodoptera* spp.) in a number of different countries (González and Zocco 1996; Singh 2004; Yaseen et al. 1981). *Telenomus remus* was successfully established following inundative releases, with parasitism levels exceeding 80% in India (Joshi et al. 1982; Singh 2004), the Caribbean islands (Cock 1985; Yaseen et al. 1981), Guiana and Suriname (Cock 1985; Yaseen et al. 1981), Venezuela (Ferrer 2001; González and Zocco 1996; Hernández et al. 1989,), and Colombia (García-Roa et al. 2002). Cave (2000) demonstrated that *T. remus*, in laboratory conditions, can parasitize 80–100% of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) eggs.

Yet, efforts to biologically manage *S. frugiperda*, commonly known as the fall armyworm, in Brazil with releases of *T. remus* have not proven as successful as in other countries (Varella et al. 2015). We suspect that these failures might be at least partially attributed to the continuous rearing of *T. remus* prior to releases. In fact, *T. remus* has been continuously reared in the Applied Ecology Laboratory at São Paulo State University (UNESP, Jaboticabal, São Paulo [SP], Brazil) since 2004. This colony was initiated using insects obtained from a laboratory colony maintained at EMBRAPA Maize and Sorghum, Sete Lagoas, MG, Brazil. Thus, we estimated that the *T. remus* colony used for releases had been reared in the laboratory (both locations) for almost 600 generations without any evidence of adding wild sources of the parasitoid to the colony.

The study reported herein was undertaken to assess the possible effects of the long-term mass rearing of *T. remus* on parasitoid performance. We compared the EMBRAPA laboratory-reared colony of *T. remus* with a field-collected wild strain of *T. remus* from Venezuela. According to the International Organization for Biological Control, parasitoid strains need to be selected based on characteristics such as fecundity, longevity, development time, sex ratio, behavior, and locomotion (Hassan 1994). Therefore, we assessed (a) genetic diversity, (b) reproductive parameters and fertility life tables, (c) parasitic behavior, and, (d) flight activity of the wild strain from Venezuela and from the EMBRAPA laboratory-reared strain.

Materials and Methods

Insects. The laboratory strain was obtained from EMBRAPA Maize and Sorghum Research Center (Sete Lagoas-MG, Brazil) and had been in continuous laboratory culture for approximately 600 generations. The wild strain was introduced from Venezuela (Ministry of Agriculture, Livestock, and Supply permit number 21052.010177/2011-40) to the Costa Lima Quarantine Laboratory (EMBRAPA, Jaguariúna, SP). The colony was initially established with 650,000 individuals collected from S. frugiperda infesting maize in Barquisimeto, Lara State, Venezuela. The insects remained in quarantine for six generations as prescribed by the importation process, during which the colony was screened for contaminants, adapted to laboratory conditions, and initially screened for host range to nontarget

organisms (Sá et al. 2000). The colony was maintained on *S. frugiperda* eggs according to Oliveira et al. (2006). After establishment, wild strain individuals were sent to Instituto Biológico, Campinas-SP, for hyperparasitoid screening and taxonomic identification. Voucher specimens were deposited in the quarantine laboratory collection (number 23/2012 – LQC).

Molecular analysis. The mitochondrial DNA fragment *16S* rRNA and internal transcribed spacer 1 (ITS1) of ribosomal RNA (rDNA) for both strains were extracted, amplified, and analyzed. These fragments are often used in analysis of population genetic variability (Hartl and Clark 2010; Weekers et al. 2001). Twenty-five randomly selected females from both strains were placed in absolute alcohol. Extraction was performed using the Wizard Genomic DNA Purification Kit (Promega Corp., Fichburg, WI) following the protocol supplied by the manufacturer. After extraction, DNA was quantified using the Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA).

The *16S* rRNA gene and ITS1 region were amplified by polymerase chain reaction (PCR) performed in a thermocycler Mastercycler® (Eppendorf, Hamburg, Germany) with a final volume of 25 μl (12.5 μl of GoTaq® Green Master Mix 2x [Promega Corp.], 0.2–0.6 μM of each primer, and approximately 40 ng DNA total). The products were electrophoresed in 1% agarose gel and stained with ethidium bromide to confirm the amplification.

The primers LR-J-12887 (5′ CCGGTTTGAACTCAGATCATGT 3′) and SR-N-13398 (5′ CRCYTGTTTAWCAAAAACAT 3′) were used for amplification of the *16S* rRNA gene (Simon et al. 1994) under the following conditions: initial denaturation at 95°C (3 min) followed by 5 cycles of 15 s at 92°C, 45 s at 48°C, 2 min and 30 s at 62°C, 29 cycles of 15 s at 92°C, 45 s at 52°C, 2 min at 62°C, and a final extension step of 62°C (7 min). The ITS1 region was amplified using the primers CS249_F (5′ TCGTAACAAGGTTTCCG 3′) (Schlötterer and Tautz 1994) and DT421_R (5′ GCTGCGTTCTTCATCG 3′) (White et al. 1990) under the following conditions: 27 cycles at 94°C (1 min), 2 min at 45°C, 2 min, and 30 s at 72°C.

Eleven restriction endonucleases (AluI, DpnI, DraI, EcoRI, HaeIII, HincII, NlaIII, MboI, MseI, MspI, and TaqI) were used for restriction fragment length polymorphism (RFLP) of *16S* rRNA and ITS1, resulting in 22 molecular markers. The digestion reactions were performed according to the manufacturer's recommendations (Thermo Fisher Scientific). The digested products were visualized on 2% agarose gel stained with ethidium bromide.

Reproductive parameters. Reproductive performance of each colony was assessed by quantifying the number of host eggs parasitized during the female parasitoid lifespan as well as by survivorship of progeny of the ensuing generation. For these assessments, 20 randomly selected *T. remus* females (<24 h old), mated and without previous oviposition experience (i.e., naïve) from each colony, were placed individually in glass tubes (2 cm diameter × 8 cm long) containing a drop of honey. One *S. frugiperda* egg mass (<24 h old) with approximately 250 eggs was offered to each female for 24 h. Egg masses were replaced daily for the duration of the life of each female. Egg masses and parasitoids were held in an environmental chamber maintained at 25 ± 1°C, 70 ± 10% relative humidity, and on 12 L:12 D photophase.

Egg masses exposed to the females were transferred to glass tubes maintained under identical conditions until parasitoid emergence. These were inspected daily to

remove *S. frugiperda* neonates that could damage parasitized eggs. Number of parasitized eggs, cumulative parasitism, total number of parasitized eggs per female (total fecundity), and female longevity were recorded for each female. Developmental time was established based on the interval between oviposition and parasitoid emergence. Once the parasitoids emerged (F1 progeny), they were fed with honey and observed daily to assess their survival. After death, the gender of each individual was determined with antennal morphology (Cave 2000).

The survivorship (*lx*: survival rates in *x* age), age-specific fecundity (*mx*: offspring produced per female at age *x* that are female), and sex ratio in relation to female age (*x*) were calculated through life table analyses. Based on this information, the following parameters were calculated: net reproductive rate (R_o); generation duration (T); intrinsic rate of increase (r_m); daily finite rate of increase (λ); and the population doubling time (Dt).

Parasitoid behavior. No-choice tests compared the host searching efficiency and oviposition behavior of each colony. The behavioral sequence was described in an ethogram using one naïve female per replication ($n = 30$) of each strain, in the same conditions as previously described. Females were released in glass tubes, approximately 3 cm from an egg mass containing approximately 100 *S. frugiperda* eggs. Oviposition time and parasitoid behavior events were observed and recorded for 10 min as per Vet et al. (1983). The host searching efficiency was estimated by recording the time for females to find the egg mass.

Flight activity. Flying test units, similar to the model developed by Prezotti et al. (2002), were used to assess parasitoid flight activity. Each unit consisted of a PVC cylinder (10 cm diameter $\times$ 21.5 cm high) internally painted with black acrylic paint. The upper opening of the cylinder was sealed with a circular yellow sticky trap lid (Biotrap®, Greenlid Enviroscienses, Toronto, Canada) and clear tape. An opening (2 cm diameter) in the center of the lid was constructed to allow light access and induce a positive phototropic response in the parasitoids. The bottom of the cylinder was sealed with black plastic. A strip of yellow sticky trap (5 mm wide) was placed 3.5 cm from the lower end of PVC cylinder on the inner wall, forming an adhesive barrier to entrap walking individuals.

Spodoptera frugiperda egg masses (<24 h old) with approximately 100 eggs each were offered to naïve and mated *T. remus* females for 24 h. One parasitized egg mass was placed in a glass tube (8 $\times$ 2 cm) on the bottom of the test unit 24 h before emergence. This was repeated 10 times. Honey was provided in droplets on the walls of the glass tubes. The test units were maintained in environmental chambers as previously described for 48 h after emergence. After this period, the insects were frozen and counted. Parasitoids trapped in the adhesive barrier (walkers), on the lid (fliers), and on the bottom (nonfliers) were counted under a stereoscopic microscope. Data were expressed as percentage of emerged adults.

Statistical analysis. The average parasitism, longevity, development time, and oviposition time of each of the colonies were subjected to analysis of variance and a Fisher's test. F1 survival was compared with the Kaplan-Meier test with the multiple comparisons method using the log-rank (Laumann et al. 2008) available in the 2011 IBM SPSS Statistics program (IBM 2011). Fertility life table parameters and their associated standard errors and confidence intervals were estimated using the PROC LIFETEST (SAS Institute 2013) with the "jackknife" technique, and means were calculated by one- or two-tailed *t*-tests ($P < 0.05$) (Maia et al. 2000). The

Table 1. RFLP fragment sizes of *Telenomus remus* 16S rRNA gene and ITS-1 region. LS: Laboratory-reared strain. WS: Wild strain.

	16S rRNA		ITS-1	
Enzymes	**LS**	**WS**	**LS**	**WS**
*Alu*I	*	*	550 bp	550 bp
			400 bp	400 bp
*Dpn*I	*	*	550 bp	550 bp
			400 bp	400 bp
	450 bp	450 bp		
*Taq*I	75 bp	75 bp	200 bp	200 bp
			150 bp	150 bp
			100 bp	100 bp
*Mbo*I + *Mse*I	100 bp		650 bp	650 bp
	50 bp	100 bp		
		50 bp		
*Dra*I + *Hin*cII	250 bp	250 bp	500 bp	500 bp
	200 bp	200 bp	400 bp	400 bp
	100 bp	100 bp		
	50 bp	50 bp		

* Not used in the analysis bp: base pairs

Kruskal-Wallis test was used to compare the search efficiency between the two strains. Flight capacity data were analyzed by the Kruskal-Wallis test and the Games-Howell test ($\alpha = 0.05$) for multiple comparisons among means using the IBM SPSS Statistics (2011) program.

Results

Molecular analysis. Both strains showed the same cut-off sites for all the enzymes used in the two gene markers analyzed, generating the same restriction fragments pattern (Table 1). The *16S* rRNA gene and ITS1 region generated a sequence of approximately 550 base pairs (bp) and 750 bp, respectively (Table 1). For *16S* rRNA, TaqI was cut only in two sites. The combined digestion of MboI and MseI was cut in two restriction sites whereas the combined digestion of DraI and HincII showed three restriction sites. The enzymes AluI, DpnI, EcoRI, HaeIII, NlaIII, and MspI did not present restriction sites in the *16S* rRNA fragment analyzed.

For ITS1, the combined digestion of MboI and MseI and a simple digestion of TaqI, AluI, and DpnI showed two restriction sites each whereas the combined

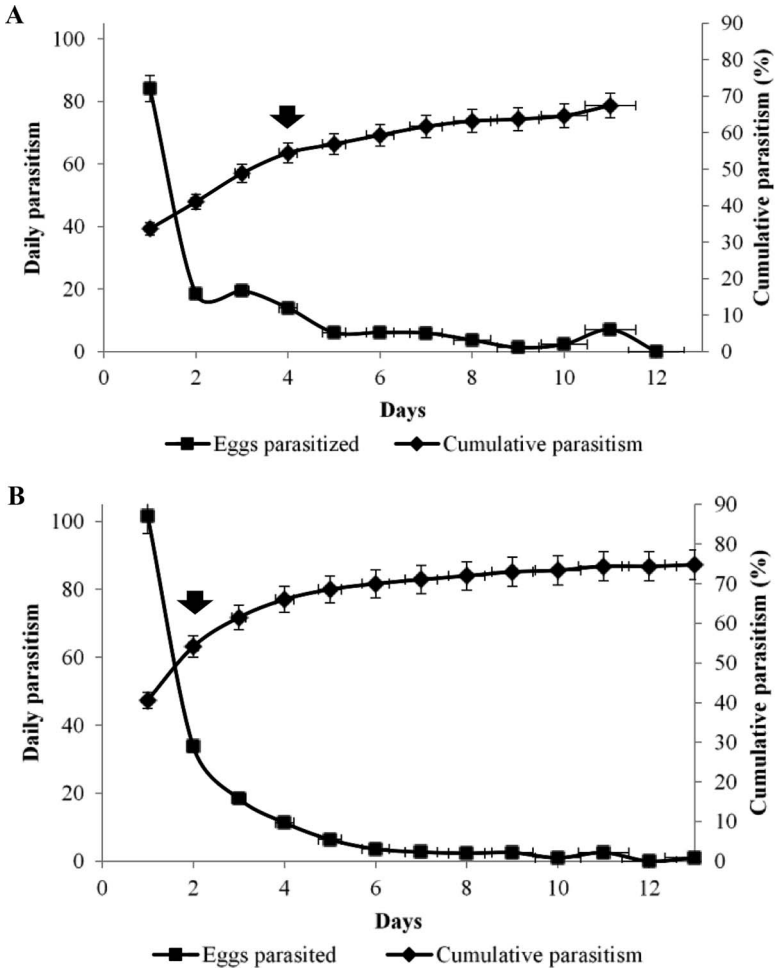

Fig. 1. Daily and cumulative parasitism of *Telenomus remus* strains on *Spodoptera frugiperda* eggs (mean ± SD). Arrow indicates 80% parasitism level. (A) LS: Laboratory reared strain. (B). WS: Wild strain.

digestion of DraI and HincII showed one restriction site. The enzymes EcoRI, HaeIII, NlaIII, and MspI did not present restriction sites in the ITS1 fragment analyzed.

Reproductive parameters. Daily parasitism of *T. remus* decreased with female age. The highest parasitism was observed on the first day with both strains (Fig. 1). The average number of eggs parasitized in the first 24 h was 84.1 ± 2.0 for the laboratory strain and 101.5 ± 2.8 for the wild strain. The wild strain showed maximum parasitism (80%) on the second day whereas the laboratory strain reached this percentage 2 d later on the 4th day). Cumulative parasitism differed significantly between the two strains ($F = 4.76$; df = 1, 38; $P = 0.035$). The

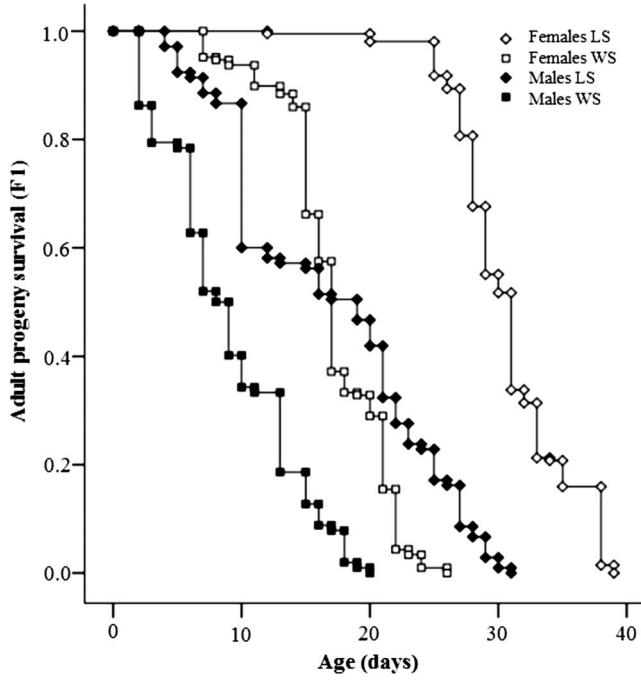

Fig. 2. Adult progeny (F1) survival of laboratory reared and wild *Telenomus remus* strains reared on *Spodoptera frugiperda* eggs. LS: Laboratory-reared strain. WS: Wild strain.

reproductive period was significantly shorter in the laboratory strain than in the wild strain ($F = 4.158$; df = 1, 38; $P = 0.048$). The average oviposition period was 6.6 d for the laboratory strain and 7.7 d for the wild strain. The wild strain also exhibited a higher total fecundity than did the laboratory strain.

Female longevity ($F = 0.11$; df = 1, 38; $P = 0.74$) and developmental time ($F = 0.44$; df = 1, 38; $P = 0.67$) did not differ significantly between the two strains, but F1 longevity ($F = 788.18$; df = 1, 462; $P < 0.0001$) and gender ($F = 496.02$; df = 1, 462; $P < 0.0001$) differed significantly. There also were significant differences in the lifespan of solitary females (mothers) and F1 individuals (females and males) that remained grouped. F1 females lived, on average, 52% and 48% longer than the F1 males in the laboratory strain and the wild strain, respectively. F1 laboratory females showed lifespans 61% longer than the F1 wild females (Table 2).

Survivorship of F1 adults also differed significantly with gender and strain ($S = 732.96$; df = 1, 38; $P < 0.001$), and survival of F1 females and males was higher in the laboratory strain than in the wild strain (Fig. 2). We observed a pronounced decrease in survival (l_x) of both strains from the 7th day onward. However, the average survival rate was greater for the wild strain (Fig. 3A). We also observed an age-specific fertility (m_x) reduction (Fig. 3B) and a decrease in F1 female proportion depending on the mother's age for both strains (Fig. 3C).

Table 2. Biological parasitism parameters (±SD) of laboratory reared (LS) and wild (WS) *Telenomus remus* strains parasitizing *Spodoptera frugiperda* eggs.

	Strain	
Biological Parameters	**LS**	**WS**
Total parasitized eggs/ female	145.8 ± 4.2	173.5 ± 3,8*
Female longevity (days)	8.7 ± 2.6	8.4 ± 3.0
Development time	12.0 ± 0.2	11.2 ± 0.4
F1 female longevity (days)	30.8 ± 0.3	18.6 ± 0.3*
F1 male longevity (days)	16.0 ± 0.6	9.0 ± 0.6*

* Means followed by asterisk are significantly different according to the Fisher test ($P \leq 0.05$).

The net reproductive rate (R_o) and the intrinsic rate of increase (r_m) calculated from the fertility life tables were significantly greater for the wild strain than for the laboratory strain (Table 3). The population doubling time (*Dt*) was significantly lower for the wild strain than for the laboratory strain. By contrast, there were no significant differences in the finite rate of increase (λ) and in the generation time (days) (T) between the strains. Overall, the laboratory strain increases 65.4 females/female in one generation and 1.6 females/day in 12.1 d while the wild strain increases 120.8 females/female in one generation and 6.2 females/day in 11.4 d.

Parasitoid behavior. The sequence of events leading to oviposition is shown in Figure 4. The behavioral patterns were similar for laboratory and wild females and are defined as follows. (a) Walking; Females initially walk rapidly with the antennae touching the arena surface at varying time intervals. (b) Host patch encounter; Females locate the host patch (laboratory = 67%, wild = 80%) or not (laboratory = 33%, wild = 20%). During the searching, wasps kept moving their antennae and made oscillating movements over the inspected area. There were significant differences in the time spent by both strain females in search efficiency ($\chi^2 = 18.43$; df = 59; $P = 0.001$). Wild females took only 28% of the total time spent by laboratory strains females to find the egg masses (122.2 ± 18.6 s and 442.1 ± 13.9, respectively). (c) Drumming; once females found the patch, movements slowed and eggs were examined with the antennae. (d) Probing; the female inserted the ovipositor into an egg, testing for a suitable host. (e) Host acceptance/rejection and oviposition; females can reject the host, withdrawing the ovipositor quickly and walking away (laboratory = 27%, wild = 27%) or they can accept the egg and oviposit. In both strains, females accepted the host and oviposited in 73% of observations. When accepted, females laid approximately 1 egg in 50 s (laboratory = 50.9 ± 14.0, wild = 53.0 ± 22.9) with antennae spread to 90° and producing trembling movements with antennae and abdomen. Statistical differences were observed for this behavior ($F = 0.64$; df = 1, 227; $P = 0.42$). (f) Marking host; once oviposition was completed, females rubbed the ovipositor over the egg surface for

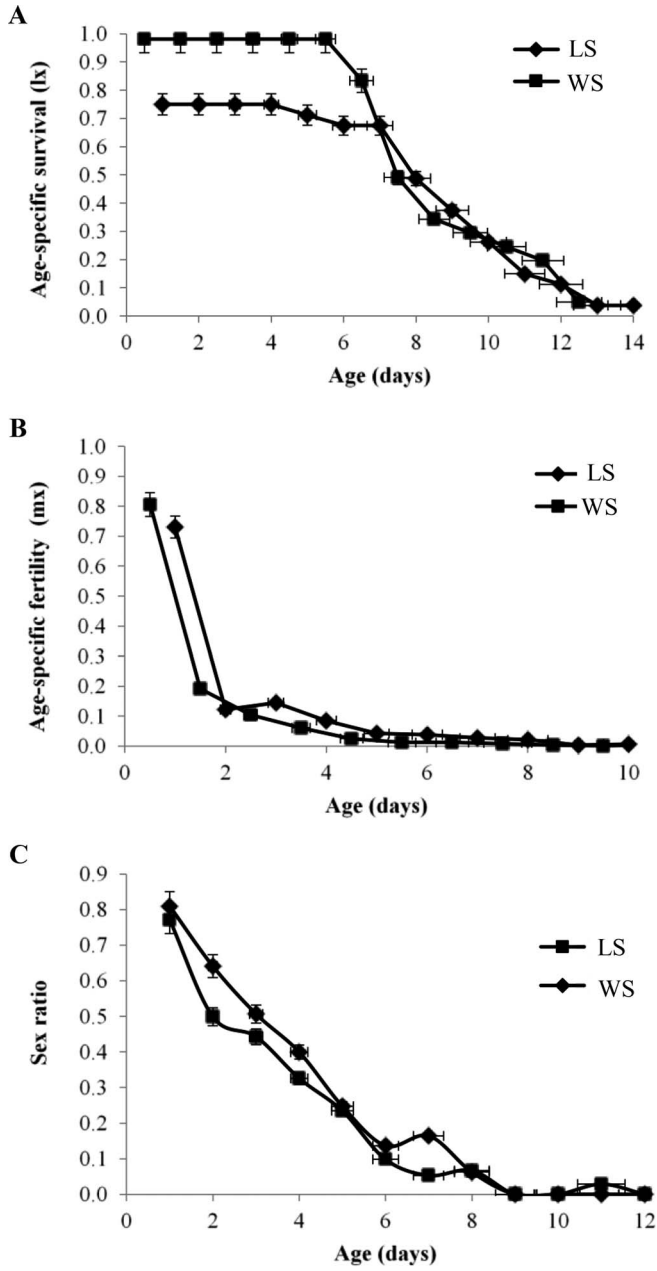

Fig. 3. Reproductive potential of *Telenomus remus* on *Spodoptera frugiperda* eggs (mean ± SD). (A) Survival rate in age *x* (l_x). (B) Age-specific fertility (m_x). (C) Sex ratio in relation to female age. LS: Laboratory-reared strain. WS: Wild strain.

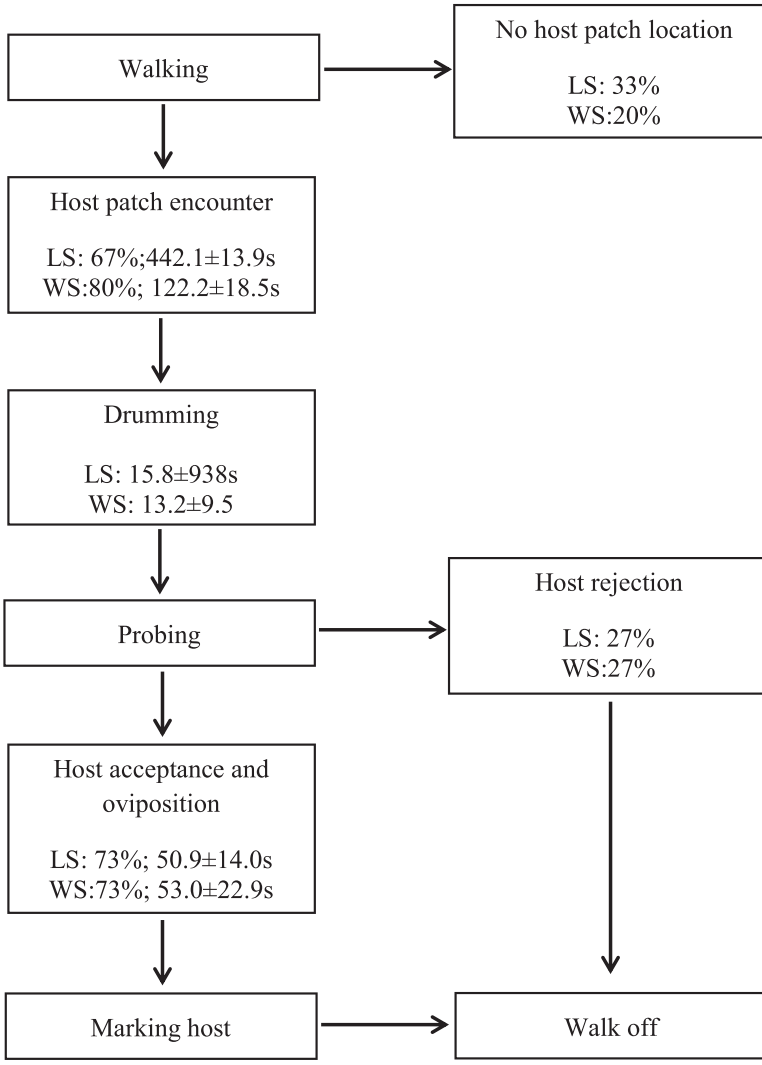

Fig. 4. Host location and oviposition ethogram of laboratory reared (LS) and wild (WS) *Telenomus remus* females on *Spodoptera frugiperda* eggs. Arrows connect sequential behaviors. Values indicate percentages of individuals displaying each behavior, the mean duration (s), and standard deviation (SD).

marking parasitized hosts. (g) Walk off; females cleaned their antennae and legs before continuing their searching for other hosts.

Flight activity. We found differences between the strains in relation to individuals captured at different locations in the flying test units. On average, laboratory-strain fliers (34.4 ± 3.1%), that is, individuals that reached the upper lid

Table 3. Parameters associated to fertility life tables: jackknife estimation (mean ± SD), 95% confidence intervals, and *p* bicaudal (P_b) and *p* unicaudal (P_u) values.

Parameters	Jackknife Estimation (Confidence Interval)		*P*-Values	
	Laboratory Strain (LS)	Wild Strain (WS)	P_b	P_u
Net reproductive rate[a] – R_o	65.4 ± 5.5 (53.9–76.8)	120.8 ± 5.9 (108.5–133.0)	0.0005	0.0002
Intrinsic rate of increase[a] – r_m	2.8 ± 0.2 (2.3–3.2)	4.2 ± 0.3 (3.6–4.8)	0.0268	0.9865
Finite rate of increase[b] – λ	1.6 ± 3.5 (0.8–2.2)	6.2 ± 1.9 (2.2–10.3)	0.0003	0.9998
Generation time[b] (days) – *T*	12.1 ± 0.1 (10.6–17.7)	11.4 ± 0.1 (9.9–13.0)	<0.0001	1.0000
Population doubling time [a] – *Dt*	1.5 ± 0.01 (0.2–0.3)	0.2 ± 0.01 (0.1–0.2)	0.0141	0.0071

[a] compared by the one-tailed *t* test
[b] compared by the two-tailed *t* test

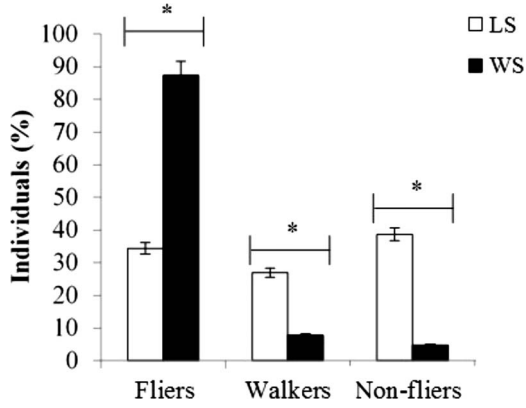

Fig. 5. Mean percentage (±SD) of fliers, walkers, and nonfliers individuals of laboratory reared (LS) and wild (WS) *Telenomus remus.* Means with asterisk have significant differences according to the Tukey test ($P \leq 0.05$).

of the flying test units, were significantly fewer ($\chi^2 = 14.32$; df = 142; $P < 0.0001$) than were wild-strain fliers (87.3 ± 1.4%). Consequently, the percentage of nonfliers (individuals that remained in testing tube) or walkers (individuals trapped on the inner wall) was higher for the laboratory strain (26.9 ± 3.1 and 38.7 ± 1.5%) than for the wild strain (7.87 ± 1.14 and 4.83 ± 0.73%) (Fig. 5).

Discussion

The DNA analysis confirmed the genetic similarity of the two colonies. No genetic changes had occurred in the rearing of *T. remus* in the laboratory for 600 generations.

The size of the *16S* rRNA fragment was consistent with fragments reported for other platygastrids (e.g., approximately 470 bp for *Baryconus* spp., *Sparsion* spp., and *Inostema* spp. (Dowton and Austin 2001). We found no reports on the amplification of the intergenic region ITS1 for *Telenomus* or other platygastrids. The ITS1 fragment we obtained, however, was similar with that of other egg parasitoids such as *Trichogramma*, with varying reports of 430 bp (Chang et al. 2000) to 570 bp (Jeng et al. 2013).

Despite the two colonies being subjected to different selection pressures, those pressures were insufficient to cause genetic differentiation in the ITS1 and *16S* regions between the two groups. Thus, we deduce that both strains are closely related and have a recent common ancestor. Furthermore, the genetic similarity between the laboratory colony and a wild colony could guarantee the successful introduction of wild reproductive individuals into the laboratory colony, which would expand the gene pool of laboratory strain.

Our results also demonstrated that the foraging and flying abilities of *T. remus* can be reduced by mass rearing in the laboratory over long periods of time and, thus, could negatively impact the performance and success of individuals released

into the wild for biological control programs. Indeed, parasitism capacity, fertility, search efficiency, and flight activity can be altered in mass rearing conditions of egg parasitoids (Baitha 2005; Nagarkatti and Nagaraja 1978; Oliveira et al. 2005). Pomari-Fernandes et al. (2015, 2016) showed that *T. remus* colonies reared on a factitious host *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and on its natural host, *S. frugiperda*, do not lose their ability to parasitize eggs until 19 generations in the laboratory. They also saw no significant difference between the number of "flyers," "walkers," or "deformed" individuals between the colonies until 250 generations in laboratory rearing.

Parasitism of eggs was highest on the first day after adult emergence for both *T. remus* strains in our study. This also has been reported with *Telenomus podisi* Ashmead (Pacheco and Corrêa-Ferreira 1998) and *Telenomus calvus* Johnson (Orr et al. 1986). Similarly, Bueno et al. (2010) and Morales et al. (2000) reported that the highest offspring and female production occur during the first days of oviposition and decreases with time, which is characteristic of typical provigenic species such as platygastrid parasitoids (Flanders 1950).

As expected, our wild strain exhibited the highest parasitism performance, producing significantly more offspring than did the laboratory strain in a shorter period of time. Higher fecundity was also observed in wild *Trichogramma* spp. when compared with strains reared for several generations in the laboratory (Baitha 2005; Nagarkatti and Nagaraja 1978; Oliveira et al. 2005).

Schwartz and Gerling (1974) reported that solitary *T. remus* females lived approximately 8 d while females in a group lived approximately 18 d. Males, on the other hand, lived approximately 8 d, whether living in a group or alone. The higher longevity and survival of F1 females may be due to the absence of the host. Orr et al. (1986) found that longevity of honey-fed *T. calvus* females in the absence of the host was 33.7 d. However, this period was reduced to 5.7 d when the parasitoid was placed with its host, *Podisus maculiventris* Say (Hemiptera: Pentatomidae). This is recognized as an adaptive strategy that allows parasitoids to store metabolic resources and convert them into energy when the host is experiencing unfavorable conditions or the host is not available (Bell and Koufopanou 1986) for parasitisim.

Similarly, Baitha (2005) and Nagarkatti and Nagaraja (1978) found that laboratory strains of *Trichogramma confusum* Viggiani lived longer than the wild strains, despite producing fewer offspring. The longer lifespan is related to the reproductive period, which was higher in the wild strain than in the laboratory-reared strain. In other words, the effective life (reproductive period) of laboratory-reared strains is shorter than that of wild strains, and the parameters R_o, r_m, and λ indicate that the wild strain had a higher potential for a population increase over a shorter period of time.

The search efficiency was lower for the laboratory colony than for the wild strain in our study. Interestingly, in an earlier study conducted in our laboratory with the same laboratory colony, Carneiro et al. (2010) reported that *T. remus* females took, on average, 275.4 $\pm$ 0.9 s to find the host in 9-cm-diameter Petri dishes. In our study reported herein (4 yr and 122 generations later), we observed the period of the host patch encounter for the laboratory colony was more than 2.5-fold longer, even with a reduced foraging area (1.5 cm diameter). This result clearly indicates that laboratory rearing over time affects the *T. remus* host search time.

Oviposition time is an important process for successful parasitism, particularly in specialist parasitoids such as *T. remus* (Cave 2000). The mean time of *T. remus* parasitizing *S. frugiperda* eggs, calculated by Carneiro et al. (2010), was 40.6–41.8 s/egg. We observed approximately 10 s longer in both strains. This discrepancy may be related to the host characteristics, as the *S. frugiperda* individuals used by Carneiro et al. (2010) were obtained from a different colony. Schwartz and Gerling (1974) reported that the average oviposition time for *T. remus* females parasitizing *Spodoptera litorallis* Boisduval eggs was 37 s/egg.

The wild strain showed superior flight ability in our study as evaluated in the flying test units. The observation that the laboratory colony had a higher percentage of nonfliers and walking individuals than did the wild colony may indicate this strain has lost the natural ability or impetus to fly soon after emergence. This result was also observed by Prezotti et al. (2002), evaluating the flight ability of three *Trichogramma pretiosum* Riley strains over 21 generations in the laboratory. Dutton and Bigler (1995) also found a loss in the ability to fly in two strains of *Trichogramma brassicae* Bezdenko evaluated after 39 generations in laboratory. Wild individuals could disperse more satisfactorily when released in the field, an important attribute in successful searching, dispersal, and establishment (Dutton and Bigler 1995).

To the contrary, Soares et al. (2012) found that *Tr. pretiosum* and *Trichogramma atopovirilia* Oatman and Platner strains collected in the field presented lower percentages of fliers than their mass-reared counterparts. This is likely attributed to the former adapting to laboratory study conditions. With our results, we can conclude that, at least until the sixth generation, laboratory conditions did not limit the successful adaptation of our wild strain.

The parameters evaluated in this study are essential features for natural enemies that will be used for biological control programs because they affect the foraging and dispersal patterns in the field (Gardner and van Lenteren 1986). The wild strain in this study yielded greater numbers of offspring over shorter periods of time than did the laboratory colony. It also had a higher search efficiency and flight capacity than did the laboratory strain, even after six generations of mass rearing.

In summary, a problem encountered in mass rearing of biological control agents is the lack of techniques to avoid selection pressures that can reduce efficiency of the parasitoids. The conditions provided in a laboratory lead to domestication because the genotypes are adapted to a new environment, thus inducing genetic drifting in the population, inbreeding, and selection (van Lenteren 2003). Therefore, it is important to emphasize that long-term rearing of *T. remus* requires care that limits the loss of potential for biological control. Subsequent quality evaluations can be completed during the rearing process that would allow immediate actions (Prezotti et al. 2002). Regarding rearing strategies, van Lenteren (2003) suggested variation in laboratory conditions (e.g., range of time and space, different regimes of light, temperature, and humidity) using genetic infusion (introduction of wild individuals or different lineages periodically) and semicaptive rearing systems. Nunney (2003) recommended maintenance of diverse inbred lines (Isofemale lines) in the laboratory as a possible solution.

Relatively few studies have compared the loss of desirable characteristics for egg parasitoids and documented the consequences to biological control. Considering the biological and commercial potential of *T. remus* (Cave 2000),

and the lack of information on its mass rearing, this research contributes to the knowledge about monitoring quality. In addition, this study provides a useful tool for identifying strains that may have desirable traits for biological control.

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