Male Reproductive Potential of *Aphidius colemani* (Hymenoptera: Aphidiinae) Exposed to Constant or Fluctuating Thermal Regimens

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ABSTRACT Prolonged exposure to low temperature generally induces deleterious effects on survival and reproduction of insects. Reproduction costs are well documented in cold-exposed female parasitoids, but there is little information concerning males. In some species, low temperature is suspected to cause male sterility. Mummies of the aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae) were exposed to either fluctuating thermal regimens (FTR: 4°C, 22 h; 20°C, 2 h) or constant low temperature (CLT: 4°C) for 15 d. We verified whether cold exposure can sterilize males and evaluated treatment-related survival, reproductive potential, and mobility parameters. Sterility trials showed that cold-exposed males were all fertile. Survival and reproductive potential of males (e.g., mating success, premating period, and competition for mating) were negatively affected when individuals were exposed to CLT. These alterations were associated with a reduction in locomotion performances during premating period. When parasitoids were exposed to FTR, survival, reproductive potential, and mobility parameters were unaffected. The reduced survival and mobility under CLT, probably results physiological perturbations: processes that may have a limited impact on individuals exposed to FTR. The consequence of mobility reduction on partner acceptance and competitive mating ability is discussed.

KEY WORDS low temperature, parasitoids, males, mating, fertility, mobility

Exposure to prolonged low temperature is known to have detrimental effects on the survival of parasitoids (Langer and Hance 2000, Lysyk 2004, Tezze and Botto 2004, Levie et al. 2005, Colinet et al. 2006a, 2007a). When the dose of cold exposure (i.e., a combination of exposure time and temperature) exceeds a specific threshold, chilling injuries accumulate, become progressively irreversible, and eventually lethal (Bale 1996, 2002; Koštál et al. 2006).

Several studies emphasize that exposing insects to fluctuating thermal regimens (FTRs) (i.e., cold exposure interrupted by periodic short pulses to high temperature) versus constant low temperatures (CLT), significantly reduced mortality in most species tested to date (Chen and Denlinger 1992, Leopold et al. 1998, Nedvěd et al. 1998, Renault et al. 2004, Colinet et al. 2006b, Koštál et al. 2007). Under FTRs, the low mortality results from periodic opportunities to repair and recover from accumulated chilling injuries during brief high temperature intervals (Colinet et al. 2007b, 2007c; Koštál et al. 2007, Lalouette et al. 2007).

Exposure to constant suboptimal temperatures usually negatively affected reproductive potential of insect parasitoids (Hance et al. 2007). The deleterious effects of cold exposure on reproduction are well documented in females (Hance et al. 2007), but there is little information concerning reproductive costs in males (Lacoume et al. 2007). Cold-induced male sterility has already been reported in several insect species. In drosophilids for example, exposure to low temperature below a specific threshold during development induces male sterility, observable by an absence of mobile sperm in seminal vesicles (Chakir et al. 2002, Araripe et al. 2004). Whether this phenomenon is general in drosophilids and other insects is poorly documented. Dissection of male genital tracts of drosophilids exposed to sub- or supraoptimal rearing temperatures showed abnormalities corresponding to an atrophy or absence of the testes (Araripe et al. 2004). In *Triatoma infestans* Klug (Hemiptera: Reduviidae), suboptimal rearing temperature induces male sterility caused by a decrease of gametic cell proliferation, an inhibition of spermatid production and a reduction in accessory gland secretion (Giojalas and Catala 1993). More recently, Giojalas (2005) showed that prolonged exposure to low temperature (12°C for 10 d) caused abnormal changes in the spermiogenic cells of *T. infestans* males.

In parasitoids, male sterility also has been reported as a potential consequence of cold exposure. In *Euchalcidia caryobori* Hanna (Hymenoptera: Chalcidinae), tissues of testes were more sensitive to low

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temperature than tissues of ovaries, and it was observed that low temperature causes retardation in spermatogenesis and degeneration of premetamorphic cells resulting in male sterility (Hanna 1935). Recently, Lacoume et al. (2007) showed that sperm production of *Dinarmus basalis* Rond. (Hymenoptera: Pteromalidae) was affected by cold shocks, inducing a delay in sperm production and seminal vesicle replenishment. In parasitoid wasps, reproduction occurs by arrhenotokous parthenogenesis: males are haploid while females are diploid, a system known as haplodiploid sex determination (King 1987). Therefore, unfertilized eggs develop into males. Some studies have shown that after being paired with cold-stored males, some female parasitoids only produced male progeny (i.e., unfertilized eggs), and therefore, the males were suspected to be sterile (Rigaux et al. 2000, Levie et al. 2005, Pandey and Johnson 2005). However, in these studies, mating behaviors (e.g., copulation) were not observed.

In parasitoids, acceptance or refusal of the male by female depends largely on whether or not the male displays the proper courtship behavior (Mackauer 1969). Alteration of the behavior sequence leading to copulation could reduce female's receptivity. There are no studies that address how mobility impacts mating success or failure in parasitoids; however, male courtship success in *Drosophila* is controlled by the readiness of the courtship (Laudien and Seifert 1983) and the running speed of males (Partridge et al. 1987). Low temperature is known to physiologically damage the neuromuscular system (Yocum et al. 1994, Kelty et al. 1996), which can result in behavioral alterations (e.g., grooming ability) (Kelty et al. 1996). Koštál et al. (2006) also found that prolonged cold exposure disturbs locomotion behavior (e.g., defects in crawling and uncoordinated movements). In parasitoids, perturbations in mobility have also been reported after cold storage (Tezze and Botto 2004). Compared with cold-exposed males, healthy males may thus appear more attractive to females and may also be more able to dynamically pursue females. Cold storage of parasitoids is frequently used in the context of industrial production (Leopold 1998). Mass production may encounter problems in ratio of males to females (e.g., male-biased sex ratio) because of a lower mating incidence. Because females mate only once (Mackauer 1969), mass rearing may become difficult if females are not readily fertilized because of cold-induced male sterility.

In this study, we focused on the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae). *A. colemani* is commercially produced as a biological control agent for suppression of the aphid *Myzus persicae* Sulzer (Homoptera: Aphididae) in many European countries. Cold exposure under CLT is known to be very detrimental to survival of *A. colemani*, whereas FTR greatly reduces the negative effects of low temperatures (Colinet et al. 2006b, 2007b). We conducted tests to evaluate whether, in addition to the differential impact on survival, reproductive potential may also be differently affected by thermal treatments. We verified if cold exposure sterilizes males. We also evaluated the treatment-related reproductive potential of males using different criteria: mobility during premating periods, competitive mating ability, mating success, and finally fertility or sterility.

Materials and Methods

Rearing Aphids and Parasitoids. The green peach aphid, *M. persicae*, was used as the host for the parasitoid rearing. Laboratory cultures were established from individuals collected in fields during 2000 at Louvain-la-Neuve, Belgium (50.3° N latitude, 4.3° E longitude). Aphids were reared in 0.3-m^3 cages on sweet pepper (*Capsicum annuum* L.) under 18 ± 1°C, ±60% RH, and L-D 16:8 h. *A. colemani*, originally obtained from Viridaxis SA. (Belgium), were subsequently reared in the laboratory under the same conditions.

To obtain standard parasitoid mummies for cold exposure, batches of 50 standardized 3-d-old aphids were offered to a female parasitoid wasp for 4 h.Parasitoid were <48 h old, naïve, and mated. The resulting parasitized aphids were reared under laboratory conditions (18 ± 1°C, ±60% RH, and LD 16:8 h) until mummification. Newly formed mummies were left to develop for 1 d, under the same rearing conditions, before cold exposure.

Cold Exposure and Survival Assay. The parasitoid mummies were exposed to 4°C, a temperature known to affect *A. colemani* survival (Colinet et al. 2006a). One-day-old mummies were placed in small plastic petri dishes. Mummies were exposed to low temperature inside thermo-regulated cooled incubators (model 305; LMS, Sevenoaks, Kent, UK) with saturated relative humidity and complete darkness. Mummies were randomly assigned to either constant low temperature (CLT: continuous exposure at 4°C) or fluctuating temperature regimes (FTR: the 4°C exposure was interrupted daily by a transfer to 20°C for 2 h) (see Colinet et al. 2006b). As a control, groups of mummies were allowed to continue their development until emergence under standard conditions (18 ± 1°C, ±60% RH, and LD 16:8 h). To confirm the differential impact of thermal treatments on parasitoid survival, three batches of 50 mummies were removed from each experimental condition and kept at 18 ± 1°C. The survival after 15 d of cold exposure, expressed as the emergence rate, was assessed as the number of adults that successfully emerged from the mummies when replaced at 18 ± 1°C.

For all the assays, groups of mummies were removed from incubators after 15 d of cold exposure (under FTR or CLT) and kept at 18 ± 1°C. Before emergence, all mummies were individually placed in 1.5-ml Eppendorf tubes to avoid any contact between sexes before the assays. The same procedure was applied to untreated control mummies.

Mobility During Premating Period. The mobility during premating period was measured for males coming from the different treatments (i.e., CLT, FTR, and control). A male was introduced under the top of a

sterile glass petri dish (5 cm diameter) and left for 5 min to acclimate. The arena was placed under standard conditions to avoid any influence on male activity (Mackauer 1969): on a glass light table (2500 LUX) and in a constant temperature chamber (22 ± 1°C). A camera coupled to a computer was used to film the experimental arena. A control virgin female was released in the arena to stimulate the male courtship behavior. As soon as the male became aware of the female presence (antennae held forward, wing spread out and flapping; Mackauer 1969), the female was removed. Males usually continued to actively search for females. Male activity was recorded continuously during a period of 60 s, and mobility parameters were analyzed using The Observer 5.0 (Noldus Technology, Wageningen, The Netherlands). Parasitoids used were all 6–24 h old, fed (honey:water 50:50), and randomly chosen. Because mating experience can significantly reduce locomotion activity in parasitoids (Pompanon et al. 1999), all tested males were virgin. Different mobility parameters were recorded: (1) the maximal instantaneous velocity (maximal walking distance per time), (2) the mean walking velocity during time of activity (periods of rest were not taken into account), and (3) the total walking distance during time of experiment. Twelve males were tested for each experimental condition.

Competitive Mating Ability. The first approach to evaluate the reproductive potential of males consisted of measuring their competitive mating ability. Males of virtually all parasitoid species can mate immediately on emergence (Quimio and Walter 2000). However, in *Aphidius ervi*, newly emerged males are able to perform their courtship display but fail to mate until they are 4 h old (He et al. 2004). For that reason, individuals used were at least 6 h old when tested. Because females mate only once (Mackauer 1969), we only used virgin females of 6–48 h old for each trial. All individuals were fed (honey:water 50:50). We observed male pairs consisting of one control male and one cold-exposed male (either CLT or FTR) during a 10-min contest for a control virgin female. Competing males were randomly chosen among treatments. We used a plastic arena (2.5 by 1 by 1 cm) divided in three connected compartments and covered with a glass slide. The two males were left to acclimate for 5 min in the opposite compartments to avoid contacts between them before the female release. The female was introduced in the center compartment. When the access to female was allowed, interactions were observed continuously to determine which male successfully mated with the female. As observed in many insect species, temperature during ontogeny can affect body pigmentation, resulting in a cuticle darkening at low temperature (David et al. 1990, Sehnal 1991). Distinguishing between competitors was thus possible because cold-exposed males (CLT and FTR) were clearly darker than control males. Forty-one replicates were performed to test "control male versus CLT male" and 40 replicates to test "control male versus FTR male."

Mating and Fertility Trials. The second approach to evaluate male reproductive potential consisted of measuring mating success, premating period, and fertility or sterility. A control virgin female was transferred directly into a 1.5-ml Eppendorf tube containing an isolated virgin male coming from the different treatments (e.g., CLT, FTR, and control). The males were at least 6 h old, fed (honey:water 50:50), and randomly chosen among treatments. The tube was placed on a light table (2,500 LUX) in a constant temperature chamber (22 ± 1°C). The proportion of individuals that successfully mate was calculated. The premating period was measured during 15 min, and mating was considered unsuccessful if no copulation occurred at the end of this experimental period. Fifty, 50, and 37 trials were performed for treatment control, FTR, and CLT, respectively. After mating, control females were removed and male sterility/fertility was assessed by observing the female progeny. If a male is sterile, the female will only lay unfertilized eggs that will develop into males. The mated females were released during 4 h in a plastic petri dish (4.5 cm diameter) containing 50 *M. persicae* larvae (L_2). After 4 h, the females were removed, and the aphids were maintained on artificial diet to continue their development until mummification, as described in Colinet et al. (2005). The presence or absence of females in the progeny indicates that males were fertile or sterile.

Statistics. Difference in survival rate (i.e., emergence) between treatments were analyzed using simple one-way ANOVA followed by Student–Newman–Keuls multiple comparisons (Proc GLM; SAS Institute, Cary, NC). Arcsin square-root transformation was required to normalize the distribution of emergence data. Mobility data were also compared between treatments using simple one-way ANOVA followed by Student–Newman–Keuls multiple comparisons (Proc GLM; SAS Institute). For competitive mating ability, χ^2 goodness-of-fit statistic was used to test the hypothesis that the distribution of partner choices of responding females deviated from a null model where both competitors should be chosen with an equal frequency (i.e., expected proportion of 0.5; Proc FREQ; SAS Institute). To compare homogeneity of mating success proportions, Pearson's χ^2 test, or Fisher exact test in case of counts less than five in a category were used (Proc FREQ; SAS Institute). Premating periods were compared between treatments using simple one-way ANOVA followed by Student–Newman–Keuls multiple comparisons (Proc GLM; SAS Institute). A log-transformation was required to normalize premating period data. Normality was verified before each analysis of variance (ANOVA) using the Shapiro-Wilk statistics (Proc UNIVARIATE; SAS Institute). Data presented in figures are untransformed. A significance level of $\alpha = 0.05$ was used for all tests.

Results

Chill Susceptibility Assay. Under CLT, only 43% of individuals could successfully emerge after 15 d of

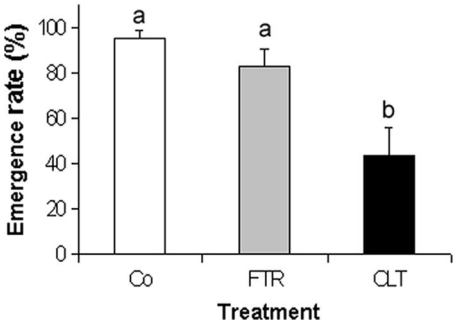

Fig. 1. Emergence rate (mean ± SE, n = 3 by 50) of control mummies (Co) left under standard conditions (18°C) and of mummies exposed to either fluctuating thermal regimen (FTR: 4°C, 22 h; 20°C, 2 h) or constant low temperature (CLT: 4°C) for 15 d. Different letters indicate significant differences (Student–Newman–Keuls test, α = 0.05).

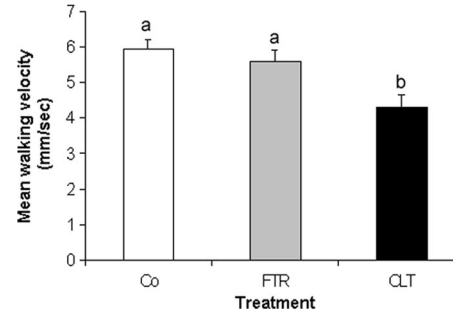

Fig. 3. Mean velocity (mean ± SE, n = 12) recorded during period of activity in control excited males (Co) left under standard conditions (18°C) and in excited males exposed to either fluctuating thermal regimen (FTR: 4°C, 22 h; 20°C, 2 h) or constant low temperature (CLT: 4°C) for 15 d. Different letters indicate significant differences (Student–Newman–Keuls test, α = 0.05).

cold exposure, whereas 83% of parasitoids survived under FTR (Fig. 1). In treatment CLT, some emerging individuals appeared weak, showed uncoordinated movements, and had wing deformations. As expected, the survival expressed as emergence rate (Fig. 1) was significantly affected by treatments (F = 28.46, P < 0.001). Student–Newman–Keuls tests showed that emergence rate in treatment FTR was similar to control, whereas it was significantly lower than control in treatment CLT.

Mobility. All the mobility parameters measured during premating period seemed to be affected by treatments. The maximal instantaneous velocity varied significantly among treatments (F = 10.30; P < 0.001), Student–Newman–Keuls tests showed that it was significantly lower in CLT males than in control males, whereas it was similar between control and FTR males (Fig. 2). The mean walking velocity during time of activity was significantly affected by treatments (F = 7.14; P = 0.003), pairwise comparisons indicated that it was lower in CLT males than in control males, whereas it was similar between control and FTR males (Fig. 3). Finally, the total walking distance also varied significantly among treatments (F = 6.75; P = 0.004), Student–Newman–Keuls tests showed that it was shorter in CLT males than in control males, whereas FTR males walked a total distance similar to control males (Fig. 4).

Competitive Mating Ability. When two partners were simultaneously placed with a receptive control female, successful mating was observed in the majority of trials (37/40 for control versus FTR and 37/41 for control versus CLT). Control and FTR males were accepted with similar frequency (χ^2 = 0.98; P = 0.32), providing no evidence of unequal mating ability between males from both treatments (Fig. 5). However, control and CLT males were not accepted with an equal frequency (χ^2 = 6.92; P = 0.008); in the majority of cases (27/37), control males were able to mate before CLT males (Fig. 5). Double-mounting was frequent as might be expected, given direct contest conditions, but the first male was considered as "the win-

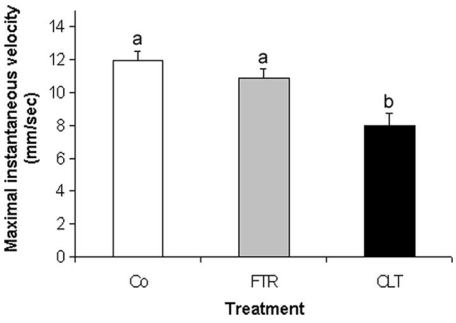

Fig. 2. Maximal instantaneous velocity (mean ± SE, n = 12) recorded in control excited males (Co) left under standard conditions (18°C) and in excited males exposed to either fluctuating thermal regimen (FTR: 4°C, 22 h; 20°C, 2 h) or constant low temperature (CLT: 4°C) for 15 d. Different letters indicate significant differences (Student–Newman–Keuls test, α = 0.05).

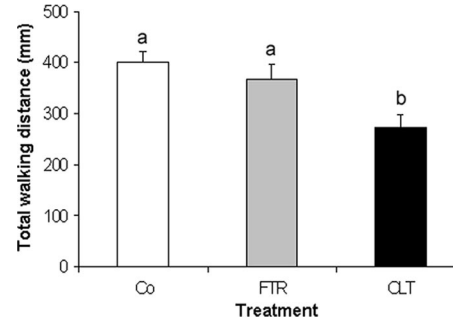

Fig. 4. Total walking distance (mean ± SE, n = 12) recorded during 60 s in control excited males (Co) left under standard conditions (18°C) and in excited males exposed to either fluctuating thermal regimen (FTR: 4°C, 22 h; 20°C, 2 h) or constant low temperature (CLT: 4°C) for 15 d. Different letters indicate significant differences (Student–Newman–Keuls test, α = 0.05).

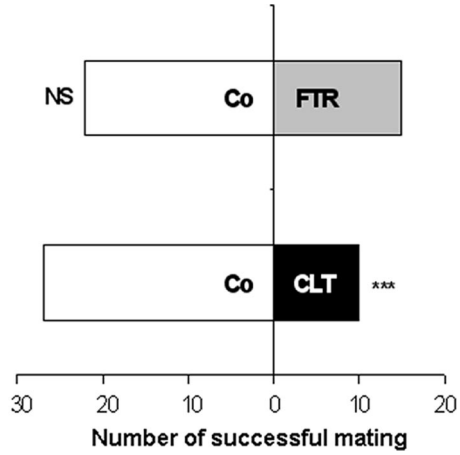

Fig. 5. Number of successful mating in male to male contests. In the contests "Co male versus FTR male," mating ability was similar between competitors. In the contests "Co male versus CLT male," mating ability was superior for the control (Co) males (χ^2 test, $\alpha = 0.05$).

ner," because displacement of the first male is rarely successful in *Aphidius* sp. (Cloutier et al. 2000).

Mating and Fertility Trials. When control males were individually placed with virgin control females, most of individuals could successfully mate with females (mating success 94%) and premating period was relatively short (60 ± 16 s; Figs. 6 and 7). Males exposed to FTR mated in similar proportion than control males ($\chi^2 = 1.65$; $P = 0.199$), but males exposed to CLT mated proportionally less than control males (Fisher exact test; $P = 0.006$; Fig. 6). All males exhibited sexual activity, at least the early stages of orienting toward the female and wing flapping. Mating failure seemed to be more related to lack of female interest rather than male interest in mating. Premating periods varied significantly according to treatments ($F = 8.34$, $P < 0.001$). Compared to control value, the increased of a fold factor 1.8 and 2.6 in treatment FTR and mean premating periods CLT respectively (Fig. 7). Finally, all the males that successfully mated with a virgin

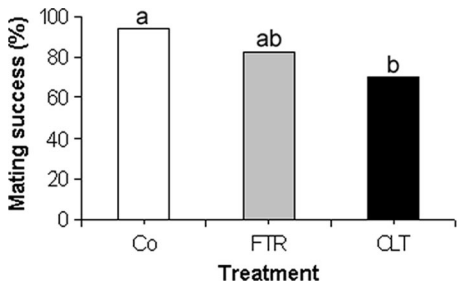

Fig. 6. Mating success (i.e., proportion of successful mating reported to the number of pairs tested) of control (Co) males ($n = 50$) left under standard conditions (18°C) and of males exposed to either fluctuating thermal regimen (FTR: 4°C, 22 h; 20°C, 2 h; $n = 50$) or constant low temperature (CLT: 4°C, $n = 37$) for 15 d. Different letters indicate significant differences (χ^2 test, $\alpha = 0.05$).

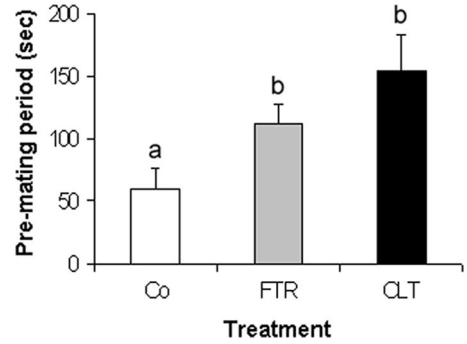

Fig. 7. Duration of premating period (mean ± SE) of control (Co) parasitoids ($n = 47$) left under standard conditions (18°C), and of parasitoids exposed to either fluctuating thermal regimen (FTR: 4°C, 22 h; 20°C, 2 h; $n = 41$) or constant low temperature (CLT: 4°C, $n = 26$) for 15 d. Different letters indicate significant differences (Student–Newman–Keuls test, $\alpha = 0.05$).

female were able to transfer sperm and fertilized eggs, because females were found in the progeny in all the cases (100% of fertility in control, FTR and CLT).

Discussion

Aphidius colemani is known to be severely affected (e.g., reduction in emergence and longevity) by prolonged constant cold exposure, even at temperatures well above the supercooling point (Colinet et al. 2006a, 2007a). However, as shown in previous studies, the mortality of *A. colemani* mummies is markedly reduced under fluctuating thermal regimens because of the periodic opportunities to recover from chilling injuries under these conditions (Colinet et al. 2006b, 2007b).

Acute temperature stress (i.e., heat or cold shock) is known to affect reproductive potential of insects (Krebs and Loeschcke 1994, Rinehart et al. 2000). In parasitic wasps, heat shock strongly reduced male fertility, leading to production of atypical sperm or empty seminal vesicles (Chihrane and Lauge 1997). Cold shock can also reduce male fertility in parasitoids by decreasing sperm production and seminal vesicle replenishment (Lacoume et al. 2007). The reproductive cost of acute temperature stress has been largely documented; however, chronic or prolonged stress to moderately low temperature has received less attention. In females, prolonged exposure to low temperature is known to reduce reproductive potential by affecting fecundity, longevity, oviposition behavior, and sex allocation (reviewed by Hance et al. 2007); however deleterious effects on male reproductive potential is less documented. We assumed that, compared with cold-exposed males, healthy males may appear more attractive to females and may be more able to dynamically pursue females.

In this study, we showed that reproductive potential of males exposed to CLT for 15 d was negatively affected; a significant proportion of individuals was unable to mate with females. However, mating success

of FTR males was similar to control males. The mechanism controlling partner acceptance during the mating behavioral sequences includes two sequential steps: (1) female recognition by male, mediated by sex pheromone, and (2) male recognition and acceptance by female after antennal contact, mediated by a contact pheromone produced by the male (Battaglia et al. 2002). In this study, the first step was apparently not perturbed, because presence of a virgin female always triggered the behavioral courtship sequence. Therefore, mating failure seems to be related more to the lack of female interest rather than male interest in mating. Acceptance or refusal of a male depends on whether or not it displays the proper courtship behavior. In *Aphidius* sp. females, receptivity is a major determinant of mating. Its influence is mainly on the duration of premating period and on the final success or failure of mating (Mackauer 1969). In Aphidiinae, duration of premating interval is generally short, ranging from 25 to 159 s depending on species (Mackauer 1969, Battaglia et al. 2002). In this study, the length of premating period was short in control males (59 ± 15 s), whereas it was more than the double of time in CLT males (154 ± 28 s). Compared with control males, the increased premating period and the higher proportion of mating failure in CLT males may result from different alterations in some individuals, which would consequently appear less attractive to females: (1) behavioral alteration of the courtship sequence events leading to copulation. Indeed, prolonged exposure to constant low temperature can produce behavioral alterations in parasitoids (Van Baaren et al. 2005): (2) morphological alteration of cold-stressed individuals. We observed that some CLT males had deformed wings. Morphological abnormalities have been reported in parasitoids exposed to constant cold exposure (Tezze and Botto 2004, Bourdais et al. 2006): (3) reduction of activity and state of readiness of stressed males as observed in this study.

The reduction in reproductive ability of CLT males is also supported by mating contests, which showed that CLT males were significantly less efficient than control males. However, control and FTR males were accepted with similar frequency, providing evidence of an equal mating ability. It was found in *Drosophila* that male courtship success correlates with running speed (Partridge et al. 1987). Therefore, we assume that reduction in mating performances in parasitoid exposed to CLT may be, at least in part, a consequence of a reduced mobility. Low temperature can strongly affect insect locomotion performances. For example, exposure to low temperature reduces running speed by 23% in *Chrysomela aeneicollis* Schaeffer (Coleoptera: Chrysomelidae) (Rank et al. 2007). In this study, walking capacities of control males were similar than those observed in other *Aphidius* sp. (Langer et al. 2004). Compared with control males, mobility parameters of CLT males were all significantly reduced (by ≈ 30%), whereas FTR males were not affected. The physiological mechanisms responsible for cold-induced mobility perturbations have been characterized by previous studies. Chilling is known to disturb muscular resting potentials, neuronal conduction velocities, and neuromuscular coordination (Kelty et al. 1996). Koštál et al. (2006) found that chill-injured insects (by prolonged cold exposure) showed defects in crawling and uncoordinated movements. This chilling injuries were correlated with changes in metal ion concentrations (i.e., increased $[K^+]$ with cold-exposure duration), leading to a gradual dissipation of equilibrium potentials across the muscle cell membranes (Koštál et al. 2004, 2006). Under FTR, a physiological recovery is possible during warm intervals, allowing a repair of accumulated chilling injuries (Colinet et al. 2007b, Lalouette et al. 2007). Recently, Koštál et al. (2007) found that ion pumping systems were allowed to re-establish the perturbed ion gradients during the recovery periods under FTR but not under CLT. The reduced mobility under CLT may thus result form physiological perturbations (e.g., ion homeostasis), a process that may have a limited impact on individuals exposed to FTR. Our results also support previous observations where individual mobility was affected by constant cold exposure in *Trichogramma nerudai* (Hymenoptera: Trichogrammatidae) (Tezze and Botto 2004).

In this study, we showed that survival, as well as parameters related to male reproductive potential (e.g., mating success, premating period, and competitive ability for mating), were negatively affected when individuals were exposed to CLT. These alterations were associated with decreased mobility. When parasitoids were exposed to FTR, survival, reproductive potential, and mobility were unaffected. We assume that mobility reduction may affect mating potential by decreasing partner acceptance by females and competitive ability for mating of cold-exposed males.

Because females generally copulate only once (Mackauer 1969), mating with a sterile male would result in unsuccessful fertilization. Consequently, it may alter the offspring sex ratio, and this may be considered as a major problem in the context of a commercial mass rearing. Rigaux et al. (2000) and Levie et al. (2005) supposed that cold storage induced male sterility and emphasized the needed to further study that topic. In this study, when cold-exposed males were able to mate, insemination and fertilization was effective, because females were always found in the progeny. This suggests that the suspected male sterility caused by absence of female progeny in *Aphidius rhopalosiphi* was likely related to mating failure rather than physio-anatomical perturbations in sperm production.

Finally, from an applied point of view (e.g., for cold storage), the benefits of using FTR are underlined again. In addition to the beneficial impact on survival, FTR allows the preservation of male reproductive ability. This is important for mass production because females have to be readily fertilized. FTR also allows the conservation of individual mobility, which is particularly important for females. In parasitoids, locomotion activity is mostly driven by search for mates in males and by host searching in females (Pompanon et

al. 1999). Therefore, the consequence of mobility reduction should be further studied regarding host searching capacities. Walking is indeed an important part of intrapatch searching behavior, and any reduction in mobility could be costly for pest control efficiency. In a natural environment where temperatures often fluctuate, prolonged cold stress may induce the accumulation of physiological perturbations; however, insects probably profit from periodic opportunities to recover from these injuries at the return of optimal conditions.

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