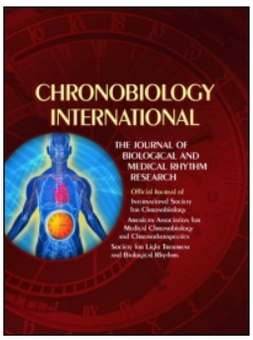

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Consequence of emergence pattern on inbreeding risk in the aphid parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae)

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ABSTRACT

In insect parasitoids, mating strategy depends on mate availability and is influenced by the spatial and temporal emergence patterns of adults. For quasi-gregarious species, simultaneous emergence favors local mating and reduces search costs for partners while increasing the risk of inbreeding, particularly when only one female parasitizes the initial host patch. Consequently, in inbreeding sensitive species, mating on the place of adult emergence (patch mating) between siblings should be counter selected. In practice, the timing of male and female emergence and of their dispersal influences mate availability and can limit on patch mating. To test the role of these two factors, we analyzed the daily distribution of emergence and patch residence time of a cohort in the aphid parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae). We observed that adult emergence is concentrated on the morning with males emerging on average before females with some overlaps. A more precise evaluation of emergence pattern within a brood suggests that brothers and sisters rarely emerge at the same time and rapid dispersal of males and females favors off-patch mating. The relationships between timing of emergence including differences between sex and consequences on inbreeding probability in these species are thus discussed.

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Mate availability; protandry; local mate competition; timing of emergence; post emergence dispersal; inbreeding avoidance

Introduction

In insect parasitoids, numerous life history traits are host-constrained (Hance et al. 2007). For instance, mating depends on the spatial distribution of the host and the way female distributes eggs inside the host population (Boulton et al. 2015; Godfray 1994; Wajnberg 2006). Females of solitary species usually lay a single egg in each solitary host and newly emerged adults must disperse from their natal patch to find a mate (Godfray and Cook 1997). In such species, competition for mates is ubiquitous for all individuals throughout the whole population. Conversely, in solitary parasitoids that develop in a quasi-gregarious fashion (when females lay a single egg per host and hosts are clumped) and gregarious parasitoids (when females lay several eggs in each solitary host), emerging together on the natal patch facilitates the finding of a partner and saves time. Thus, adults may mate totally or partially on their natal patch (Boulton et al. 2015; Hamilton 1967; Le Ralec et al. 2010; Wajnberg 2006). However, it increases the competition between brothers to mate with their sisters (Local Mate Competition, LMC, Hamilton; 1967; Werren 1980) and the probability for inbreeding (Beck 1991; Doyon and Boivin 2005), situation already described by Hamilton in 1967 as extreme biofacies. So, for species presenting a risk of inbreeding depression, selection should lead to the avoidance of mating on the emergence patch. Partners may mate with non-related individuals if they are able to distinguish them. Otherwise, we can expect a rapid dispersal of the emergence zone. The study of emergence patterns and the time spent by male and females on the place of emergence may thus provide interesting insights on the mating structure in this case.

Insects display a large number of behavioral, developmental and physiological events controlled by endogenous clock-like processes (Giebultowicz 1999; Nunes and Saunders 1999; Saunders et al. 2002). Daily behaviors such as locomotor activity, flight, mating, oviposition, egg hatching, pupation, and pupal emergence are governed by circadian oscillators (Giebultowicz 1999; Pittendrigh 1967;

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Sakai and Ishida 2001) while photoperiodic clocks mostly governed seasonal phenomena such as diapause or larval growth (Saunders 2009; Tauber and Kyriacou 2005). Concerning pupal ecdysis and emergence, the circadian system elicits the time of release of the eclosion hormone through a neural pathway (Steel and Davey 2013). Data on daily rhythm of parasitoids adult emergence show that they mostly emerge during the first hours of the day (Corrigan et al. 1995; Fanitou et al. 1998; Fauvergue et al. 1999; Hirose et al. 1988; Mazzi et al. 2011; Van Lenteren et al. 1992). Protandry is frequently observed (Wedell 1992; Pompanon et al. 1995; Morbey and Ydenberg 2001; Greeff 2002; Doyon and Boivin 2005, 2006).

Protandry allows the male to maximize mating opportunities and minimizes the female reproductive period (Doyon and Boivin 2006; Pompanon et al. 1995; Zonneveld and Metz 1991). Under this hypothesis, females should be receptive just after emergence and sib mating should not have deleterious effects. This is typically the case in the gregarious/quasi-gregarious *Trichogramma* egg parasitoids where males emerge before females and wait for their emergence on the cluster of host eggs to mate before dispersal (Doyon and Boivin 2006). However, for species with single-locus complementary sex determination (sl-CSD) as *Aphidius* species (Salin et al. 2004), mating with related individuals will increase homozygosity through generations, leading to the production of sterile diploid males (Cook and Crozier 1995; Heimpel and de Boer 2008; Salin et al. 2004; Zaviezo et al. 2018). In consequence, when no mechanism of sib-mating avoidance exists (Wiklund and Fagerstrom 1977), protandry associated with male rapid dispersal just after emergence and before mating should reduce on-site mating and the risk of inbreeding depression. Despite the importance of these aspects in quasi-gregarious and gregarious species, the emergence patterns of both sexes and their postemergence dispersal is rarely considered (Doyon and Boivin 2006; He and Wang 2008). While *Aphidius* species are widely used in biological control and mass-reared (Hance et al. 2017), very little quantitative information is available on their emergence rhythm.

Our aim was thus to analyze adult emergence pattern in the quasi-gregarious aphid parasitoid *Aphidius matricariae* (Hymenoptera, Braconidae) to determine the probability of mating and sib mating on the natal patch. *Aphidius* species are characterized by patchily distributed hosts, a female-biased sex-ratio and protandrous males (Battaglia et al. 2002; Brodeur and Rosenheim 2000; Giri et al. 1982; Hagvar and Hofsang 1991; He and Wang 2008; He et al. 2004; Le Ralec et al. 2010; Mackauer and Stary 1967). In *Aphidius rhopalosiphi* inbreeding produces a strongly biased sex-ratio towards males, probably linked to the production of diploid non-viable males (Salin et al. 2004). Moreover, *A. matricariae* individuals do not seem to avoid sib mating by any direct behavioral decisions (Bourdais and Hance 2009). Our hypothesis is that sib mating is avoided by differences in emergence pattern between males and females combined with a rapid dispersal from the natal patch before mating.

We first analyzed the daily pattern of emergence of both sexes under laboratory conditions. We then explored the consequences of light onset on emergence rhythm using various photoperiodic regimes. Using female broods, we recorded if brothers and sisters could emerge during the same time intervals, favoring their mating on the natal patch. Finally, mating and timing of dispersal of males and females from their natal patch were quantified.

Material and methods

Biological material and experimental conditions

Aphidius matricariae (Hymenoptera: Braconidae) is a solitary parasitoid wasp attacking a wide range of aphid species (Hance et al. 2017). *A. matricariae* and aphid strains were provided by Viridaxis S.A. In our laboratory, *A. matricariae* was reared on peach potato aphids *Myzus persicae* (Hemiptera: Aphididae), on turnip plants (*Brassica rapa* L. subsp. *rapa*). Aphid and parasitoid cultures were maintained in separate rooms under identical standardized conditions (Temperature: 20 ± 2C°, Relative humidity: 70 ± 5% and Photoperiod: LD16:8).

To minimize bias, experimental groups were constituted on the basis of females randomly sampled from rearing. Individuals were also randomly distributed among experimental conditions.

Preliminary tests were realized to evaluate the development time of our strain under laboratory conditions. The larval development needs 10 days before mummy formation, which represents the beginning of the pupal stage of the parasitoid. Adults begin to emerge 13 days after egg-laying (Zamani et al. 2007). To obtain time-standardized individuals, we let one young (<24 h) mated female parasitize 100 size-standardized aphids (2 days old) during 2 h in a Petri dish (Ø = 9 cm) and this was repeated with 25 different females. After 10 days of development, every parasitoid pupa (i.e., mummy) was individually kept in 1.5 ml microcentrifuge tube.

Temporal distribution of adult emergence

To understand the temporal distribution of adult emergence of a cohort (group of individuals of the same age), the standardized mummies were maintained under controlled conditions of light (light from 7:00 to 23:00). As in the preliminary experiment, we observed that emergence began after 13 days of development, we checked the mummies for the emergence of adult parasitoids every 30 min from 7:00 to 23:00 from 13 days post-oviposition until the last day of emergence and recorded the sex of emerging individuals. With this method, the chronological pattern of emergence of both sexes was determined with very good precision (30 min) for each consecutive day, which allowed to test for protandry.

The development time of males and females of the whole population was compared using Student's t-tests after checking for normality and homogeneity of variances. The emergence pattern (number of minutes needed from to onset of daylight to emergence ± 30 min) between males and females from day to day was compared using a one-way ANOVA with day of emergence as a fixed parameter followed by Tukey post-hoc tests. The numbers of males and females that emerged per day were compared to an expected distribution using X^2 test.

Influence of the onset

To determine the influence of the onset of daylight on adult emergence, two generations of parasitoids and aphids were reared under a photoperiodic regimen of 12 h of light and 12 h of dark (light from 9:00 to 21:00). Then, standardized mummies obtained in the new light conditions (light from 9:00 to 21:00) were randomly allocated to three groups. Thirteen days after oviposition (time for first emergence) each group was placed in three different photoperiodic regimens (25 reps per condition): (1) control photoperiod with light from 9:00 to 21:00, (2) advanced lighting (light from 8:00 to 20:00) and (3) delayed lighting (from 10:00 to 22:00). From day 13 to day 15 post-oviposition, male and female emergences were checked every 30 min during the entire photophase.

The average time of emergence of males and females from day to day was compared using a one-way ANOVA with a day of emergence as a fixed parameter followed by Tukey post-hoc tests. In order to compare the emergence pattern between sex, the cumulative numbers of emerged parasitoids according to time were best fitted to a sigmoidal curve (GraphPad Prism, Copeland 2000) using the formula $Y = M^* X^b/(K' + X^b)$. In this formula, Y represents the value of the cumulative number of insects at day 'X', K' is related to the inflexion point (it is equal to the inflexion point when b = 1), M is the maximum number of insects (plateau value) and b is the curve slope.

Influence of the timing of emergence on sib mating opportunities

To determine if overlaps for male and female emergence may lead to sib-mating, we analyzed the pattern of emergence of offspring as if their mothers have parasitized the same aphid patch. Nineteen females (mated, <24 h old) were individually allowed to parasitize a patch of 100 size-standardized aphids during 2 h in a Petri dish (Ø = 9 cm). After 10 days of development, each parasitoid pupa (i.e., mummy) from each female was individually kept in a 1.5 ml microcentrifuge tube until emergence. Adult emergences were observed during the photophase from 9:00to 21:00, and the maternal origin of each individual was recorded.

Two categories of individuals were then defined: (1) individuals who emerged without any individual of the opposite sex having emerged within 30 min and across the entire population (called "emergence without a partner") and (2) individuals who emerged

during the same period of at least 30 min in conjunction with other individuals of both the same sex and the opposite sex (emergence under "on-patch mate competition"). These under "on patch mate competition" individuals have been divided in three categories of potential competition: obligatory inbreeding (only males and females of the same mother emerged in the same 30-min period of time), outbreeding (no brother-sister emerged in the same 30-min period of time) or possible inbreeding (if at least one brother-sister pair emerged together in the same 30-min period of time). Using these categories, we compared the number of the brothers and sisters that emerged alone or under mate competition within the same female brood (n = 19) using X^2 tests.

Residence time before dispersal on the natal patch

To determine the time during which males and females stay on the natal patch and thus the probability they mate before dispersal, we observed their behavior after emergence from standardized mummies. Groups of 40 mummies (n = 5) were placed in a Petri dish (Ø45 mm) itself placed inside a larger one (Ø90 mm). Dispersal was effective when the parasitoid left the central zone by flying or walking. Mummies and emergence of individuals were observed from 7:00 to 15:00. For each sexed individual, we noted the time of emergence, the time of dispersal from the patch and whether it dispersed as virgin *vs* mated (leaving the patch) or not.

The numbers of individuals that dispersed virgin *vs*. mated were compared using X^2 tests. As dispersal time followed a normal distribution for males and females, we used two ways ANOVA to test the effect of sex and patch of emergence on the mean dispersal time of individuals. T-tests were used to compare dispersal time between mated and unmated individuals. The estimation of mating probabilities on the patch was calculated as the number of males and females that were present in the same time then at least one unmated individual of the opposite sex reported to the total number of individuals that emerge. Sex differences in mating probabilities were compared using X^2 tests.

Results

Temporal distribution of adult emergence

We did not observe any emergence during the dark phase (no adult present at the onset of lighting). Three days were needed for the emergence of all individuals of the cohort to reach a total of 330 adults (127 males and 203 females). The development time from oviposition to emergence was significantly shorter for males (313,95 ± 1,2 h) than for females (321,38 ± 0.82 h) with a mean difference of 7,43 h (t-test, t = 5.658, df = 328, $p < 0.0001$). Within a day, males and females began to emerge some minutes after the lighting onset (Figure 1). Despite a peak of emergence that occurred soon after onset (especially in males), there was an overlap in the emergence of both sexes during the photophase (Figure 1).

Males and females exhibited different patterns of emergence (Figure 1, Table 1). There were 70.9% of all observed males that emerged on day 13. Of these, 68.3% emerged during the first 4 h of illumination of that day. On day 14, 28.3% of males emerged in their turn, 80.1% in the first 4 h. This indicates that the peak of emergence of the males arrives soon after the onset (Table 1), (Figure 1).

The emergence pattern of females was more complex as it presented a peak during the morning of day 14 only while emergence in day 13 was concentrated on the afternoon (Figure 1). The same proportion of females emerged on day 13 and 14 ($X^2 = 0.74$, df = 1, p = 0.389; Table 1). The mean time of emergence after the lighting onset differed in males (207.6 min ± 18.62, n = 127) and females (338.30 min ± 17.45, n = 203) (t = 4.923, df = 328, $p < 0.0001$). However, for males, emergence pattern did not differ significantly from one day to another while females tended to emerge earlier over the three days (Table 1).

Influence of the lighting onset

The emergence pattern of adult parasitoids under the three different photoperiodic regimes is described in Figure 2. A total of 451 adults (235 males and 216 females) emerged. Within each

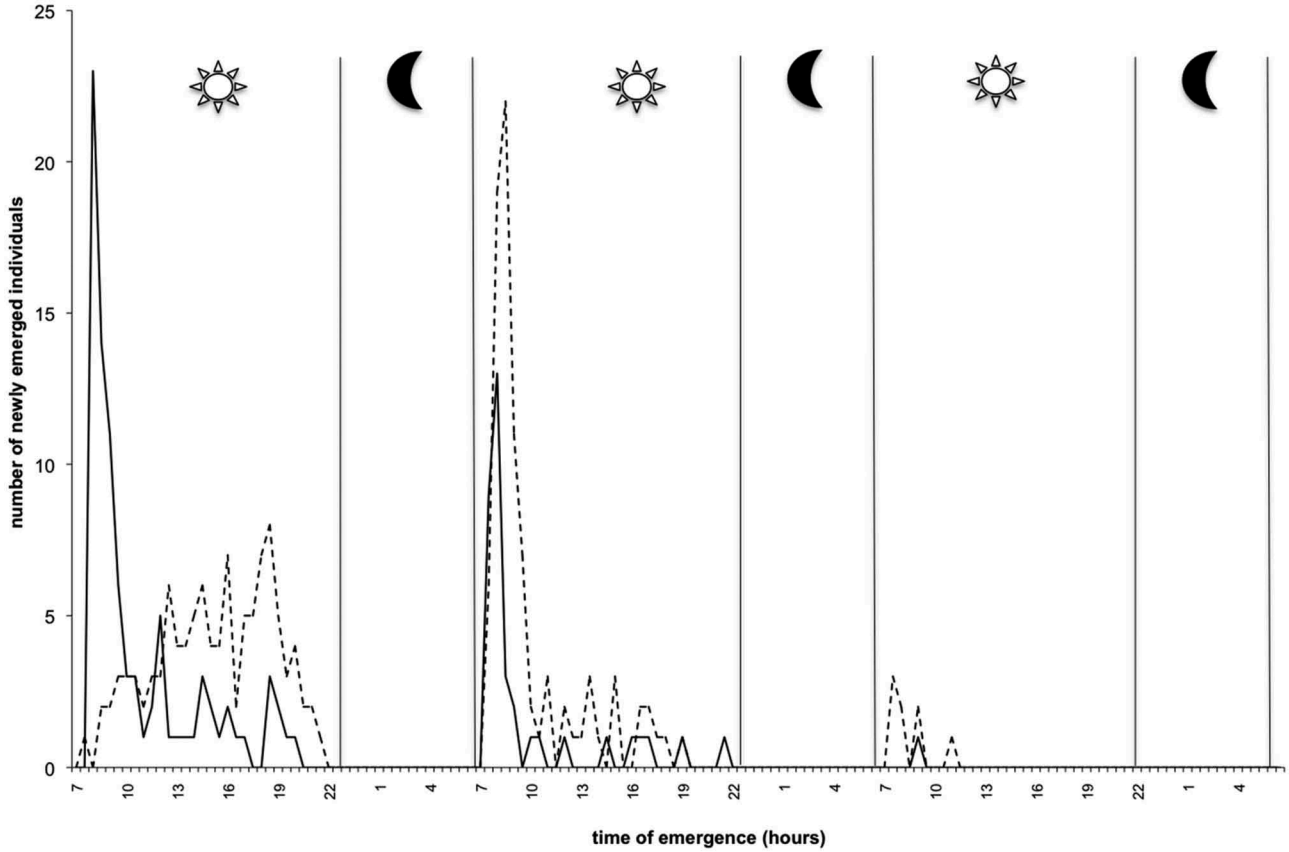

Figure 1. Distribution of adult males (dark lines, n = 127) and females (pointed lines, n = 203) emergence in *A. matricariae* under the standard photoperiodic regime (light-dark photoperiod of 16:8, from 7:00 to 23:00). Emergence was distributed over 3 days and graph represents the number of males and females newly emerged during each 30-min period of time.

Table 1. Pattern of adult emergence of *A. matricariae* males and females: distribution of emergence between days and mean time of adult emergence (number of minutes after light onset) within a day. Different letters indicate a significant difference between days for the same sex.

	% Adult emergence			Times of emergence (min) ± SE		
Number of days after oviposition	13	14	15	13	14	15
Males (n = 127)	70.9 a	28.3 b	0.78 c	223.8 ± 21.7 a	166.7 ± 37.1 a	120 a
	chi2 = 57.01, df = 2, **p < 0.0001**			ANOVA: F = 1.125, df 2–124; p = 0.3279		
Females (n = 203)	52.21 a	43.84 a	3.94 b	493.9 ± 20.0 a	175.6 ± 18.1b	86.25 ± 26.b
	chi2 = 58.30, df = 2, **p < 0.0001**			ANOVA: F = 76.86, df = 2–200, **p < 0.0001**		

photoperiodic regime, the peak of emergence of males preceded the female one (see peak values, Table 2). The rate of emergence did not differ between males and females (see b parameter, Table 2). When lighting onset is advanced or delayed of 1 h, the time required to reach the peak of emergence is not different for males (peak parameter comparison, F = 28.42, p > 0.05) and for females (peak parameter comparison, F = 12.62, p > 0.05) indicating that the start of illumination is the main signal that these species uses for the emergence. Within each day, males emerged in average 1 h30 before females for all photoperiodic regimes tested. This difference was always significant for the first day of emergence but not necessarily for the other days (Table 3).

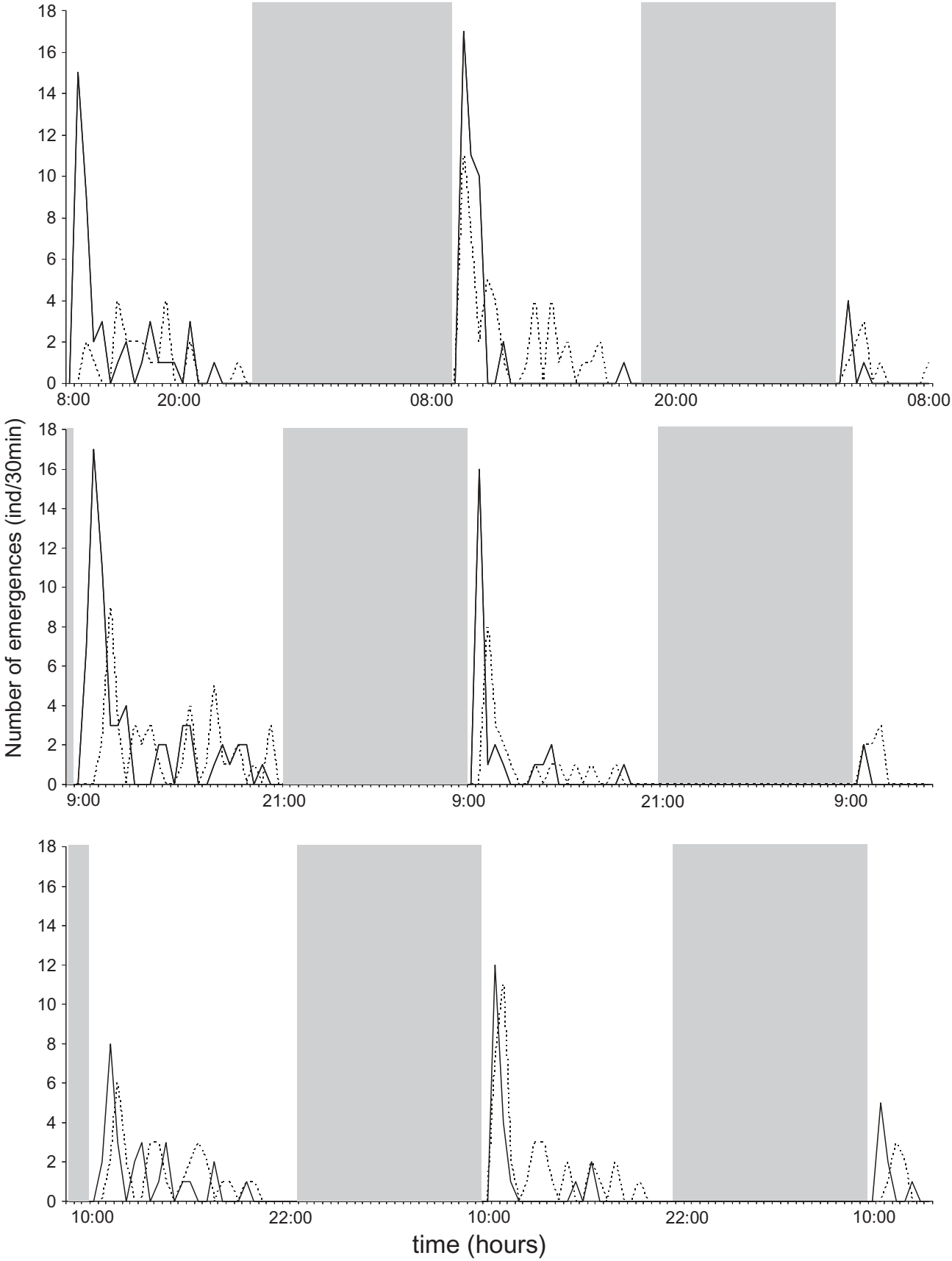

Figure 2. Distribution of males (dark lines) and females (dotted lines) adult *A. matricariae* emergences over 30-min period of time in under the three photoperiodic regimes: (1) control photoperiod with light from 9:00 to 21:00, (2) advanced lighting (light from 8:00 to 20:00) and (3) delayed lighting (from 10:00 to 22:00).

Table 2. Patterns of emergence of males and females *Aphidius matricariae* under the three photoperiodic regimens. « Top » value represents the total mean number of emerged individuals ± SE, « peak » represents the time needed in hours (h) after onset ± SE to obtain the peak of emergence, and « b » is the curve slope (rate of emergence). The experimental data were fitted to a sigmoid curve by the method of the least squares ordinary fit. The parameters were compared two by two between males and females with F tests; the values of p are indicated.

☼ 8–20		Day 1		Day 2		Day 3	
		♀	♂	♀	♂	♀	♂
Best-fit values	Top (nb)	21.84 ± 0.19	42.65 ± 0.81	46.09 ± 082	40.24 ± 0.22	7.86 ± 0.07	4.97 ± 0.03
	Peak (h)	4.63 ± 0.07	1.50 ± 0.21	2.36 ± 018	0.69 ± 0.03	1.16 ± 0.05	0.38 ± 0.07
	b	0.35 ± 0.02	0.25 ± 0.03	0.22 ± 002	1.41 ± 0.12	1.21 ± 0.12	5.07 ± 2.27
Comparisons	**Top**	**P < 0.001**		**P < 0.001**		**P < 0.001**	
(F test, p value)	**Peak**	**P < 0.001**		**P < 0.001**		**P < 0.001**	
	b	**NS**		**P < 0.001**		**P < 0.001**	
☼ 9–21 (Control)		**d1**		**d2**		**d3**	
		♀	♂	♀	♂	♀	♂
Best-fit values	Top (nb)	42.29 ± 0.60	63.80 ± 0.71	20.37 ± 0.41	24.03 ± 0.43	7.02 ± 0.03	2.00 ± 0.
	Peak (h)	3.72 ± 0.13	1.34 ± 0.12	1.83 ± 0.19	0.65 ± 0.15	0.84 ± 0.02	0.26 ± 0
	b	0.27 ± 0.02	0.28 ± 0.03	0.36 ± 0.06	0.61 ± 0.14	1.57 ± 0.11	60.43 ± 0.3
Comparisons	**Top**	**P < 0.001**		**P < 0.001**		**P < 0.001**	
(F test, p value)	**Peak**	**P < 0.001**		**P < 0.001**		**P < 0.001**	
	b	**Ns**		**Ns**		**P < 0.001**	
☼ 10–22		**d1**		**d2**		**d3**	
		♀	♂	♀	♂	♀	♂
Best-fit values	Top (nb)	26.04 ± 0.36	24.85 ± 0.33	35.53 ± 0.61	24.45 ± 0.95	6.01 ± 0.01	7.91 ± 0.05
	Peak (h)	3.05 ± 0.13	2.00 ± 0.13	1.91 ± 0.17	1.15 ± 0.47	1.35 ± 0.01	0.43 ± 0.03
	b	0.26 ± 0.02	0.27 ± 0.02	0.27 ± 0.03	0.15 ± 0.004	2.15 ± 0.06	3.13 ± 0.96
Comparisons	**Top**	**P < 0.05**		**P < 0.001**		**P < 0.001**	
(F test, p value)	**Peak**	**P < 0.001**		**Ns**		**P < 0.001**	
	b	**Ns**		**P < 0.05**		**ns**	

Influence of the timing of emergence on sib mating opportunities

The pattern of emergence for all offspring of the 19 females tested was similar to the previous experience and distributed over 3 days, from day 13 to 15 after oviposition. First emergence of male offspring occurred 45 min after the lighting onset and lasted to 174 min, while for females it began after 83 min and lasted to 352 min. No difference between mothers appeared in progeny emergence either for their male or female offspring (one-way ANOVA for males: f = 1.079, df = 18,194, p = 0.3763, for females f = 1.30, df = 18,184, p = 0.1918).

If we consider that the offspring of the 19 females represent a standardized field population, fewer males than females emerged in the absence of any partner (Figure 3a). None of the males or females that emerged in this population had to obligatory mate with a sib (Figure 3a). Inbreeding was possible for only 20% of males and 18% of females whereas 71.74% of males and 63.05% of females had no possibilities to mate with a sibling (Figure 3a). If we suppose that an aphid colony was parasitized by only one female within a brief time interval, around 80% of her sons and daughters will emerge without any mating opportunities on the patch (Figure 3b, n = 19 aphid colonies), sib-mating on the natal patch was only possible in around 20% of the cases (Figure 3b).

Residence time before dispersal on the natal patch

We observed the post-emergence behavior of a total of 25 females and 63 males that had emerged from five different patches. The sex ratio of the patches did not differ significantly ($X^2 = 6.924$, df = 4, p = 0.139) with a mean proportion of males of 73.99%.

Females stayed longer on the natal patch (1664 ± 153 s) than males (876 ± 60 s) before dispersing (ANOVA II: patch effect: p = 0.81, df = 4, f = 0.39, sex effect: p = 0.0011, df = 1, f = 11.49, interaction p = 0.66, df = 4, f = 0.60). Mated females did not disperse more rapidly than unmated ones (t-test, p = 0.13, t = 1.576, df = 19). However, unmated males dispersed 793 ± 87 s after emergence (n = 58), more rapidly than mated ones (1710 ± 146 s, n = 5) (t-test, p = 0.0036, t = 3.02, df = 61).

Table 3. Meantime emergence ± SE (min) of *A. matricariae* in relation to light onset under the same light/dark duration (LD12:12). Differences between sexes within a day were tested using Student's t-test. The mean differences between days within sex were tested with ANOVA followed by Tukey multiple comparison tests (different letters in each column indicate significant differences between days at $p < 0.05$ within a photoperiodic regime).

Photoperiodic regimen		Females	Males	Comparisons between sexes
7:00–19:00	Day 1	271 ± 29min (n = 22) a	145 ± 22min (n = 43) a	*t = 3.287, df = 63,* ***p = 0.0017***
	Day 2	185 ± 24min (n = 46) b	74 ± 15min (n = 41) ac	*t = 3.792, df = 85,* ***p = 0.0003***
	Day 3	112.5 ± 33.4min (n = 8) ab	42 ± 12min (n = 5) bc	*t = 1.603, df = 11, p = 0.1371*
		Anova, f = 4.209 df = 2,86	*Anova f = 4.03 df = 2,73*	
		p = 0.018	***P = 0.0217***	
8:00 –20:00	Day 1	307 ± 31.6min (n = 42) a	169.2 ± 24.3min (n = 64) a	*t = 3.51, df = 104,* ***p = 0.0007***
	Day 2	172.5 ± 35.5min (n = 20) b	100.8 ± 26.7min (n = 25) a	*t = 1.64, df = 43, p = 0,1078*
	Day 3	64.3 ± 10min (n = 7) b	30 ± 0min (n = 2) a	*t = 1.686, df = 7, p = 0.1356*
		Anova f = 7.745, df = 2,66	*Anova, f = 1.747, df = 2,88*	
		P = 0.0012	*P = 0.18*	
9:00–21:00	Day 1	236.8 ± 31min (n = 29) a	150 ± 28.4min (n = 27) a	*t = 2.081, df = 54,* ***p = 0.0422***
	Day 2	165 ± 25.7min (n = 36) a	90 ± 27.3min (n = 20) a	*t = 1.86, df = 54, p = 0.0676*
	Day 3	95 ± 9.2min (n = 6) a	52.5 ± 14.7min (n = 8) a	*t = 2.24, df = 12,* ***p = 0.0441***
		Anova, f = 2.99, df = 2,68	*Anova, f = 2.31, df = 2,52*	
		P = 0.0564	*P = 0.1092*	

Concerning mating opportunities, we found that 43.85% of males and 85.7% of females were present on the natal patch with at least one individual of the opposite sex, making mating possible more often for females than males ($X^2 = 11.63$, df = 1, p = 0.0006). From these individuals, more males left the patch still virgin than females did (X2 = 4.108, df = 1, p = 0.0427). We observed that 32% of females (n = 8) and 11% of males (n = 7) dispersed mated from their natal patch.

Discussion

In the present study, we analyzed emergence rhythm of the aphid parasitoid *Aphidius matricariae* to determine the risk of inbreeding. Our results showed that the combination of partial protandry, emergence distribution within a brood and post-emergence dispersal reduce considerably the risk of sib mating on the natal patch, even if these species does not avoid sib mating under laboratory conditions (Bourdais and Hance 2009). Moreover, lighting onset trigger emergence and favors synchronization.

Emergence rhythm has frequently been monitored in insect species (Møller 2004; Wedell 1992; Zijlstra et al. 2002; Zonneveld 1996;) including parasitic wasps (Doyon and Boivin 2005, 2006; Hirose et al. 1988; Le Ralec et al. 2010; Pompanon et al. 1995; Tentelier et al. 2006) showing a high frequency of protandry. We observed that emergence of *A. matricariae* presents similarities with other parasitoids: synchronization due to an exogenous signal (light), protandry, and peak in the few hours following the lighting onset (Doyon and Boivin 2005; Sakai and Ishida 2001; Zaslavski et al. 1999). Males emergence begin with light onset without any sign of activity at the darkness-light transition, in contrast with *Drosophila melanogaster* (Helfrich-Föster 2001) but similar to most parasitoid species (Doyon and Boivin 2005; Karpova 2006; Wiklund and Fagerstrom 1977; Zaslavski et al. 1999). Moreover, when light onset was advanced or delayed by 1 h, both sexes wait for light to emerge, suggesting that lighting onset is the cue for emergence in these species, probably mediated by photoreceptor and eclosion hormone activation. No significant differences appeared between the three regimens, even if a delayed or an advance of lighting seems to reduce the time for the peak of emergence. However, the proportion of females that emerged on day 2 was lower in the control than in the case of advanced or delayed lighting onset.

This kind of daily distribution of emergence restricted to certain parts of the photophase cycle was already mentioned in *Trichogramma semifumatum* (Rounbehler and Ellington 1973), *T. minutum* (Corrigan et al. 1995), *T. embryophagum* (Karpova 2006; Reznik et al. 2008), *Nasonia vitripennis* males (Bertossa et al. 2010) and *Aphidius ervi* (He et al. 2004). Emergence during the first hours after the onset coincides with more favorable conditions, as

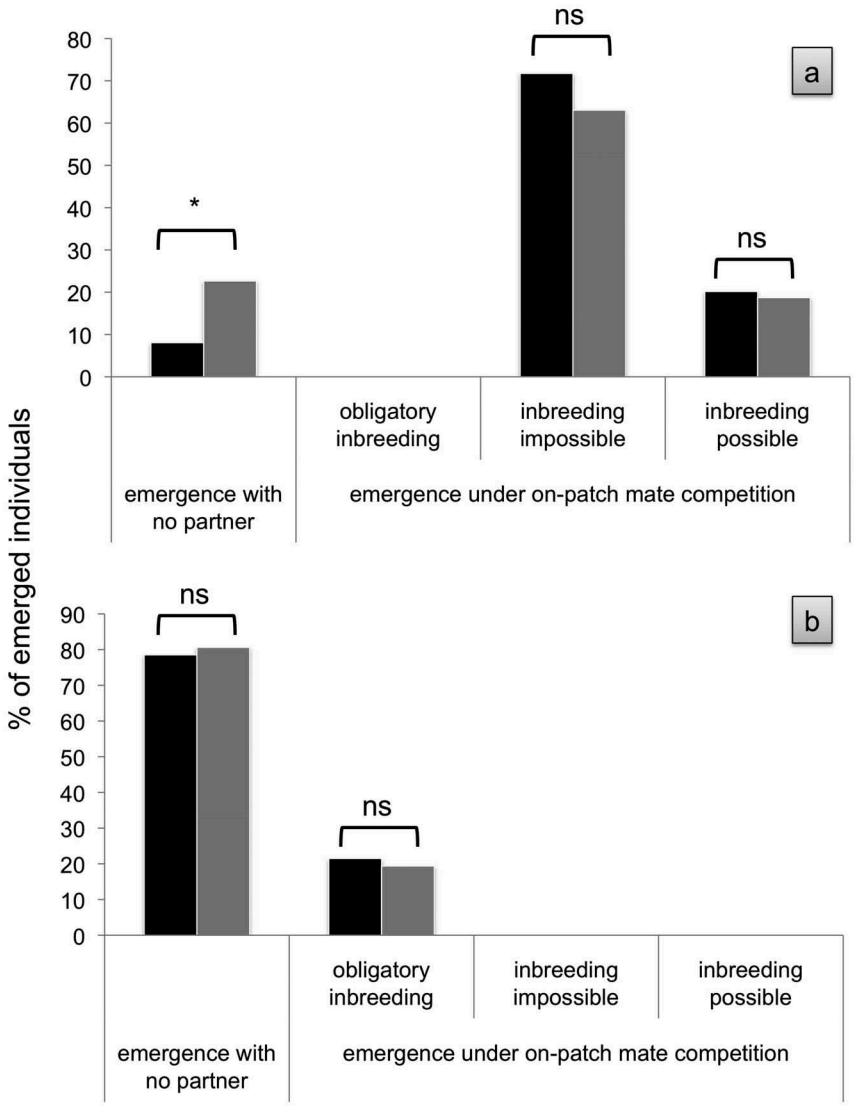

Figure 3. Inbreeding risk of the offspring: (a) considering the time of emergence of offspring from the 19 female tested lines, (b) data for each mother brood and mean percentage of emergence. 1) Emergence with no partner corresponds to the percentage of males (dark bars) and females (grey bars) that emerged during a time when no individual of the other sex has emerged, 2) Obligatory inbreeding corresponds to an emergence at the same time of partners from the same mother (only inbreeding is possible), 3) Percentage of individuals that emerged in the same time than partners coming only from an unrelated mother, 4) percentage of individuals that emerged in the same time than both related and unrelated partners (possible inbreeding). Fisher exact tests were done to compare males and females, * indicates a significant difference at $p < 0.05$.

morning temperatures are usually milder and humidity higher than during the mid-day, reducing water loss risk through the cuticle of newly emerged individuals (Lankinen 1986) and allowing insects to spread their wings (Skopik and Pittendrigh 1967). The emergence at the beginning of the daylight period had been suggested to favor reproductive behavior and activity in parasitoids (Karpova 2006; Karpova and Reznik 2002; Pompanon et al. 1995).

In this experiment, we did not test the importance of the dark phase duration. However, in the first experiment where mummies underwent LD16:08 condition, females significantly emerged more rapidly on day 2 (175,6 ± 18,1 min after onset) than on first day (493,9 ± 20,0 min after onset). This difference is probably due to the spread over in maturation of individuals. Indeed, in most cases, hatching is gated by the endogenous circadian system to a restricted period of the day (Myers 2003) and individuals that reach maturity outside that time window will emerge during the next appropriate period, usually 24 h later. As both

sexes do not emerge during the night, individuals that are near to emerge in the afternoon perhaps wait for the appropriate signal the next morning (Bertossa et al. 2010; Myers 2003). Indeed, under LD12:12 conditions, most females emerged quite early during the morning (Figure 2). So, a shorter dark phase seems to postpone the emergence pattern during day 1. The same trend was observed for males but as the number of individuals that emerged on day 2 and 3 was quite low and the time for the peak was shorter than for females, no significant differences could be underlined. Future experiments should thus investigate the influence of the duration of the dark phase on the timing of emergence.

If insect developmental rates and emergence rhythms tend to be similar for most individuals under the same conditions (Beck 1991; Saunders et al. 2002), development time plasticity can still arise (Doyon and Boivin 2005; Shaffer 1983). In our case, brood emergence was spread during three consecutive days even if they were all laid in 2 h and reared in the same conditions. This plasticity could be linked to a genetic polymorphism for that trait probably favored by sib mating avoidance.

Aphidius matricariae males usually emerge before females, but with overlaps reducing the protandrous status of the species. In *Trichogramma evanescens*, males emerge before females in an actual protandrous pattern (Doyon and Boivin 2006) with little overlaps. More or less synchronized temporal patterns of emergence have been observed for some parasitoid species, mostly *Trichogramma* species (Reznik et al. 2008). *Trichogramma* species are short-lived species (Boivin and Lagacé 1999) for which dispersal capacities are quite low (Smith 1996) and that mate mostly between relatives on the emergence patch (Martel and Boivin 2004; Martel et al. 2010). In that case, the timing of emergence favors a rapid mating before dispersal, as mating between sibs does not have detrimental consequences (Doyon and Boivin 2006).

A. matricariae behave more like *A. ervi* (He et al. 2004) and as the gregarious larval parasitoid, *Cotesia glomerata*, of the small white butterfly *Pieris rapae* (Mazzi et al. 2011). In these three species, male and female's emergence overlaps much more than in *Trichogramma* even if on average, males emerge before females. He et al. (2004) unfortunately did not discuss their results in terms of mating strategies but Mazzi et al. (2011) interpreted the emergence pattern of *Cotesia glomerata* regarding the mating opportunities within a patch and the inbreeding costs. It appears that *Cotesia glomerata* does not suffer so much from inbreeding (Elias et al. 2010) because inbred males are still able to mate and to produce offspring contrary to many species with single-locus complementary sex determination (Heimpel and de Boer 2008). It is thus the post-emergence behavior of males that determine whether the significant difference in the timing of emergence of the two sexes limits the occurrence of sib mating (Mazzi et al. 2011). In *A. matricariae*, considering different females broods, brothers and sisters do not emerge together in 80% of cases. This pattern of emergence decreases the probability of mating with a sib on the natal patch and could have been selected to increase outbreeding. No behavioral mechanism of sib mating avoidance had been found in these species (Bourdais and Hance 2009) even if mating with a sib could have deleterious effects on hymenopteran species with complementary sex determination (Heimpel and de Boer 2008), including closely related species (Salin et al. 2004). Our observations about the post-emergence behavior tend to favor this hypothesis because males disperse more rapidly than females and often disperse still virgin. However, rapid male dispersion also reduces local competition with other males. Complementary experiments had shown that *A. matricariae* females need 30 min of the pre-mating period while males do not need any time after emergence before mating (DB unpublished data). This could explain why few females disperse mated from the patch and why most of the males do not wait for females.

Conclusions

These results give new insights about the relationship with plasticity in development time, protandry, local mate competition, and inbreeding risks. Particular strategies to avoid inbreeding were selected: (1) When a female lays eggs in an aphid colony even in a short time, offspring emerges

during three consecutive days, diluting the probabilities of kin mating on the natal patch. (2) Brothers and sisters seldom emerge at the same time and thus have little chances to mate. (3) Male and female's maximum of emergence differs within a population reducing emergence overlap, making outbreeding more probable than inbreeding. (4) Finally, the tendency of males and females to disperse out of the patch before mating increases their probability to mate with non-kin than with a sib. *Aphidius matricariae* thus presents a low partial and asymmetric mate competition resulting in a low risk of sib-mating. Emergence at the same time as conspecifics and/or sib could be possible only in cases of a high population density of aphids coupled with a high rate of parasitism. This is notably the case for industrial rearing of parasitoids for mass release biological control and inbreeding may occur frequently under these conditions. However, mating systems of aphid parasitoids remain still poorly understand and future studies would benefit by considering how aphid patches are distributed in the environments and how parasitized aphid behave in their colony after being attacked by parasitoid females.

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References

Battaglia D, Isidoro N, Romani R, Bin F, Pennacchio F. 2002. Mating behaviour of *Aphidius ervi* (Hymenoptera: braconidae): the role of antennae. Eur J Entomol. 99:451–456.

Beck SD. 1991. Thermoperiodism. In: Lee RE, Denlinger DL, editors. Insects at low temperature. New York (NY): Chapman and Hall. p. 199–228.

Bertossa RC, van Dijk J, Beersma DG, Beukeboom LW. 2010. Circadian rhythm of adult emergence and activity but no eclosion in males of the parasitic wasp *Nasonia vitripennis*. J Insect Physiol. 56:805–812.

Boivin G, Lagacé M. 1999. Effet de la taille sur la fitness de *Trichogramma evanescens* (Hymenoptera: trichogrammatidae). Ann Soc Entomol Fr. 35:371–378.

Boulton RA, Collins LA, Shuker DM. 2015. Beyond sex allocation: the role of mating systems in sexual selection in parasitoid wasps. Biol Rev. 90(2):599–627.

Bourdais D, Hance T. 2009. Lack of behavioural evidence for kin avoidance in mate choice in a hymenopteran parasitoid (Hymenoptera: braconidae). Behav Process. 81:92–94.

Brodeur J, Rosenheim JA. 2000. Intraguild interactions in aphid parasitoids. Entomol Exp Appl. 97:93–108.

Cook JM, Crozier RH. 1995. Sex determination and population biology in the hymenoptera. Trends Ecol Evol. 10:281–286.

Copeland RA. 2000. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis. New York (NY): Wiley and Sons.

Corrigan JE, Laing JE, Zubricky JS. 1995. Effects of parasitoid to host ratio and time of day of parasitism on development and emergence of *Trichogramma minutum* (Hymenoptera: trichogrammatidae) parasitizing eggs of *Ephestia kuehniella* (Lepidoptera: pyralidae). Ann Entomol Soc Am. 88:773–780.

Doyon J, Boivin G. 2005. The effect of development time on the fitness of female *Trichogramma evanescens*. J Insect Sci. 5:4–9.

Doyon J, Boivin G. 2006. Impact of the timing of male emergence on mating capacity of males *Trichogramma evanescens* westwood. Biocontrol. 51:703–713.

Elias J, Dorn S, Mazzi D. 2010. Inbreeding in a natural population of the gregarious parasitoid wasp *Cotesia glomerata*. Mol Ecol. 19:2336–2345.

Fanitou AA, Alexandr MP, Tsitsipis JA. 1998. Adult emergence rhythm of the egg parasitoid *Telenomus busseolae*. BioControl. 43:141–151.

Fauvergue X, Fleury F, Lemaitre C, Allemand R. 1999. Parasitoid mating structures when hosts are patchily distributed: field and laboratory experiments with *Leptopilina boulardi* and *L. heterotoma*. Oikos. 86:344–356.

Giebultowicz JM. 1999. Insect circadian clocks: is it all their heads? J Insect Physiol. 45:791–800.

Giri MK, Pass BC, Yeargan KV, Parr JC. 1982. Behaviour, net reproduction, longevity and mummy-stage survival of *Aphidius matricariae*. Entomophaga. 27:147–153.

Godfray HCJ. 1994. Parasitoids: behavioural and evolutionary ecology. Princeton (NJ): Princeton University Press.

Godfray HCJ, Cook JM. 1997. Mating systems of parasitoid wasps. In: Choe JC, Crespi BJ, editors. The evolution of mating systems in insects and Arachnids. Cambridge (UK): Cambridge University Press. p. 211–225.

Greeff JM. 2002. Alternative mating strategies, partial sibmating and split sex ratios in haplodiploid species. J Evol Biol. 9:855–869.

Hagvar EB, Hofsang T. 1991. Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. Biocontrol News Inf. 12:13–42.

Hamilton WD. 1967. Extraordinary sex ratios. Science. 156:477–488.

Hance T, Kohandani-Trafresh F, Munaut F. 2017. Biological control. In: van Emden HF, Harrington R, editors. Aphids as crop pests. 2nd ed. Wallingford (UK): CABI. p. 448–493.

Hance T, Van Baaren J, Vernon P, Boivin G. 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annu Rev Entomol. 52:107–126.

He XZ, Wang Q. 2008. Reproductive strategies of *Aphidius ervi* Haliday (Hymenoptera Aphidiidae). Biol Control. 45:281–287.

He XZ, Wang Q, Teulon DAJ. 2004. Emergence, sexual maturation and oviposition of *Aphidius ervi* (Hymenoptera: aphidiidae). N Z Plant Prot. 57:214–220.

Heimpel GE, de Boer JG. 2008. Sex determination in the hymenoptera. Annu Rev Entomol. 53:209–230.

Helfrich-Föster C. 2001. The locomotor activity rhythm of *Drosophila melanogaster* in controlled by a dual oscillator system. J Insect Physiol. 47:877–887.

Hirose Y, Vinson SB, Hirose Y. 1988. Protandry in the parasitoid *Cardiochiles nigripes*, as related to its mating system. Ecol Res. 3:217–226.

Karpova SG. 2006. The role of endogenous and exogenous factors in regulation of synchronous emergence of *Trichogramma embryophagum* Harting and *T. principium* Sug. Et Sor. (Hymenoptera, Trichogrammatidae). Ent Rev. 86:267–282.

Karpova SG, Reznik SY. 2002. Interaction of exogenous factors (light and temperature) in their influence on the daily pattern of adult eclosion in *Trichogramma embryophagum* (Hymenoptera: trichogrammatidae). Eur J Entomol. 99:427–436.

Lankinen P. 1986. Geographical variation in circadian eclosion rhythm and photoperiodic adult diapause in *Drosophila littoralis*. J Comp Physiol. 159:123–142.

Le Ralec A, Anselme C, Outreman Y, Poirié M, van Baaren J, Le Lann C, van Alphen JJM. 2010. Evolutionary ecology of the interactions between aphids and their parasitoids. CR Biologies. 333:554–565.

Mackauer M, Stary P. 1967. Hymenoptera. Ichneumonoidae. World Aphidiidae. In: Delluchi V, Remaudière G, editors. Index of Entomophagous Insects. Paris (France): Le François. p. 1–195.

Martel V, Boivin G. 2004. Premating dispersion in the egg parasitoid *Trichogramma* (Hymenoptera: trichogrammatidae). Environ Entomol. 33:855–859.

Martel V, Doyon J, Boivin G. 2010. Partial local mate competition in the wasp *Trichogramma euproctidis*: the role of emergence sex ratio on female mating behaviour. Ecol Entomol. 35(6):698–703.

Mazzi D, Hatt F, Hein S, Dorn S. 2011. Ladies last: diel rhythmicity of adult emergence in a parasitoid with complementary sex determination. Physiol Entomol. 36:47–53.

Møller AP. 2004. Protandry, sexual selection and climate change. Glob Chang Biol. 10:2028–2035.

Morbey YE, Ydenberg RC. 2001. Protandrous arrival timing to breeding areas: a review. Ecol Lett. 4:663–673.

Myers EM. 2003. The circadian control of eclosion. Chronobiol Int. 20:775–794.

Nunes MV, Saunders D. 1999. Photoperiodic time measurement in insects: a review of clock models. J Biol Rhythm. 14:84–104.

Pittendrigh K. 1967. Circadian systems: I. The driving oscillation and its essay in *Drosophila pseudoobscura*. Proc Nat Acad Sci USA. 58:1762–1767.

Pompanon F, Fouillet P, Bouletreau M. 1995. Emergence rhythm and protandry in relation to daily patterns of locomotor activity in trichogramma species. Evol Ecol. 9:467–477.

Reznik SY, Voinovich ND, Vaghina NP, Karpova SG. 2008. Arrhythmic adult ecdysis but rhythmic emergence from the host chorion in *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae). Eur J Entomol. 105 (1):81–85.

Rounbehler MD, Ellington JJ. 1973. Some biological effects of selected light regimes on *Trichogramma semifumatum* (Hymenoptera; Trichogrammatidae). Ann Entomol Soc Am. 66:6–10.

Sakai T, Ishida N. 2001. Circadian rhythm of female mating activities governed by clock genes in *Drosophila*. Proc Natl Acad Sci USA. 98:9221–9225.

Salin C, Deprez B, van Bockstaele DR, Mahillon J, Hance T. 2004. Sex détermination mechanism in the hymenopteran parasitoid *Aphidius rhopalosiphi* de strephani perez (Braconidae, Aphidiinae). Belg J Zool. 134:15–21.

Saunders DS. 2009. Circadian rhythm and the evolution of photo periodic timing in insects. Physiol Entomol. 34:301–308.

Saunders DS, Steel CGH, Vafopoulou X, Lewis RD. 2002. Insect Clocks. Amsterdam (Netherlands): Elsevier. 560 p.

Shaffer PL. 1983. Prediction of variation in development period of insects and mites reared at constant temperatures. Environ Entomol. 12(4):1012–1019.

Skopik SD, Pittendrigh CS. 1967. Circadian systems. II. The oscillation in the individual Drosophila pupa; its independence of developmental stage. Proc Nat Acad Sci USA. 58:1862–1869.

Smith SM. 1996. Biological control with *Trichogramma*: advances, successes, and potential for their use. Annu Rev Entomol. 41:375–406.

Steel CGH, Davey KG. 2013. Integration in the insect endocrine system. In: Kerkut GA, Gilbert LI, editors. Comprehensive insect physiology, pharmacology and biochemistry. Endocrinology II. Vol. 8. Oxford (UK): Pergamon Press. p. 1–36.

Tauber E, Kyriacou CP. 2005. Molecular evolution and population genetics of circadian clock genes. Meth Enzymol. 393:797–817.

Tentelier C, Desouhant E, Fauvergue X. 2006. Habitat assessment by parasitoids: mechanisms for patch use behavior. Behav Ecol. 17:515–521.

Van Lenteren JC, Szabo P, Huisman PWT. 1992. The parasite-host relashioship between Encasia formosa Gahan (Hymenoptera APhelinidae) and *Trialeurodes vaporarium* (Homoptera, Aleyrodidae) (Westwood) XXXVII Adult emergence and initial dispersal pattern of *E. formosa*. J Appl Entomol. 114:392–399.

Wajnberg E. 2006. Time allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioral mechanisms. Behav Ecol Sociobiol. 60:589–611.

Wedell N. 1992. Protandry and mate assessment in the wart-biter *Decticus verrucivorus* (Orthopetra, Tettigoniidae). Behav Ecol Sociobiol. 31:301–308.

Werren JH. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. Science. 208(4448):1157–1159.

Wiklund C, Fagerstrom T. 1977. Why do males emerge before females? An hypothesis to explain the incidence of protandry in butterflies. Oecologia. 31:153–158.

Zamani AA, Talebi A, Fathipour Y, Baniameri V. 2007. Effect of temperature on life history of *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: braconidae), two parasitoids of *Aphis gossypii* and *Myzus persicae* (Homoptera: aphididae). Environ Entomol. 36(2):263–271.

Zaslavski VA, Zinovjeva KB, Umarova TY, Reznik SY. 1999. Interaction of circadian rhythm synchronized by photoperiod and thermoperiod with direct influence of light and temperature as factor determining rhythm of adult eclosion in two species of *Trichogramma* (Hymenoptera, Trichogrammatidae). Entomol Rev. 79:1–10.

Zaviezo T, Retamal R, Urvois T, Fauvergue X, Blin A, Malausa T. 2018. Effects of inbreeding on a gregarious parasitoid wasp with complementary sex determination. Evol Appl. 11(2):243–253.

Zijlstra WG, Kesbeke F, Zwaan BJ, Brakefield PM. 2002. Protandry in the butterfly *Bicyclus anynana*. Evol Ecol Res. 4:1229–1240.

Zonneveld C. 1996. Sperm competition cannot eliminate protandry. J Theor Biol. 178:105–112.

Zonneveld C, Metz JAJ. 1991. Models on butterfly protandry: virgine females are at risk to die. Theor Popul Biol. 40:308–321.