

CHAPTER 4

Mating with Sperm-Depleted Males Does Not Increase Female Mating Frequency in the Parasitoid *Lariophagus distinguendus*

Abstract In parasitic Hymenoptera, males have been reported to mate multiple times whereas many females copulate just once. Thus, accepting only a single mate might be costly when monandrous females copulate with a sperm-depleted male. In this study we investigated the mating characteristics and reproductive performance of the solitary ectoparasitoid *Lariophagus distinguendus* (Förster) (Hymenoptera: Pteromalidae). In the bioassays males were able to mate with up to 17 females offered in rapid succession within a 10 h test period. Sperm transfer remained constant during the first seven matings and then decreased significantly with increasing number of copulations of the males. Experimentally sperm-depleted males continued to mate even if they transferred only small amounts or no sperm at all. Unlike males, the majority of *L. distinguendus* females mated only once during a 192 h test period. A second copulation was observed only in a few cases (15%). The frequency of second matings did not depend on the mating status of the males with which the females had copulated the first time, suggesting that these events are not controlled by sperm deficiency of the females. Furthermore, we investigated the pheromone-mediated male courtship behaviour towards mated females. In general, male courtship intensity towards mated females decreased with increasing age of females. Females that had mated with a sperm-depleted male did not release stronger or longer lasting behavioural responses in courting males than those that received a full sperm load. We discuss these results with respect to a possible mating strategy of sperm-depleted males.

Key words Parasitoid, *Lariophagus distinguendus*, Pteromalidae, multiple mating, offspring production, sex ratio, sperm depletion.

Introduction

With respect to reproductive success, male and female insects have evolved divergent mating interests, often resulting in sexual conflicts (Chapman et al., 2003). In parasitic Hymenoptera, the fitness of males is primarily related to their ability to gain access to receptive females and fertilise more eggs than other males in the population. Since many parasitoid species are characterised by arrhenotokous parthenogenesis (unfertilised eggs develop into males, fertilised eggs into females), male reproduction success is determined by the quantity of daughters produced by their mates (Godfray, 1994; Quicke, 1997). In addition, males can maximise their reproductive success by limiting other males' access to females and thus preventing rivals from mating with them (Damiens and Boivin, 2006). Therefore, males have evolved a variety of mechanisms to manipulate female receptivity at the costs of female fecundity or longevity (Chapman et al., 1995; Snook, 2001). When ejaculating, insect males do not only transfer sperm but also a variety of bioactive molecules (mainly peptides) secreted by the accessory glands. These have been shown to induce in the females physiological responses like refractoriness (rejection of courting males), cessation of pheromone production or stimulation of egg development (Gillott, 2003). In species where refractoriness proceeds gradually after copulation, males often increase their chance of paternity behaviourally by guarding their mates to prevent further copulations with competitors (Field and Keller, 1993).

By contrast, a female's reproductive success is more restricted to her entire offspring production and less to the number of matings (Wedell et al., 2002). Sperm received from a single insemination is often sufficient for a female to fertilise a lifetime supply of eggs (Thornhill and Alcock, 1983). Though there are several examples of parasitoid species that exhibit females mating multiple times (Chevrier and Bressac, 2002; Jacob and Boivin, 2005), majority of parasitic Hymenoptera females observed so far mate just once (Gordh and DeBach, 1976; Ridley, 1993; Quicke, 1997). However, monandrous females copulating with multi-mated males that transfer insufficient sperm to fertilise all eggs may decrease their reproductive success. Since these females do not replenish their sperm supply by a second mating, they produce more sons than are optimal and thus waste resources that could have been used for the production of female offspring (Damiens and Boivin, 2005).

The solitary ectoparasitoid *L. distinguendus* (Förster) (Hymenoptera: Pteromalidae) parasitises larval and prepupal stages of several stored product infesting beetle species which lay their eggs in clumps (Steidle and Schöller, 1997). The mating system is characterised by local mate competition (Hamilton, 1967) because (1) mating commonly occurs at the emergence site, (2) males emerge first and then wait for females to copulate (protandry), (3) there is a high degree of sibling mating and (4) offspring sex ratios are female-biased. *L. distinguendus* females are supposed to copulate only once during their lifetime (Ruther et al., 2000), whereas males are able to mate several times (van den Assem et al., 1989). When encountering unmated females, *L. distinguendus* males perform a characteristic courtship behaviour including wing fanning, mounting and antennal stroking (van den Assem, 1970; Ruther et al., 2000). In response of the male courtship behaviour, females demonstrate sexual receptivity by lowering their head and almost simultaneously exposing their genitalia. Male arrestment and courtship behaviour is mediated by a female-derived sex pheromone (Ruther et al., 2000; Steiner et al., 2005). Although the sex pheromone of *L. distinguendus* females is still perceived by males several days after female mating, frequency of courtship elements shown by males towards mated females decreases with increasing time (Ruther et al., 2000) suggesting a cessation of pheromone activity after mating.

The objective of the present study was to acquire basic data (mating frequency, offspring number, sex ratio, development time) on the reproductive performance of male and female *L. distinguendus* and to investigate whether mating with sperm-depleted males influences the female's tendency to mate a second time. Furthermore, we studied the intensity of male courtship behaviour (as an indicator for the presence of the female courtship pheromone) towards mated females and asked whether females that mate with a sperm-depleted male maintain a higher pheromone activity.

Methods and materials

Insect rearing and general methods The *L. distinguendus* strain used in this study originated from Uzwill, Switzerland and was reared on larvae of the granary weevil *Sitophilus granarius* (Coleoptera: Curculionidae), maintained on wheat

grains (*Triticum aestivum* L., variety Batis) at a constant temperature of 25°C, a relative humidity of 60% and a photoperiod of 16:8 h light:dark (for details see Steidle and Schöller, 1997). One day before emergence, parasitised grains were individually isolated in 1.5 ml microcentrifuge tubes (Sarstedt, Nürnberg, Germany) to obtain virgin males and females for the following experiments. Freshly emerged parasitoids of either sex were held in groups of at most 15 individuals in Petri dishes with moistened filter paper. Experiments were performed in a bioassay chamber (10 mm diameter x 3 mm height) as described by Ruther et al. (2000) using a stereo microscope under illumination of a microscope light. Behavioural recordings were done using the computer software The Observer 3.0 (Noldus, Wageningen, The Netherlands).

Experiment 1: Male and female offspring production The first experiment was performed to estimate male and female offspring production and daily sex ratio of *L. distinguendus* when females are held under optimal conditions. For this purpose, virgin females were placed individually in separate tubes directly after emergence and mated with unmated males ($N = 21$). After mating, female parasitoids were transferred individually into Petri dishes and provided with weevil-infested wheat grains *ad libitum* for oviposition. Hosts were renewed every 24 h until death of the parasitoids. The first appearance as well as the daily and total number of emerging male and female offspring was recorded and sex ratio (proportion of daughters) was calculated.

Experiment 2: Male mating potential The following experiment was done to investigate the effect of rapid mating with several females on sperm depletion of *L. distinguendus* males during a 10 h test period. For this purpose, every half hour a single virgin female was presented to a male parasitoid in a bioassay chamber and male copulation success was observed for 10 min. The amount of sperm transferred with every successive copulation was estimated by counting the female offspring of all females with which the test males had copulated. For this purpose, individual females were placed directly after mating in Petri dishes containing weevil-infested grains *ad libitum* and allowed to lay eggs until their death. Female offspring were counted as a measure of male fitness and sex ratios were calculated ($N = 20$).

Experiment 3: Second mating of females This experiment addressed the question of whether the tendency of a second mating is increased in those *L. distinguendus* females which mate with a sperm-depleted male. Virgin females were mated with (a) a sperm-depleted male or (b) an unmated male as control. To obtain sperm-depleted males, single unmated males were placed in Petri dishes with 20 virgin females and allowed to copulate during the next 10 h. Subsequently, females of both treatments were transferred individually in a bioassay chamber and allowed to mate a second time with a virgin male 0 (< 1 min), 1, 15, 48, 96 and 192 h after their first copulation ($N = 27$ for every treatment). Between experiments, females were held in Petri dishes on host-infested wheat grains at rearing conditions. Second matings of the females were observed for 10 min. Parasitoids which had copulated twice were excluded from further matings. Additionally, the frequency of key elements of male courtship behaviour (wing fanning, antennal stroking and copulation attempts) were recorded to investigate whether females that have copulated with sperm-depleted males continue to release the sex pheromone to maintain the chance for a second mating in comparison to those females that received sufficient sperm.

Statistical analysis Statistical analysis was performed using Statistica 4.5 scientific software (StatSoft, Hamburg, Germany). All data in text and figures are given as means \pm standard errors (SE). Spearman correlations were used to investigate the relation between the number of offspring and female longevity (experiment 1), the number of copulations and the sex ratio (proportion of females) (experiment 2), as well as the relation between the time after first copulation and the number of key elements of the male mating behaviour (wing fanning, antennal stroking and copulation attempts) (experiment 3). Differences in the frequency of behavioural elements shown by males towards mated females of the two types (experiment 3) as well as comparison of the development time between male and female offspring (experiment 1) were analysed by a *t*-test. Proportions of second matings performed by differently treated females were compared by a $2 \times 2 \chi^2$ test.

Results

Experiment 1: Male and female offspring production Mated *L. distinguendus* females had a lifetime of 19.7 ± 1.2 d and produced an offspring of 123.3 ± 5.6 individuals. There was a positive correlation between offspring production and female longevity ($r_s = 0.739$; $P < 0.001$). Females produced male and female offspring from the first day on. Development time of males (20.1 ± 0.2 d) was significantly shorter compared to females (21.7 ± 0.2 d) ($t_{2556} = 34.818$; $P < 0.001$). Mean sex ratio was 0.61 ± 0.03 (proportion of females). Females produced male offspring at a constant low level that reached the maximum on day ten (3.3 ± 0.5 individuals), whereas more than 50% of the female offspring were produced between day two and seven, maximising on day six (9.5 ± 0.4 individuals) (Fig. 1). From day 14 on, few parasitoids of both sexes were produced. The results of this experiment show that protandry in *L. distinguendus* is due to a shorter male development time rather than the result of the oviposition strategy of the mothers.

Experiment 2: Male mating potential During the 10 h test period, *L. distinguendus* males mated with 13 ± 0.7 out of 20 females presented. Thereby, number of copulations varied between 6 and 17. Though no relationship was found between the total number of offspring and the number of copulations a male achieved during this experiment ($r_s = 0.222$; $P = 0.41$), the proportion of female offspring remained constant during the first seven copulations and then decreased gradually with successive matings of males ($r_s = -0.658$; $P < 0.001$) (Fig. 2). Thus, females which had copulated with unmated or rare-mated (i.e., 1-7 previous copulations) males produced offspring with a high proportion of females (up to 66.4%), whereas individuals which had mated with multi-mated males (> 11 previous copulations) produced few females and, therefore, had strongly male-biased sex ratios (up to 95.7% males). In some cases (4.9%) a copulation but no female offspring was observed, indicating that no sperm was transferred at all.

Experiment 3: Second mating of females Regardless of whether *L. distinguendus* females copulated first with an unmated (UM) or a multi-mated (MM) male, the majority of females mated only once during the 192 h test period (Fig. 3). However, in both treatments 4 out of 27 females (14.8%) copulated a

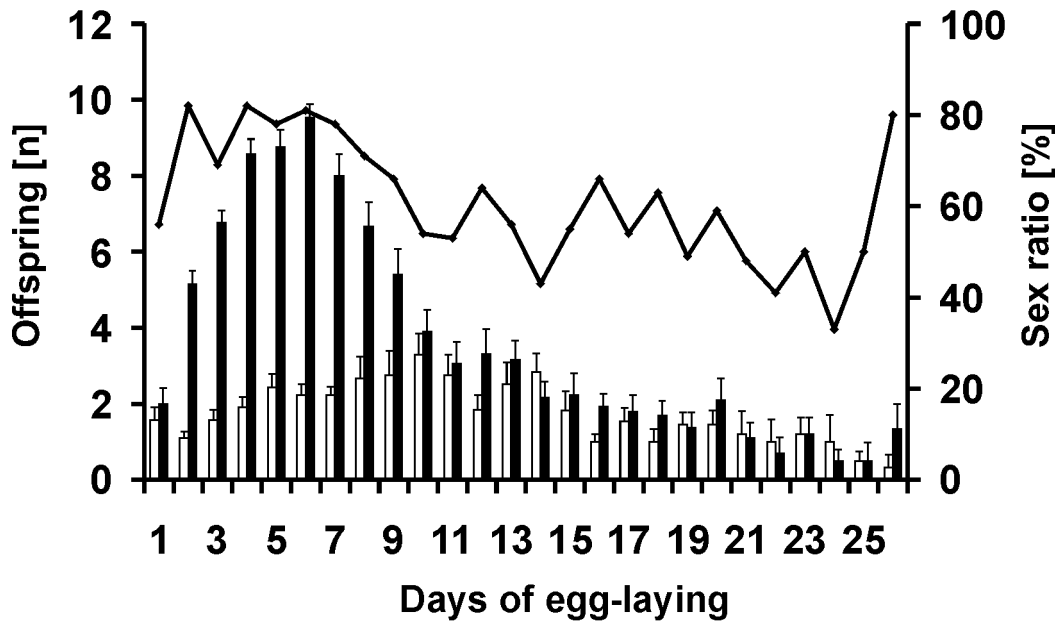


Fig. 1 Male (white bars) and female (black bars) offspring production (mean \pm SE) and daily sex ratio (proportion of females) of mated *L. distinguendus* females ($N = 21$).

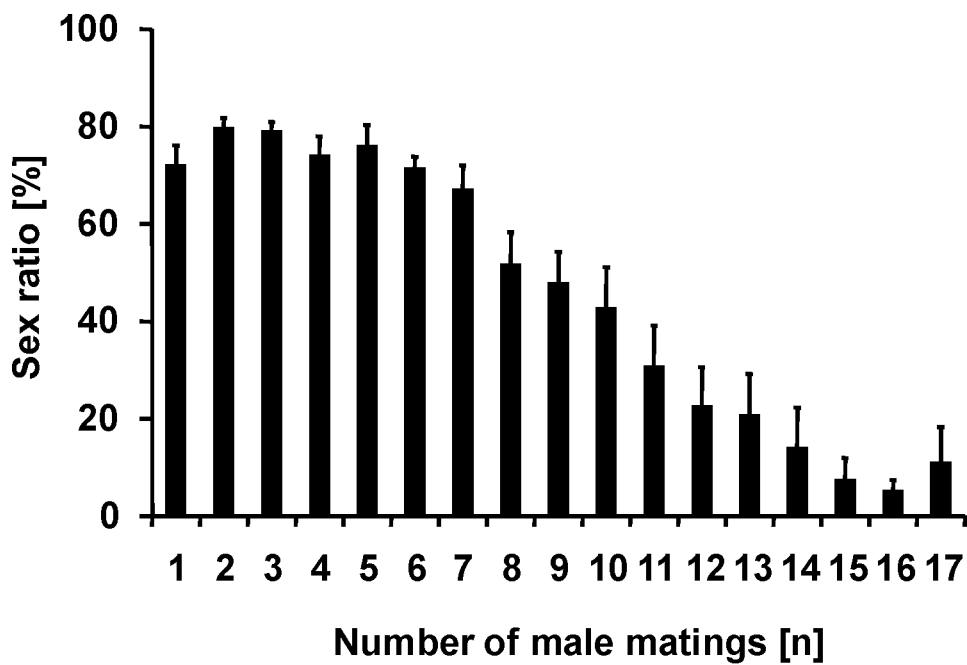


Fig. 2 Relation between the number of copulations by *L. distinguendus* males and the sex ratio (proportion of females) produced by their mates ($N = 20$).

second time. Second matings occurred only within the first 15 h after the initial copulation. Furthermore, elements of the male courtship behaviour were recorded indicating the level of female pheromone activity throughout the test period. Number of wing fanning (UM: $r_s = -0.749$; $P < 0.001$ / MM: $r_s = -0.747$; $P < 0.001$), antennal stroking (UM: $r_s = -0.720$; $P < 0.001$ / MM: $r_s = -0.845$; $P < 0.001$) and copulation attempts (UM: $r_s = -0.693$; $P < 0.001$ / MM: $r_s = -0.781$; $P < 0.001$) performed by *L. distinguendus* males towards the once-mated females decreased with increasing time after initial copulation but were not significantly different between the two treatments (t -test, $P > 0.05$ for all) (Fig. 4a-c). Results demonstrate that mated females did not induce a higher degree of male courtship after having copulated initially with a sperm-depleted male.

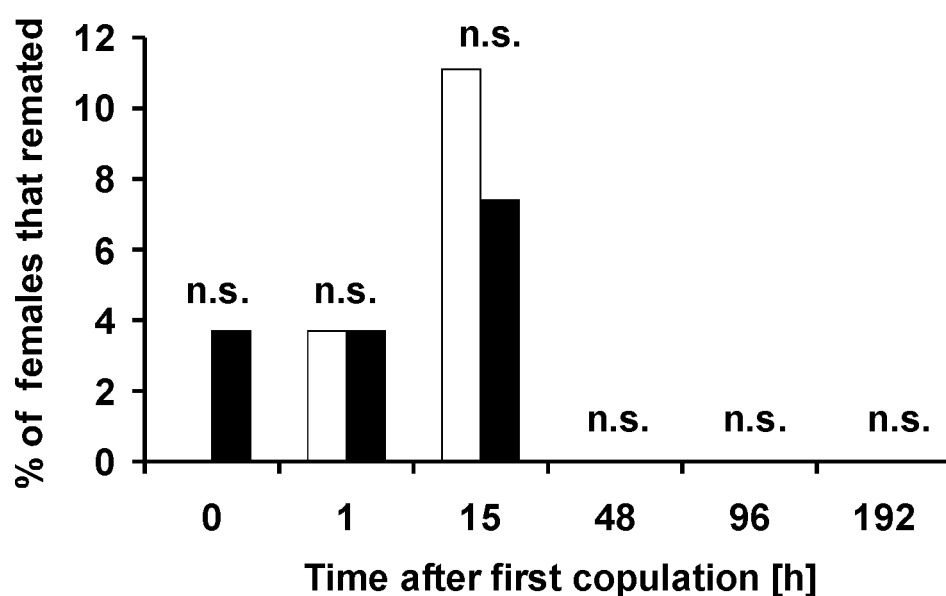


Fig. 3 Number of second matings of *L. distinguendus* females 0, 1, 15, 48, 96, 192 h after first copulation (black bars = females mated initially with virgin males; white bars = females mated initially with multi-mated males). N.s. = not significant (analysed by χ^2 test, $N = 27$).

Discussion

Results in this study demonstrate that *L. distinguendus* males transferred a relatively constant amount of sperm to females during their first seven copulations. However, the proportion of daughters produced by their mates decreased with further matings of males, suggesting that the amount of sperm is a limiting factor for

the fitness of *L. distinguendus* males. In some cases, no sperm was transferred to the females, resulting in the production of only male offspring. Reduction in sperm transfer with successive inseminations occurs in several other parasitic Hymenoptera (Nadel and Luck, 1985; King, 2000; Damiens and Boivin, 2005). Males of the pteromalid *Nasonia vitripennis*, for example, are able to copulate with hundreds of females, however, the full amount of sperm is transferred only for a few copulations (Barras, 1961; van den Assem, 1986). In *L. distinguendus*, males did not stop copulating with females even when they transferred little or no sperm. Most *L. distinguendus* females mated only once in their lifetime. Only a few females copulated twice. However, mating with a sperm-depleted male did not increase the tendency of *L. distinguendus* females to mate a second time. Thus, in *L. distinguendus* remating does not occur as a result of sperm deficiency as shown for other insect species (Leatemia et al., 1995; Foster and Ayers, 1996; Damiens and Boivin, 2006). After receiving a reduced amount of sperm by a multi-mated male, females did not release stronger or longer lasting behavioural responses in courting males than those females that received a full sperm supply. This suggests that *L. distinguendus* females no longer invest in pheromone biosynthesis after mating irrespective of whether they received sufficient sperm or not. Both female refractoriness and termination of pheromone synthesis can be induced by bioactive proteinous molecules secreted by male accessory glands and transferred together with sperm during ejaculation (Gillot, 2003). Thus, sperm-depleted males may “switch off” virgin females by these molecules and thereby may increase their relative fitness without actually transferring sperm because these females are no longer available for their male competitors. Furthermore, sperm-depleted males increase the proportion of males in the next generation and thus the insemination probabilities of their daughters (Damiens and Boivin, 2006). Such mating behaviour often discussed in terms of spite is also known from other parasitic wasps. In *Habrobracon lineatella*, freshly emerged males copulate with females even before they are sexually mature (Laing and Caltagirone, 1969). Damiens and Boivin (2006) reported in a recent study that sperm-depleted males of *Trichogramma evanescens* do not prevent their mates from further copulations but reduce the female’s ability to store sperm from other males. However, the problem of whether spiteful behaviour in animals can evolve under evolutionary pressure has been controversially discussed since many years (Foster et al., 2001; Vickery et al., 2003).

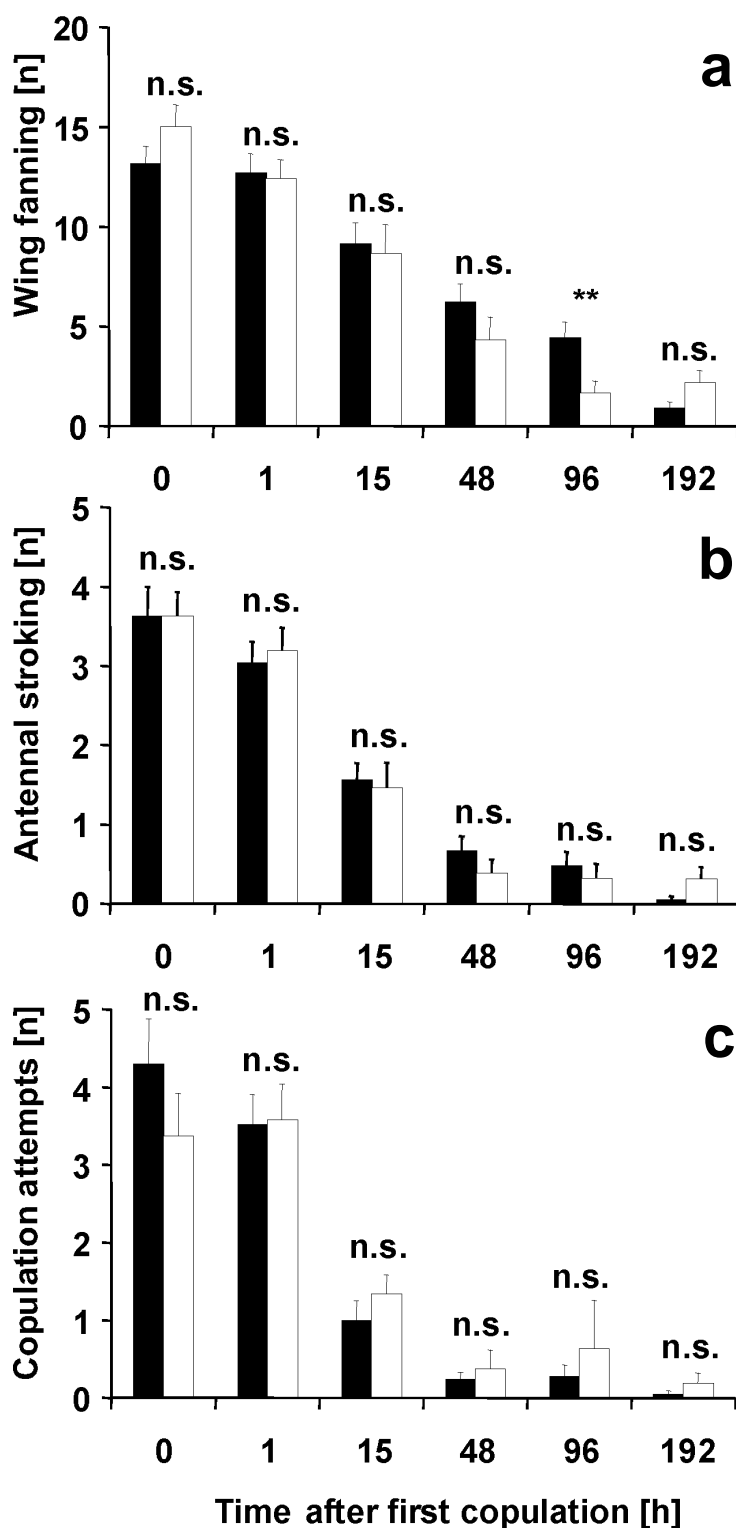


Fig. 4 Behavioural key elements of mating behaviour (mean \pm SE) shown by unmated *L. distinguendus* males towards mated females 0, 1, 15, 48, 96, 192 h after copulation (black bars = females mated initially with virgin males; white bars = females mated initially with multi-mated males). (a) Mean number of wing fanning, (b) antennal stroking and (c) copulation attempts during a 10 min observation time. N.s. = not significant; ** = $P < 0.01$ (analysed by a *t*-test, $N = 27$).

From the female's perspective, mating with a sperm-depleted male may result in sperm limitation and hence in a reduction of female offspring. Thus, monandrous females are expected to evolve antagonistic mechanisms against the described male mating strategy (Chapman et al., 2003). One possibility is that females prevent mating with sperm-depleted males. In our experiments, however, virgin *L. distinguendus* females never rejected a courting male irrespective of its sperm status suggesting that *L. distinguendus* females are not able to recognise the mating status of males. Thus, the question arises why females are not able to discriminate between sperm-depleted males and those with a full batch of sperm. A possible reason is that life span of parasitoids in nature is assumed to be shorter than under experimental conditions (Nadel and Luck, 1985) and, hence, mated *L. distinguendus* females may not live long enough to exhaust their sperm supply even when they were not fully inseminated by a multi-mated male. Furthermore, females that run out of sperm are nevertheless able to gain fitness by producing sons from unfertilised eggs (Godfray, 1994). Another possibility is that even successful males are rarely sperm-depleted under natural conditions. Our results demonstrate that after receiving the ejaculate from an unmated male *L. distinguendus* females produce a female-biased offspring (61% females) in which calculatively two males copulate with three mates to inseminate all females in a given patch. Under these circumstances, *L. distinguendus* females would never encounter sperm-depleted males since we showed that even seven copulations do not result in a significant decrease of the number of fertilised eggs (i.e., female offspring). However, male mating success is often not uniformly distributed among the males of a patch. Some males might copulate with a high number of females while others do not mate at all. In *L. distinguendus* size is a crucial factor of male mating success since large males are able to outrival smaller ones when directly competing for a female (van den Assem et al., 1989). In the quasi-gregarious parasitoid *T. evanescens*, 20% of the males which left the emergence site were sperm-depleted whereas others still had a full batch of sperm (Damiens and Boivin, 2006). Another aspect is that the majority of females often emerge within the first days and thus, males emerging shortly before may have drastically more mating opportunities than later ones. In this study, the sex ratio of *L. distinguendus* (proportion of females) during the first days is clearly above the mean sex ratio. Consequently, early emerging males should have the chance to mate much more frequently than later ones. However, it remains unclear whether this may actually lead to significant sperm depletion. Therefore, it should be investigated in future

studies whether sperm-depletion occurs in *L. distinguendus* in natural habitats and whether this leads to a sexual conflict between both genders due to the ongoing mating activities of multi-mated males.

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References

- Assem, J. van den (1970) Courtship and mating in *Lariophagus distinguendus* (Först.) Kurdj. (Hymenoptera, Pteromalidae). *Netherl. J. Zool.* 20:329-352.
- Assem, J. van den (1986) Mating behavior in parasitic wasps. In: Waage, J. and Greathead, D. (eds.) *Insect parasitoids*, pp. 137-167. Academic Press, London.
- Assem, J. van den, Iersel, J.J.A. van and Los-den Hartogh, R.L. (1989) Is being large more important for female than male parasitic wasps? *Behaviour* 108:160-195.
- Barras, R. (1961) A quantitative study of the behavior of the male *Mormoniella vitripennis* towards two constant stimulus situations. *Behaviour* 18:288-312.
- Chapman, T., Arnqvist, G., Bangham, J. and Rowe, L. (2003) Sexual conflict. *Trends Ecol. Evol.* 18:41-47.
- Chapman, T., Little, L.F., Kalb, J.M. Wolfner, M.F. and Partridge, L. (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory-gland products. *Nature* 373:241-244.
- Chevrier, C. and Bressac, C. (2002) Sperm storage and use after multiple mating in *Dinarmus basalis* (Hymenoptera: Pteromalidae). *J. Insect Behav.* 15:385-398.
- Damiens, D. and Boivin, G. (2005) Male reproductive strategy in *Trichogramma evanescens*: Sperm production and allocation to females. *Physiol. Entomol.* 30:241-247.
- Damiens, D. and Boivin, G. (2006) Why do sperm-depleted parasitoid males continue to mate? *Behav. Ecol.* 17:138-143.
- Field, S.A. and Keller, M.A. (1993) Alternative mating tactics and female mimicry as post-copulatory mate-guarding behaviour in the parasitic wasp *Cotesia rubecula*. *Anim. Behav.* 46:1183-1189.
- Foster, S.P. and Ayers, R.H. (1996) Multiple mating and its effects in the lightbrown apple moth, *Epiphyas postvittana* (Walker). *J. Insect Physiol.* 42:657-667.
- Foster, K.R., Wenseleers, T. and Ratnieks, F.L.W. (2001) Spite: Hamilton's unproven theory. *Ann. Zool. Fenn.* 38:229-238.

- Gillott, C. (2003) Male accessory gland secretions: Modulators of female reproductive physiology and behavior. *Annu. Rev. Entomol.* 48:163-184.
- Godfray, H.C.J. (1994) Parasitoids – Behavioral and evolutionary ecology. Monographs in behavior and ecology. Princeton University Press, Princeton, New Jersey.
- Gordh, G. and DeBach, P. (1976) Male inseminative potential in *Aphytis lingnamensis* (Hymenoptera: Aphelinidae). *Can. Entomol.* 108:583-589.
- Hamilton, W.D. (1967) Extraordinary sex ratios. *Science* 156:477-488.
- Jacob, S. and Boivin, G. (2005) Costs and benefits of polyandry in the egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). *Biol. Control* 32:311-318.
- King, B.H. (2000) Sperm depletion and mating behavior in the parasitoid wasp *Spalangia cameroni* (Hymenoptera: Pteromalidae). *Gt. Lakes Entomol.* 33:117-127.
- Laing, D.R. and Caltagirone, L.E. (1969) Biology of *Habrobracon lineatella* (Hymenoptera, Braconidae). *Can. Entomol.* 101:135-142.
- Leatemia, J.A., Laing, J.E. and Corrigan, J.E. (1995) Production of exclusively male progeny by mated, honey-fed *Trichogramma minutum* Riley (Hym., Trichogrammatidae). *J. Appl. Entomol.* 119:561-566.
- Nadel, H. and Luck, R.F. (1985) Span of female emergence and male sperm depletion in the female-biased, quasi-gregarious parasitoid, *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae). *Ann. Entomol. Soc. Am.* 78:410-414.
- Quicke, D.L.J. (1997) Parasitic wasps. Chapman and Hall, London.
- Ridley, M. (1993) Clutch size and mating frequency in parasitic Hymenoptera. *Am. Nat.* 142:893-910.
- Ruther, J., Homann, M. and Steidle, J.L.M. (2000) Female derived sex pheromone mediates courtship behaviour in the parasitoid *Lariophagus distinguendus*. *Entomol. Exp. Appl.* 96:265-274.
- Snook, R. (2001) Sexual selection: Conflict, kindness and chicanery. *Curr. Biol.* 11:337-341.
- Steidle, J.L.M. and Schöller, M. (1997) Olfactory host location and learning in the granary weevil parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *J. Insect Behav.* 10:331-342.
- Steiner, S., Steidle, J.L.M. and Ruther, J. (2005) Female sex pheromone in immature insect males – A case of pre-emergence chemical mimicry? *Behav. Ecol. Sociobiol.* 58:111-120.
- Thornhill, R and Alcock, J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge.
- Vickery, W.L., Brown, J.S. and FitzGerlad, G.J. (2003) Spite: Altruism's evil twin. *Oikos* 102:413-416.
- Wedell, N., Gage, M.J.G. and Parker, G.A. (2002) Sperm competition, male prudence and sperm-limited females. *Trends Ecol. Evol.* 17:313-320.

