

# THE EFFECT OF SEX RATIO AND GROUP DENSITY ON THE MATING SUCCESS OF TWO LINES OF *DELIA PLATURA* (DIPTERA: ANTHOMYIIDAE)

Allen Bush-Beaupré<sup>1</sup>, Marc Bélisle<sup>1</sup>, Anne-Marie Fortier<sup>2</sup>, François Fournier<sup>3</sup>, and Jade Savage<sup>4</sup>

<sup>1</sup>Université de Sherbrooke Département de Biologie

<sup>2</sup>Compagnie de recherche Phytodata Inc.

<sup>3</sup>Collège Montmorency

<sup>4</sup>Bishop's University Department of Biology

November 16, 2022

## Abstract

Many control methods target agricultural pests' reproductive traits and so, knowledge of these traits is crucial. The seedcorn maggot, *Delia platura* (Diptera: Anthomyiidae), is reported as a cosmopolitan polyphagous pest species which can be found in high numbers in numerous crops. Two morphologically identical genetic lines of *D. platura* (H- and N- lines) with distinct distribution ranges were recently discovered. While many biological traits have been described for *D. platura*, no study to date has been conducted on the life history strategies and reproductive behaviors of its two lines. Using laboratory-reared colonies originating from the Montérégie region in Québec, this project investigates the effect of group composition (sex-ratio and density) on the mating success and pre-oviposition period of the two *D. platura* lines. We found a substantial increase in mating success with increasing proportion of males within mating groups for both lines while group density had negligible effects. These results corroborate reports of *D. platura* high-density swarms in which sex ratios are usually male-biased. The pre-oviposition period decreased as the ratio of males to female increased at low density only for the N-line while the opposite trend was observed at high density for both lines. These results suggest differences between the mating systems of these two lines, with the H-line females being choosier towards potential mates than those of the N-line. We also describe reproductive traits of both lines along with their implications for integrated pest management strategies such as the sterile insect technique or the release of individuals carrying pathogens, both of which must take into account the high degree of sexual selection present in such group contexts. Keywords: reproductive biology, mating dynamics, seedcorn maggot, swarms

**THE EFFECT OF SEX RATIO AND GROUP DENSITY ON THE MATING SUCCESS OF TWO LINES OF *DELIA PLATURA* (DIPTERA: ANTHOMYIIDAE)** Authors: Allen Bush-Beaupré<sup>1,2\*</sup>, Marc Bélisle<sup>1,3</sup>, Anne-Marie Fortier<sup>4</sup>, François Fournier<sup>5</sup>, Jade Savage<sup>2,1</sup> Université de Sherbrooke, Département de biologie, Sherbrooke, Canada<sup>2</sup>Bishop's University, Department of biology, Canada<sup>3</sup>Centre d'étude de la forêt (CÉF)<sup>4</sup>Compagnie de recherche Phytodata inc, Sherrington, Canada<sup>5</sup>Collège Montmorency, Laval, Canada \*Corresponding author Allen Bush-Beaupré Université de Sherbrooke, Département de biologie 2500 Boulevard de l'Université Sherbrooke, Québec, Canada J1K 2R1 Email: allen.bush-beaupre@usherbrooke.ca **Cover letter** Describing and comparing mating systems is an essential part of species delimitation. Considering that many Diptera species mate in swarms, investigating the effect of factors that are known to influence swarming dynamics such as sex ratio and group density can serve to describe and contrast the dynamics of such mating system. The seedcorn maggot, *Delia platura* (Diptera: Anthomyiidae), is reported as a cosmopolitan polyphagous pest species which may be found in

high numbers in numerous crops. Two morphologically identical genetic lines of *D. platura* (H- and N- lines) with distinct distributions were recently discovered. While many biological traits have been described for *D. platura*, no study to date has been conducted on the life history strategies and reproductive behaviors of its two lines. This article investigates the effects of sex ratio and group density on the H- and N- lines of *Delia platura* mating success, pre-oviposition period and fecundity. The benefits of conducting such study are three-fold. First, differences in mating dynamics between the two lines may serve as evidence for pre-mating isolation between them. Second, a better understanding of the group composition that optimises mating probability can then serve to provide such a setting to each line when conducting reproductive compatibility experiments to maximise the probability that mating would occur in the first place. Third, describing the mating and egg-laying dynamics of the two *D. platura* lines will serve to ensure that future studies pertaining to their biology will take into account their respective reproductive traits. This article is thus relevant to Ecology and Evolution as it pertains to the reproductive behavior, life history and inter-individual interactions.

**Abstract** Many control methods target agricultural pests' reproductive traits and so, knowledge of these traits is crucial. The seedcorn maggot, *Delia platura* (Diptera: Anthomyiidae), is reported as a cosmopolitan polyphagous pest species which can be found in high numbers in numerous crops. Two morphologically identical genetic lines of *D. platura* (H- and N- lines) with distinct distribution ranges were recently discovered. While many biological traits have been described for *D. platura*, no study to date has been conducted on the life history strategies and reproductive behaviors of its two lines. Using laboratory-reared colonies originating from the Montérégie region in Québec, this project investigates the effect of group composition (sex-ratio and density) on the mating success and pre-oviposition period of the two *D. platura* lines. We found a substantial increase in mating success with increasing proportion of males within mating groups for both lines while group density had negligible effects. These results corroborate reports of *D. platura* high-density swarms in which sex ratios are usually male-biased. The pre-oviposition period decreased as the ratio of males to female increased at low density only for the N-line while the opposite trend was observed at high density for both lines. These results suggest differences between the mating systems of these two lines, with the H-line females being choosier towards potential mates than those of the N-line. We also describe reproductive traits of both lines along with their implications for integrated pest management strategies such as the sterile insect technique or the release of individuals carrying pathogens, both of which must take into account the high degree of sexual selection present in such group contexts.

**Keywords:** reproductive biology, mating dynamics, seedcorn maggot, swarms

**Introduction** Mating systems vary greatly between and even within animal taxa. Multiple factors influence the dynamics of these systems such as life history traits, the distribution and availability of resources, and parental investment. These factors in turn affect the temporal and spatial distribution of sexually receptive individuals which determine the adult and operational sex ratio. The sex ratio ultimately determines the number of available mates which, in interaction with sexual selection, will directly affect the dynamics within a given mating system (reviewed in Klug, 2011). Thus, mating system dynamics are greatly affected by variations in the total number of individuals and the relative proportions of females and males within a group or population. Additionally, the dynamics involved in mating systems, especially those relating to mate choice, are believed to influence the rate of speciation through the formation of reproductive barriers (Wilkinson & Johns, 2005). A mating systems' dynamics are influenced by the sex ratio in multiple ways. Firstly, male-biased sex ratios lead to an increase in male-male competition. In many cases, males accentuate their courtship behaviors when other males are present to increase their competitiveness (Carrillo et al., 2012; Leftwich et al., 2012). However, the mating consequences of increased competitiveness appear to be species-specific. In cases where females can remate such as seen in medflies (*Ceratitis capitata* (Diptera: Tephritidae)), it is suggested that sperm competition or female cryptic choice leads to a decrease paternity for any one male (Leftwich et al., 2012). Conversely, an increase in male courtship behavior due to competitive settings led to a reduction in remating probability for female housefly (*Musca domestica* (Diptera: Muscidae)) and increased the number of eggs laid in the first clutch along with higher offspring survivorship. Carrillo et al. (2012) speculate that male houseflies transfer accessory seminal products during mating, which stimulate females to utilize the male's sperm rather than remate. Another method by which males attempt to limit competition is by increasing copulation time and mate guarding (Clark, 1988; Otronen, 1996). However, there appears to be a threshold

at which the type of competition between males switches as the group sex-ratio becomes more male-biased. Among both vertebrate and invertebrates, there is an increasing trend in contest competition at sex-ratios between 1 to 2 males per female followed by a switch to scramble competition as male proportions increase past 2 males per female (Weir et al., 2011). An individual's mating probability is directly related to the availability of potential mates and the abundance of individuals of the same sex. Either through male competition or cryptic female choice, a higher proportion of males within a group leads to a decrease in an individual male's mating probability (Carrillo et al., 2012; Leftwich et al., 2012) and, conversely, a higher proportion of females within a group decreases an individual female's mating probability (Guo et al., 2014). Male-biased sex ratios increase female mating probability and frequency in many insect species (Lauer et al., 1996; Punzalan & Rowe, 2013; Vahl et al., 2013). However, in some cases, female mating success is influenced by its age rather than group sex ratio (Makee & Saour, 2001). Group density also has varying effects on mating dynamics. An increased number of individuals causes an increase in remating opportunities and thus increases female fertility in some species (Parry et al., 2017). Males, however, seem adversely affected by density as increased crowding reduces their ability to form leks (Miyatake & Haraguchi, 1996) and increases inter-male fighting leading to a decrease in multiple matings (House et al., 2019). In addition to the role it plays in mating dynamics, group density can also affect females' post-mating oviposition behavior. The presence and oviposition of conspecific females affect individual females' own oviposition variably. In certain species, female crowding can increase the length of the preoviposition period (Ambrose et al., 1988) and ovipositional substrates with the presence of conspecific eggs may deter females (Elsensohn et al., 2021). However, these trends can be reversed in some species (Desurmont et al., 2014; Judd & Borden, 1992; Ulmer et al., 2003) such as the viburnum leaf beetle, *Pyrrhalta viburni* (Coleoptera: Chrysomelidae), where females spent 21% less time searching and selecting an oviposition site when conspecific egg masses were present on twigs, thus reducing the preoviposition period. Although the effects of sex ratio and density can be observed separately, it is of interest to observe how these two demographic factors may interact to affect mating dynamics. When evaluated in interaction, sex ratio and density appear to affect mating dynamics variably depending on the taxa. For example, one eco-type of the isopod *Asellus aquaticus* (Isopoda: Asellidae) had an increased mating frequency in male-biased sex ratios but was not affected by density whereas another eco-type's mating propensity was not affected by either of these factors (Karlsson et al., 2010). A higher proportion of mated females was observed when in high density conditions no matter the sex ratio in the almond moth, *Cadra cautella* (Lepidoptera: Pyralidae) (Sammani et al., 2020). However, for the same species, female copulation frequency increased in male-biased conditions no matter the density (Mcnamara et al., 2008). In two *Drosophila* (Diptera: Drosophilidae) species, female mating probability increased to a greater extent when exposed to high density and male-biased sex ratios (Vahl et al., 2013). True flies (order Diptera) display a broad range of mating systems, including the congregation of many individuals in mating swarms (Wilkinson & Johns, 2005). Swarming behavior is thought to increase mate encounter rates and mate choice opportunities (Downes, 1969). For example, *Anopheles gambiae* (Diptera: Culicidae) male mating success was observed to increase with swarm size (Diabaté et al., 2011). Within mating swarms, sex ratios typically tend to be male-biased, offering female flies increased mating opportunities. Females typically join the swarm to mate and leave once mated while males stay in the swarm. As a result, female flies' mating success is influenced both by the aggregation's density (as expressed by the number of individuals per unit volume) and male to female proportions (Rhains, 2010). Additionally, along with being variable in terms of density, fly swarms vary in shape and height depending on the species, swarm marker (conspicuous object or location where swarms occur) and female behavior (Sullivan, 1981). Among its diverse functions, swarming behavior can serve as reproductive isolation between diverged populations. When the swarming behavior of *Anopheles gambiae* and *Anopheles coluzzii* was investigated as potential reproductive isolation strategies in Burkina Faso, segregated swarms were found to be more common than swarms composed of both species and no inter-breeding was detected (Sawadogo et al., 2014). Similar swarm segregation, associated with different swarm markers, was observed in these species in Mali (Diabaté et al., 2009). The daily timing at which the swarms of these two species were formed appeared to further contribute to their reproductive isolation (Sawadogo et al., 2013). Studying swarms as mating systems thus has the potential to uncover gene flow barriers between populations. Considering the prominent and variable effects of density and sex ratio in

mating system and swarming dynamics, different relative effect sizes of these variables on mating success and post-mating behavior may indicate differences in mating systems between populations. The seedcorn maggot, *Delia platura* (Meigen) (Diptera: Anthomyiidae) is currently recognized as an agricultural pest with a near cosmopolitan range and a high diversity of larval hosts, both living and dead (Griffiths, 1993). Being highly polyphagous, the larvae of *D. platura* will feed on many species of cultivated vegetables and field crops (Griffiths, 1993; Hough-Goldstein & Hess, 1984; Howard et al., 1994; Soroka & Dosdall, 2011). The seedcorn maggot can act as a primary or secondary invader of host plants and it can also complete its development in decaying organic matter (Finch, 1989). It is a member of the Seed Maggot Complex (SMC) along with *Delia florilega* (Zetterstedt) whose females are morphologically similar and larvae identical to those of *D. platura* (Brooks, 1951; Savage et al., 2016). In Ontario (Canada), mixed swarms of *Delia platura* and *Delia florilega* have been observed and although the identification of females to species was not positive, a much higher proportion of males was observed (Miller & McClanahan, 1960). We identified two genetic clusters within *D. platura*, separated by a minimum p-distance of 4.45% for the barcoding gene COI (Folmer region), and exhibiting different geographical distributions; the provisionally named H-line having a primarily Holarctic range while the N-line is restricted to the Nearctic and Central American regions. Eastern Canada currently appears to be the only region where these two lines overlap (Savage et al. 2016). Biological differences have also been reported in southwestern Québec, with Van der Heyden et al. (2020) showing that N-line larvae appeared in sampled crops almost 2.5 weeks before the H-line, and Savage et al. (2016) finding the H-line to be 2.5 times more abundant than the N-line in cruciferous crops (*Brassica*) while the trend was reversed in onions (*Allium*). Since intraspecific distance for COI in muscoid flies, (including *Delia*) is typically below 2.5% (Renaud et al., 2012; Savage et al., 2016), and considering that other named cryptic species with distribution and/or life history differences typically exhibit less inter-specific distance (Derocles et al., 2016), we suspect that, in spite of their identical morphology, the two genetic lines of *D. platura* represent distinct biological entities. A better understanding of the biological attributes of the two genetic lines of *D. platura* is especially relevant considering that a vast body of regional literature has been produced on various aspects of the natural history and control of this pest species; data and recommendations based on local studies involving one line (or even a mix of both) may, however, not be transferable to other settings or localities. The sterile insect technique (SIT) is a control method that has been successfully applied to other *Delia* species in Canada and abroad (Fortier, 2021; Ticheler et al., 1980). As the effectiveness of SIT relies on knowledge of certain characteristics including mating patterns and spatial distribution (Barclay, 2005; Ikegawa & Himuro, 2017; Oléron Evans & Bishop, 2014), we are especially interested in the mating habits of the two genetic lines. Hough-Goldstein et al. (1987) studied the effect of *D. platura* group size (density) and sex ratio on female insemination and fecundity (egg hatchability) and noted an increased proportion of inseminated females in male-biased sex ratios while fecundity seemed unaffected by group composition. However, the authors did not assess whether the effect of sex ratio depended upon group size, and if it did, by how much. Additionally, the identity of the genetic line(s) studied by remains unknown. As the H- and N- lines have only recently been identified, describing and comparing their mating systems will ensure that future experimental work on their life history traits and mating compatibility is properly designed to account for potential differences. The specific aim of our study was therefore to investigate the effect of group density and sex ratio, along with their interaction, on *D. platura* H- and N- line female mating probability, pre-oviposition period, and egg hatchability. **Methods** *Delia platura* colonies Colonies of the N- and H- lines of *D. platura* were established from wild flies collected in the Montérégie region of southern Québec (Canada) and maintained under constant conditions (20°C, RH 60%, 16:8 h L:D) at Collège Montmorency (Laval, QC, Canada) for approximately two years prior to the experiment. Wild gravid females were isolated in individual arenas and allowed to lay eggs on a substrate of soil and germinating bean seeds for approximately one week. Female COI haplotype was determined using a high-resolution melting PCR (HRM) assay following Van der Heyden et al. (2020) to determine the identity of their offspring. Each colony was thus established with the offspring of several females. HRM was also used to periodically test random individuals from each colony to ensure no cross contamination had taken place. Colonies were maintained with an artificial larval diet similar to Ishikawa et al. (1983), and adults were supplied with distilled water, a diet which consisted in a dry mixture of milk powder, icing sugar, autolyzed yeast extract, brewer's yeast and soy flour in a 10:10:1:1:1 ratio, respective-

ly, and a rutabaga (*Brassica napus*) oviposition site. *Experimental stocks* Eggs from each main colony were harvested periodically (17 and 16 times for the N- and H- lines, respectively) over the course of 10 months and reared on artificial diet containers. Following 16-18 days of development, pupae were harvested, sieved with 1.7-mm mesh to remove small individuals, and placed in individual plastic vials to be used as adults for the experiment (see Appendix Figure 1 for explanation of sieve size choice). Voucher specimens were deposited in the Bishop’s University Insect Collection (Sherbrooke, QC, Canada). *Experimental design* A single experiment was conducted to evaluate the effect of group composition on female mating probability, egg hatchability and time to first fertile egg-laying (pre-oviposition period from here on). For the experimental design described below, each treatment was replicated 10 times for each of the 2 lines. The treatments were composed of different group compositions, consisting of 4 sex ratios in either low- or high-density groups (hence, 8 treatments x 2 lines x 10 replicates = 160 experimental units; Table 1). Groups were formed of individuals having emerged within 24 hours of each other and placed in a mating arena of approximately 1000 cm<sup>3</sup> (Appendix Figure 2). Flies were supplied with distilled water via a dental wick and adult diet *ad libitum*. An ovipositional substrate consisting of a 2.0 to 2.5-g piece of rutabaga placed on damp filter paper was supplied and replaced every 2 days. *Oviposition* Starting from the day on which groups were formed (day 0), oviposition was evaluated every two days by transferring the eggs laid on the ovipositional site to a petri dish with a humid filter paper and counted. Each evaluation day, dead males were replaced with virgin males of variable age (average number of males replaced for each treatment is shown in Appendix Figure 3). If dead females were found, evaluation was cancelled, and another replicate of that group composition was formed as a replacement. *Mating probability* As soon as fertile egg-laying was confirmed, all females within a treatment replicate were euthanized (placed in a freezer at -20°C for approximately 24 hours). Females were then stored in 70% ethanol while awaiting dissection. To obtain a measure of the proportion of mated females within the group (mating probability), all three spermathecae of each female within a group were dissected to confirm the presence of sperm masses (Avanesyan et al., 2017). *Egg hatchability and pre-oviposition period* Throne & Eckenrode (1986) observed nearly 100% egg hatchability following 2-3 days of development at 20°C for *D. platura*. The identity of the line involved in their study is unknown, however. To consider possible variation in egg developmental time between the lines, we evaluated egg hatchability (# of eggs hatched/ # of eggs laid) following six days of incubation in a petri dish. Eggs were deemed fertile if they had hatched. As oviposition was evaluated every two days and egg hatchability evaluated six days after being laid, three additional egg-laying events were recorded between the first fertile egg-laying day and the end of the evaluation for each group, totaling four measures of egg hatchability once mating had occurred. If no fertile eggs were laid, evaluation ceased after 42 days. *Statistical Analysis* Some treatment replicates were lost due to sampling errors or dead females and had to be repeated, therefore not all treatments could be conducted following a formal randomized complete block design. As a result, replicate (block) ID was not included as a random variable in the following models, which were all fitted within the R environment (v.4.1.1; R Core Team, 2021). *Mating probability* Female mating probability was modeled using a generalized linear model with a binomial error distribution and complementary log-log (cloglog) link function. *D. platura* line (N and H), density (high and low) and sex ratio (number of males per female) and their three-way interaction were included as covariates. As the number of males inherently increases female mating probability (more males = more mating opportunity), the log(number of males) was included as an offset term. The model was fitted using the glmmTMB library (v.1.2.2; Brooks et al., 2017) and model diagnostics were inspected using the DHARMA library (v.0.4.3; Hartig, 2021). *Pre-oviposition period* The length of the pre-oviposition period was modeled using a Cox proportional hazards model. *Delia platura* line (N and H), density (high and low) and sex ratio (number of males per female) and their three-way interaction were included as covariates. The model was fitted using the coxph formula within the survival library (v.3.2-13; Therneau, 2021). Model diagnostics were inspected using the cox.zph, ggcox.zph and ggcoxdiagnostics functions from the survminer library (v.0.4.9; Kassambara et al., 2021). *Egg hatchability* As the number of replicates with fertile egg laying were reduced by the number of replicates where mating did not occur (see Results section), we considered the effective number of replicates to be too low to represent the population and thus no inferential statistical analyses on egg hatchability were conducted. **Results** *Mating probability* The overall mean proportion of mated females was 63.18% ± 40.21% (SD). The N-line had a higher proportion of mated females compared

to the H-line along with an approximately 20% higher baseline mating probability (intercept; Figure 1 A; Appendix Table 1). Whereas none of the females in the H-line 1:1 group mated, 40% of N-line females mated within the same treatment. At both low and high densities, the proportion of mated females of each line increased similarly with the proportion of males to females, reaching between 90 and 100% at a ratio of 10 males to 1 female for the N-line and around 60% for the H-line at the same ratio. *Pre-oviposition period* The overall mean of the pre-oviposition period was  $9.60 \pm 4.63$  days with a mean of  $9.38 \pm 3.72$  and  $9.88 \pm 5.59$  days for the N- and H- line, respectively. While the Cox proportional hazards model supported the fact that groups of N-line females generally laid a first fertile egg more rapidly (higher hazard ratio) than those of the H-line, the pre-oviposition period also varied with both the sex ratio and group size, yet differently between lines (Figure 1 B; Appendix Table 1). Groups took more time to lay a first fertile egg (lower hazard ratio) in the low-density treatments, yet in the N-line, the pre-oviposition period tended to decrease as the male to female sex ratio increased. In the high-density treatment, laying time increased with increasing sex ratios, especially for the N-line, which resulted in similar laying times for both density treatments at the highest male to female sex ratio. *Egg hatchability* Considerable variation in the proportion of eggs hatched was observed for the N-line (mean  $\pm$  SD:  $79.48\% \pm 26.91\%$ ). The overall median was 91.20%. Most group compositions' medians did not deviate substantially from the overall median with the exception of the 1:1 group composition (Figure 1 C). Only four effective replicates were obtained for this group composition which may explain the low median (75.68). The H-line had a similar overall mean proportion of eggs hatched compared to the N-line (mean  $\pm$  SD:  $84.69\% \pm 26.66\%$ ). The overall median for the H-line was 96.36% with no group compositions' median deviating substantially from this value with the exception of the 1:1 group composition for which no eggs hatched after 6 days of incubation (Figure 1 C). No obvious trend in the proportion of eggs hatched was observed in relation to sex ratio or density in either the N or the H-line. **Discussion** The effect of group density and sex ratio on female mating probability, pre-oviposition period and egg hatchability was investigated for the N- and H- lines of *D. platura*. Female mating probability increased as the number of males per female increased for both lines but females from the N-line had a higher mating probability than those of the H-line under all treatments. The pre-oviposition period decreased as the ratio of males to female increased at low density only for the N-line while the opposite trend was observed at high density for both lines. These results suggest differences between the mating systems of these two lines, with the H-line females being choosier towards potential mates than those of the N-line. Egg laying dynamics were also different, with group composition having a stronger effect on the N-line. The differences in the mating system dynamics may result in reproductive isolation between the two lines of *D. platura* which may consequently affect future control methods such as the sterile insect technique which relies on mating compatibility between released individuals and the target population. *Mating probability* Within mating swarms, sex ratios typically tend to be male-biased, offering females increased mating opportunities. While females typically fly in to mate and promptly leave once mated, males stay in the swarms (Downes, 1969). Female mating probability was increased in both *D. platura* lines with increased male to female ratios rather than group density, a result congruent with trends observed in other arthropod species (Karlsson et al., 2010; Vahl et al., 2013). These trends also corroborate field observations of *D. platura* forming mating swarms ; A. Bush-Beaupré unpublished). An increase in female mating probability under male-biased sex ratios could be due to an increase in male mating-related activity, such as locomotion (Bahrndorff et al., 2012) and courtship (Leftwich et al., 2012; Marie-Orleach et al., 2019). Although male-male interactions, and thus aggressions, are more likely to occur under male-biased sex ratios, thereby reducing male mating success (Enders, 1993), male-male competition in our group treatments for both genetic lines could have switched from interference (aggression) to scramble competition as the group sex ratio became increasingly male-biased (as reviewed in Weir et al., 2011). While male-biased sex ratios may cause an increase in female resistant behavior (Carrillo et al., 2012) a decrease in such behavior may also be observed as male insistence increases (Lauer et al., 1996). If male insistence and female resistance are relevant factors in our study system, a lack of female resistance is a more probable explanation for the increased mating probability of females from both lines when exposed to male-biased sex ratios. Indeed, if female resistance had increased to the point of overcoming male insistence, mating probability would have decreased. This may be particularly true for the N-line as mating probability plateaued near 100%. However, mating probability plateaued at around 60% for H-line females

which may be considered as evidence of an increase in female resistance proportional to the overall increase in male insistence assuming that male-male competition did not hinder their mating behavior. It is, however, possible that male behavior may not have been the defining factor in the mating interactions we observed. The increase in female mating probability could simply be due to an increase in the number of available males to choose from, a main characteristic of swarming behavior. Female mate choice can be affected by multiple factors. For example, males in mating pairs tended to be larger than males sampled at random in swarms of the mayfly *Epeorus longimanus* (Ephemeroptera: Heptageniidae) (Flecker et al., 1988), and in the yellow dung fly, *Scathophaga stercoraria* (Diptera: Scathophagidae) (Otronen, 1996), larger males copulated and mate-guarded more under male-biased sex ratios. Even though we sieved pupae with 1.7-mm mesh for our experimental stocks, this did not entirely eliminate size variability. Females of both *D. platura* lines could therefore have chosen the larger male to mate with if they were more successful in winning male-male competitions as observed in the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) (Benelli et al., 2016). The low proportions of mated females in our smallest group composition (1 male and 1 female) were unexpected, especially for the H-line where no mating occurred within 42 days in all 10 replicas compared to 4 out of 10 mated females for the N-line. This result is surprising as females remaining unmated even in the presence of a male would appear to be disadvantageous. However, lifelong female virginity is not uncommon. According to a review by Rhains (2019), a higher rate of lifelong virginity is observed in females that are flightless, short-lived, choosy, small, and that have a long pre-reproductive maturation period (when coupled with a high rate of mate encounters leading to an increased rate of mating rejection). Lifetime female virginity is also affected by reproductive asynchrony, female-biased sex ratios, low population density at a large scale (Allee effect), and high population density at a fine scale (signal jamming). In our study, *D. platura* females could fly, were relatively large and long-lived (over 42 days), and some had a short pre-oviposition period (minimum of 6 days). Furthermore, the long duration of the experiment mitigated the potential effects of reproductive asynchrony, the sex ratio in the low-density treatment was not female-biased, the size of the arena likely negated a putative Allee effect, and fine scale population density was low. These facts suggest that H-line females are choosy towards the male with which they mate. The long duration of the trial additionally enforces this supposition as females are predicted to reduce their choosiness as they age as so to ensure fertilization during their lifetime (Kokko & Mappes, 2005). As 6 out of 10 N-line females remained unmated in this group composition, it may be suggested that females of this line are also choosy but to a lesser extent than H-line females. While the results obtained here suggest high mate selectivity in H-line females, this choosiness was not explicitly evaluated. As such, laboratory experiments in which females are offered a choice between males with different trait values (such as size or wing length) are needed to support or refute this hypothesis. It is pertinent to note, however, that dense swarms (exceeding the volume of the arenas used in our experimental treatments) were frequently observed in the main colony cage of the H-line but never witnessed for the N-line (A. Bush-Beaupré, pers. obs.). It is hence possible that the arena size was too small to allow H-line males to form a swarm thus leading to a low mating probability. Additionally, it is likely that the single male in the 1 male with 1 female group treatment was not stimulated into courtship due to a lack of other males with which to form a swarm. A similar study conducted on *D. platura* by investigated the effect of group composition on mating dynamics and reported a mating probability of around 70% in a 15:15 male:female group composition as well as some successful mating in their 1:1 treatment. These results are highly congruent with those reported here for the N-line and while the authors did not deposit voucher specimens or specify the capture locality of their founding stocks, we suspect that they worked with that genetic line. Considering that the two lines of *D. platura* differ in their mating systems (and possibly other biological traits), there are clear limitations to the extrapolation and application of results obtained in studies where the line(s) identity was not determined and voucher specimens unavailable for a posteriori determination. We therefore recommend that future work on *D. platura* involves the determination of the genetic line(s) under study *Pre-oviposition period* In both *D. platura* lines, the fastest fertile oviposition (highest hazard ratio; Fig. 2B) was observed in the 16:16 group composition. A decrease in the time to first fertile oviposition was observed as the number of females within a group increased at high densities for both lines whereas in the low-density treatment, the delay to first fertile egg seemed to decrease slightly as the number of males per female increased for the N-line. Although a higher number of females could have incre-

ased the probability that females with an intrinsically faster rate of oviposition were present in the group, the difference in the pre-oviposition period between the two lines suggests that this was not the main factor affecting the speed at which females lay their first fertile eggs. Additionally, if the preoviposition period was fully explained by the number of females within a group, we would expect a much shorter preoviposition period in the group with 16 females compared to the group with only 5 which was not the case for either line in our study. As such, the total number of females within a group does not fully explain the rate at which they lay their first fertile eggs. The presence and oviposition of conspecific females can have different effects on oviposition behavior depending on the group. For certain species, female crowding can increase the preoviposition period (Ambrose et al., 1988) and ovipositional substrates with the presence of conspecific eggs may deter females (Elsensohn et al., 2021). However, opposite trends can be observed depending on the species (Desurmont et al., 2014; Judd & Borden, 1992; Ulmer et al., 2003). In our study, the group with the most females (16:16) had the shortest pre-oviposition period in both lines. In the viburnum leaf beetle, *Pyrrhalta viburni*, females spent 21% less time searching and selecting for an oviposition site when conspecific egg masses were present on twigs, thus reducing the preoviposition period. Since we included a single oviposition site per experimental arena regardless of treatment, the presence of conspecific eggs may have been one of the factors explaining the shorter pre-oviposition period we observed at high densities in the two lines of *D. platura*. Since neither of the *D. platura* lines laid fertile eggs before the interval of day 4 to 6, we can infer that ovipositional maturity was reached no sooner than the first 4-6 days of their lifespan. This result is comparable to the preoviposition period measured in the closely related *Delia florilega* (6.5 days) (Kim & Eckenrode, 1987). The average preoviposition period for both lines of *D. platura* was approximately 10 days, concurring with results obtained by McClanahan & Miller (1958), which ranged between 10 and 20 days. The minimum length of the preoviposition period observed here (between 4 and 6 days) suggests that the absence of oviposition reported by Mlynarek et al. (2020) for *D. platura* females aged 2-4 days exposed to different developmental stages of onion (*Allium cepa*) for 48 hours in a no-choice experiment may not have been due to a rejection of the oviposition substrate but caused instead by the fact that females had not yet reached ovipositional maturity.

**Egg hatchability** Group composition has been known to influence egg hatchability. For example, in the flea beetle, *Agasicles hygrophila* (Coleoptera: Chrysomelidae), hatchability was highest when the group composition consisted of 66% female proportions (Guo et al., 2014) whereas in *Musca domestica*, it was highest in male-biased sex ratios (Carrillo et al., 2012). In three out of four blowfly species (Diptera: Calliphoridae) Parry et al. (2017) found an increase in egg hatchability when increasing group density. However, the examples listed above are all species in which remating is possible and so, group composition likely affected remating opportunities and thus egg hatchability. *Delia platura* is generally thought to mate only once like its close relative the onion maggot (*Delia antiqua*) (as *Hylemya antiqua* in Martin & McEwen, 1982). Egg hatchability can also reflect a female's mate choice as higher quality males could confer a higher egg hatchability (McLain, 1998). However, no obvious trend in egg hatchability was observed between group compositions for either line of *D. platura*, which reinforces the assumptions that either mating occurs only once in females of both lines or that female mate choice does not affect egg hatchability.

**Reproductive compatibility & isolation** The group composed of 30 males and 2 females had the highest mating probability for the H-line. As this group composition also had a high mating probability for the N-line, and to take into account the possible involvement of male activity in the mating dynamics (Bahrndorff et al., 2012; Carrillo et al., 2012; Enders, 1993; Lauer et al., 1996; Leftwich et al., 2012; Marie-Orleach et al., 2019; Weir et al., 2011), inter-line crosses should be conducted with a 30:2 group composition to evaluate the reproductive compatibility of the two lines of *D. platura*. Considering that H-line females appear highly selective for the male with which they mate, they could be better at discriminating heterospecifics than the N-line, thereby reducing the probability that female H- and male N-line crosses would occur even if the lines are genetically compatible. As our results suggest that some of the aspects of the mating systems differ between the N- and H-lines (with H-line females being more selective), mating behavior could be a form of reproductive isolation between the two lines, at least under laboratory conditions. Further field studies should therefore be carried out in localities where the two lines of *D. platura* co-occur to determine if levels of physical and temporal segregation in swarms are as high as those reported for other swarming insects (Diabaté et al., 2009; Savolainen et al., 1993; Sawadogo et al., 2014).

**Applications** The results we obtained highlight the need

to further investigate differences in biological and behavioral traits of the two lines of *D. platura*. Such trait differences may indeed influence the methods required for efficient control of either line in crop productions. Considering that mating is optimal in male-biased groups for both lines of *D. platura*, control methods such as the sterile insect technique or the release of individuals carrying pathogens, must take into account the high degree of sexual selection present in such group contexts (Cator et al., 2020), especially considering the high degree of choosiness of H-line females. As such, special care must be taken to ensure that released males will join and compete within swarms (Hassan et al., 2014; Hendrichs et al., 2002) and be attractive to females (Shelly et al., 1994). Future studies should determine the localities that are conducive to swarm formation for the two *D. platura* lines along with the capability of released sterile males to join and compete within these along with being selected by natural females of the target line(s).

**Acknowledgements** We are grateful to the following students, research technician and research professional for helping with data collection: Maria Magdalena Virlan, Marianne Allard, Marc-André Villeneuve and Chelsey Paquette along with Andrew MacDonald for statistical consultations. This work was funded by Agriculture and Agri-Food Canada, The Fruit & Vegetable Growers of Canada (Canadian AgriScience Cluster for Horticulture program) #ASC-18/19 – Activity 8 and Bishop’s University.

**References** Ambrose, D. P., Sahaya Rani, M. R., & Vennison, S. J. (1988). Effect of Crowding on the Camouflaging Behaviour, Size, Development, Oviposition Pattern and Hatchability of Offspring of *Acanthaspis pedestris* Stål, A Potential Predator of Insect Pests (Heteroptera Reduviidae). *Monitore Zoologico Italiano - Italian Journal of Zoology*, 22(2), 111–120. Avanesyan, A., Jaffe, B. D., & Guédot, C. (2017). Isolating spermathecae and determining mating status of *Drosophila suzukii*: A protocol for tissue dissection and its applications. *Insects*, 8(1). Bahrndorff, S., Kjaersgaard, A., Pertoldi, C., Loeschcke, V., Schou, T. M., Skovgård, H., & Hald, B. (2012). The effects of sex-ratio and density on locomotor activity in the house fly, *Musca domestica*. *Number 71 Journal of Insect Science*, 12(71). Barclay, H. J. (2005). Mathematical models for the use of sterile insects. In V. A. Dyck, J. Hendrichs, & A. Robinson (Eds.), *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management* (pp. 147–174). Springer. Dordrecht Benelli, G., Donati, E., Romano, D., Ragni, G., Bonsignori, G., Stefanini, C., & Canale, A. (2016). Is bigger better? Male body size affects wing-borne courtship signals and mating success in the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). *Insect Science*, 23(6), 869–880. Brooks, A. R. (1951). Identification of the Root Maggots (Diptera: Anthomyiidae) Attacking Cruciferous Garden Crops in Canada, with Notes on Biology and Control. *The Canadian Entomologist*, 83(5), 109–120. Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. (2017). GlmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal*, 9(2), 378–400. Carrillo, J., Danielson-François, A., Siemann, E., & Meffert, L. (2012). Male-biased sex ratio increases female egg laying and fitness in the housefly, *Musca domestica*. *Journal of Ethology*, 30(2), 247–254. Cator, L. J., Wyer, C. A. S., & Harrington, L. C. (2020). Mosquito Sexual Selection and Reproductive Control Programs. *Trends in Parasitology*, 2020, 1–10. Clark, S. J. (1988). The effects of operational sex ratio and food deprivation on copulation duration in the water strider (*Gerris remigis* Say). *Behavioral Ecology and Sociobiology*, 23, 317–322. Derocles, S. A. P., Plantegenest, M., Rasplus, J. Y., Marie, A., Evans, D. M., Lunt, D. H., & Le Ralec, A. (2016). Are generalist Aphidiinae (Hym. Braconidae) mostly cryptic species complexes? *Systematic Entomology*, 41(2), 379–391. Desurmont, G. A., Weston, P. A., & Agrawal, A. A. (2014). Reduction of oviposition time and enhanced larval feeding: Two potential benefits of aggregative oviposition for the viburnum leaf beetle. *Ecological Entomology*, 39(1), 125–132. Diabaté, A., Dao, A., Yaro, A. S., Adamou, A., Gonzalez, R., Manoukis, N. C., Traoré, S. F., Gwadz, R. W., & Lehmann, T. (2009). Spatial swarm segregation and reproductive isolation between the molecular forms of *Anopheles gambiae*. *Proceedings of the Royal Society B: Biological Sciences*, 276(1676), 4215–4222. Diabaté, A., Yaro, A. S., Dao, A., Diallo, M., Huestis, D. L., & Lehmann, T. (2011). Spatial distribution and male mating success of *Anopheles gambiae* swarms. *BMC Evolutionary Biology*, 11(1). Downes, J. A. (1969). The Swarming and Mating Flight of Diptera. *Annual Review of Entomology*, 14(1), 271–298. Elsensohn, J. E., Aly, M. F. K., Schal, C., & Burrack, H. J. (2021). Social signals mediate oviposition site selection in *Drosophila suzukii*. *Scientific Reports*, 11(3796), 1–10. Enders, M. M. (1993). The effect of male size and operational sex ratio on male mating success in the common spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Animal Behaviour*, 46(5), 835–846. Finch, S. (1989). Ecological considerations in the

management of *Delia* pest species in vegetable crops. *Annual Review of Entomology*, 34, 117–137. Flecker, A. S., Allan, J. D., & McClintock, N. L. (1988). Male body size and mating success in swarms of the mayfly *Epeorus longimanus*. *Holarctic Ecology*, 11, 280–285. Fortier, A.-M. (2021). *Utilisation et maintien de l'emploi de mouches stériles en remplacement du chlorpyrifos, chez les producteurs d'oignons de la Montérégie*. doi: [https://prisme.ca/wpcontent/uploads/2022/02/rapport\\_final\\_2.1\\_2021.pdf](https://prisme.ca/wpcontent/uploads/2022/02/rapport_final_2.1_2021.pdf) Griffiths, G. C. D. (1993). Anthomyiidae. In *Flies of the Nearctic Region* (pp. 1417–1632). E. Schweizerbart. Stuttgart Guo, Ji. Y., Fu, J. W., Shi, M. Z., Li, J. Y., & Wan, F. H. (2014). Sex ratio effects on copulation, fecundity and progeny fitness for *Agasicles hygrophila*, a biological control agent of alligator weed. *Biocontrol Science and Technology*, 24(11), 1321–1332. Hartig, F. (2021). *DHARMA: Residual Diagnostics for Hierarchical (Multi-level / Mixed) Regression Models*. Hassan, M. M., Zain, H. M., Basheer, M. A., Elhaj, H. E. F., & El-Sayed, B. B. (2014). Swarming and mating behavior of male *Anopheles arabiensis* Patton (Diptera: Culicidae) in an area of the Sterile Insect Technique Project in Dongola, northern Sudan. *Acta Tropica*, 132(Supplement), S64–S69. Hendrichs, J., Robinson, A. S., Cayol, J. P., & Enkerlin, W. (2002). Medfly Areawide Sterile Insect Technique Programmes for Prevention, Suppression or Eradication: The Importance of Mating Behavior Studies. *The Florida Entomologist*, 85(1), 1–13. Hough-Goldstein, J. A., & Hess, K. A. (1984). Seedcorn Maggot (Diptera: Anthomyiidae) Infestation Levels and Effects on Five Crops. *Environmental Entomology*, 13, 962–965. Hough-Goldstein, J. A., Hess, K. A., & Cates, S. M. (1987). Group Effect on Seedcorn Maggot (Diptera: Anthomyiidae) Mating Behavior. *Annals of the Entomological Society of America*, 80(4), 520–523. House, C. M., Rapkin, J., Hunt, J., & Hosken, D. J. (2019). Operational sex ratio and density predict the potential for sexual selection in the broad-horned beetle. *Animal Behaviour*, 152, 63–69. Howard, R. J., Allan, J., & Seaman, W. L. (1994). *Diseases and Pests of Vegetable Crops in Canada* (R. J. Howard, J. Allan, & W. L. Seaman, Eds.). The Canadian Phytopathological Society and The Entomological Society of Canada. Ottawa Ikegawa, Y., & Himuro, C. (2017). Limited mobility of target pests crucially lowers controllability when sterile insect releases are spatiotemporally biased. *Journal of Theoretical Biology*, 421, 93–100. Ishikawa, Y., Mochizuki, A., Ikeshoji, T., & Matsumoto, Y. (1983). Mass-rearing of the Onion and Seed-corn Flies, *Hylemya antiqua* and *H. platura* (Diptera: Anthomyiidae), on an artificial diet with antibiotics. *Applied Entomology and Zoology*, 18(1), 62–69. Judd, G. J. R., & Borden, J. H. (1992). Aggregated Oviposition in *Delia antiqua* (Meigen): A Case for Mediation by Semiochemicals. *Journal of Chemical Ecology*, 18(4), 621–635. Karlsson, K., Eroukhmanoff, F., & Svensson, E. I. (2010). Phenotypic plasticity in response to the social environment: Effects of density and sex ratio on mating behaviour following ecotype divergence. *PLoS ONE*, 5(9), 1–6. Kassambara, A., Kosinski, M., & Biecek, P. (2021). *survminer: Drawing Survival Curves using “ggplot2”*. Kim, T. H., & Eckenrode, C. J. (1987). Bionomics of the Bean Seed Maggot, *Delia florilega* (Diptera: Anthomyiidae), Under Controlled Conditions. *Environmental Entomology*, 16(4), 881–886. Klug, H. (2011). Animal Mating Systems. *ELS*. 1-7. Kokko, H., & Mappes, J. (2005). Sexual selection when fertilization is not guaranteed. *Evolution*, 59(9), 1876–1885. Lauer, M. J., Sih, A., & Krupa, J. J. (1996). Male density, female density and inter-sexual conflict in a stream-dwelling insect. *Anim. Behav*, 52, 929–939. Leftwich, P. T., Edward, D. A., Alphey, L., Gage, M. J. G., & Chapman, T. (2012). Variation in adult sex ratio alters the association between courtship, mating frequency and paternity in the lek-forming fruitfly *Ceratitis capitata*. *Journal of Evolutionary Biology*, 25, 1732–1740. Makee, H., & Saour, G. (2001). Factors influencing mating success, mating frequency, and fecundity in *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Environmental Entomology*, 30(1), 31–36. Marie-Orleach, L., Bailey, N. W., & Ritchie, M. G. (2019). Social effects on fruit fly courtship song. *Ecology and Evolution*, 9, 410–416. Martin, J. S., & McEwen, F. L. (1982). Frequency of Mating in the Onion Maggot *Hylemya antiqua* (Diptera: Anthomyiidae). *Canadian Entomologist*, 114, 647–648. McClanahan, R. J., & Miller, L. A. (1958). Laboratory Rearing of the Seed-corn Maggot, *Hylemya cilicrura* (Rond.) (Diptera: Anthomyiidae). *The Canadian Entomologist*, 90(6), 372–374. McLain, D. K. (1998). Non-genetic benefits of mate choice: Fecundity enhancement and sexy sons. *Animal Behaviour*, 55, 1191–1201. McNamara, K. B., Elgar, M. A., & Jones, T. M. (2008). Causes and Consequences of Variation in Female Mating Frequency in the Almond Moth, *Cadra cautella*. *Behaviour*, 145(6), 779–793. Miller, L. A., & McClanahan, R. J. (1960). Life-History of the Seed-Corn Maggot, *Hylemya cilicrura* (Rond.) And of *H. liturata* (Mg.) (Diptera: Anthomyiidae) in Southwestern Ontario. *The Canadian Entomologist*, 92(3), 210–221. Miyatake, T., & Haraguchi, D. (1996). Mating success in *Batrocera cucurbitae* (Diptera: Tephritidae)

under different rearing densities. *Annals of the Entomological Society of America*, 89(2), 284–289. Mlynarek, J. J., Macdonald, M., Sim, K., Hiltz, K., McDonald, M. R., & Blatt, S. (2020). Oviposition, feeding preferences and distribution of *Delia* species (Diptera: Anthomyiidae) in eastern Canadian onions. *Insects*, 11, 1–10. Oléron Evans, T. P., & Bishop, S. R. (2014). A spatial model with pulsed releases to compare strategies for the sterile insect technique applied to the mosquito *Aedes aegypti*. *Mathematical Biosciences*, 254(1), 6–27. Otronen, M. (1996). Effects of seasonal variation in operational sex ratio and population density on the mating success of different sized and aged males in the yellow dung fly, *Scathophaga stercoraria*. *Ecology and Evolution*, 8(4), 399–411. Parry, N. J., Pieterse, E., & Weldon, C. W. (2017). Longevity, Fertility and Fecundity of Adult Blow Flies (Diptera: Calliphoridae) Held at Varying Densities: Implications for Use in Bioconversion of Waste. *Journal of Economic Entomology*, 110(6), 2388–2396. Punzalan, D., & Rowe, L. (2013). Ecological correlates of daily mating frequency in a wild population of ambush bugs. *Ecological Entomology*, 38(4), 429–432. R Core Team. (2021). *R: A language and environment for statistical computing*. URL <https://www.R-project.org/>. Renaud, A. K., Savage, J., & Adamowicz, S. J. (2012). DNA barcoding of Northern Nearctic Muscidae (Diptera) reveals high correspondence between morphological and molecular species limits. *BMC Ecology*, 12(1), 24. Rhainds, M. (2010). Female mating failures in insects. *Entomologia Experimentalis et Applicata*, 136(3), 211–226. Rhainds, M. (2019). Ecology of female mating failure/lifelong virginity: A review of causal mechanisms in insects and arachnids. *Entomologia Experimentalis et Applicata*, 167(1), 73–84. Sammani, A. M. P., Dissanayaka, D. M. S. K., Wijayarathne, L. K. W., & Morrison, W. R. (2020). Effect of Pheromone Blend Components, Sex Ratio, and Population Size on the Mating of *Cadra cautella* (Lepidoptera: Pyralidae). *Journal of Insect Science*, 20(6), 1–7. Savage, J., Fortier, A.-M., Fournier, F., & Bellavance, V. (2016). Identification of *Delia* pest species (Diptera: Anthomyiidae) in cultivated crucifers and other vegetable crops in Canada. *Canadian Journal of Arthropod Identification*, 29, 1–40. Savolainen, E., Saura, A., & Hantula, J. (1993). Mode of Swarming in Relation to Reproductive Isolation in Mayflies. *Evolution*, 47(6), 1796–1804. Sawadogo, P. S., Namountougou, M., Toé, K. H., Rouamba, J., Maïga, H., Ouédraogo, K. R., Baldet, T., Gouagna, L. C., Kengne, P., Simard, F., Costantini, C., Gibson, G., Diabaté, A., Lees, R. S., Gilles, J. R. L., & Dabiré, K. R. (2014). Swarming behaviour in natural populations of *Anopheles gambiae* and *An. Coluzzii*: Review of 4 years survey in rural areas of sympatry, Burkina Faso (West Africa). *Acta Tropica*, 132(1), S42–S52. Sawadogo, S. P., Costantini, C., Pennetier, C., Diabaté, A., Gibson, G., & Dabiré, R. K. (2013). Differences in timing of mating swarms in sympatric populations of *Anopheles coluzzii* and *Anopheles gambiae* s.s. (formerly *An. Gambiae* M and S molecular forms) in Burkina Faso, West Africa. *Parasites and Vectors*, 6(275), 1–14. Shelly, T. E., Whittier, T. S., & Kaneshiro, K. Y. (1994). Sterile Insect Release and the Natural Mating System of the Mediterranean Fruit Fly, *Ceratitidis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, 87(4), 470–481. Soroka, J. J., & Dossdall, L. M. (2011). Coping with root maggots in Prairie canola crops. *Prairie Soils and Crop Journal*, 4, 1–247. Sullivan, R. T. (1981). Insect Swarming and Mating. *The Florida Entomologist*, 64(1), 44–65. Therneau, T. M. (2021). *survival: A Package for Survival Analysis in R*. Throne, J. E., & Eckenrode, C. J. (1986). Development Rates for the Seed Maggots *Delia platura* and *D. florilega* (Diptera: Anthomyiidae). *Environmental Entomology*, 15(5), 1022–1027. Ticheler, J., Loosjes, M., & Noorlander, J. (1980). Sterile-insect technique for control of the onion maggot, *Delia antiqua*. In A. K. Minks & P. Gruys (Eds.), *Integrated control of insect pests in the Netherlands* (pp. 93–98). Centre for Agricultural Publishing and Documentation. Wageningen Ulmer, B., Gillott, C., & Erlandson, M. (2003). Conspecific Eggs and Bertha Armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), Oviposition Site Selection. *Environmental Entomology*, 32(3), 529–534. Vahl, W. K., Boiteau, G., de Heij, M. E., MacKinley, P. D., & Kokko, H. (2013). Female Fertilization: Effects of Sex-Specific Density and Sex Ratio Determined Experimentally for Colorado Potato Beetles and *Drosophila* Fruit Flies. *PLoS ONE*, 8(4). Van der Heyden, H., Fortier, A.-M., & Savage, J. (2020). A HRM Assay for Rapid Identification of Members of the Seedcorn Maggot Complex (*Delia florilega* and *D. platura*) (Diptera: Anthomyiidae) and Evidence for Variation in Temporal Patterns of Larval Occurrence. *Journal of Economic Entomology*, October, 1–11. Weir, L. K., Grant, J. W. A., & Hutchings, J. A. (2011). The influence of operational sex ratio on the intensity of competition for mates. *American Naturalist*, 177(2), 167–176. Wilkinson, G. S., & Johns, P. (2005). Sexual selection and the evolution of mating systems in flies. In *The evolutionary biology of flies* (pp. 312–339). Columbia University Press, New York

## Tables

Table 1. Treatments for the sex ratio and density experiment Treatments (group compositions) tested for their effect on female mating probability, pre-oviposition period and egg hatchability in the N- and H- lines of *Delia platura*.

Sex Ratio (:)	Number of individuals (:)	Number of individuals (:)
	Low Density	High Density
1:1	1:1	16:16
5:1	5:1	25:5
10:1	10:1	30:3
15:1	15:1	30:2

## Figure legend

**Figure 1. Effect of *Delia platura* line, density and sex ratio on mating success, pre-oviposition period and egg hatchability** Model predictions of effect of *Delia platura* line, density and sex ratio on proportion of mated females (A) and hazard ratio (HR) of pre-oviposition period (B). A higher HR indicates a shorter pre-oviposition period. Error bars depict 95% confidence intervals. C) Displays the raw data as raincloud plots of the proportion of eggs hatched as a function of group composition. Dashed lines depict overall average. Solid lines depict overall median. 8 treatments x 2 lines x 10 replicates = 160 experimental units

## Appendix figure legends and tables

**Appendix Figure 1. Rationale for mesh size used for sieving *Delia platura* pupae** Pupal weight (left panel) and pupal diameter of ‘normal’ weight pupae for the H- and N- lines of *D. platura*. ‘Normal’ pupal weight was calculated as the inter-quartile range (salmon portion of distribution, left panel). Pupae with weight corresponding to this range were measured at the wider-most region of pupal casing to obtain a distribution of pupal diameters (right panel). The green line represents the mesh size for the sieve used to remove the smaller individuals from the experiments. As small individuals tend to have decreased mating success (Benelli et al., 2016; Rhainds, 2019), we aimed for removing smaller individuals from both *D. platura* lines in an attempt to control for size effects on mating probability. To determine the mesh size required to sieve out the smaller pupae of both lines, we measured the diameter of pupae of ‘normal’ weight (interquartile range; Sup. Fig. 1 left panel; pupal weight data supplied by F. Fournier). We then chose an available mesh size (1.7mm) closest to the minimal pupal diameter of both lines (Sup. Fig. 1 right panel). While this mesh size was successful in retaining all ‘normal’ size pupae for the N-line, it eliminated some of the smaller H-line pupae of ‘normal’ size. As such, by retaining only a larger proportion large H-line pupae, we would expect a higher and less variable mating success within this line. However, we observed the opposite trend; H-line females had a lower mating probability than N-line females. Thus, sieving a greater proportion of small-sized H-line individuals further enforces the amplitude of the effect of sex ratio and density measured in our study. Appendix Figure 2. Arena used in the group sex ratio and density experiment Arena used to evaluate the effect of group sex ratio and density on mating probability, pre-oviposition period and egg hatchability for the H- and N- lines of *D. platura*. Appendix Figure 3. Average ( $\pm$  SD) number of dead males replaced for each treatment of the group sex ratio and density experiment Average ( $\pm$  SD) number of dead males replaced for each treatment evaluating the effect of group sex ratio and density on mating probability, pre-oviposition period and egg hatchability for the H- and N- lines of *D. platura*. A higher number of substituted males did not correspond proportionally to the size of the effects observed in the mating probability and pre-oviposition period analyses. Appendix Table 1. Output of statistical analyses for sex ratio and density experiment Estimates/Hazard ratios and corresponding 95% confidence intervals (CI) of the effects of *D. platura* line (H and N), group sex ratio, group density and their interactions on mating probability (Generalized Linear Model) and pre-oviposition period (Cox Proportional Hazards). 8

treatments x 2 lines x 10 replicates = 160 experimental units

<i>Covariates</i>	Female Mating Probability		Time to First F
	<i>Estimates</i>	95% <i>CI</i>	<i>Estimates</i>
Intercept	-3.92	-4.19 – -3.65	
Line [N]	1.16	0.83 – 1.49	0.93
Sex Ratio	0.05	0.00 – 0.09	-0.04
Density [Low]	2.33	1.34 – 3.32	-2.56
Line [N] : Sex Ratio	-0.04	-0.09 – 0.02	-0.06
Line [N] : Density [Low]	-0.51	-1.85 – 0.82	-0.17
Sex Ratio : Density [Low]	-0.14	-0.25 – -0.03	0.11
Line [N] : Sex Ratio : Density [Low]	0.14	-0.06 – 0.34	0.09
R <sup>2</sup>	0.393	0.393	0.427

### Data accessibility statement

Data and R script will be available in Dryad upon acceptance of the manuscript.

### Competing interests statement

The authors declare no conflict of interest.

### Author contributions

**Allen Bush-Beaupré:** Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing – Original draft preparation (lead); Writing – review & editing (lead). **Jade Savage:** Conceptualization (equal); Funding acquisition (equal); Supervision (equal); Writing – review & editing (equal). **Anne-Marie Fortier:** Conceptualization (equal); Funding acquisition (equal); Supervision (equal); Writing – review & editing (equal). **François Fournier:** Conceptualization (equal); Funding acquisition (equal); Supervision (equal); Resources (lead); Writing – review & editing (equal). **Marc Bélisle:** Conceptualization (equal); Supervision (equal); Writing – review & editing (equal).





