

ARTICLE

The effect of sex ratio and group density on the mating success of two lines of *Delia platura* (Diptera: Anthomyiidae)

Allen Bush-Beaupré^{1,2} , Marc Bélisle^{1,3}, Anne-Marie Fortier⁴, François Fournier⁵, and Jade Savage²

¹Département de biologie, Université de Sherbrooke, 2500 Boulevard de l'Université, Sherbrooke, Québec, J1K 2R1, Canada,

²Department of Biology and Biochemistry, Bishop's University, 2600 College St, Sherbrooke, Québec, J1M 1Z7, Canada,

³Centre d'étude de la forêt (CEF), Pavillon des Sciences biologiques, Université du Québec à Montréal, 141 Président-Kennedy, SB-2987, Montréal, Québec, H2X 1Y4, Canada, ⁴Compagnie de recherche Phytodata Inc., 291 rue de la Coopérative, Sherrington, Québec, J0L 2N0, Canada, and ⁵Collège Montmorency, 475 Boulevard de l'Avenir, Laval, Québec, H7N 5H9, Canada

Corresponding author: Allen Bush-Beaupré; Email: allen.bush-beaupre@usherbrooke.ca

(Received 28 March 2023; accepted 25 August 2023)

Abstract

The seedcorn maggot, *Delia platura* (Meigen) (Diptera: Anthomyiidae), is reported as a polyphagous pest species found in numerous crops, including onion, corn, crucifers, and soy. Two morphologically identical genetic lines of *D. platura* (H- and N-lines) with distinct distribution ranges were recently discovered. Although many biological traits have been described for *D. platura*, no study to date has been conducted on the life history strategies and reproductive behaviours of its two lines. Using laboratory-reared colonies, this project investigates the effect of group composition (sex ratio and density) on the mating success and preoviposition 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, whereas we found group density had negligible effects. However, the H-line had a lower average mating probability across treatments compared to the N-line. The preoviposition period decreased as the ratio of males to female increased at low density only for the N-line, and the opposite trend was observed at high density for both lines. These results suggest differences between the mating systems of these two lines, thereby highlighting the need for further research into the factors that influence their respective mating systems.

Introduction

True flies (Diptera) display a broad range of mating systems, including the congregation of many individuals in mating swarms (Wilkinson and Johns 2005). Swarming behaviour occurs in several families of flies and is thought to increase mate encounter rates and mate choice opportunities (Downes 1969). 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 (Downes 1969). As a result, female flies' mating success is influenced by both the aggregation's density (as expressed by the number of individuals per unit volume) and the male-to-female proportions (Rhainds 2010). In addition to the role it plays in mating dynamics, group density can also affect females' postmating oviposition behaviour. In certain species, female crowding can increase the length of the preoviposition period (Ambrose *et al.* 1988), and ovipositional substrates where conspecific eggs are present may deter

Subject editor: Hervé Colinet

© The Author(s), 2023. Published by Cambridge University Press on behalf of the Entomological Society of Canada

females (Elsensohn *et al.* 2021). However, these trends can be reversed in some species (Judd and Borden 1992; Ulmer *et al.* 2003; Desurmont *et al.* 2014).

The seedcorn maggot, *Delia platura* (Meigen) (Diptera: Anthomyiidae), is currently recognised 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, with infestations that can reach nearly 100% of sampled plants, depending on the crop (Hough-Goldstein and Hess 1984; Griffiths 1993; Howard *et al.* 1994; Soroka and Dossall 2011; Guerra *et al.* 2017; Erazo-Garcia *et al.* 2021). The species can act as a primary or secondary invader of host plants and can even complete its development in decaying organic matter (Finch 1989). *Delia platura* is a member of the Seedcorn Maggot Complex, along with *Delia florilega* (Zetterstedt), which has females that are morphologically similar and larvae that are identical to those of *D. platura* (Brooks 1951; Savage *et al.* 2016). In Ontario, Canada, mixed swarms of *D. platura* and *D. florilega* have been observed, and although the identification of females to species was not possible due to morphological similarities, a much higher proportion of males was observed (Miller and McClanahan 1960).

Savage *et al.* (2016) identified two genetic clusters within *D. platura* that were separated by a minimum p-distance of 4.45% for the barcoding gene, cytochrome *c* oxidase 1 (CO1; Folmer region), and exhibiting different geographical distributions; the provisionally named H-line has a primarily Holarctic range, whereas 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; Van der Heyden *et al.* (2020) showed that N-line larvae appeared in sampled crops almost 2.5 weeks before the H-line, and Savage *et al.* (2016) found the H-line to be 2.5 times more abundant than the N-line in cruciferous crops (*Brassica spp.* Linnaeus (Brassicaceae)), but the opposite trend was observed in onions (*Allium spp.* Linnaeus (Amaryllidaceae)).

Because intraspecific distance for CO1 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 or life history differences typically exhibit less interspecific distance (Derocles *et al.* 2016), we suspect that, despite their identical morphology, the two genetic lines of *D. platura* represent distinct biological entities (*i.e.*, lines with different biological traits). 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.

A more thorough understanding of the biological traits of the two genetic lines of *D. platura* would facilitate the development of production (breeding and rearing) and control methods tailored to each line. For example, the sterile insect technique is a control method that has been successfully applied to other *Delia* species in Canada and abroad (Ticheler *et al.* 1980; Fortier 2021). *Delia platura* is generally thought to mate only once, similar to its close relative, the onion maggot, *Delia antiqua* (Meigen) (Martin and McEwen 1982), which makes *D. platura* a prime candidate for the use of the sterile insect technique. Additionally, the optimal radiation dose using Cobalt60 has been determined for *D. platura* (Kim *et al.* 2001). However, to our knowledge, no field trials have yet been conducted to evaluate the performance of the sterile insect technique for the control of the seedcorn maggot. As the effectiveness of this technique relies on knowledge of certain characteristics of the target insect, including mating patterns and spatial distribution (Barclay 2005; Oléron Evans and Bishop 2014; Ikegawa and Himuro 2017), 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,

whereas fecundity seemed to be 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 Hough-Goldstein *et al.* (1987) remains unknown.

Because the H- and N-lines of *D. platyura* 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 the present study was therefore to investigate the effect of group density, sex ratio, and their interaction on *D. platyura* H- and N-line female mating probability and preoviposition period.

Methods

Delia platyura colonies

Colonies of the H- and N-lines of *D. platyura* (one colony per line) were established from wild flies collected in the Montérégie region of southern Québec, Canada and maintained under constant conditions (20 °C, 60% relative humidity, 16:8-hour light:dark photoperiod) at Collège Montmorency (Laval, Québec, Canada) for approximately two years before 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. The female CO1 haplotype was determined using a high-resolution melting polymerase chain reaction assay, following Van der Heyden *et al.* (2020), to determine the identity of their offspring. In this way, each colony was established with the offspring of several females. High-resolution melting 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 that consisted of a dry mixture of milk powder, icing sugar, autolysed yeast extract, brewer's yeast, and soy flour in a 10:10:1:1:1 ratio, and a rutabaga (*Brassica napus* Linnaeus) oviposition site.

Experimental stocks

Eggs from each main colony were harvested periodically (16 and 17 times for the H- and N-lines, respectively) over the course of 10 months and reared in containers of artificial diet. 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 Supplemental material, Fig. S1 for an explanation of the sieve-size choice). Voucher specimens were deposited in the Bishop's University Insect Collection (Sherbrooke, Québec, Canada).

Experimental design

A single experiment was conducted to evaluate the effect of group composition on female mating probability and time to first fertile egg-laying (called “preoviposition period” hereafter). For the experimental design described below, each treatment was replicated 10 times for each of the two lines. The experiment initially was designed as a randomised complete block design, but because replicates of certain treatments were lost due to manipulation errors or females dying, the randomised complete block design could not be entirely respected. The replicates that were lost were re-evaluated to ensure a balanced experimental design. The treatments comprised different group compositions, consisting of four sex ratios in either low- or high-density groups (hence, 8 treatments × 2 lines × 10 replicates = 160 experimental units; Table 1). Groups were formed of individuals having emerged within 24 hours of each other and placed in a cylinder-shaped, approximately 1-L mating arena (Supplemental material, Fig. S2). Flies were supplied with

Table 1. Treatments (group compositions) tested for their effect on female mating probability and preoviposition period in the N- and H-lines of *Delia platura*.

Sex ratio (♂:♀)	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

distilled water *via* a dental wick and with 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 two days. The experiment was conducted under the same rearing conditions as the main colonies (20 °C, 60% relative humidity, 16:8-hour light:dark photoperiod).

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 Supplemental material, Fig. S3). 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 euthanised (placed in a freezer at –20 °C for approximately 24 hours). Females were then stored in 70% ethanol until 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) for all 600 females in the experiment.

Preoviposition period

Throne and 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 times between the lines, we evaluated egg fertility following six days of incubation in a petri dish. Eggs were deemed fertile if they had hatched. 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 randomised 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 (version 4.1.1; <https://www.R-project.org/>). All code used for data handling and statistical analyses can be found in Supplementary material, Script 1.

Table 2. Output of statistical analyses for sex ratio and density experiment. Estimates/hazard ratios and corresponding 95% confidence intervals of the effects of *Delia platura* line (H and N), group sex ratio, group density and their interactions on mating probability (generalised linear model) and preoviposition period (Cox proportional hazards). 8 treatments × 2 lines × 10 replicates = 160 experimental units

Covariates	Female mating probability		Time to first fertile egg	
	Estimates	95% confidence interval	Estimates	95% confidence interval
Intercept	-3.92	-4.19 – -3.65		
Line [N]	1.16	0.83 – 1.49	0.93	0.15 – 1.71
Sex ratio	0.05	0.00 – 0.09	-0.04	-0.11 – 0.04
Density [Low]	2.33	1.34 – 3.32	-2.56	-3.59 – -1.52
Line [N]: Sex ratio	-0.04	-0.09 – 0.02	-0.06	-0.15 – 0.03
Line [N]: Density [low]	-0.51	-1.85 – 0.82	-0.17	-1.48 – 1.14
Sex ratio: Density [low]	-0.14	-0.25 – -0.03	0.11	0.00 – 0.22
Line [N]: Sex ratio: Density [low]	0.14	-0.06 – 0.34	0.09	-0.05 – 0.23
R^2	0.393		0.427	

Mating probability

Female mating probability was modelled using a generalised linear model with a binomial error distribution and a complementary log–log (cloglog) link function. *Delia platura* line (H, N), density (high, low), 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 package, version 1.2.2 (Brooks *et al.* 2017), and model diagnostics were inspected using the DHARMA package, version 0.4.3 (Hartig 2021).

Preoviposition period

The length of the preoviposition period was modelled using a Cox proportional hazards model. *Delia platura* line (H, N), density (high, low), 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 package, version 3.2-13 (Therneau 2021). Model diagnostics were inspected using the cox.zph, ggcoxzph, and ggcoxdiagnostics functions from the survminer package, version 0.4.9 (Kassambara *et al.* 2021).

Results

Regression coefficients and their 95% confidence intervals for mating probability and preoviposition period are reported in Table 2. Confidence intervals of coefficients are statistically significant if they do not overlap zero. However, individual effects with confidence intervals overlapping zero are also deemed statistically significant if they are part of a statistically significant interaction. Given that the effect size of a variable cannot be interpreted without taking into account the interactions in which it occurs, we focussed on the interpretation of the biologically significant effects displayed by the plotted model predictions in Figure 1.

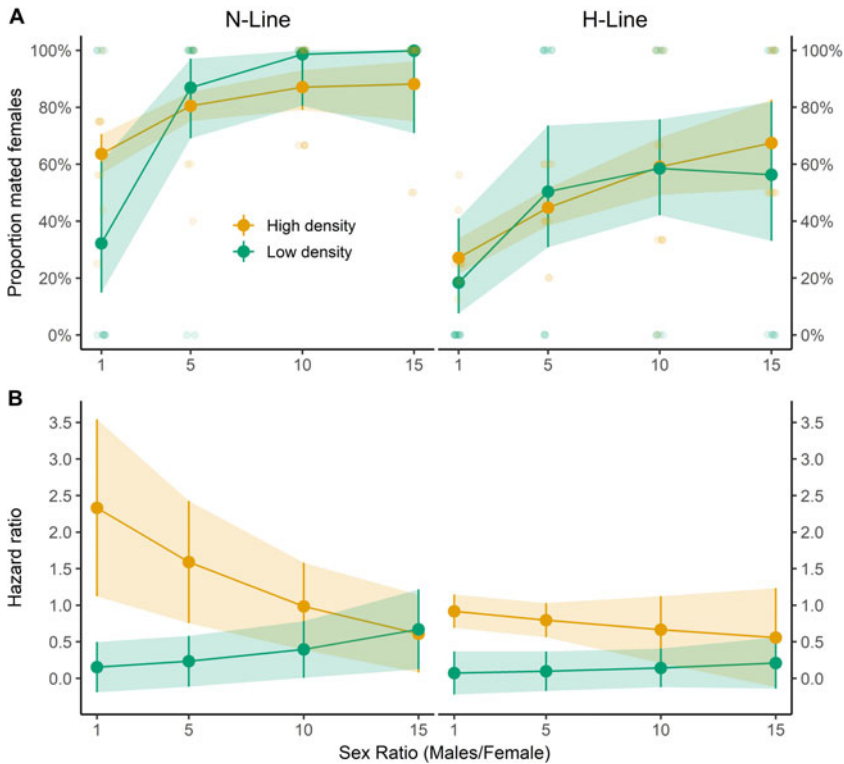


Figure 1. Effect of *Delia platura* line, density, and sex ratio on mating success and preoviposition period: **A**, model predictions of effect of *Delia platura* line, density, and sex ratio on proportion of mated females and **B**, hazard ratio of the preoviposition period. A higher hazard ratio indicates a shorter preoviposition period. Error bars depict 95% confidence intervals.

Mating probability

The overall mean proportion of mated females was $63.18 \pm 40.21\%$ (standard deviation). The N-line had a higher proportion of mated females compared to the H-line and also had an approximately 20% higher baseline mating probability (intercept; Fig. 1A; Table 2). 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 one female for the N-line and around 60% at the same ratio for the H-line.

Preoviposition period

The overall mean of the preoviposition period was 9.60 ± 4.63 days, with a mean of 9.38 ± 3.72 days and 9.88 ± 5.59 days for the N-line and H-line, respectively. Although 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 did those of the H-line, the preoviposition period also varied with both the sex ratio and group size, albeit differently between lines (Fig. 1B; Table 2). Groups took more time to lay a first fertile egg (lower hazard ratio) in the low-density treatments, but in the N-line, the preoviposition 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.

Discussion

The effect of group density and sex ratio on female mating probability and preoviposition period 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 preoviposition period decreased as the ratio of males to female increased at low density only for the N-line, whereas the opposite trend was observed at high density for both lines. These results suggest differences between the mating systems of the two lines that could possibly act as reproductive barriers and which will need to be accounted for in the development of control techniques that rely on mating compatibility, such as the sterile insect technique. The development of new seedcorn maggot control methods, such as the sterile insect technique, must take these potential barriers into account as the success of the approach 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. In our study, female mating probability was increased in both *D. platura* lines in response to 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 (Miller and McClanahan 1960; A. Bush-Beaupré, unpublished data).

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 (Weir *et al.* 2011).

Although male-biased sex ratios may cause an increase in female resistance behaviour (Carrillo *et al.* 2012), a decrease in such behaviour may also be observed as male insistence increases (Lauer *et al.* 1996). If female resistance had increased to the point of overcoming male insistence in male-biased mating groups, mating probability would have decreased. This may be particularly true for the N-line because mating probability plateaued near 100%. However, mating probability plateaued at around 60% for H-line females, possibly indicating an increase in female resistance proportional to the overall increase in male insistence, assuming that male–male competition did not hinder their mating behaviour.

It is, however, possible that male behaviour may not have been the defining factor in the mating interactions 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 behaviour (Downes 1969). 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* (Eaton) (Ephemeroptera: Heptageniidae) (Flecker *et al.* 1988), and in the yellow dung fly, *Scathophaga stercoraria* Linnaeus (Diptera: Scathophagidae), larger males copulated and mate-guarded more under male-biased sex ratios (Otronen 1996). 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 males to mate with if these latter were more successful in winning male–male competitions, as Benelli *et al.* (2016) observed in the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae).

The low proportions of mated females in our smallest group composition (1 male and 1 female) were unexpected, especially for the H-line, in which no mating occurred within 42 days in all 10 replicates compared to 4 of 10 females for the N-line. This result is surprising because females remaining unmated even in the presence of a male would appear to be disadvantageous. However, lifelong female virginity is not uncommon in insects. According to a review by Rhainds (2019), a higher rate of lifelong virginity is observed in females that are flightless, short-lived, choosy, small, and have a long prereproductive 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 the present study, *D. platura* females could fly, were relatively large, and were long-lived (over 42 days), and some had a short preoviposition period (an average of 10 days out of the 42-day trial period). Furthermore, the long duration of the experimental trials (up to 42 days) mitigated the potential effects of reproductive asynchrony, the sex ratio in the low-density treatment (1:1) was not female-biased, the relatively small size of the experimental arenas 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 because females are predicted to reduce their choosiness as they age as so to ensure fertilisation during their lifetime (Kokko and Mappes 2005). With 6 of 10 N-line females remaining unmated in the 1:1 group composition, females of this line may also be choosy but to a lesser extent than H-line females are. Although 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 and were rarely witnessed for the N-line (A. Bush-Beaupré, unpublished data). It is therefore possible that the arena size was too small to allow H-line males to form a swarm, thus leading to a low mating probability. In addition, it is possible that the single male in the 1:1 male: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 Hough-Goldstein *et al.* (1987) 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 ranging from 0 to 25% in their 1:1 treatments, depending on the experiment. These results are highly congruent with those reported in the present study for the N-line and, although Hough-Goldstein *et al.* (1987) 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 in 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 are unavailable for *a posteriori* determination. We therefore recommend that future work on *D. platura* involves the determination of the genetic line(s) under study.

Preoviposition period

In both *D. platura* lines, the fastest fertile oviposition (highest hazard ratio; Fig. 1B) 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 increased the probability that females with an intrinsically faster rate of oviposition were

present in the group, the difference in the preoviposition 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 five females. This 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 behaviour, depending on the group. In some species, female crowding can increase the preoviposition period (Ambrose *et al.* 1988). In the present study, the group with the most females (16:16) had the shortest preoviposition period in both lines. In the viburnum leaf beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae), females spent less time searching and selecting for an oviposition site when conspecific egg masses were present, thus reducing the preoviposition period (Desurmont *et al.* 2014). Because 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 preoviposition period that we observed at high densities in the two lines of *D. platura*.

Because neither of the *D. platura* lines laid fertile eggs before the interval of day 4–6, 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 average preoviposition period measured in the closely related *Delia florilega* (6.5 days; Kim and Eckenrode 1987). The average preoviposition period for both lines of *D. platura* was approximately 10 days, concurring with results obtained by McClanahan and Miller (1958), which ranged between 10 and 20 days. The minimum length of the preoviposition period observed in the present study (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.

Our results are congruent with results obtained by a companion experiment conducted on the reproductive compatibility of the two *D. platura* lines (Bush-Beaupré *et al.* 2023). In the aforementioned study, the mating probability of intraline crosses (H-line males with H-line females and N-line males with N-line females) was compared to that of interline crosses (H-line males with N-line females and vice-versa) using a group composition of 30 males with two females. The mating probability of both lines in Bush-Beaupré *et al.* (2023) was equivalent to the mating probability obtained in the 30 males to two females group composition of the present study (approximately 80 and 70% for the N- and H-lines, respectively). Additionally, females of both lines in Bush-Beaupré *et al.* (2023) also had a mean preoviposition period of approximately 10 days. The congruence between the results reported in both studies emphasises their replicability and adds robustness to our estimate of the conditions that maximise mating probability in *D. platura*.

Applications

The results presented here highlight the need for further investigation into differences in biological and behavioural traits of the two lines of *D. platura*. In addition, the results emphasise the importance of considering the effects of sex ratio and density on the insect's mating dynamics when designing lab and field experiments. Such trait differences may 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*, the development of control methods such as the sterile insect technique or the release of individuals carrying pathogens would need to account for the high degree of sexual selection present in such group contexts (Cator *et al.* 2020), especially

considering the apparent 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 (Hendrichs *et al.* 2002; Hassan *et al.* 2014) and be attractive to females (Shelly *et al.* 1994). To further develop the potential for the sterile insect technique as a control method for the seedcorn maggot, we recommend that future studies investigate what landscape markers and environmental conditions influence swarm formation in each *D. platura* line, as well as the ability of released sterile males to join and compete within swarms of each target line. The results obtained in the present study also highlight the need for additional research into the mating dynamics of the two *D. platura* lines, including mate choice, competition for mates, and the effect of conspecifics on egg-laying dynamics.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.4039/tce.2023.21>.

Data accessibility statement. Data used for this manuscript can be found at: <https://doi.org/10.5683/SP3/83WSVT>.

Acknowledgements. The authors are grateful to the following students, research technician, and research professional, Maria Magdalena Virlean, Marianne Allard, Marc-André Villeneuve, and Chelsey Paquette, for help with data collection, as well as Andrew MacDonald for statistical consultations. The authors also thank the reviewers for suggestions that improved the manuscript. This work was funded by Agriculture and Agri-Food Canada, the Fruit and Vegetable Growers of Canada (Canadian AgriScience Cluster for Horticulture program) #ASC-18/19 – Activity 8, and Bishop’s University.

Author contributions. **Allen Bush-Beaupré:** conceptualisation (lead), data curation (lead), formal analysis (lead), investigation (lead), methodology (lead), visualisation (lead), writing – original draft preparation (lead), and writing – review and editing (lead); **Jade Savage:** conceptualisation (equal), funding acquisition (equal), supervision (equal), and writing – review and editing (equal); **Anne-Marie Fortier:** conceptualisation (equal), funding acquisition (equal), supervision (equal), and writing – review and editing (equal). **François Fournier:** conceptualisation (equal), funding acquisition (equal), supervision (equal), resources (lead), and writing – review and editing (equal); **Marc Bélisle:** conceptualisation (equal), supervision (equal), and writing – review and editing (equal).

Competing interests. The authors declare they have no conflict of interest.

References

- Ambrose, D.P., Sahaya Rani, M.R., and 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**: 111–120. <https://doi.org/10.1080/00269786.1988.10736546>.
- Avanesyan, A., Jaffe, B.D., and Guédot, C. 2017. Isolating spermathecae and determining mating status of *Drosophila suzukii*: a protocol for tissue dissection and its applications. *Insects*, **8**: 32. <https://doi.org/10.3390/insects8010032>.
- Bahrndorff, S., Kjaersgaard, A., Pertoldi, C., Loeschcke, V., Schou, T.M., Skovgård, H., and Hald, B. 2012. The effects of sex-ratio and density on locomotor activity in the house fly, *Musca domestica*. *Journal of Insect Science*, **12**: 71. <https://doi.org/10.1673/031.012.7101>.
- Barclay, H.J. 2005. Mathematical models for the use of sterile insects. *In* Sterile insect technique: principles and practice in area-wide integrated pest management. *Edited by*

- V.A. Dyck, J. Hendrichs, and A. Robinson. Springer, Dordrecht, The Netherlands. Pp. 147–174. https://doi.org/10.1007/1-4020-4051-2_6.
- Benelli, G., Donati, E., Romano, D., Ragni, G., Bonsignori, G., Stefanini, C., and 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**: 869–880. <https://doi.org/10.1111/1744-7917.12253>.
- 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**: 109–120. <https://doi.org/10.4039/Ent83109-5>.
- Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., *et al.* 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, **9**: 378–400.
- Bush-Beaupré, A., Bélisle, M., Fortier, A.-M., Fournier, F., MacDonald, A., and Savage, J. 2023. Reproductive compatibility of two lines of *Delia platura* (Diptera: Anthomyiidae) [preprint]. *Biorxiv*. <https://doi.org/10.1101/2023.08.01.551352>.
- Carrillo, J., Danielson-François, A., Siemann, E., and Meffert, L. 2012. Male-biased sex ratio increases female egg laying and fitness in the housefly, *Musca domestica*. *Journal of Ethology*, **30**: 247–254. <https://doi.org/10.1007/s10164-011-0317-6>.
- Cator, L.J., Wyer, C.A.S., and Harrington, L.C. 2020. Mosquito sexual selection and reproductive control programs. *Trends in Parasitology*, **2020**: 1–10. <https://doi.org/10.1016/j.pt.2020.11.009>.
- Derocles, S.A.P., Plantegenest, M., Rasplus, J.Y., Marie, A., Evans, D.M., Lunt, D.H., and Le Ralec, A. 2016. Are generalist Aphidiinae (Hym. Braconidae) mostly cryptic species complexes? *Systematic Entomology*, **41**: 379–391. <https://doi.org/10.1111/syen.12160>.
- Desurmont, G.A., Weston, P.A., and 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**: 125–132. <https://doi.org/10.1111/een.12073>.
- Downes, J.A. 1969. The swarming and mating flight of Diptera. *Annual Review of Entomology*, **14**: 271–298. <https://doi.org/10.1146/annurev.en.14.010169.001415>.
- Elsensohn, J.E., Aly, M.F.K., Schal, C., and Burrack, H.J. 2021. Social signals mediate oviposition site selection in *Drosophila suzukii*. *Scientific Reports*, **11**: 1–10. <https://doi.org/10.1038/s41598-021-83354-2>.
- 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**: 835–846.
- Erazo-García, M.P., Sotelo-Proañó, A.R., Ramírez-Villacis, D.X., Garcés-Carrera, S., and Leon-Reyes, A. 2021. Methyl jasmonate-induced resistance to *Delia platura* (Diptera: Anthomyiidae) in *Lupinus mutabilis*. *Pest Management Science*, **77**: 5382–5395. <https://doi.org/10.1002/ps.6578>.
- Finch, S. 1989. Ecological considerations in the management of *Delia* pest species in vegetable crops. *Annual Review of Entomology*, **34**: 117–137. <https://doi.org/10.1146/annurev.ento.34.1.117>.
- Flecker, A.S., Allan, J.D., and 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 [Use and continued use of sterile flies as a replacement for chlorpyrifos, among onion producers in Montérégie]. Available from https://prisme.ca/wpcontent/uploads/2022/02/rapport_final_2.1_2021.pdf [accessed 6 July 2022].
- Griffiths, G.C.D. 1993. Anthomyiidae. In *Flies of the Nearctic Region*. Volume 3, Part 2, Number 15. Edited by G.C.D. Griffiths. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, Germany. Pp. 1417–1632.

- Guerra, P.C., Keil, C.B., Stevenson, P.C., Mina, D., Samaniego, S., Peralta, E., *et al.* 2017. Larval performance and adult attraction of *Delia platura* (Diptera: Anthomyiidae) in a native and an introduced crop. *Journal of Economic Entomology*, **110**: 186–191. <https://doi.org/10.1093/jee/tow237>.
- Hartig, F. 2021. DHARMA: residual diagnostics for hierarchical (multi-level/mixed) regression models (R package, version 0.4.3). Available from <https://CRAN.R-project.org/package=DHARMA>.
- Hassan, M.M., Zain, H.M., Basheer, M.A., Elhaj, H.E.F., and 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. <https://doi.org/10.1016/j.actatropica.2013.11.013>.
- Hendrichs, J., Robinson, A.S., Cayol, J.P., and Enkerlin, W. 2002. Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. *The Florida Entomologist*, **85**: 1–13.
- Hough-Goldstein, J.A. and Hess, K.A. 1984. Seedcorn maggot (Diptera: Anthomyiidae) infestation levels and effects on five crops. *Environmental Entomology*, **13**: 962–965. <https://doi.org/10.1093/ee/13.4.962>.
- Hough-Goldstein, J.A., Hess, K.A., and Cates, S.M. 1987. Group effect on seedcorn maggot (Diptera: Anthomyiidae) mating behavior. *Annals of the Entomological Society of America*, **80**: 520–523. <https://doi.org/10.1093/aesa/80.4.520>.
- Howard, R.J., Allan, J., and Seaman, W.L. 1994. Diseases and pests of vegetable crops in Canada. *Edited by* R.J. Howard, J. Allan, and W.L. Seaman. Canadian Phytopathological Society and Entomological Society of Canada, Ottawa, Canada. Available from <https://phytopath.ca/publications/diseases-of-vegetable-crops-in-canada/> [accessed 23 June 2022].
- Ikegawa, Y. and 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. <https://doi.org/10.1016/j.jtbi.2017.03.026>
- Ishikawa, Y., Mochizuki, A., Ikeshoji, T., and 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**: 62–69.
- Judd, G.J.R. and Borden, J.H. 1992. Aggregated oviposition in *Delia antiqua* (Meigen): a case for mediation by semiochemicals. *Journal of Chemical Ecology*, **18**: 621–635.
- Karlsson, K., Eroukhmanoff, F., and 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**: 1–6. <https://doi.org/10.1371/journal.pone.0012755>.
- Kassambara, A., Kosinski, M., and Biecek, P. 2021. survminer: drawing survival curves using “ggplot2.” (R package, version 0.4.9). Available from <https://CRAN.R-project.org/package=survminer>.
- Kim, H.S., Cho, J.R., Kim, J.J., Lee, M., and Byun, M.W. 2001. Optimal radiation dose of cobalt-60 to improve the sterile insect technique for *Delia antiqua*, and *Delia platura*. *Journal of Asia-Pacific Entomology*, **4**: 11–16. [https://doi.org/10.1016/s1226-8615\(08\)60095-3](https://doi.org/10.1016/s1226-8615(08)60095-3).
- Kim, T.H. and Eckenrode, C.J. 1987. Bionomics of the bean seed maggot, *Delia florilega* (Diptera: Anthomyiidae), under controlled conditions. *Environmental Entomology*, **16**: 881–886. <https://doi.org/10.1093/ee/16.4.881>.
- Kokko, H. and Mappes, J. 2005. Sexual selection when fertilization is not guaranteed. *Evolution*, **59**: 1876–1885.
- Lauer, M.J., Sih, A., and Krupa, J.J. 1996. Male density, female density and inter-sexual conflict in a stream-dwelling insect. *Animal Behaviour*, **52**: 929–939.

- Leftwich, P.T., Edward, D.A., Alphey, L., Gage, M.J.G., and 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. <https://doi.org/10.1111/j.1420-9101.2012.02556.x>.
- Marie-Orleach, L., Bailey, N.W., and Ritchie, M.G. 2019. Social effects on fruit fly courtship song. *Ecology and Evolution*, **9**: 410–416. <https://doi.org/10.1002/ece3.4759>.
- Martin, J.S. and McEwen, F.L. 1982. Frequency of mating in the onion maggot *Hylemya antiqua* (Diptera: Anthomyiidae). *The Canadian Entomologist*, **114**: 647–648. <https://doi.org/10.4039/Ent114647-7>.
- McClanahan, R.J. and Miller, L.A. 1958. Laboratory rearing of the seed-corn maggot, *Hylemya cilicrura* (Rond.) (Diptera: Anthomyiidae). *The Canadian Entomologist*, **90**: 372–374. <https://doi.org/10.4039/Ent90372-6>.
- Miller, L.A. and 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**: 210–221. <https://doi.org/10.4039/Ent92210-3>.
- Mlynarek, J.J., Macdonald, M., Sim, K., Hiltz, K., McDonald, M.R., and Blatt, S. 2020. Oviposition, feeding preferences and distribution of *Delia* species (Diptera: Anthomyiidae) in eastern Canadian onions. *Insects*, **11**: 1–10. <https://doi.org/10.3390/insects11110780>.
- Oléron Evans, T.P. and 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**: 6–27. <https://doi.org/10.1016/j.mbs.2014.06.001>.
- 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*. *Ethology Ecology and Evolution*, **8**: 399–411. <https://doi.org/10.1080/08927014.1996.9522912>.
- Renaud, A.K., Savage, J., and Adamowicz, S.J. 2012. DNA barcoding of northern Nearctic Muscidae (Diptera) reveals high correspondence between morphological and molecular species limits. *BMC Ecology*, **12**: 24. <https://doi.org/10.1186/1472-6785-12-24>.
- Rhainds, M. 2010. Female mating failures in insects. *Entomologia Experimentalis et Applicata*, **136**: 211–226. <https://doi.org/10.1111/j.1570-7458.2010.01032.x>.
- Rhainds, M. 2019. Ecology of female mating failure/lifelong virginity: a review of causal mechanisms in insects and arachnids. *Entomologia Experimentalis et Applicata*, **167**: 73–84. <https://doi.org/10.1111/eea.12759>.
- Savage, J., Fortier, A.-M., Fournier, F., and 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. <https://doi.org/10.3752/cjai.2016.29>.
- Shelly, T.E., Whittier, T.S., and Kaneshiro, K.Y. 1994. Sterile insect release and the natural mating system of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, **87**: 470–481.
- Soroka, J.J. and Dossall, L.M. 2011. Coping with root maggots in Prairie canola crops. *Prairie Soils and Crop Journal*, **4**: 1–247.
- Therneau, T.M. 2021. survival: a package for survival analysis in R. (R package, version 3.2-13.) Available from <https://CRAN.R-project.org/package=survival>.
- Throne, J.E. and Eckenrode, C.J. 1986. Development rates for the seed maggots, *Delia platura* and *D. florilega* (Diptera: Anthomyiidae). *Environmental Entomology*, **15**: 1022–1027. <https://doi.org/10.1093/ee/15.5.1022>.
- Ticheler, J., Loosjes, M., and Noorlander, J. 1980. Sterile-insect technique for control of the onion maggot, *Delia antiqua*. In *Integrated control of insect pests in the Netherlands*. Edited by A.K. Minks and P. Gruys. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands. Pp. 93–98.

- Ulmer, B., Gillott, C., and Erlandson, M. 2003. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. *Environmental Entomology*, **32**: 529–534.
- Vahl, W.K., Boiteau, G., de Heij, M.E., MacKinley, P.D., and 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**: e60381. <https://doi.org/10.1371/journal.pone.0060381>.
- Van der Heyden, H., Fortier, A.-M., and 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*, **113**: 2920–2930. <https://doi.org/10.1093/jee/toaa230>.
- Weir, L.K., Grant, J.W.A., and Hutchings, J.A. 2011. The influence of operational sex ratio on the intensity of competition for mates. *The American Naturalist*, **177**: 167–176. <https://doi.org/10.1086/657918>.
- Wilkinson, G.S. and Johns, P. 2005. Sexual selection and the evolution of mating systems in flies. *In* *The evolutionary biology of flies*. Columbia University Press, New York, New York, United States of America. Pp. 312–339.