Effect of density and species preferences on collective choices: an experimental study on maggot aggregation behaviours

Running title: Maggot aggregation behaviours

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SUMMARY STATEMENT

This study highlights the effect of density and interspecific interactions on collective decision-making in blow fly larvae, providing new insights into the behaviour of mixed-species groups.

ABSTRACT

Collective decisions have been extensively studied in arthropods, but they remain poorly known in heterospecific groups. This study was designed to (1) assess the collective behaviours of blow fly larvae (Diptera: Calliphoridae) in groups varying in density and species composition, and (2) to relate them to the costs and benefits of aggregating on fresh or decomposed food. First, experiments testing conspecific groups of *Lucilia sericata* and *Calliphora vicina* larvae, two species feeding at the same time on fresh carcasses, demonstrated decreases in growth and survival on rotten beef liver compared to fresh liver. However, mixing species together reduced this adverse impact of decomposition by increasing the mass of emerged adults. Second, larval groups were observed in binary choice tests between fresh and rotten liver (*i.e.*, optimal and sub-optimal food sources). The results showed that larvae interacted with each other and that these interactions influenced

their food preferences. We observed that (1) larvae were able to collectively choose the optimal food, (2) their choice accuracy increased with density, and (3) the presence of another species induced a reversal in larval preferences towards rotten food. These results highlight the ubiquity of collective decision properties in gregarious insects. They also reveal an unexpected effect of interspecific association, suggesting the colonization of new resources through a developmental niche construction.

KEYWORDS

collective decision, aggregation, preference, mixed-species group, blow fly larvae

INTRODUCTION

Evolution of animal species has resulted in various forms of sociality, differing in complexity. In the simple case, social interactions involve only inter-attractions that eventually lead to group formation (*i.e.*, aggregation) (Parrish and Edelstein-Keshet, 1999). These aggregations can reduce the risks of predation, improve foraging, and protect individuals against environmental variations. Aggregation can also facilitate information transfer between individuals (Parrish and Edelstein-Keshet, 1999; Krause and Ruxton, 2002) and lead to collective decision-making, *i.e.*, the selection of an alternative among several through social interactions (Conradt and Roper, 2005; Jeanson et al., 2012).

Collective decisions usually result in the maintenance of group cohesion and the increase of decision speed and/or accuracy (*i.e.*, the probability to choose the best alternative) (Conradt and Roper, 2005; Jeanson et al., 2012). While arthropod species with complex social structures (*e.g.*, eusocial insects) use active recruitment and differential signalling (*e.g.*, trail-laying in ants), collective decisions can also emerge through self-organized processes, involving only local interactions with no need for elaborate perceptive or cognitive abilities (Bonabeau et al., 1997; Sumpter, 2005; Jeanson et al., 2012). For instance, collective decision-making for a shelter in cockroaches relies only on the modulation of individual resting time as a function of shelter quality and the number of conspecifics (Deneubourg et al., 2002; Lihoreau et al., 2010).

Collective decisions can thus be strongly influenced by group size and inter-individual variability (Jeanson et al., 2012). An increase in group size generally leads to an increase in

decision speed and/or accuracy (Lihoreau et al., 2010, 2016; Canonge et al., 2011; Szopek et al., 2013), but can also trap individuals on sub-optimal alternatives (Dussutour et al., 2007). Inter-individual variability (*e.g.*, personality) can also affect both decision speed and accuracy (Planas-Sitjà et al., 2018) and can reduce the risk of choosing a sub-optimal option (Despland, 2013). This assumption could apply to groups composed of several species, which are frequently observed in arthropods and are known to provide adaptive benefits (Boulay et al., 2017; Goodale et al., 2020). However, the influence of species on collective decisions remains understudied (Leoncini and Rivault, 2005; Boulay et al., 2016; Broly et al., 2016).

The goal of this study was to investigate collective decision-making processes in necrophagous blow fly larvae (Diptera: Calliphoridae). In blow flies, adults lay eggs on vertebrate cadavers, on which newly hatched larvae feed until reaching a critical size, allowing pupation. During the feeding stage, larvae form groups including dozens to thousands of individuals (Rivers et al., 2011). These maggot masses mostly result from attractive and/or retentive social cues (Boulay et al., 2013; Fouche et al., 2018). Blow flies are also attracted by other species, resulting in interspecific aggregates (Woodcock et al., 2002; Joy et al., 2006; Slone and Gruner, 2007; Fouche et al., 2018; Komo et al., 2019). Feeding aggregations can be advantageous for larvae: they generate heat (larval-mass effect; Charabidze et al., 2011) and increase exodigestion (Wilson et al., 2016; Scanvion et al., 2018), which speeds metabolic activity and facilitates food assimilation. These effects reduce mortality and accelerate development, resulting in an Allee effect (Scanvion et al., 2018).

The intense competition between microbes, necrophagous arthropods and vertebrate scavengers for carrion consumption leads to rapid nutrient depletion and the risk of failing complete development (Campobasso et al., 2001; Devault et al., 2003; Benbow et al., 2015). In this context, the ability to make collective decisions, observed in both conspecific and heterospecific groups of blow fly larvae (Boulay et al., 2016), could allow fast and efficient location of the best food sources on the cadaver and confer a significant competitive advantage. This study aimed to test three hypotheses: (1) blow fly larvae are able to choose the best food source in their environment, (2) their decision speed and/or accuracy increases with increasing density, and (3) these factors are modulated by interspecific differences. Two species were studied, *Lucilia sericata* (Meigen) and *Calliphora vicina* (Linnaeus) (Diptera: Calliphoridae). In a first step, the growth and survival of larval groups of different size and species composition were measured on fresh or rotten beef liver. In a second step, groups of *L. sericata* and *C. vicina* larvae were tested in binary choice experiments opposing fresh and

rotten liver, and collective foraging dynamics were compared between groups of different density and species composition.

MATERIAL AND METHODS

Biological material

Flies of *L. sericata* and *C. vicina* were collected in the surroundings of Lille (Hauts-de-France, France) and then reared separately in the laboratory. Rearings were composed of tulle cages (50 x 50 x 50 cm), stored at room temperature (25 ± 2 °C) in daylight, in which water and caster sugar were provided *ad libitum*. New individuals from the field were added to the cages four times a year. To provide proteins required for oocyte maturation, pork heart was placed inside the cage during a week, and then placed daily for only 2 h to trigger egg laying. Eggs were deposited on 10 g of fresh minced beef liver in a plastic box (108 x 83 x 64 mm) and stored in an incubator (Pol-Eko-Aparatura, model ST BASIC) at either 25° ± 1 °C (for development tests) or 30 ± 1 °C (for binary choice tests). Young second instar larvae used for experiments were collected 24 ± 2 h after egg laying. For binary choice tests, *C. vicina* larvae were used 30 ± 2 h after egg laying, as preliminary observations showed a high mortality for younger larvae (*C. vicina* being better adapted to cooler temperatures; Donovan et al., 2006).

Development tests

Development tests were adapted from Scanvion et al. (2018). In short, larvae were placed in plastic boxes (108 x 83 x 64 mm) with 50 g of minced beef liver stored in an incubator (Pol-Eko-Aparatura, model ST BASIC) in the darkness at 25 ± 1 °C. Post-feeding larvae were transferred on a 2-cm layer of sand in new small boxes and kept in the incubator until fly emergence. Flies were left inside the box until their natural death and were then collected, counted, dried and all weighed simultaneously (Kern ALT 310-4 precision balance).

Three larval densities were tested: 100, 250 and 500 individuals per box (respectively labelled as "d100", "d250" and "d500"). For conspecific groups, all larvae were of the same species, either *L. sericata* or *C. vicina*. For heterospecific groups, each density included half of *L. sericata* and half of *C. vicina* larvae (*i.e.*, 50 *L. sericata* + 50 *C. vicina*, 125 *L. sericata* + 125 *C. vicina*, and 250 *L. sericata* + 250 *C. vicina*). Liver (*i.e.*, larval food) was initially kept in a freezer and either directly used after thawing (fresh liver) or left at 25 ± 1 °C in vacuum packaging for 7 days (rotten liver). This last treatment allowed decomposition in anaerobic conditions, which was previously shown to slow down larval development and growth of *C.*

vicina (Richards et al., 2013). All conditions (18 in total) were repeated 9 times, except d500 conditions, which were repeated 6 times.

Binary choice tests

The spatial distribution of larvae over time was observed in a circular arena showing two food spots of different quality. The arena, a glass Petri dish (Pyrex®, 2 cm in height, 20 cm in diameter), was filled with an agar solution (4 %, 1 cm in height; Fig. 1). To create food spots, two holes were dug in the agar, with 7.5 cm between both and at equal distance from the edge of the arena. Each spot had an equal size of 1 cm in height and either 2 cm (small spots) or 3.45 cm (big spots) in diameter. Both holes were filled with 3.14 mL (small spots) or 9.42 mL (big spots) of a mixture made of 10 g of minced beef liver and 3 mL of saline solution (0.9 % of NaCl). One hole was filled with fresh liver (fresh spot) and the other with rotten liver (rotten spot). The agar was then covered with a piece of tulle, which prevented larvae from digging into the food (while allowing them to feed). The whole device was placed in an incubator (Pol-Eko-Aparatura, model ST BASIC) at 30 ± 1 °C in darkness. At the beginning of the experiment, larvae were evenly spread on the middle line of the arena at equal distance from the two spots (Fig. 1). Their spatial distribution was followed by taking one picture every hour for 24 h or 48 h (Canon, model EOS 750D; preliminary observations showed the flash did not affect spatial distribution of larvae).

The conditions tested are summarized in Table 1. For each condition, the number of larvae on each spot was counted from pictures taken hourly. When a single larva was tested, the time spent on each spot throughout the experiment was calculated. It was assumed that when located on one spot at a time t, the larva spent the following hour on this spot. When a group of larvae was tested, analyses focused on the spatial distribution of larvae at the beginning of experiment. Two parameters were calculated to characterize this distribution:

- (1) latency: the time (t_{75}) before 75 % of larvae had made their first spot choice;
- (2) mean larval number: the mean number of larvae on the fresh spot observed during the time interval between t_{75} and the moment when the percentage of larvae on fresh spot exceeded 25 % variation (only minimum intervals of 5 h were considered); this number was computed from the values measured every hour.

Table 1. Conditions tested in binary choice tests.

Spot area	3 cm² (small spots)			9 cm² (big spots)	
Number of larvae	1	15	40	40	120
Density (number/cm²)	0.3	5	13	4.4	13
L. sericata	n = 20	n = 10	n = 10	n = 10	n = 10
C. vicina	n = 10		n = 10		
L. sericata + *C. vicina*			n = 10		

Number of replicates (n) is indicated as a function of spot area (cm²), number of larvae, larval density (number of larvae/spot area), and species. All experiments lasted 24 h, except the one with 40 *L. sericata* larvae on small spots, which lasted 48 h.

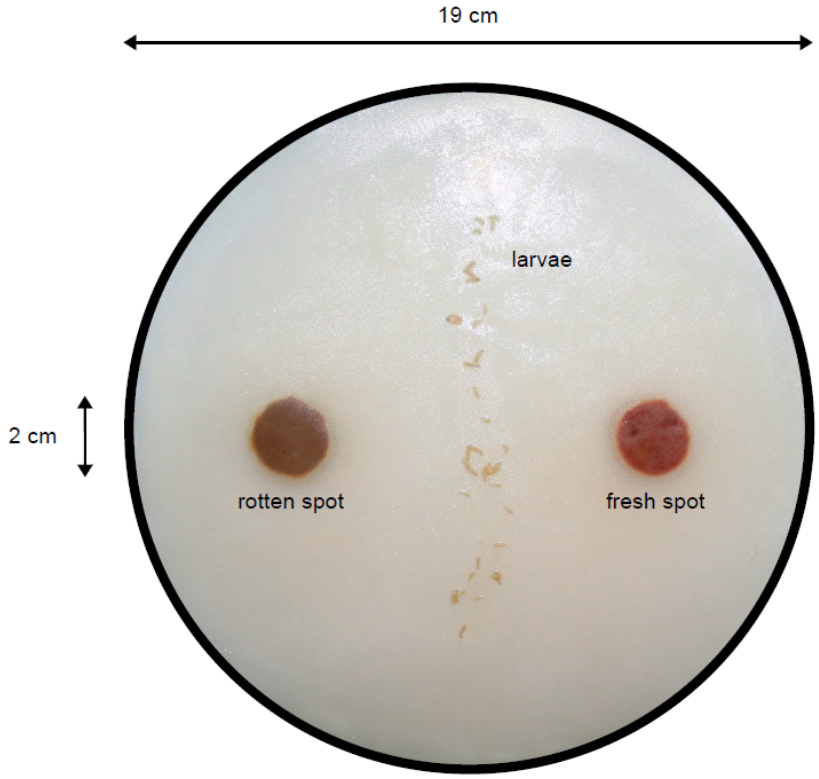

Fig. 1. Binary-choice setup at the start of one experiment. Rotten spot was made of 7 days old liver, the other of fresh liver. Larvae were placed at the middle line of the arena at equal distance of both spots, then photographed every hour for 24 h or 48 h. The picture represents 40 *L. sericata* larvae with small spots (2 cm of diameter).

Data analysis

For development tests, the percentage of emerged adults and their mean individual dry weight were analysed by mean comparisons between conditions. The Kruskal-Wallis test and Nemenyi post hoc test were used to compare conditions of different densities, and the Mann-

Whitney test was used to compare conspecific and heterospecific groups and fresh and rotten food. Comparisons of mean dry weight were performed only when a minimum of 4 values were measured in the condition. The significance level was set at $\alpha = 0.05/n$, with n being the number of statistical comparisons performed for one given condition.

For binary choice tests, when a single larva was tested, the percentage of time spent was compared between spots using the Wilcoxon test. The percentage of time spent on the fresh spot was then used to analyse results of group tests: it was considered as the probability to observe one larva on a fresh spot at a time t under the assumption that larvae did not interact with each other (henceforth named the "no group effect hypothesis").

When a group of larvae was tested, the latency of larvae first choices were compared between conditions using the Kruskal-Wallis test and the Nemenyi post hoc test. Then, binomial tests were used to analyse the group effect on larval preferences, using the probability assessed from the individual tests. When the mean larval number on the fresh spot was significantly different than expected under the no group effect hypothesis, the label "aggregation on the rotten spot" (lower number) or "aggregation on the fresh spot" (higher number) was attributed to the replicate. Then, binomial tests were used to test (1) the total number of aggregations, (2) the number of aggregations on the rotten spot, and (3) the number of aggregations on the fresh spot, with the respective probabilities of success expected under the no group effect hypothesis, that is, (1) 0.05, (2) and (3) 0.025. Finally, binomial tests were used again to test the number of positive group effects in relation to the total number of group effects (probability of success of 0.5). The significance level was set at $\alpha = 0.05$ for all these analyses. Development and binary choice test analyses were performed with R software (Version 3.6.2). Datasets are available in Table S1 and Table S2.

RESULTS

Development tests

Effect of density

On either fresh or rotten liver and in either the conspecific or the heterospecific group, the percentages of emerged adults of both *L. sericata* and *C. vicina* were not significantly different between densities d100, d250 and d500 (Table 2). Furthermore, no significant difference in mean adult weight was observed between densities in *L. sericata*. A significant difference was observed in *C. vicina* conspecific group on fresh liver: the adult weight was significantly lower at d500 than at d250 and d100 (Table 2). Accordingly, percentages and

weights of emerged adults at different densities were pooled for subsequent analysis, but comparisons of weights were detailed at each density for *C. vicina* in conspecific group on fresh liver (Table 3).

Table 2. Statistical comparisons of percentage of emerged adults and mean adult dry weight between densities.

Life history trait	Species	(1) fresh liver - conspecific: d100 vs d250 vs d500	n	(2) rotten liver - conspecific: d100 vs d250 vs d500	n	(3) fresh liver - heterospecific: d100 vs d250 vs d500	n	(4) rotten liver - heterospecific: d100 vs d250 vs d500	n
Percentage of emerged adults	*L. sericata*	62 ± 11 % 65 ± 9 % 67 ± 13 %	9 9 6	5 ± 4 % 5 ± 2 % 6 ± 3 %	9 9 6	54 ± 9 % 55 ± 8 % 47 ± 12 %	9 9 6	14 ± 9 % 14 ± 4 % 7 ± 6 %	9 9 6
		H = 0.11 NS		H = 4.08 NS		H = 0.31 NS		H = 1.91 NS	
	C. vicina	53 ± 2 % 55 ± 4 % 33 ± 6 %	9 9 6	6 ± 2 % 8 ± 3 % 14 ± 3 %	9 9 6	55 ± 7 % 53 ± 6 % 53 ± 9 %	9 9 6	4 ± 3 % 16 ± 4 % 11 ± 7 %	9 9 6
		H = 7.55 NS		H = 4.13 NS		H = 0.66 NS		H = 7.15 NS	
Mean adult dry weight	*L. sericata*	4.36 ± 0.71 mg 4.73 ± 0.69 mg 3.12 ± 0.40 mg	9 9 6	- 2.65 ± 0.24 mg 2.20 ± 0.19 mg	6 6	3.81 ± 0.60 mg 3.65 ± 0.48 mg 3.60 ± 0.81 mg	9 9 6	- 4.57 ± 0.48 mg -	7
		H = 2.29 NS		W = 26 NS		H = 0.07 NS		-	
	C. vicina	8.27 ± 0.62 mg 8.29 ± 0.32 mg 6.15 ± 0.58 mg	9 9 6	6.26 ± 0.68 mg 5.42 ± 0.29 mg 6.88 ± 0.45 mg	4 9 6	7.69 ± 0.52 mg 7.73 ± 0.55 mg 6.78 ± 0.68 mg	9 9 6	- 5.84 ± 0.34 mg 6.61 ± 0.61 mg	7 4
		H = 8.61 *		H = 6.01 NS		H = 1.02 NS		W = 8 NS	

For both *L. sericata* and *C. vicina*, comparisons were done in the conspecific group on (1) fresh liver and (2) rotten liver, and in the heterospecific group on (3) fresh liver and (4) rotten liver. For each comparison, mean values (± s.e.m.) are reported first (first line corresponding to d100, second to d250, and third to d500), and values of statistical tests are on the last line ("n": number of replicates). Asterisks (in bold) indicate a significant difference (Kruskal-Wallis test (H) and Mann-Whitney test (W); * $P < 0.05$; NS: non-significant difference). For *C. vicina*, a significant difference was observed in conspecific group on fresh liver: adult weight was significantly lower at d500 than at d250 and d100 (Kruskal-Wallis test, H = 8.22, $P = 0.016$; Nemenyi post-hoc test, d100 vs d250, $P = 0.99$, d100 vs d500, $P = 0.026$, d250 vs d500, $P = 0.030$).

Table 3. Statistical comparisons of percentage of emerged adults and mean adult dry weight between fresh and rotten liver and between conspecific and heterospecific groups.

Life history trait	Species	(1) conspecific: fresh vs rotten liver	n	(2) heterospecific: fresh vs rotten liver	n	(3) fresh liver: conspecific vs heterospecific	n	(4) rotten liver: conspecific vs heterospecific	n
Percentage of emerged adults	*L. sericata*	64 ± 6 % 5 ± 2 % **W = 567 *****	24 24	53 ± 5 % 12 ± 4 % **W = 528 *****	24 24	64 ± 6 % 53 ± 5 % W = 372 NS	24 24	5 ± 2 % 12 ± 4 % W = 249 NS	24 24
	C. vicina	49 ± 3 % 9 ± 2 % **W = 567 *****	24 24	54 ± 4 % 10 ± 3 % **W = 550 *****	24 24	49 ± 3 % 54 ± 4 % W = 214 NS	24 24	9 ± 2 % 10 ± 3 % W = 314 NS	24 24
Mean adult dry weight	*L. sericata*	4.19 ± 0.38 mg 2.66 ± 0.21 mg **W = 256 ****	24 14	3.70 ± 0.32 mg 4.36 ± 0.31 mg W = 107 NS	24 13	4.19 ± 0.38 mg 3.70 ± 0.32 mg W = 332 NS	24 24	2.66 ± 0.21 mg 4.36 ± 0.31 mg **W = 16 *****	14 13
	C. vicina	7.75 ± 0.34 mg 6.06 ± 0.26 mg **W = 366 ***** d100: W = 30 NS **d250: W = 81 ***** d500: W = 12 NS	24 19 9 9 6	7.48 ± 0.31 mg 6.00 ± 0.27 mg **W = 248 ****	24 13	7.75 ± 0.34 mg 7.48 ± 0.31 mg W = 325 NS	24 24	6.06 ± 0.26 mg 6.00 ± 0.27 mg W = 127 NS	19 13

For both *L. sericata* and *C. vicina*, comparisons were done between fresh and rotten liver in (1) the conspecific and (2) heterospecific groups, and between conspecific and heterospecific groups on (3) fresh liver and (4) rotten liver. Values of different densities were pooled for each condition. For each comparison, mean values (± s.e.m.) are reported first (first line corresponding to first compared condition), and values of statistical tests are on the last line ("n": number of replicates). Mean adult dry weight of *C. vicina* in the conspecific groups were also compared between fresh and rotten liver at each density (d100, d250, d500). Asterisks (in bold) indicate a significant difference (Mann-Whitney test; ** P < 0.01; *** P < 0.001; NS: non-significant difference).

Effect of decomposition

The percentage of emerged adult was significantly lower on rotten than fresh liver in either the conspecific or the heterospecific group and for both species (Fig. 2, Table 3). The mean adult weight of *L. sericata* was also significantly lower on rotten liver in the conspecific group but was not different in the heterospecific group (Fig. 3, Table 3). For *C. vicina*, adult weight was significantly lower on rotten liver in both groups (Fig. 3, Table 3).

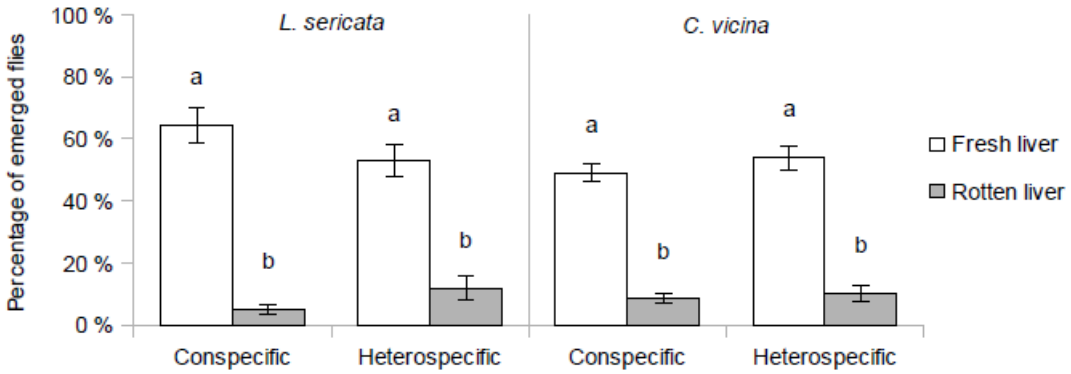

Fig. 2. Effect of food type and species composition on the percentage of emerged flies. Percentages (mean ± s.e.m.) of emerged adult flies of *L. sericata* and *C. vicina* on fresh (white) and rotten (grey) liver in the conspecific and heterospecific groups (n = 24 for each condition). The percentage of emerged adults was always significantly lower on rotten than fresh liver, with no differences between conspecific and heterospecific groups. Bars with different letters are significantly different (separate analyses for each species; Mann-Whitney test, $P < 0.05$).

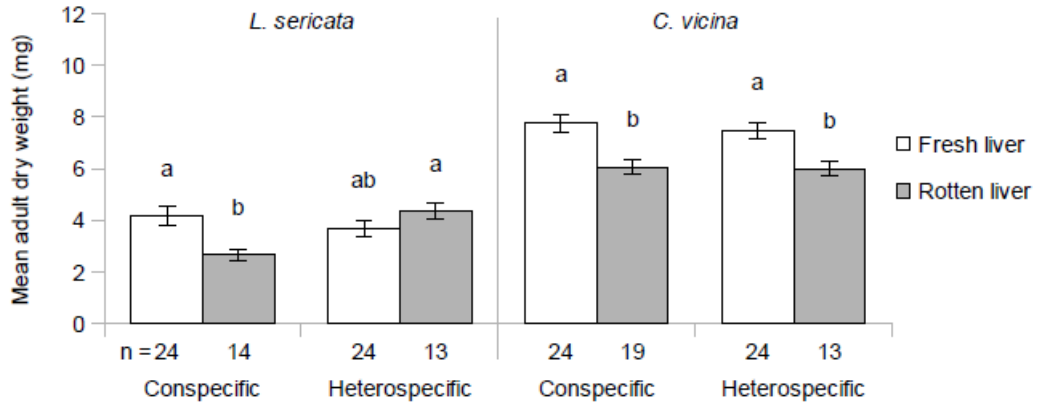

Fig. 3. Effect of food type and species composition on the mean adult dry weight. Mean adult dry weight (mean ± s.e.m.) of *L. sericata* and *C. vicina* on fresh (white) and rotten (grey) liver in conspecific and heterospecific groups. For a given food type, the mean adult weight did not significantly differ between conspecific and heterospecific groups, except for *L. sericata* on rotten liver (higher weight in the heterospecific group). Bars with different letters are significantly different (separate analyses for each species; Mann-Whitney test, $P < 0.05$).

Effect of species composition

On either fresh or rotten liver and for both species, the percentage of emerged adult was not significantly different between the conspecific and heterospecific groups (Fig. 2, Table 3). The mean adult dry weight of *L. sericata* was also not significantly different between these groups on fresh liver (Fig. 3, Table 3). However, on rotten liver, it was significantly higher in the

heterospecific than the conspecific group (Fig. 3, Table 3). No significant difference in mean adult dry weight between groups was observed for *C. vicina*, regardless of food type (Fig. 3, Table 3).

Binary choice tests

Preferences in the single-larva experiments

The estimated probabilities to observe one larva on the fresh spot at a time t were 0.73 for *L. sericata* (95 % confidence interval (CI95): [0.51, 0.91]) and 0.67 for *C. vicina* (CI95: [0.35, 0.93]). *L. sericata* larvae spent significantly more time on the fresh spot than on the rotten spot (Wilcoxon test, m_{fresh} = 73 %, m_{rotten} = 26 %, V = 168, *P* = 0.021), contrary to *C. vicina* (Wilcoxon test, m_{fresh} = 67 %, m_{rotten} = 30 %, V = 43, *P* = 0.14). Both spots were occupied by the larva in only 1 out of 20 tests for *L. sericata* and 3 out of 10 tests for *C. vicina*.

Collective dynamics over 48 h

When 40 *L. sericata* larvae where simultaneously tested in an arena with small food spots, 74 % of larvae were on the fresh spot and 23 % were on the rotten spot during the first 18 h. Then, the distributions balanced until the end of the experiment, with 40 to 60 % of larvae located on each spot (Fig. 4A). In 3 out of 10 replicates, regular shifts of larvae distribution were observed during the last 24 h of the experiment. Larvae stayed all on one spot from 15 to 60 min, moved collectively to the other spot for the same duration, then came back and started again, with several successive switches (Fig. 4B,C, Movie 1).

Effect of density

The latency of larvae first choices were not significantly different between high- and low-density conditions (Kruskal-Wallis test, H = 2.42, *P* = 0.49; Fig. 5). The number of replicates showing a group effect is summarized in Fig. 6 (Table S3). There were significantly more aggregations than expected under the no group effect hypothesis in all conditions at high density, but only in one condition at low density ("40 *L. sericata* on big spots"; Table 4). Moreover, there were significantly more aggregations on the fresh spot than on the rotten spot at high density (binomial test, n_{total} = 15, n = 12, *P* = 0.035) but not at low density (binomial test, n_{total} = 9, n = 6, *P* = 0.51). Finally, although the number of aggregations on the rotten spot was still higher than expected at high density, it was not significantly higher in the largest group (120 larvae; Fig. 6, Table 4).

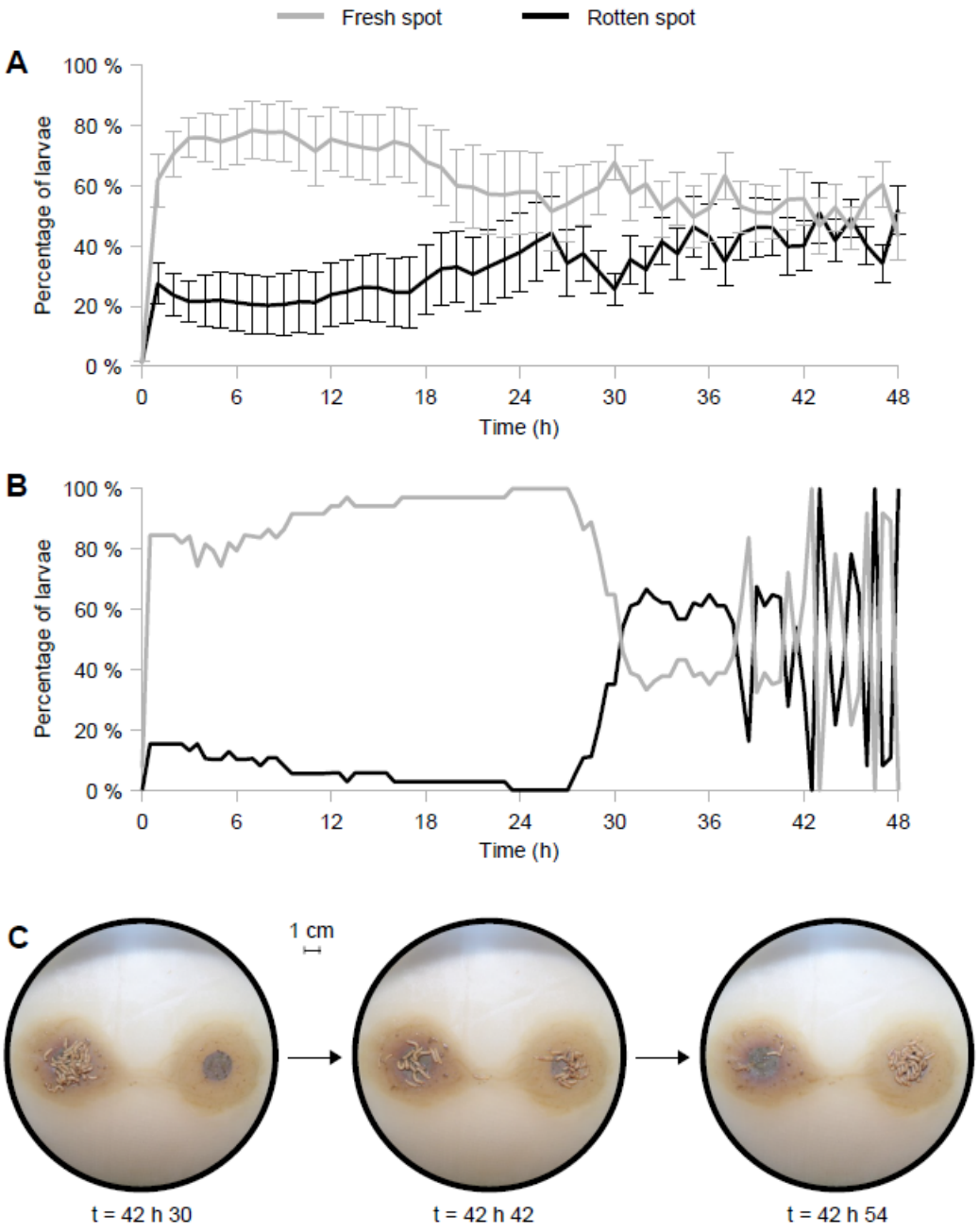

Fig. 4. Collective dynamics over 48 h. A. Percentages (mean ± s.e.m.) of *L. sericata* larvae on fresh (grey) and rotten (black) spots over time in experiments testing 40 *L. sericata* larvae in an arena with small spots (n = 10). **B.** Percentage of *L. sericata* larvae on fresh (grey) and rotten (black) spots over time in one replicate of the experiments displayed in A. Curve patterns between 38 h and 48 h illustrate regular successive shifts in location of larvae from one spot to the other (Movie 1). **C.** Switch of 40 larvae from the fresh (left) to the rotten spot (right), starting at 42 h 30 and lasting 24 min (pictures from the replicate described in B).

Table 4. Statistical comparisons between numbers of replicates showing an aggregation on the rotten spot, no aggregation, or an aggregation on the fresh spot.

Condition		Binomial tests			
		ntotal = total number of replications n = number of aggregations p = 0.05	ntotal = total number of replications n = number of aggregations on the fresh spot p = 0.025	ntotal = total number of replications n = number of aggregations on the rotten spot p = 0.025	ntotal = number of aggregations n = number of aggregations on the fresh spot p = 0.5
15 Ls / SS	ntotal	10	10	10	2
	n	2	1	1	1
	P	0.086	0.22	0.22	1.00
40 Ls / BS	ntotal	10	10	10	6
	n	6	2	4	2
	P	**< 0.001**	**0.025**	**< 0.001**	0.69
40 Ls / SS	ntotal	10	10	10	8
	n	8	6	2	6
	P	**< 0.001**	**< 0.001**	**0.025**	0.29
120 Ls / BS	ntotal	10	10	10	7
	n	7	6	1	6
	P	**< 0.001**	**< 0.001**	0.22	0.13
40 Cv / SS	ntotal	10	10	10	6
	n	6	3	3	3
	P	**< 0.001**	**0.002**	**0.002**	1.00
2 x 20 / SS	ntotal	10	10	10	7
	n	7	1	6	1
	P	**< 0.001**	0.22	**< 0.001**	0.13

The first column shows the binomial test of the number of aggregations with a probability of success (p) of 0.05; second, the number of aggregations on the fresh spot with p = 0.025; third, the number of aggregations on the fresh spot with p = 0.025; and fourth, the number of aggregations on the fresh spot in relation to the total number of aggregations with p = 0.5. Significance is highlighted in bold. Abbreviations: "Ls": *L. sericata*, "Cv": *C. vicina*, "2 x 20": 20 *L. sericata* and 20 *C. vicina*, "SS": small spots, "BS", big spots (*e.g.*, "15 Ls/SS": 15 *L. sericata* larvae on small spots).

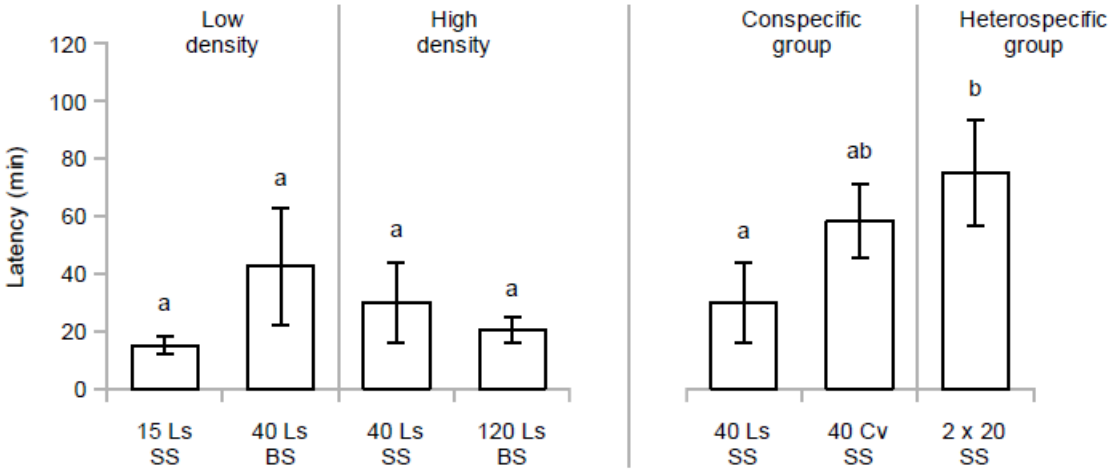

Fig. 5. Effect of density and species composition on choice speed. Latency (min; mean ± s.e.m.) to observe 75 % of larvae on both spots, in conditions of both low and high density and in conspecific and heterospecific groups (n = 10 for each condition). Bars with different letters are significantly different (Kruskal-Wallis test and Nemenyi post hoc test, $P < 0.05$). In the heterospecific group, latency was significantly higher than in *L. sericata*'s conspecific group. Abbreviations: "Ls": *L. sericata*, "Cv": *C. vicina*, "2 x 20": 20 *L. sericata* and 20 *C. vicina*, "SS": small spots, "BS": big spots (*e.g.*, "15 Ls/SS": 15 *L. sericata* larvae on small spots).

Effect of species composition

The latency of larvae first choices was significantly higher in the heterospecific group than in the *L. sericata* group (Kruskal-Wallis test, H = 10.46, $P = 0.005$; Nemenyi post hoc test, 40 *L. sericata* vs 40 *C. vicina*, $P = 0.054$, 40 *L. sericata* vs [20 *L. sericata* + 20 *C. vicina*], $P = 0.006$, 40 *C. vicina* vs [20 *L. sericata* + 20 *C. vicina*], $P = 0.71$; Fig. 5). In all conditions, there were significantly more aggregations than expected under the no group effect hypothesis (Table 4). However, the number of aggregations on the rotten spot was significantly higher than expected in conspecific groups, but not in heterospecific group (Fig. 6, Table 4). Finally, no significant difference was observed when comparing numbers of aggregations on the fresh spot with those on the rotten spot (Table 4).

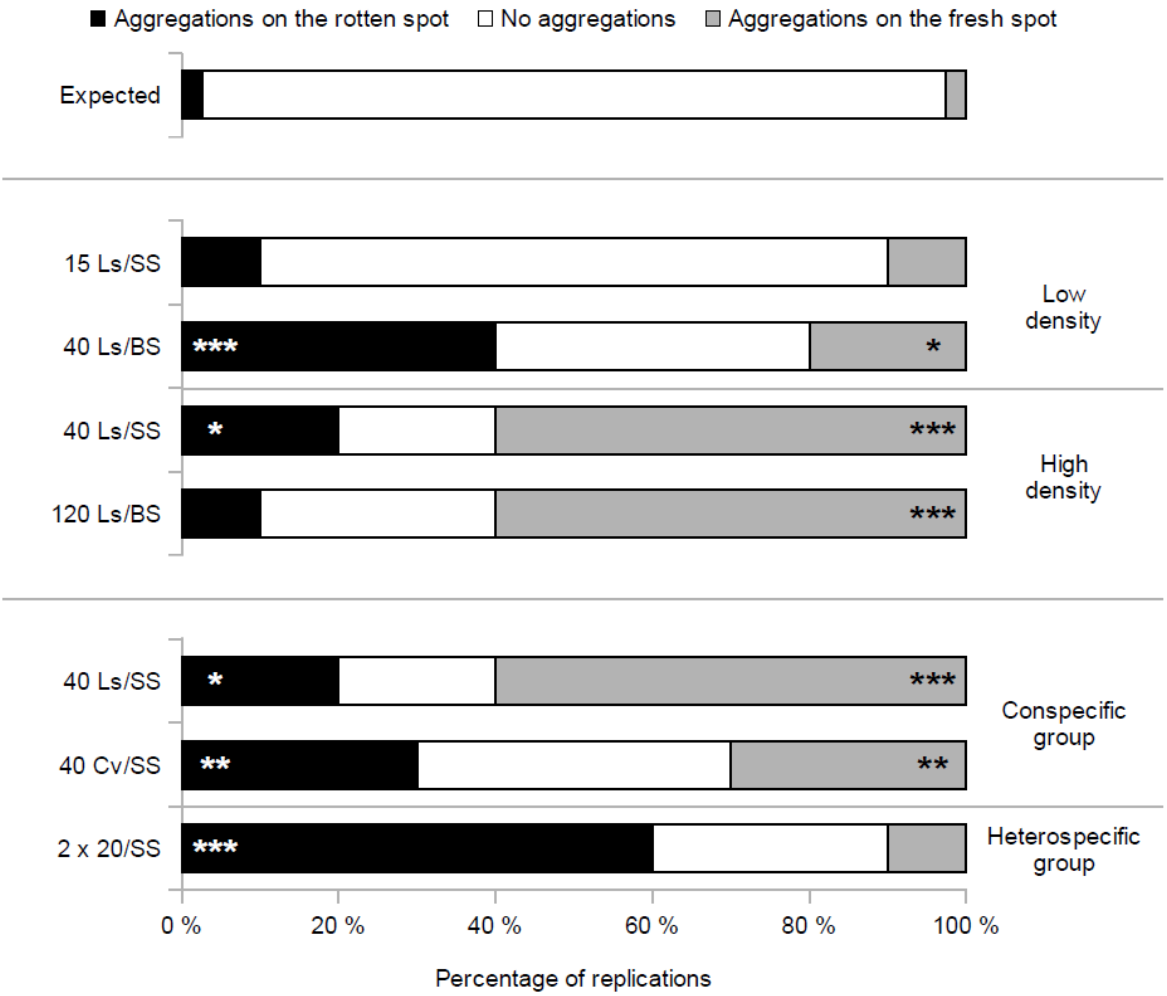

Fig. 6. Effect of density and species composition on larval aggregations. Percentage of replicates showing significantly more aggregations on the rotten spot (black), no aggregations (white), or significantly more aggregations on the fresh spot (grey), for each experimental condition (n = 10 per condition). The first bar, "Expected", indicates the probability to observe one (p = 0.05) or no (p = 0.95) group effect if larvae did not interact with each other. Asterisks indicate a significantly higher percentage of aggregations on one spot than expected (binomial test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). For instance, in groups of 120 *L. sericata* on big spots ("120 Ls/BS"), there were significantly more aggregations on the fresh spot than expected, but not more on the rotten spot. Abbreviations: "Ls": *L. sericata*, "Cv": *C. vicina*, "2 x 20": 20 *L. sericata* and 20 *C. vicina*, "SS": small spots, "BS", big spots (*e.g.*, "15 Ls/SS": 15 *L. sericata* larvae on small spots).

DISCUSSION

This study was designed to assess the costs and benefits of food decomposition, larval density and group species composition on larval development and to investigate the effects of these three factors on larval collective behaviour. The results of development tests showed (1) a negative effect of decomposition on both survival and growth of larvae, (2) no benefit of

increasing larval density, and (3) a benefit on *L. sericata* larval growth of developing in a heterospecific group on decomposed food.

Liver decomposed under anaerobic conditions is a sub-optimal food source for both blow fly species (as previously evidenced in *C. vicina*; Richards et al., 2013). The adverse effect of decomposition could be due to two main factors: (1) an alteration of nutritional value with putrefaction (Carter et al., 2007; Metcalf et al., 2015) and (2) a modification of food microbial community, causing either nutrient depletion, the proliferation of pathogenic bacteria and/or the decrease in the relative abundance of beneficial bacteria (Ahmad et al., 2006; Andersen et al., 2009; Lam et al., 2009a,b; Metcalf et al., 2015). Despite the single observed benefit of the heterospecific group, both increasing density and species number did not allow larvae to reach a fitness on rotten liver as high as that on fresh liver. Therefore, the following observed effects of density and species number in binary choice tests can not be explained solely by changes in food quality induced by density or species. Indeed, according to the ideal free distribution (Giraldeau and Caraco, 2000), larvae would always benefit most from aggregating on fresh than decomposed food (assuming that the food quantity is high enough to prevent competition).

The results of binary choice tests with single larvae are consistent with a fresh-food-associated benefit (first hypothesis): *L. sericata* larvae spent significantly more time (73 %) on the fresh than the rotten spot. *C. vicina* larvae also tended to spend more time (67 %) on the fresh spot, although not significantly. These results indicate that larvae were able to assess the quality of the food and preferred fresh to rotten meat. This preference is consistent with the natural behaviour of blow fly larvae on carrion: flies can lay eggs in a few minutes after death (*e.g.*, Payne, 1965), larvae feed on fresh food and leave it at the post-feeding stage when the tissues gets decomposed. However, larvae can sometimes be laid on several day old carcasses and feed only on decomposed food (e.g., Matuszewski et al., 2010). Repulsion from rotten food is a common phenomenon among animals: it refers to the spoiling theory, which supposed that microbes release repellent compounds allowing them to compete with animals (Janzen, 1977; Ruxton et al., 2014). For *L. sericata*, the fact that almost no larvae (5 %) visited both spots suggests they first oriented themselves preferentially towards the fresh odour source. Then, the food exerted a strong retention effect, preventing larvae on the rotten spot to switch to the fresh one. For *C. vicina*, a few more larvae visited both spots (30 %), suggesting species-specific preferences or increased tolerance.

Experiments testing groups of *L. sericata* showed that larvae interacted with each other and

that these interactions influenced their food preferences. The fact that aggregations occurred either on the fresh spot or on the rotten spot demonstrate that this effect of congeners can lead to both optimal and sub-optimal choices. However, there were more aggregations on the fresh spot than on the rotten spot at high density. Moreover, in groups of 120 larvae, the number of aggregations on the rotten spot was not significantly different than expected if larvae did not interact with each other. These two results validate the second hypothesis of the study: choice accuracy increases with increasing density, with effects of both the absolute larval number and the ratio between larval number and food quantity. The absence of a significant difference in latency shows, however, that the speed of larval choices was not affected by either group size or density. This result suggests that the increase in choice accuracy results not from a density-dependent increase in larval movement (as such increase would lead to finding the spots more quickly) but rather from larval inter-attractions.

Social influences occurring in collective decisions are often mediated by short-range cues, such as trail pheromones and contact cues (Jeanson et al., 2012). In cockroaches, the tactile perception of compounds left on the ground induces an increase in resting time, which can trigger a collective choice (Deneubourg et al., 2002; Lihoreau et al., 2010). The same mechanism is likely to occur in blow fly collective decisions, as they are known to leave retentive chemical cues (Boulay et al., 2013; Fouche et al., 2018). This mechanism could also explain the alternating of spot selection observed between 38 h and 48 h with 40 *L. sericata* (Movie 1). A spatial saturation of the most-crowded spot could induce a shift of some larvae to the less-crowded spot. This shift could also be due to a group effect, such as waste accumulation (*e.g.*, ammoniac), to a decrease in food quantity, or to a decrease in food quality through a change in microbiome, induced or not by larvae (Johnson et al., 2013; Metcalf et al., 2015). Then the formation of a trail would rise the probability to leave one spot for the other (Boulay et al., 2013; Fouche et al., 2018). The decrease in larvae number on the initial aggregate increased the probability of the remaining larvae to leave, leading to the collective movement. Further studies using modelling remain necessary to investigate these hypotheses.

In the present study, social effects led to both optimal and sub-optimal larval choices. Optimal collective decisions are usually achieved by the amplification of individual preferences for the best resource (Canonge et al., 2011; Jeanson et al., 2012). However, the amplification mechanism can also induce trapping on sub-optimal locations (Dussutour et al., 2007). Such apparently non-adaptive behaviours can be caused by a lack in flexibility in the response to congeners. In forest tent caterpillars, although individuals increased their exploration level on the unbalanced food, this increase was too weak to counterbalance the trail-following

behaviour and induce a switch in group decision (Dussutour et al., 2007). In the present study, the strong food retention effect also suggests a lack of behavioural plasticity: larvae trapped on the rotten spot were not able to leave even when other larvae were on the fresh spot. Nevertheless, an increase in density offers a way to limit sub-optimal choices: when density increases, the influence of social cues is enhanced, and initial preferences for the best resource get more chances to be amplified by the group (Canonge et al., 2011; Szopek et al., 2013).

Finally, the results showed that larval preferences were affected by the group's species composition, confirming the third hypothesis of this study. Although conspecifc groups of both species made both optimal and sub-optimal choices, when these species were mixed together, they were unable to make optimal collective choices. Moreover, *L. sericata* larvae took significantly more time to make their first decision in the heterospecific than the conspecific group. Therefore, the presence of another species led to a cost in choice accuracy through a reversal of larval preferences towards rotten liver, in addition to a cost in choice speed for *L. sericata*. Former studies reported a change in individual preferences when environmental conditions were modified, such as by adding predation-related cues (*e.g.*, spiny lobsters: Eggleston and Lipcius, 1992; snails: Gerald and Spezzano, 2005). This change was shown to be adaptive, leading to a decrease in predation risk. Preferences for food can also switch depending on the microbial community, as shown with *L. sericata* larvae (Rhinesmith-Carranza et al., 2018). In the present study, development tests did not show equal or greater benefit of developing on rotten than fresh liver when species were together. However, such a benefit could arise in a natural environment, as discussed thereafter.

On field carcasses, larval masses can largely exceed the highest density studied here and often contain more than two species (Rivers et al., 2018). Larvae also feed on various tissue types, which results in different impacts on larval fitness (Kaneshrajah and Turner, 2004; Clark et al., 2006). When feeding on hard tissues such as muscles, *L. sericata* larvae develop faster and survive better at higher density (*i.e.*, Allee effect; Scanvion et al., 2018). Furthermore, benefits of interspecific aggregation were observed for both *L. sericata* and *C. vicina* larvae when reared together on such food (Komo et al., 2019). Finally, external selective pressures such as predation (Goodbrod and Goff, 1990) and parasitism (Frederickx et al., 2013) can be limited by group living (Krause and Ruxton, 2002; Rivers et al., 2012). It is therefore possible that the group behaviours observed in the present study could be adaptive under natural conditions.

For example, being trapped on a sub-optimal food source also leads to maintaining group

cohesion and the associated benefits (*e.g.*, larval-mass effect; Rivers et al., 2011). Furthermore, the adult mass increase observed for *L. sericata* grown in the heterospecific groups demonstrates that species association can provide benefits on decomposed food. Such benefits could result from the pooling of species-specific antibacterial compounds (Barnes et al., 2010) that would select a microbiome more favourable for development. Under this scenario, aggregating together on rotten food would make new food sources available for participant species, reducing local competition and creating a new developmental niche (Odling-Smee et al., 2013; Schwab et al., 2017; Goodale et al., 2020). In a similar way, collective decisions of *Drosophila* larvae towards a food spot infected by a pathogenic fungus were explained by the ability of larvae to collectively inhibit fungal growth and improve their development, enabling the colonisation of new food spots (Rholfs, 2005; Rholfs et al., 2005). In blow flies, interspecific aggregation on rotten food would also be more advantageous when larval competition on fresh spots is high, inducing increased mortality and decreased adult mass and fecundity (Kamal, 1958; Saunders and Bee, 1995; Smith and Wall, 1997). Such higher competition on fresh than rotten spots could occur at the end of the bloat stage, when the skin cracks and larvae get access to the uncolonized internal organs decomposed by anaerobic bacteria (Hyde et al., 2013; Metcalf et al., 2013; Pechal et al., 2014). Further experiments on the fitness consequences of interspecific aggregations are needed to confirm or not these hypotheses.

Finally, the aggregation behaviour of larvae may be affected by other factors not studied here. For instance, the presence of predators and parasitoids could increase the attraction between larvae such that the group becomes more cohesive and provides greater protection (Rivers et al., 2012). The local temperature has also a critical influence on larval aggregation: the two species studied here differ widely in their optimum temperatures (*C. vicina* having lower optima than *L. sericata*; Grassberger and Reiter, 2001; Donovan et al., 2006) and *C. vicina* aggregate preferentially at 22°C when placed in an thermic gradient (against 33°C for *L. sericata*; Aubernon et al., 2016). Bacterial growth is slower at cooler temperatures, which could explain the slightly lower tendency of *C. vicina* to choose the fresh food. It also suggests that *C. vicina* is usually exposed to a lower competition level with microorganisms and would have evolved less efficient antibacterial secretions compared to *L. sericata*, as observed in Barnes et al. (2010). However, this would lead to a benefit for *C. vicina* to be associated with a greater competitive species such as *L. sericata*, while the opposite has been observed in this study.

To conclude, this study has demonstrated that (1) blow fly larvae are able to collectively choose the best resource in their environment, (2) their choice accuracy is weak at low

density but increases with increasing density, and (3) larval preferences are modulated by the presence of other species, leading to a choice reversal in heterospecific groups. These results provide new evidence of the ubiquity of collective decision properties in non-eusocial insects. They show that blow fly larval behaviour results from the perception and integration of environmental cues (food decomposition level), social cues (density) and species-specific cues. Their implications in the ecology of interspecific aggregations stress the importance of studying further collective decision-making in heterospecific groups.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Q.F. and D.C. conceived and designed the experiments, Q.F. conducted the experiments, Q.F. and D.C. analysed the results, and Q.F. and D.C. wrote the manuscript. All authors reviewed the manuscript.

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