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Abstract: Several studies have highlighted the impact of environmental factors such as food type or larval density on the development of blowfly larvae. We investigated how changes in development speed (due to larval density and group composition) are divided among feeding and post-feeding stages. Even if these parameters impinge only on feeding larvae, they may ultimately also affect their subsequent development, and especially metamorphosis duration. Therefore, this study analysed the effect of larval density and group composition on the rhythm of necrophagous blowfly development. Based on laboratory studies, we highlighted that *Calliphora vicina* individuals with a fast development during their feeding phase developed slower in the later post-feeding phase (*i.e.,* they had a compensatory effect). *Lucilia sericata*, a calliphorid species also frequently found on carrion at the same time as *C. vicina*, showed a different developmental strategy by not making its post-larval development speed dependent on the larval development speed. Finally, while a compensatory effect may exist, variations in the development rate more often accumulate through life-stages and resulted in a larger variability for later development instars. In this respect, the inclusion of detailed development duration covering all life stages, including post-feeding, is recommended in future studies, especially those dedicated to forensic entomology.

Keywords: Calliphoridae, maggots, wandering larvae, eclosion, development rate

1. Introduction

Necrophagous larvae of blowflies (*i.e.*, maggots) live and feed on vertebrate carrion. However, they generally leave this food source in the subsequent development stages to hide and pupate (Gomes et al., 2006). How developmental conditions encountered during feeding stages affect these later post-feeding stages is the topic of this study. Below, the life cycle of maggots as well as the influence of biotic and abiotic factors on their development is explained in detail.

1.1. Life cycle of necrophagous maggots

The life cycle of a blowfly is defined by four morphologically distinct life-stages: egg, larva, pupa and imago. Eggs and pupae constitute immobile development stages, whereas larvae and adults are mobile (*i.e.*, moving) stages. However, while flies can fly up to 9 km/h and cover a flying distance of several kilometres to find carrion, feeding larvae barely move from the place they were laid. Only post-feeding larvae disperse around carrion (Bomphrey et al., 2009; Braack, 1981; Charabidze et al., 2008; Green, 1951). In other words, flies select carrion for their eggs, larvae their feeding site at the carrion and post-feeding the pupariation site around carrion.

Between each larval instar, a maggot sheds its cuticle, until finally the last outer cuticle shrinks and hardens to a puparium (Castner, 2001; Gunn, 2009). To enter the next development stage, a threshold size and weight must be met. Consequently, sufficient food intake during the larval feeding stages is mandatory (Hightower et al., 1972; Shaaya and Levenbook, 1982). Once peak feeding is reached, calliphorid maggots usually wander away from their food source and burrow in soil for pupariation (Gomes et al., 2006). This transition represents a behavioural stage called the *post-feeding stage*.

Inside the puparium, the insect completes metamorphosis, until finally the adult fly ecloses. Responsible for initiating metamorphosis is a pulse of the moulting hormone ecdysone at the end of larval life (Nijhout et al., 2006). Whether this profound transformation will be completed successfully largely depends on the initial developmental efficiency of the larva (Denlinger, 1994; Mohr, 2012).

1.2. Influence of biotic and abiotic factors

Necromass can be regarded as a harsh environment to develop on (Brown and Gaugler, 1997; Cornwallis et al., 2017; Lewis and Shapiro-Ilan, 2002; Trumbo, 1997). In addition to natural decomposition processes and spoiling by bacteria (Benbow et al., 2015b), competition with scavengers can result in a sudden food depletion and the death of larvae (Erincçlioğlu, 1996). DeVault *et al.* (2004) observed a mean time of rodent carrion removal by scavengers of 2.6 days: larvae with a longer feeding time risk being eaten. High larval densities can also lead to intense conspecific and heterospecific competition for resources (Denno and Cothran, 1976; Rivers et al., 2011). Furthermore, predatory larvae such as those of *Chrysomya* (Diptera: Calliphoridae) can significantly deplete blowfly larvae populations (Flores et al., 2017). Last, parasitoids wasps can induce high mortality rates in blowflies, reaching 90% under certain conditions (Frederickx et al., 2013). Given all these biotic pressures on survival, the feeding speed and development rate of larvae determine their probability of survival (Levot et al., 1979).

The development rate of necrophagous larvae is determined by abiotic (notably temperature) but also biotic factors (Benbow et al., 2015a). As an example, food moisture and pupation substrate were shown to have significant influence on the growth of *L. sericata*, producing a developmental difference of up to 7.4 days (Tarone and Foran, 2006). Other scientists have also observed repeatedly that different soft and protein-rich nutrition affects larval development duration (Clark et al., 2006; El-Moaty and Kheirallah, 2013; Ireland and Turner, 2006). Previous studies also demonstrated that blowfly larvae reared under certain heterospecific conditions had faster and better development than conspecific groups, with higher survival (Komo et al., 2019, 2020a, 2020b). However, larval development that is too rapid can result in nutrient deficiency, with profound and permanent effects on later stages (*e.g.*, reduced immunity and lower adult longevity) (Cotter et al., 2004; Sevenster and van Alphen, 1993). Chippindale et al. (1997), who performed an experimental study on development speed in Drosophila, concluded that developmental trade-offs are not confined to single stages of a lifecycle.

Accordingly, the hypothesis raised in the present study is that the variability in development duration during larval feeding stages has an impact on the duration of the postfeeding stages. To test this hypothesis, the effects of species composition and larval

density on pre- and post-feeding durations of *Calliphora vicina* Robineau-Desvoidy, 1830 and *Lucilia sericata* (Meigen, 1826) have been analysed.

2. Materials and Methods

2.1. Insect rearing

Larvae of *L. sericata* and *C. vicina* (Diptera: Calliphoridae) were obtained from flies bred in Lille (Nord, France). These colonies, which were replenished with new flies every month, were kept in separate cages (50×50×50 cm) at room temperature (20±2°C) and daylight at their natural times. Caster sugar and water *ad libitum* were available throughout the flies' lifetime. Pieces of pork heart were used as the protein supply and oviposition media. For the latter purpose, they were placed in the cages for 2 h, guaranteeing an oviposition time with a maximum deviation of ±1 h. Blowfly eggs as well as larvae and pupae were kept in a climatic chamber (ST4, POL-EKO Aparatura®, Poland) at 25°C.

2.2. Preparation and start of experiments

The setup used to monitor larval development within mono- and heterospecific groupings was adapted from Scanvion *et al.* (2018). The previously frozen meat (50 g of fresh minced beef steak: 100% muscle with 15% fat content, Cora®) was thawed overnight and mixed with 15 ml of a 0.9% NaCl solution in order to offer larvae in the first stage a very soft (slightly liquefied) medium. This prepared nutrient medium was used to fill a small plastic box (100×75×63 mm), which was placed in a large breeding container (180×135×195 mm), the bottom of which was covered with sand. First instar larvae were homogeneously distributed on the food 22 h (*L. sericata*) or 24 h (*C. vicina*) after oviposition.

2.3. Experimental procedures

The number of migrating larvae in the sand was counted three times a day (10 a.m., 4 p.m. and 10 p.m.) and used as a measure of the development speed. At each measurement time, the migrating larvae from each box were transferred to a new sand-filled box with a sheltered place to pupate and were raised to adulthood (also at

25°C). Finally, the number of eclosed flies was counted at the same measurement times (*i.e.*, three times a day). Each development event (post-feeding and eclosion) was considered to have been reached when it was achieved by the first 10% of individuals. This threshold is often used in forensic studies and considers the first larval wave without considering rapid outliers (Clarkson et al., 2004). The relative development rate was calculated by the following formula: time of specific individual to reach a given instar divided by mean time in the population to reach this instar.

This procedure was carried out for five different conditions: 100 and 250 individuals of *L. sericata* or *C. vicina* in conspecific groups, along with 125 individuals of both species (250 individuals in total) in the heterospecific group. Six repetitions, which never ran simultaneously, were performed for each condition (*i.e.*, 5 boxes with 5 different conditions were used per test run). Of these, another Lille study by Scanvion *et al.* (2018) provided the developmental data of *L. sericata* in conspecific conditions.

Experimental developmental data were analysed separately for the two studied species and at two different scales. First, development rate during feeding and post-feeding stages were compared at an individual scale. This allowed a qualitative approach on the developmental rhythm for each individual larva and direct comparison across all the larvae studied. Second, average trends were quantitatively analysed (i.e., at the scale of the whole experimental population) to test our hypothesis of a compensatory effect between feeding and post-feeding stages. For the statistical analysis paired Wilcoxon test and ANOVA followed by Tukey's range test if necessary were performed.

3. Results

3.1. *Calliphora vicina* at individual level

The development rate of each larva before and after migration was compared at the individual level (see Tables S1 and S2 in the supplementary material for the proportions of eclosed flies). The observation of *C. vicina* individual development rates before and after larval migration revealed that individuals with a slow development during the feeding phase had a fast development in the post-feeding phases (Wilcoxon rank sum test: V = 137738, p < 2.2e-16; Fi□ure 1 lower right quarter). However, such a slow (*i.e.*, a rate >

1.05, right side on Figure 1) development during the feeding phase was only rarely observed, and only in conspecific condition. In contrast, if the development during the feeding phase was faster than the median (Figure 1, left side), many individuals had a comparatively slower development in the post-feeding phases (upper left quarter). This was especially true in heterospecific groups: no individual developed much slower than the median during the feeding phase, but many had a slow post-feeding development (Figure 1, upper left white quarter). Thus, many *C. vicina* larvae compensated for an initially very fast development with a slower development later. Larval development rates of 0.8 (*i.e.,* fast) could not be maintained after leaving the food. At the most, these individuals showed a development rate of 0.9, though they mostly only developed on average or even slower than the median. A few exceptions to this compensatory effect were shown by individuals with an average larval development rate (*i.e.*, 1.0) but a slow development in the post-feeding phases (*i.e.*, between 1.05 and 1.2). However, individuals with a slow feeding and post-feeding development were almost never observed (*cf.* empty upper right light orange quarter on Figure 1).

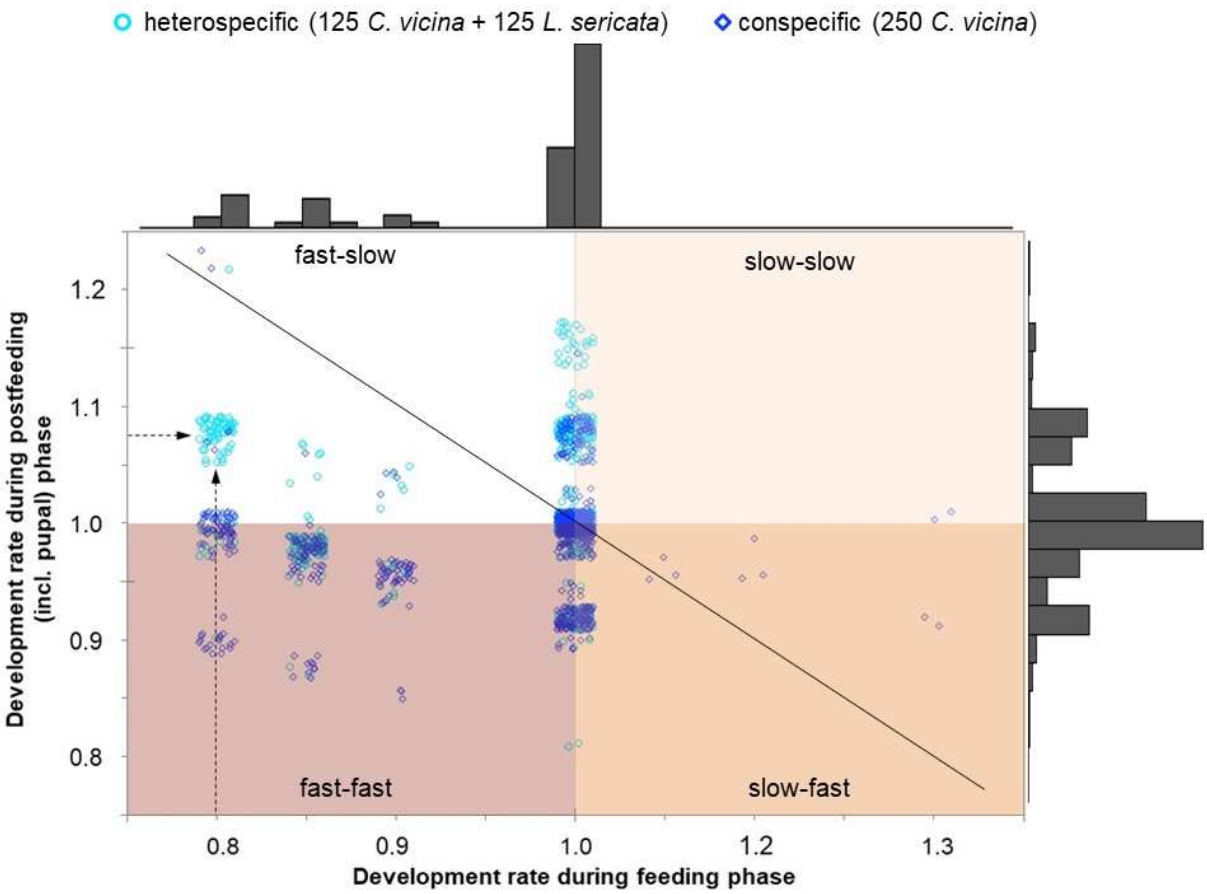


Figure 1. Development rates of *Calliphora vicina*

Individual development rates of *C. vicina* during the feeding and post-feeding phases in heterospecific (turquoise circles) and conspecific groups (blue rhombuses). Values below 1.0 indicate a faster development than the median, and above 1.0 a slower development. Those individuals that show a value of 1.0 on the x-axis grew had a median value during the feeding phase, which were most of the individuals (highlighted on the height of the columns of the histogram). The arrows point to individuals with a very fast larval development rate during the feeding phase (0,8) and a slower rate during the post-feeding phase (1,1). The middle of the graph captures those individuals that grow at median values during both development phases.

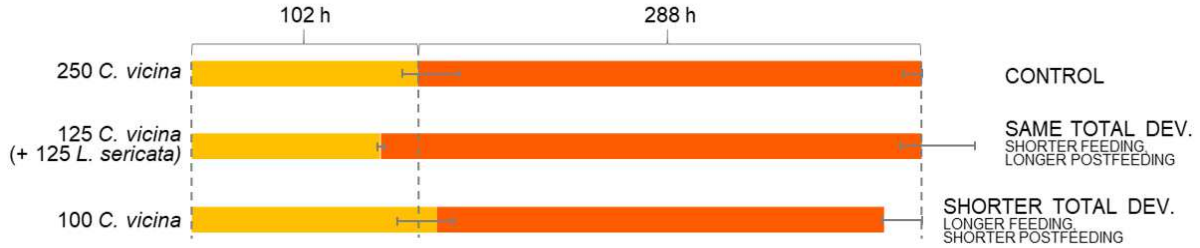
3.2. *Calliphora vicina* at group level

The average development rates during the first developmental phase (*i.e.,* feeding) and the second (*i.e.,* post-feeding) were then compared at the group level between the different conditions (*i.e.*, high- and low-density as well as conspecific and heterospecific

groups). In doing so, the heterospecific group of *C. vicina* displayed a 12-h faster development in the feeding phase and a 12-h slower development in the post-feeding phase, resulting in the same total development time as the control group. In contrast, the low-density group developed 6-h slower during the feeding phase but 18-h faster in the post-feeding phase, representing a huge compensatory that resulted in a shorter total development time (Figure 2, Table 1 as well as Tables S1 - 8 in supplementary). Although these trends are clearly visible in Figure 2, the high variability observed between repetitions does not show a significant difference between the groups (ANOVA for mi□ration: $F = 0.21$, $p = 0.65$; eclosion: $F = 0.49$, $p = 0.49$).

Table 1. Proportion of 250 *C. vicina*, 125 *C. vicina* (+125 *L. sericata*) and 100 *C. vicina* reaching postfeeding stage (migration) and ending pupal stage (eclosing) at 25 °C. Both development events were considered to have been reached when it was achieved by the first 10% of individuals. Hours are recorded from egg deposition for each repetition.

repetition	condition	migration	eclosion
1	250	16 % after 96 h	± 12 % after 378 h
2	250	16 % after 102 h	11 % after 384 h
3	250	13 % after 108 h	21 % after 390 h
4	250	10 % after 102 h	18 % after 390 h
5	250	18 % after 96 h	11 % after 384 h
6	250	± 15 % after 114 h	11 % after 384 h
1	125 + 125	± 15 % after 90 h	10 % after 384 h
2	125 + 125	± 15 % after 90 h	36 % after 390 h
3	125 + 125	± 15 % after 90 h	44 % after 390 h
4	125 + 125	± 15 % after 90 h	45 % after 390 h
5	125 + 125	± 15 % after 90 h	17 % after 390 h
6	125 + 125	± 15 % after 90 h	13 % after 408 h
1	100	± 15 % after 114 h	16 % after 372 h
2	100	± 15 % after 114 h	16 % after 372 h
3	100	13 % after 108 h	19 % after 384 h
4	100	25 % after 108 h	13 % after 372 h
5	100	17 % after 96 h	± 15 % after 378 h
6	100	10 % after 102 h	± 15 % after 378 h



198

199 **Figure 2. Effects of group size and composition on the development duration of *C.***
200 ***vicina***. Using the conspecific high-density group as the control group, the heterospecific
201 group displayed a 12-h faster larval development until migration and the low-density
202 group a 6-h slower larval development. During the post-feeding (including pupal) phase,
203 this ratio was reversed, *i.e.*, the heterospecific group developed 12 h slower and the
204 low-density group 18 h faster than the high-density group. Error bars represent standard
205 deviations of group means.

206

207 3.3. *Lucilia sericata* at individual level

208 At the individual level, many *L. sericata* larvae experienced a slow development time in
209 both pre- and post-feeding phases in heterospecific conditions (Figure 3, upper right
210 quarter). However, some larvae also showed a compensatory effect, with both slow-fast
211 and fast-slow individuals.

212

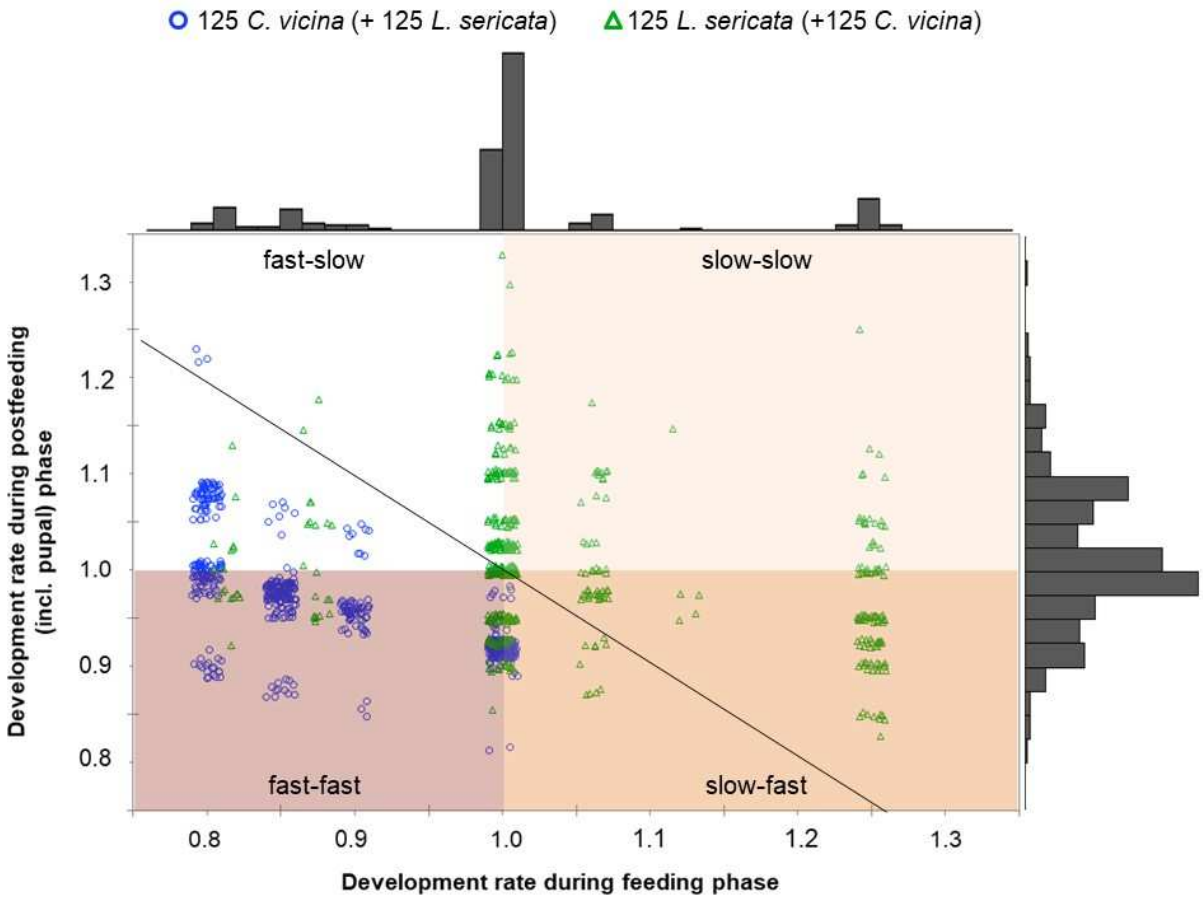


Figure 3. Development rates of *Lucilia sericata*

Development rates of *L. sericata* in heterospecific groups during the feeding and post-feeding phases. Each individual of the more than 1400 tested is represented by one triangle. Those individuals that show a value of 1.0 on the x-axis grew at median values during the feeding phase (which were most of the individuals highlighted on the height of the columns of the histogram). All triangles in the upper right square represent individuals that developed slower than the median in both development phases, that is, the feeding and the post-feeding phases (pupal phase included). The line displays a theoretical compensatory effect, *i.e.*, the development speed during post-feeding that is inversely proportional to the feeding phase.

3.4. *Lucilia sericata* at group level

Comparing the average development rates during the feeding and post-feeding development phases at group level, we observed that *L. sericata* larvae reached the post-feeding phase in the heterospecific and low-density group on average 12 h later

than in the control group (Figure 4¸ ANOVA: F = 19.56, p = 6.62e-05; TukeyHSD: $p_{250\text{-}125}$ = 0.002). In contrast to *C. vicina*, no accelerated development followed in post-feeding phases, but the time lag either remained constant (heterospecific) or increased further to 18 h (low-density group; ANOVA: F = 5.735; p = 0.0141; TukeyHSD: $p_{250\text{-}125}$ = 0.079, $p_{250\text{-}100}$ = 0.013, $p_{125\text{-}100}$ = 0.625).

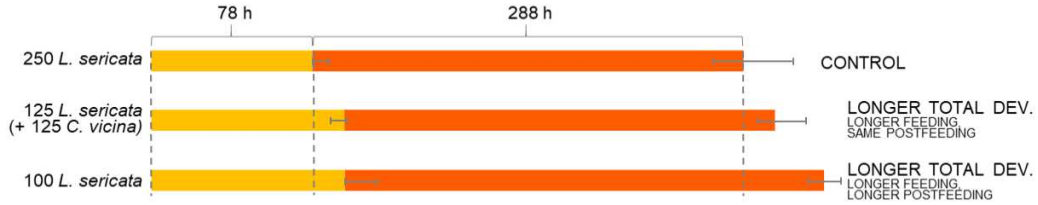


Figure 4. Effects of group size and composition on the development of *L. sericata*

Using the conspecific high-density group as a control group, both the heterospecific and the low-density group showed a 12-h slower larval development until migration. During the post-feeding (including pupal) phase, this time lag was maintained until eclosion in the heterospecific group and increased to 18 h in the low-density group [data for conspecific groups from Scanvion *et al. (2018)*, 25°C].

4. Discussion

We hypothesized that the variability in development duration during larval feeding phase may also have an impact on the duration of the postfeeding phases. Our results confirm this hypothesis and demonstrate that events impacting larval development of calliphorids have consequences through stages, both accelerating and slowing-down post-feeding development. Thus, even if key developmental parameters (e.g., competition) impinged on individuals only during the feeding stages, they ultimately also affected the metamorphosis duration. Most likely, blow fly larvae compensate for an initially too fast or too slow development in the later development stages to avoid long-term consequences (Metcalfe and Monaghan, 2001; Faris et al., 2020).

In many insects, final adult size depends on the development rate and is controlled by mechanisms that terminate growth when the individual reaches a species-specific size (Mirth and Riddiford, 2007, Nijhout et al., 2014). Both the development rate and critical weight can differ between environmental conditions (Nijhout & al., 2006). On carrion, selection pressures can act directly on larvae (e.g., parasitism, predation pressure, *etc.)* but also indirectly through feeding disturbances, food limitation or bacterial accumulation (Arendt, 1997; Gruszka, 2020; Munch and Conover, 2003; Prasad et al., 2001; Wertheim et al., 2002). Indeed, while blowfly larvae are specialized for feeding on carrion, they avoid decayed carcasses and prefer fresh ones (same principle as in other food webs; Burkepile et al., 2006). The increase in microbes caused by the decomposition process alters the quality of the food and disrupts larval development (Benbow et al., 2019; Pechal et al., 2014; Richards et al. 2013). In this context, the selection pressure mostly acts on the timing of larval migration, suggesting a benefit of a short feeding phase (Prasad et al., 2001; Rivers, 2011). According to this goal, necrophagous blow fly larvae aggregate on large interspecific groups gathering thousands of individuals, a social mechanism facilitating feeding and accelerating their development (Komo et al., 2019; Scanvion et al., 2018). This behavioural adaptation thus reduces the feeding phase duration and consequently the exposition of larvae to environmental selection pressure.

However, fast development and larval competition can lead to undernourishment and a lack of reserves, resulting in smaller individuals and low survival rates (Arendt, 1997; Fox, 1997; Munch and Conover, 2003; Richner, 1992; Sinervo and Doughty, 1996). Indeed, the shorter the feeding period is, the less energy that can be stored, and fewer resources remain available for later phases (Khelifa et al., 2019). To limit mortality during post-feeding stages, physiological control involving Target Of Rapamycin (TOR, a core component of the nutrient-responsive pathway) can delay the moulting hormone ecdysone secretion, which otherwise terminates larval development (Layalle et al. 2008). This consequently extends the duration of larval growth, giving maggots an opportunity to attain a bigger body size. Recently, Dombrovski et al. (2021) observed that the developmental delay in clustered Drosophila larvae was accompanied by an increase in adults' wing size, suggesting this developmental retardation finally resulted in fitness

benefits. Thus, the consequences of larval aggregation, development speed and feeding can entail a wide range of costs and benefits throughout the individuals life-stages (Blanckenhorn, 1998).

4.1 Different strategies between species

The influence of the developmental conditions (i.e., group composition or group size) triggered different compensatory effects for the two different fly species of this study. It has already been argued that larval aggregation can be costly or beneficial: whether the advantages or disadvantages finally prevail depends on stages, initial densities (of each species population), amount of resources, temperature and species (Hans & VanLaerhoven 2021). It has also been suggested that costs of interspecific aggregation may be from decreased quantity of food resource and availability of nutrients as well as increased risk of pathogens and disease (Rivers et al. 2011). On the opposite, known benefits of interspecific aggregation include cooperative feeding, enzyme activity, reduced risk of predation and parasitism as well as protection from fluctuations in environmental factors (Hans and VanLaerhoven 2021; Rivers et al., 2011).

In theory, there are several possibilities of development balance through developmental stages. Compared to a theoretical control group, individuals placed under favourable conditions (e.g., low competition) may take less time for both the feeding stage and nymphosis (Figure 5). However, a shorter feeding stage could also have no effect on metamorphosis duration or be "compensated for" by a longer metamorphosis (Chippindale et al., 1997; Holmes et al., 2020; Horváth and Kalinka, 2016; Krittika et al., 2019). The same applies to a longer feeding stage, which can increase, decrease or not affect the following pupal development.

313 **Figure 5. Developmental duration of metamorphosis**

314 Possible effects on the developmental duration of metamorphosis (including post-feeding
315 phase, orange bars) by either slower or faster larval development (during the feeding
316 phase, yellow bars) compared to a hypothetical control group.

317
318 During our experiments, larvae of the species *C. vicina* never suffered from a slow initial
319 development (Figure 3). However, in the case of interspecific groups entailing an initially
320 fast development, larvae showed a compensatory effect and took longer for the
321 subsequent post-feeding development processes. Even though such a compensatory
322 effect cannot be generalized without further investigation, it is clearly related to the given
323 developmental conditions. As an example, Richards *et al.* (2013) reported a
324 compensatory effect on *C. vicina* larvae reared under certain food substrate (Figure S1).
325 Comparing minced to whole fresh liver, they observed feeding larvae had an initial
326 developmental delay of 14 h that finally decreased to 11 h during post-feeding phases.
327 However, on rotten food, the 42-h delay in reaching maximum length finally increased to
328 58 h during post-feeding phases. In other words, decomposed liver not only extended the
329 feeding time but also the metamorphosis duration. Thus, even if toxic decomposition
330 waste impinged on individuals only during the feeding stages, it ultimately also increased
331 the post-feeding phases duration.

332
333 However, the influence of the underlying conditions (*i.e.,* group composition and group
334 size) triggered different compensatory effects for the two different fly species. While a
335 clear compensatory effect was observed at *C. vicina* population scale, unfavourable
336 developmental conditions (*i.e.,* low conspecific population) during *L. sericata* larvae
337 feeding phase increased both feeding and post-feeding phase duration. Furthermore, a

greater inter-individual variability was observed, suggesting a high individual plasticity in this later species. This highly flexible responses of *L. sericata* actually suggest that these larvae can better adapt to environmental changes than the less versatile *C. vicina*, which is reflected in the (often) higher abundance of *L. sericata* in fresh-carrion ecosystems (Arnaldos et al., 2001; Tomberlin et al., 2015). These findings are in agreement with previous observations demonstrating that *C. vicina* is more sensitive to temperature changes and has a higher oxygen consumption (*i.e.*, metabolic rate) during the feeding stage than *L. sericata* (Komo et al., 2020b; Meyer and Schaub, 1973). Thus, in contrast to the assumption of Smith and Wall (1997), our results suggest that *C. vicina* may be a weaker competitor against *L. sericata* as soon as the abiotic and biotic conditions do not meet their restricted needs.

4.2. Outcomes in a forensic context

Determining the age of maggots sampled on a corpse is one common method in forensic entomology to estimate the minimum post-mortem interval (mPMI) (Adams and Hall 2003; Amendt et al. 2007; Amendt et al. 2011). One applied approach to larval age estimation is determining the beginning of a new development event (*e.g.,* larval migration or eclosion) and calculating back to the time of oviposition (Adams and Hall 2003; Amendt et al. 2007; Amendt et al. 2011). While the age of a larva can be estimated from any developmental stage, the migration of third instar larvae and the eclosion of flies are both points in time that are easy to observe, for example, using video-recording devices, and represent valuable events for development-time calculations (Byrd and Butler 1997; Wang JW. et al. 1997; Gibson et al. 2014). In this context, the existence of various compensatory effect highlighted in the present study suggests that mPMI estimations based on fly eclosion time are less accurate and/or reliable than those based on the occurrence of wandering larvae. In view of this, the report of stage-by-stage developmental data in future forensic entomology studies is recommended. The more developmentally relevant factors are studied in forensically important blowfly species, the more confidence can be achieved in the interpretation of insect evidence in the legal system (Hans and VanLaerhoven 2021).

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Conflicts of Interest: The authors declare no conflict of interest.

Figures: All figures are single-column fitting images. Colour does not have to be used in printing.

References

Adams, Z. J., & Hall, M. J., 2003. Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length. Forensic Science International, 138(1-3), 50-61. https://doi.org/10.1016/j.forsciint.2003.08.010.

Amendt, J., Campobasso, C. P., Gaudry, E., Reiter, C., LeBlanc, H. N., & Hall, M. J., 2007. Best practice in forensic entomology—standards and guidelines. International journal of legal medicine, 121(2), 90-104. https://doi.org/10.1007/978-3-540-71111-7_11

Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R., & Hall, M. J., 2011. Forensic entomology: applications and limitations. Forensic science, medicine, and pathology, 7(4), 379-392. https://doi.org/10.1007/s12024-010-9209-2.

Arendt, J.D., 1997. Adaptive Intrinsic Growth Rates: An Integration Across Taxa. The Quarterly Review of Biology 72 (2), 149–177. https://doi.org/10.1086/419764.

Arnaldos, I., Romera, E., García, M.D., Luna, A., 2001. An initial study on the succession of sarcosaprophagous Diptera (Insecta) on carrion in the southeastern Iberian peninsula. International journal of legal medicine 114 (3), 156–162. https://doi.org/10.1007/s004140000146.

Benbow, M.E., Barton, P.S., Ulyshen, M.D., Beasley, J.C., DeVault, T.L., Strickland, M.S., Tomberlin, J.K., Jordan, H.R., Pechal, J.L., 2019. Necrobiome framework for bridging decomposition ecology of autotrophically and heterotrophically derived organic matter. Ecol Monogr 89 (1), e01331. https://doi.org/10.1002/ecm.1331.

Benbow, M.E., Pechal, J.L., Mohr, R.M., 2015a. Community and Landscape Ecology of Carrion, in: Benbow, M.E., Tomberlin, J.K., Tarone, A.M. (Eds.), Carrion Ecology, Evolution, and Their Applications. CRC Press, Hoboken, pp. 151–185.

Benbow, M.E., Tomberlin, J.K., Tarone, A.M., 2015b. Carrion Ecology, Evolution, and Their Applications. CRC Press, Hoboken, 596 pp.

Blanckenhorn, W.U., 1998. Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. Evolution; international journal of organic evolution 52 (5), 1394–1407. https://doi.org/10.1111/j.1558-5646.1998.tb02021.x.

Bomphrey, R.J., Walker, S.M., Taylor, G.K., 2009. The typical flight performance of blowflies: measuring the normal performance envelope of Calliphora vicina using a novel corner-cube arena. PloS one 4 (11), e7852. https://doi.org/10.1371/journal.pone.0007852.

Braack, L., 1981. Visitation patterns of principal species of the insect-complex at carcasses in the Kruger National Park. Koedoe 24 (1), 33–49. https://doi.org/10.4102/koedoe.v24i1.617.

Brown, I.M., Gaugler, R., 1997. Temperature and Humidity Influence Emergence and Survival of Entomopathogenic Nematodes. Nematologica 43 (5), 363–375. https://doi.org/10.1163/005025997X00102.

Burkepile, D.E., Parker, J.D., Woodson, C.B., Mills, H.J., Kubanek, J., Sobecky, P.A., Hay, M.E., 2006. Chemically mediated competition between microbes and animals: microbes as consumers in food webs. Ecology 87 (11), 2821–2831. https://doi.org/10.1890/0012-9658(2006)87[2821:CMCBMA]2.0.CO;2.

Byrd, J. H., & Butler, J. F., 1997. Effects of temperature on Chrysomya rufifacies (Diptera: Calliphoridae) development. Journal of Medical Entomology, 34(3), 353-358. https://doi.org/10.1093/jmedent/34.3.353.

Castner, J.L., 2001. General Entomology and Arthropod Biology, in: Byrd, J.H., Castner, J.L. (Eds.), Forensic entomology: The utility of arthropods in legal investigations. CRC Press, Boca Raton, pp. 17–41.

Charabidze, D., Bourel, B., Leblanc, H., Hedouin, V., Gosset, D., 2008. Effect of body length and temperature on the crawling speed of Protophormia terraenovae larvae (Robineau-Desvoidy) (Diptera Calliphoridae). Journal of insect physiology 54 (3), 529–533. https://doi.org/10.1016/j.jinsphys.2007.11.010.

Chippindale, A.K., Alipaz, J.A., Chen, H.-W., Rose, M.R., 1997. Experimental evolution of accelerated development in drosophila. 1. developmental speed and larval survival. Evolution; international journal of organic evolution 51 (5), 1536–1551. https://doi.org/10.1111/j.1558-5646.1997.tb01477.x.

Clark, K., Evans, L., Wall, R., 2006. Growth rates of the blowfly, Lucilia sericata, on different body tissues. Forensic science international 156 (2-3), 145–149. https://doi.org/10.1016/j.forsciint.2004.12.025.

Clarkson, C. A., Hobischak, N. R., & Anderson, G. S., 2004. A comparison of the development rate of Protophormia terraenovae (Robineau-Desvoidy) raised under constant and fluctuating temperature regimes. Canadian Society of Forensic Science Journal, 37(2), 95-101. https://doi.org/10.1080/00085030.2004.10757567.

Cornwallis, C.K., Botero, C.A., Rubenstein, D.R., Downing, P.A., West, S.A., Griffin, A.S., 2017. Cooperation facilitates the colonization of harsh environments. Nature ecology & evolution 1 (3), 57. https://doi.org/10.1038/s41559-016-0057.

Cotter, S.C., Kruuk, L.E.B., Wilson, K., 2004. Costs of resistance: genetic correlations and potential trade-offs in an insect immune system. Journal of evolutionary biology 17 (2), 421–429. https://doi.org/10.1046/j.1420-9101.2003.00655.x.

Denlinger, D.L., 1994. Metamorphosis behavior of flies. Annual review of entomology 39, 243–266. https://doi.org/10.1146/annurev.en.39.010194.001331.

Denno, R.F., Cothran, W.R., 1976. Competitive Interactions and Ecological Strategies of Sarcophagid and Calliphorid Flies Inhabiting Rabbit Carrion. Annals of the

447 Entomological Society of America 69 (1), 109–113.
448 https://doi.org/10.1093/aesa/69.1.109.
449 DeVault, T.L., Brisbin, J.I.L., Rhodes, J.O.E., 2004. Factors influencing the acquisition of
450 rodent carrion by vertebrate scavengers and decomposers. Can. J. Zool. 82 (3), 502–
451 509. https://doi.org/10.1139/z04-022.
452 El-Moaty, Z.A., Kheirallah, A.E.M., 2013. Developmental variation of the blow fly Lucilia
453 sericata (Meigen, 1826) (Diptera: Calliphoridae) by different substrate tissue types.
454 Journal of Asia-Pacific Entomology 16 (3), 297–300.
455 https://doi.org/10.1016/j.aspen.2013.03.008.
456 Erincçlioğlu, Z., 1996. Blowflies. Richmond, Slough, 72 pp.
457 Faris, A.M., West, W.R., Tomberlin, J.K., Tarone, A.M., 2020. Field Validation of a
458 Development Data Set for Cochliomyia macellaria (Diptera: Calliphoridae): Estimating
459 Insect Age Based on Development Stage. Journal of Medical Entomology 57: 39–49.
460 https://doi.org/10.1093/jme/tjz156.
461 Flores, M., Crippen, T.L., Longnecker, M., Tomberlin, J.K., 2017. Nonconsumptive
462 Effects of Predatory Chrysomya rufifacies (Diptera: Calliphoridae) Larval Cues on
463 Larval Cochliomyia macellaria (Diptera: Calliphoridae) Growth and Development.
464 Journal of medical entomology 54 (5), 1167–1174. https://doi.org/10.1093/jme/tjx104.
465 Fox, C.W., 1997. The Ecology of Body Size in a Seed Beetle, Stator limbatus:
466 Persistence of Environmental Variation Across Generations? Evolution 51 (3), 1005.
467 https://doi.org/10.2307/2411176.
468 Frederickx, C., Dekeirsschieter, J., Verheggen, F.J., Haubruge, E., 2013. The community
469 of Hymenoptera parasitizing necrophagous Diptera in an urban biotope. Journal of
470 insect science (Online) 13, 32. https://doi.org/10.1673/031.013.3201.
471 Gibson, J. F., Slatosky, A. D., Malfi, R. L., Roulston, T., & Davis, S. E., 2014. Eclosion of
472 Physocephala tibialis (say)(Diptera: Cnopidae) from a Bombus (Apidae:
473 Hymenoptera) host: A video record. The Journal of the Entomological Society of
474 Ontario, 145.
475 Gomes, L., Godoy, W.A.C., Zuben, C.J. von, 2006. A review of postfeeding larval
476 dispersal in blowflies: implications for forensic entomology. Die Naturwissenschaften
477 93 (5), 207–215. https://doi.org/10.1007/s00114-006-0082-5.
478 Gosselin, M., Charabidze, D., Frippiat, C., Bourel, B., Gosset, D., 2010. Development
479 Time Variability: Adaptation of Régnière's Method to the Intrinsic Variability of Belgian
480 Lucilia Sericata (Diptera, Calliphoridae) Population. Journal of Forensic Research,
481 01(2). https://doi:10.4172/2157-7145.1000109
482 Green, A.A., 1951. The control of blowflies infesting slaughter-houses I. Field
483 observations of the habits of blowflies. Ann Applied Biology 38 (2), 475–494.
484 https://doi.org/10.1111/j.1744-7348.1951.tb07822.x.

485 Gruszka, J., Krystkowiak-Kowalska, M., Frątczak-Łagiewska, K., Mądra-Bielewicz, A.,
486 Charabidze, D., & Matuszewski, S., 2020. Patterns and mechanisms for larval
487 aggregation in carrion beetle Necrodes littoralis (Coleoptera: Silphidae). Animal
488 Behaviour, 162, 1-10. https://doi.org/10.1016/j.anbehav.2020.01.011.
489 Gunn, A., 2009. Essential forensic biology, 2nd ed. Wiley - Blackwell, Chichester, West
490 Sussex, 424 pp.
491 Hans, K. R., & Vanlaerhoven, S. L., 2021. Impact of Comingled Heterospecific
492 Assemblages on Developmentally Based Estimates of the Post-Mortem Interval—A
493 Study with Lucilia sericata (Meigen), Phormia regina (Meigen) and Calliphora vicina
494 Robineau-Desvoidy (Diptera: Calliphoridae). Insects, 12(4), 280.
495 https://doi.org/10.3390/insects12040280.
496 Hightower, B.G., Spates, G.E., Garcia, J.J., 1972. Growth and Critical Size at Pupation
497 for larvae of the Screwworm Developing in Fresh Wounds1. Journal of Economic
498 Entomology 65 (5), 1349–1352. https://doi.org/10.1093/jee/65.5.1349.
499 Holmes, L.A., Nelson, W.A., Lougheed, S.C., 2020. Food quality effects on instar-specific
500 life histories of a holometabolous insect. Ecology and evolution 10 (2), 626–637.
501 https://doi.org/10.1002/ece3.5790.
502 Horváth, B., Kalinka, A.T., 2016. Effects of larval crowding on quantitative variation for
503 development time and viability in Drosophila melanogaster. Ecology and evolution 6
504 (23), 8460–8473. https://doi.org/10.1002/ece3.2552.
505 Ireland, S., Turner, B., 2006. The effects of larval crowding and food type on the size and
506 development of the blowfly, Calliphora vomitoria. Forensic science international 159
507 (2-3), 175–181. https://doi.org/10.1016/j.forsciint.2005.07.018.
508 Khelifa, R., Zebsa, R., Amari, H., Mellal, M.K., Mahdjoub, H., 2019. Field estimates of
509 fitness costs of the pace-of-life in an endangered damselfly. Journal of evolutionary
510 biology 32 (9), 943–954. https://doi.org/10.1111/jeb.13493.
511 Komo, L., Hedouin, V., Charabidze, D., 2020a. Benefits of heterospecific aggregation on
512 necromass: Influence of temperature, group density and composition on
513 fitness-related traits. Insect science. https://doi.org/10.1111/1744-7917.12766.
514 Komo, L., Hedouin, V., Charabidze, D., 2020b. Quickie well done: no evidence of
515 physiological costs in the development race of Lucilia sericata necrophagous larvae.
516 Physiol. Entomol. 45 (1), 30–37. https://doi.org/10.1111/phen.12313.
517 Komo, L., Scanvion, Q., Hedouin, V., Charabidze, D., 2019. Facing death together:
518 heterospecific aggregations of blowfly larvae evince mutual benefits. Behavioral
519 Ecology 30 (4), 1113–1122. https://doi.org/10.1093/beheco/arz059.
520 Krittika, S., Lenka, A., Yadav, P., 2019. Evidence of dietary protein restriction regulating
521 pupation height, development time and lifespan in Drosophila melanogaster. Biology
522 open 8 (6). https://doi.org/10.1242/bio.042952.

523 Layalle S, Arquier N, Léopold P., 2008. The TOR pathway couples nutrition and
524 developmental timing in Drosophila. Developmental Cell 15: 568–577.
525 Levot, G.W., Brown, K.R., Shipp, E., 1979. Larval growth of some calliphorid and
526 sarcophagid Diptera. Bull. Entomol. Res. 69 (3), 469–475.
527 https://doi.org/10.1017/S0007485300018976.
528 Lewis, E.E., Shapiro-Ilan, D.I., 2002. Host cadavers protect entomopathogenic
529 nematodes during freezing. Journal of Invertebrate Pathology 81 (1), 25–32.
530 https://doi.org/10.1016/S0022-2011(02)00115-5.
531 Metcalfe, N.B., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later?
532 Trends in Ecology & Evolution 16 (5), 254–260.
533 https://doi.org/10.1016/S0169-5347(01)02124-3.
534 Meyer, S., Schaub, G., 1973. Der respiratorische Stoffwechsel von Calliphoridenlarven in
535 Beziehung zu Temperaturadaptation und Regulation. Journal of insect physiology 19
536 (11), 2183–2198. https://doi.org/10.1016/0022-1910(73)90134-0.
537 Mohr, R.M., 2012. Female blow fly (Diptera: Calliphoridae) arrival patterns and
538 consequences for larval development on ephemeral resources. Dissertation.
539 Munch, S.B., Conover, D.O., 2003. Rapid growth results in increased susceptibility to
540 predation in Menidia menidia. Evolution 57 (9), 2119–2127.
541 https://doi.org/10.1111/j.0014-3820.2003.tb00389.x.
542 Nijhout HF, Davidowitz G, Roff DA., 2006. A quantitative analysis of the mechanism that
543 controls body size in Manduca sexta. Journal of Biology 5: 16.
544 Pechal, J.L., Benbow, M.E., Crippen, T.L., Tarone, A.M., Tomberlin, J.K., 2014. Delayed
545 insect access alters carrion decomposition and necrophagous insect community
546 assembly. Ecosphere 5 (4), art45. https://doi.org/10.1890/ES14-00022.1.
547 Prasad, N.G., Shakarad, M., Anitha, D., Rajamani, M., Joshi, A., 2001. Correlated
548 responses to selection for faster development and early reproduction in Drosophila:
549 the evolution of larval traits. Evolution 55 (7), 1363–1372.
550 https://doi.org/10.1111/j.0014-3820.2001.tb00658.x.
551 Richards, C.S., Rowlinson, C.C., Cuttiford, L., Grimsley, R., Hall, M.J.R., 2013.
552 Decomposed liver has a significantly adverse affect on the development rate of the
553 blowfly Calliphora vicina. International journal of legal medicine 127 (1), 259–262.
554 https://doi.org/10.1007/s00414-012-0697-3.
555 Richner, H., 1992. The Effect of Extra Food on Fitness in Breeding Carrion Crows.
556 Ecology 73 (1), 330–335. https://doi.org/10.2307/1938744.
557 Rivers, D.B., Thompson, C., Brogan, R., 2011. Physiological trade-offs of forming
558 maggot masses by necrophagous flies on vertebrate carrion. Bulletin of entomological
559 research 101 (5), 599–611. https://doi.org/10.1017/S0007485311000241.

Scanvion, Q., Hédouin, V., Charabidzé, D., 2018. Collective exodigestion favours blow fly colonization and development on fresh carcasses. Animal Behaviour 141, 221–232. https://doi.org/10.1016/j.anbehav.2018.05.012.

Sevenster, J.G., van Alphen, J.J.M., 1993. A Life History Trade-Off in Drosophila Species and Community Structure in Variable Environments. The Journal of Animal Ecology 62 (4), 720. https://doi.org/10.2307/5392.

Shaaya, E., Levenbook, L., 1982. The effects of starvation and 20-hydroxy-ecdysone on feeding and pupariation of early 3rd-instar Calliphora vicina larvae. Journal of insect physiology 28 (8), 683–688. https://doi.org/10.1016/0022-1910(82)90146-9.

Sinervo, B., Doughty, P., 1996. Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. Evolution; international journal of organic evolution 50 (3), 1314–1327. https://doi.org/10.1111/j.1558-5646.1996.tb02371.x.

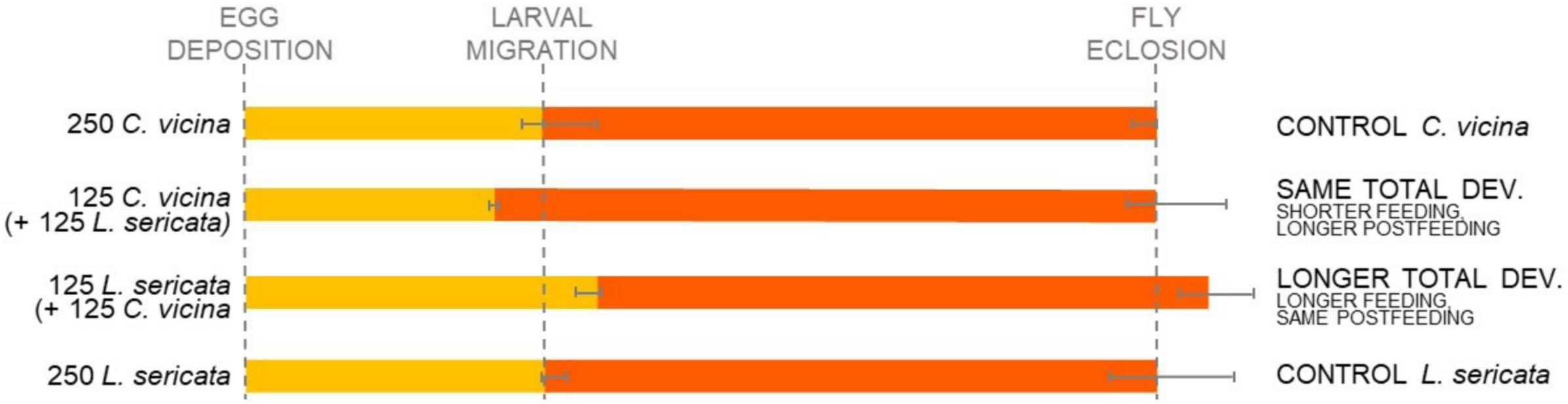
Smith, K.E., Wall, R., 1997. The use of carrion as breeding sites by the blowfly Lucilia sericata and other Calliphoridae. Med Vet Entomol 11 (1), 38–44. https://doi.org/10.1111/j.1365-2915.1997.tb00287.x.

Tomberlin, J.K., Sanford, M.R., Pimsler, M.L., VanLaerhoven, S.L., 2015. Vertebrate Carrion as a Model for Conducting Behavior Research, in: Benbow, M.E., Tomberlin, J.K., Tarone, A.M. (Eds.), Carrion Ecology, Evolution, and Their Applications. CRC Press, Hoboken, pp. 228–245.

Trumbo, S.T., 1997. Juvenile hormone-mediated reproduction in burying beetles: From behavior to physiology. Arch. Insect Biochem. Physiol. 35 (4), 479–490. https://doi.org/10.1002/(SICI)1520-6327(1997)35:4<479:AID-ARCH9>3.0.CO;2-M.

Wang, J. W., Sylwester, A. W., Reed, D., Wu, D. A. J., Soll, D. R., & Wu, C. F., 1997. Morphometric description of the wandering behavior in Drosophila larvae: aberrant locomotion in Na+ and K+ channel mutants revealed by computer-assisted motion analysis. Journal of neurogenetics, 11(3-4), 231-254. https://doi.org/10.3109/01677069709115098.

Wertheim, B., Marchais, J., Vet, L. E. M., & Dicke, M., 2002. Allee effect in larval resource exploitation in Drosophila: an interaction among density of adults, larvae, and micro-organisms. Ecological Entomology, 27(5), 608-617. https://doi.org/10.1046/j.1365-2311.2002.00449.x.


EGG DEPOSITION
LARVAL MIGRATION
FLY ECLOSION
250 C. vicina
CONTROL C. vicina
125 C. vicina (+ 125 L. sericata)
SAME TOTAL DEV.
SHORTER FEEDING,
LONGER POSTFEEDING
125 L. sericata (+ 125 C. vicina
LONGER TOTAL DEV.
LONGER FEEDING,
SAME POSTFEEDING
250 L. sericata
CONTROL L. sericata