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1 **Balance between larval and pupal development time in carrion blowflies**

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

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

27

## 28 **1. Introduction**

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

### 35 36 1.1. Life cycle of necrophagous maggots

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

45 Between each larval instar, a maggot sheds its cuticle, until finally the last outer cuticle  
46 shrinks and hardens to a puparium (Castner, 2001; Gunn, 2009). To enter the next  
47 development stage, a threshold size and weight must be met. Consequently, sufficient  
48 food intake during the larval feeding stages is mandatory (Hightower et al., 1972; Shaaya  
49 and Levenbook, 1982). Once peak feeding is reached, calliphorid maggots usually  
50 wander away from their food source and burrow in soil for pupariation (Gomes et al.,  
51 2006). This transition represents a behavioural stage called the *post-feeding stage*.

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

### 57 58 1.2. Influence of biotic and abiotic factors

59 Necromass can be regarded as a harsh environment to develop on (Brown and Gaugler,  
60 1997; Cornwallis et al., 2017; Lewis and Shapiro-Ilan, 2002; Trumbo, 1997). In addition to  
61 natural decomposition processes and spoiling by bacteria (Benbow et al., 2015b),  
62 competition with scavengers can result in a sudden food depletion and the death of larvae  
63 (Erincçlioğlu, 1996). DeVault *et al.* (2004) observed a mean time of rodent carrion removal  
64 by scavengers of 2.6 days: larvae with a longer feeding time risk being eaten. High larval  
65 densities can also lead to intense conspecific and heterospecific competition for  
66 resources (Denno and Cothran, 1976; Rivers et al., 2011). Furthermore, predatory larvae  
67 such as those of *Chrysomya* (Diptera: Calliphoridae) can significantly deplete blowfly  
68 larvae populations (Flores et al., 2017). Last, parasitoid wasps can induce high mortality  
69 rates in blowflies, reaching 90% under certain conditions (Frederickx et al., 2013). Given  
70 all these biotic pressures on survival, the feeding speed and development rate of larvae  
71 determine their probability of survival (Levot et al., 1979).

72 The development rate of necrophagous larvae is determined by abiotic (notably  
73 temperature) but also biotic factors (Benbow et al., 2015a). As an example, food moisture  
74 and pupation substrate were shown to have significant influence on the growth of *L.*  
75 *sericata*, producing a developmental difference of up to 7.4 days (Tarone and Foran,  
76 2006). Other scientists have also observed repeatedly that different soft and protein-rich  
77 nutrition affects larval development duration (Clark et al., 2006; El-Moaty and Kheirallah,  
78 2013; Ireland and Turner, 2006). Previous studies also demonstrated that blowfly larvae  
79 reared under certain heterospecific conditions had faster and better development than  
80 conspecific groups, with higher survival (Komo et al., 2019, 2020a, 2020b). However,  
81 larval development that is too rapid can result in nutrient deficiency, with profound and  
82 permanent effects on later stages (*e.g.*, reduced immunity and lower adult longevity)  
83 (Cotter et al., 2004; Sevenster and van Alphen, 1993). Chippindale et al. (1997), who  
84 performed an experimental study on development speed in *Drosophila*, concluded that  
85 developmental trade-offs are not confined to single stages of a lifecycle.

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

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

91

## 92 **2. Materials and Methods**

### 93 2.1. Insect rearing

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

102

### 103 2.2. Preparation and start of experiments

104 The setup used to monitor larval development within mono- and heterospecific groupings  
105 was adapted from Scanvion *et al.* (2018). The previously frozen meat (50 g of fresh  
106 minced beef steak: 100% muscle with 15% fat content, Cora®) was thawed overnight and  
107 mixed with 15 ml of a 0.9% NaCl solution in order to offer larvae in the first stage a very  
108 soft (slightly liquefied) medium. This prepared nutrient medium was used to fill a small  
109 plastic box (100×75×63 mm), which was placed in a large breeding container  
110 (180×135×195 mm), the bottom of which was covered with sand. First instar larvae were  
111 homogeneously distributed on the food 22 h (*L. sericata*) or 24 h (*C. vicina*) after  
112 oviposition.

113

### 114 2.3. Experimental procedures

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

119 25°C). Finally, the number of eclosed flies was counted at the same measurement times  
120 (*i.e.*, three times a day). Each development event (post-feeding and eclosion) was  
121 considered to have been reached when it was achieved by the first 10% of individuals.  
122 This threshold is often used in forensic studies and considers the first larval wave without  
123 considering rapid outliers (Clarkson et al., 2004). The relative development rate was  
124 calculated by the following formula: time of specific individual to reach a given instar  
125 divided by mean time in the population to reach this instar.  
126 This procedure was carried out for five different conditions: 100 and 250 individuals of *L.*  
127 *sericata* or *C. vicina* in conspecific groups, along with 125 individuals of both species (250  
128 individuals in total) in the heterospecific group. Six repetitions, which never ran  
129 simultaneously, were performed for each condition (*i.e.*, 5 boxes with 5 different  
130 conditions were used per test run). Of these, another Lille study by Scanvion *et al.* (2018)  
131 provided the developmental data of *L. sericata* in conspecific conditions.

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

140

### 141 **3. Results**

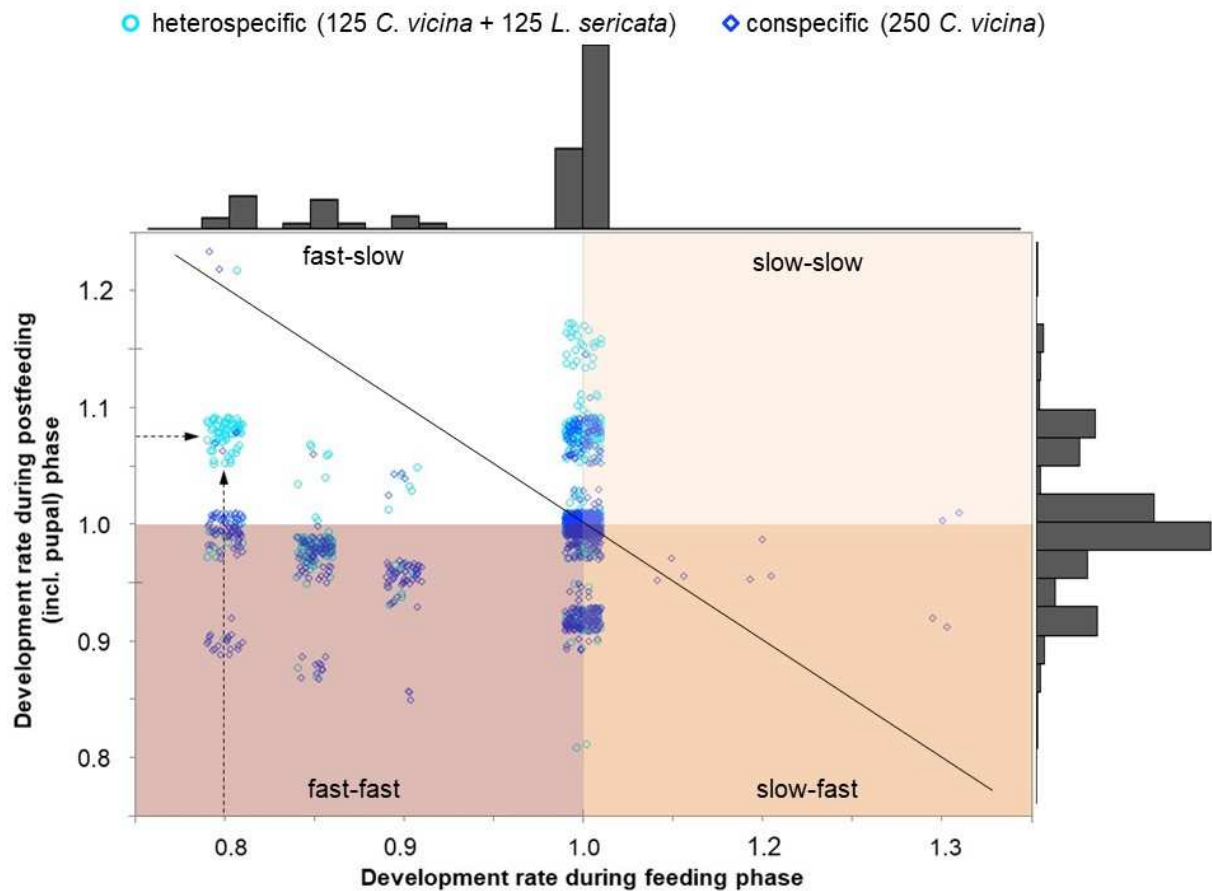
#### 142 3.1. *Calliphora vicina* at individual level

143 The development rate of each larva before and after migration was compared at the  
144 individual level (see Tables S1 and S2 in the supplementary material for the proportions  
145 of eclosed flies). The observation of *C. vicina* individual development rates before and  
146 after larval migration revealed that individuals with a slow development during the feeding  
147 phase had a fast development in the post-feeding phases (Wilcoxon rank sum test:  $V =$   
148  $137738$ ,  $p < 2.2e-16$ ; Figure 1 lower right quarter). However, such a slow (*i.e.*, a rate  $>$

149 1.05, right side on Figure 1) development during the feeding phase was only rarely  
150 observed, and only in conspecific condition. In contrast, if the development during the  
151 feeding phase was faster than the median (Figure 1, left side), many individuals had a  
152 comparatively slower development in the post-feeding phases (upper left quarter). This  
153 was especially true in heterospecific groups: no individual developed much slower than  
154 the median during the feeding phase, but many had a slow post-feeding development  
155 (Figure 1, upper left white quarter). Thus, many *C. vicina* larvae compensated for an  
156 initially very fast development with a slower development later. Larval development rates  
157 of 0.8 (*i.e.*, fast) could not be maintained after leaving the food. At the most, these  
158 individuals showed a development rate of 0.9, though they mostly only developed on  
159 average or even slower than the median. A few exceptions to this compensatory effect  
160 were shown by individuals with an average larval development rate (*i.e.*, 1.0) but a slow  
161 development in the post-feeding phases (*i.e.*, between 1.05 and 1.2). However,  
162 individuals with a slow feeding and post-feeding development were almost never  
163 observed (*cf.* empty upper right light orange quarter on Figure 1).

164





165

166 **Figure 1. Development rates of *Calliphora vicina***

167 Individual development rates of *C. vicina* during the feeding and post-feeding phases in  
 168 heterospecific (turquoise circles) and conspecific groups (blue rhombuses). Values below  
 169 1.0 indicate a faster development than the median, and above 1.0 a slower development.  
 170 Those individuals that show a value of 1.0 on the x-axis grew had a median value during  
 171 the feeding phase, which were most of the individuals (highlighted on the height of the  
 172 columns of the histogram). The arrows point to individuals with a very fast larval  
 173 development rate during the feeding phase (0,8) and a slower rate during the post-feeding  
 174 phase (1,1). The middle of the graph captures those individuals that grow at median  
 175 values during both development phases.

176

177 **3.2. *Calliphora vicina* at group level**

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

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

190

191 **Table 1. Proportion of 250 *C. vicina*, 125 *C. vicina* (+125 *L. sericata*) and 100 *C.***  
 192 ***vicina* reaching postfeeding stage (migration) and ending pupal stage (eclosing) at**  
 193 **25 °C.** Both development events were considered to have been reached when it was  
 194 achieved by the first 10% of individuals. Hours are recorded from egg deposition for each  
 195 repetition.

196

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

197



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

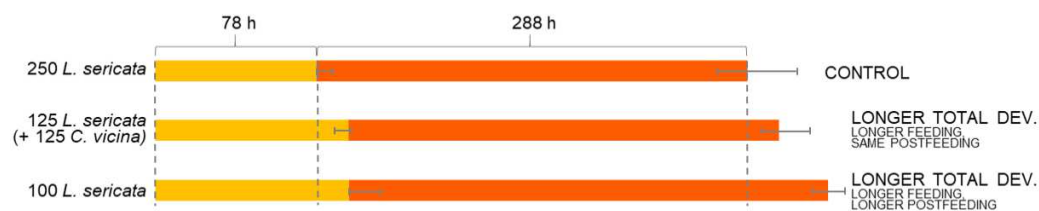
### 207 3.3. *Lucilia sericata* at individual level

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



229 than in the control group (Figure 4, ANOVA:  $F = 19.56$ ,  $p = 6.62e-05$ ; TukeyHSD:  $p_{250-125}$   
 230  $= 0.002$ ). In contrast to *C. vicina*, no accelerated development followed in post-feeding  
 231 phases, but the time lag either remained constant (heterospecific) or increased further to  
 232 18 h (low-density group; ANOVA:  $F = 5.735$ ;  $p = 0.0141$ ; TukeyHSD:  $p_{250-125} = 0.079$ ,  
 233  $p_{250-100} = 0.013$ ,  $p_{125-100} = 0.625$ ).  
 234



235  
 236 **Figure 4. Effects of group size and composition on the development of *L. sericata***

237 Using the conspecific high-density group as a control group, both the heterospecific and  
 238 the low-density group showed a 12-h slower larval development until migration. During  
 239 the post-feeding (including pupal) phase, this time lag was maintained until eclosion in  
 240 the heterospecific group and increased to 18 h in the low-density group [data for  
 241 conspecific groups from Scanvion *et al.* (2018), 25°C].  
 242  
 243

#### 244 4. Discussion

245 We hypothesized that the variability in development duration during larval feeding phase  
 246 may also have an impact on the duration of the postfeeding phases. Our results confirm  
 247 this hypothesis and demonstrate that events impacting larval development of calliphorids  
 248 have consequences through stages, both accelerating and slowing-down post-feeding  
 249 development. Thus, even if key developmental parameters (e.g., competition) impinged  
 250 on individuals only during the feeding stages, they ultimately also affected the  
 251 metamorphosis duration. Most likely, blow fly larvae compensate for an initially too fast or  
 252 too slow development in the later development stages to avoid long-term consequences  
 253 (Metcalf and Monaghan, 2001; Faris *et al.*, 2020).  
 254

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

273  
274 However, fast development and larval competition can lead to undernourishment and a  
275 lack of reserves, resulting in smaller individuals and low survival rates (Arendt, 1997;  
276 Fox, 1997; Munch and Conover, 2003; Richner, 1992; Sinervo and Doughty, 1996).  
277 Indeed, the shorter the feeding period is, the less energy that can be stored, and fewer  
278 resources remain available for later phases (Khelifa et al., 2019). To limit mortality during  
279 post-feeding stages, physiological control involving Target Of Rapamycin (TOR, a core  
280 component of the nutrient-responsive pathway) can delay the moulting hormone  
281 ecdysone secretion, which otherwise terminates larval development (Layalle et al. 2008).  
282 This consequently extends the duration of larval growth, giving maggots an opportunity to  
283 attain a bigger body size. Recently, Dombrovski et al. (2021) observed that the  
284 developmental delay in clustered *Drosophila* larvae was accompanied by an increase in  
285 adults' wing size, suggesting this developmental retardation finally resulted in fitness

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

289

#### 290 4.1 Different strategies between species

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

302

303 In theory, there are several possibilities of development balance through developmental  
304 stages. Compared to a theoretical control group, individuals placed under favourable  
305 conditions (e.g., low competition) may take less time for both the feeding stage and  
306 nymphosis (Figure 5). However, a shorter feeding stage could also have no effect on  
307 metamorphosis duration or be "compensated for" by a longer metamorphosis  
308 (Chippindale et al., 1997; Holmes et al., 2020; Horváth and Kalinka, 2016; Krittika et al.,  
309 2019). The same applies to a longer feeding stage, which can increase, decrease or not  
310 affect the following pupal development.

311

312



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



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

349

#### 350 4.2. Outcomes in a forensic context

351 Determining the age of maggots sampled on a corpse is one common method in forensic  
352 entomology to estimate the minimum post-mortem interval (mPMI) (Adams and Hall 2003;  
353 Amendt et al. 2007; Amendt et al. 2011). One applied approach to larval age estimation is  
354 determining the beginning of a new development event (*e.g.*, larval migration or eclosion)  
355 and calculating back to the time of oviposition (Adams and Hall 2003; Amendt et al. 2007;  
356 Amendt et al. 2011). While the age of a larva can be estimated from any developmental  
357 stage, the migration of third instar larvae and the eclosion of flies are both points in time  
358 that are easy to observe, for example, using video-recording devices, and represent  
359 valuable events for development-time calculations (Byrd and Butler 1997; Wang JW. et al.  
360 1997; Gibson et al. 2014). In this context, the existence of various compensatory effect  
361 highlighted in the present study suggests that mPMI estimations based on fly eclosion  
362 time are less accurate and/or reliable than those based on the occurrence of wandering  
363 larvae. In view of this, the report of stage-by-stage developmental data in future forensic  
364 entomology studies is recommended. The more developmentally relevant factors are  
365 studied in forensically important blowfly species, the more confidence can be achieved in  
366 the interpretation of insect evidence in the legal system (Hans and VanLaerhoven 2021).

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

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

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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*