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Collective exodigestion favours blow fly colonization and development on fresh carcasses

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Necrophagous flies breeding on carcasses face high selection pressures and therefore provide interesting opportunities to study social adaptations. We postulated that gregariousness in necrophagous blow fly larvae is an adaptive response to the environmental constraints of fresh carcasses. Cooperation is indeed believed to be key to the global success of social species. To test this idea, the development of *Lucilia sericata* (Diptera: Calliphoridae) larvae growing on low- or high-digestibility food substrate (control or trypsin-added ground beef muscle, respectively) at different larval densities was monitored. Results showed that larvae developed faster and had decreased mortality at high than low larval density. Furthermore, aggregation had no deleterious effect on the morphological characteristics (e.g. size) of postfeeding larvae and adult flies. We concluded that increased density positively affected population fitness, which is a conclusion consistent with the predictions of the Allee effect. Compared with those fed on regular food, larvae fed on high-digestibility food had reduced mortality and faster development on average. From these results, we postulated that collective exodigestion might be an adaptive response allowing blow flies to colonize fresh carcasses before the arrival of other insects and the multiplication of microbes. This hypothesis is consistent with the idea that cooperation may enable species to expand their niches.

Collective behaviour is a powerful adaptive strategy to cope with a harsh environment, and cooperation is believed to be key to the global success of social species (Cornwallis et al., 2017). Although eusocial Hymenoptera such as bees and ants are well-known insect societies, gregarious insects also provide interesting examples of efficient collective strategies (Choe & Crespi, 1997; Costa, 2006). Grassé (1946) defined a category of social behaviour as having a 'mass effect', that is, a group effect caused by a modification of the surrounding medium by the population itself. A striking example of this idea is provided by terrestrial crustacean woodlice, for which desiccation is a primary concern: in response to this environmental stress, aggregation offers group protection against drying (Broly, Devigne, Deneubourg, & Devigne, 2014). Deneubourg, Grégoire, and Le Fort (1990) also observed that bark beetle larvae aggregate and use communal feeding to overwhelm the reaction of the tree. Similarly, social spiders collectively inject digesting enzymes and then suck up the liquidized prey content, thereby exploiting a common resource that was jointly created (Schneider & Bilde, 2008). In flies, egg aggregations allow larvae to warm and moisten the surrounding organic material (Barnard & Geden, 1993; Bryant, 1977), improve larval ability to dig and burrow into the food (Durisko, Kemp, Mubasher, & Dukas, 2014) and limit the growth of competitive fungi (Rohlfs, Obmann, & Petersen, 2005; Zvereva, 1986).

Blow flies (Diptera: Calliphoridae) are the most widespread and abundant necrophagous species and are attracted to carcasses quickly after death (Smith, 1986). Female flies lay eggs on fresh vertebrate carcasses, on which their larvae feed until metamorphosis; larvae are confined to the carrion until they reach a sufficient weight/instar. Although some studies have reported competition among carrion fly larvae (Denno & Cothran, 1975, 1976; Feinberg & Pimentel, 1966; Hanski, 1987; Ives, 1991), Flores, Crippen, Longnecker, and Tomberlin (2017) observed that female flies do not preferentially oviposit on carcasses without larvae, and several studies have shown that ovipositing gravid blow flies enhance the attractiveness of carrion (Brodie, Wong, VanLaerhoven, & Gries, 2014; Denno & Cothran, 1975; Jiang et al., 2002; Kneidel, 1984; Wertheim, 2005). Therefore, egg aggregation is clearly not avoided and is even sought by adult blowflies. Furthermore, during the three feeding instars, calliphorid larvae actively aggregate (Boulay, Deneubourg, Hédouin, & Charabidzé, 2016; Boulay, Devigne, Gosset, & Charabidzé, 2013): masses of several thousands of larvae are commonly observed in the field (Fenton, Wall, & French, 1999; Slone & Gruner, 2007). Because females and larvae should avoid competition, whether necrophagous

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larval aggregation is advantageous is a reasonable question (Table 1). When they reach a sufficient weight, late third instars disperse to find sheltered places for pupation, a process known as postfeeding larval dispersal (Gomes, Godoy, & Zuben, 2006). Consequently, postfeeding larvae and pupae are not subject to the same selection pressure as feeding instars.

Necromass is by nature a discrete and ephemeral food source. Decomposition processes quickly alter flesh, and the probability of carcass removal by scavengers increases with time and decomposition odour (DeVault, Lehr Brisbin, & Rhodes, 2004). As pioneer species, blow flies' strategy depends on their ability to quickly colonize carrion (Denno & Cothran, 1975). Furthermore, many birds, wasps and necrophagous insects prey on maggots. Voss, Spafford, and Dadour (2009) reported an overall 11.8% parasitism of maggots, and Frederickx, Dekeirsschieter, Verheggen, and Haubruge (2013) observed that, on average, 48% of fly pupae were killed by hymenopteran parasitoids (from 3.5% in May to 90% in September). When the larvae develop rapidly, less time is spent on the carcass and the probability of food shortage, predation, parasitism and interspecific competition decreases (DeVault et al., 2004; Rivers, Kaikis, Bulanowski, Wigand, & Brogan, 2012). Higher temperatures can noticeably increase larval development speed (Aubernon, Boulay, Hédouin, & Charabidzé, 2016; Grassberger & Reiter, 2001). In this respect, a well-known adaptive strategy of blowfly larvae is the so-called 'maggot-mass effect', a local temperature increase attributed to larval metabolism (Charabidzé, Bourel, & Gosset, 2011; Slone & Gruner, 2007). Because additional heat gain reduces developmental time, aggregated larvae benefit from this maggot-mass effect with faster development than that of isolated individuals (Erzinçlioglu, 1996; Heaton, Moffatt, & Simmons, 2014; Johnson & Wallman, 2014). In the same way, aggregation can protect larvae from abrupt decreases in temperature (Huntington, Higley, & Baxendale, 2007; Magni, Dhaliwal, & Dadour, 2016; Rivers, Thompson, & Brogan, 2011). However, these thermal effects occur mostly during late feeding instars and only within large masses of more than 1000 larvae (Charabidzé et al., 2011; Heaton et al., 2014). Here, we postulated that, besides the maggot-mass effect, gregariousness also favours the feeding of necrophagous larvae and their early exploitation of fresh carcasses.

According to Disney (1986), the switch to ingesting liquid instead of solid food could be a fundamental revolution in the evolution and diversification of flies. Necrophagous larvae are unable to ingest solid particles of larger than microscopic size, and their buccal apparatus is not suitable for mastication (Guyénot, 1907). Therefore, larvae must liquefy the food before ingestion (i.e. they carry out exodigestion). The necrophagous larvae of calliphorid flies secrete various proteolytic enzymes, the main one being trypsin, as well as lipases and amylases (Chambers et al., 2003; Hobson, 1931; Rivers et al., 2011; Terra & Ferreira, 1994). The sharing of enzymes has been reported as a probable benefit of calliphorid larval aggregation, but this idea has never been demonstrated (Dos Reis, Von Zuben, & Godoy, 1999; Ireland & Turner, 2006; Rivers et al., 2011). To test the hypothesis of collective benefits through exodigestion, *Lucilia sericata* larvae were bred at different larval densities. Two hypotheses were experimentally tested: (1) larval development speed and survival increase with increasing larval density, and (2) larval development speed and survival increase with increasing food digestibility.

METHODS

Insect Rearing

Lucilia sericata larvae were obtained from adult flies bred in the laboratory and maintained in tulle cages (50×50 cm and 50 cm high) at room temperature (23 ± 2 °C). Between 100 and 200 wild flies were added every 2 months. The flies were fed finely granulated sugar and water ad libitum. After each new generation, minced beef liver was added for 7 days to provide proteins for vitellogenesis and then removed. Laying of eggs was triggered with the placement of 25 ± 5 g of beef liver for 2 h (from 1000 to 1200 hours) in cages. Oviposition time (precision ± 1 h) was noted as D_{-1} (Fig. 1).

Larval Development

A density-dependent increase in competition is found in many blow fly developmental studies (Appendix Table A1). In some studies, measurements were conducted during the feeding of instars, which probably disrupts aggregates and prevents detection of any social benefit (Martinez Sanchez, Smith, Rojo, Marcos-Garcia, & Wall, 2007; Simkiss, Daniels, & Smith, 1993). Many other studies suffer from a lack of statistical power (few replications, time between measures too long: Martinez Sanchez et al., 2007; Reigada & Godoy, 2006; Saunders & Bee, 1995; Serra, Costa, & Godoy, 2011). Finally, many authors used mixed liver or synthetic medium to feed larvae (Dos Reis, VonZuben, & Godoy, 1999; Martinez Sanchez et al., 2007; Simkiss et al., 1993; Smith & Wall, 1997). These food sources are more digestible and less

Table 1
The benefits and costs of gregariousness in necrophagous larvae of calliphorid flies

	Benefits	Costs
Temperature	The mechanisms remain hypothetical (larval metabolism, permanent friction of larvae, microbial activity), but heat production by large larval masses is clearly demonstrated (Charabidzé et al., 2011; Gruner, Slone, Capinera, & Turco, 2017; Heaton et al., 2014; Slone & Gruner, 2007). The microclimate inside the mass should increase the developmental speed of maggots	When the temperature approaches the thermal tolerance range of the larvae, self-regulation processes within the mass must be initiated, which reduce the time allocated to feeding and therefore the growth rate (Charabidzé et al., 2013)
Food availability	Cooperative exodigestion was supposed but not experimentally demonstrated (Rivers et al., 2011)	After a critical number of individuals is reached, the availability of nutrients per individual becomes insufficient, leading to decreases in the speed of development, survival, and weights and sizes (Table A1)
Physicochemical modifications	Increasing the mass may protect larvae against evaporation and temperature decreases (Broly et al., 2014; Huntington et al., 2007; Pérez et al., 2016)	Excreta rich in ammonia accumulates (Hobson, 1931)
Predators and parasites	High population densities can reduce the risk of predation/parasitism but remains to be demonstrated for necrophagous species (Hunter, 2000; Rohlfs & Hoffmeister, 2004)	Chemical signals of the larvae and metabolites increase as a result of feeding (no experimental evidence; Frederickx, Dekeirsschieter, Verheggen, & Haubruge, 2014)
Other	Repulsion of scavengers and burying beetles: female burying beetles typically exploit carcasses without blow fly larvae (Smith, 1986) Scavengers may avoid carcasses heavily colonized by larvae and/or bacteria (DeVault et al., 2004)	Increased risk of disease: many pathogens are transmitted in a density-dependent fashion, but this phenomenon has not been demonstrated in necrophagous larvae (Pöppel, Vogel, Wiesner, & Vilcinskas, 2015)

Adapted from Rivers et al. (2011).