

Competition Among Three Forensically Important Blow Fly Species (Diptera: Calliphoridae): *Phormia regina*, *Lucilia sericata*, and *Chrysomya rufifacies*

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Abstract

Avoiding competition is thought to explain insect successional patterns on carrion, but few studies have looked at competition directly. We use replacement series experiments with three species of blow flies: *Phormia regina* (Meigen) (Diptera: Calliphoridae), *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) to characterize competitive relationships. From experimental results, *P. regina* showed a significant competitive advantage over *L. sericata*. Infestation of carrion differs between *L. sericata* and *P. regina*; specifically, *L. sericata* oviposits on carrion without any delay, while *P. regina* typically delays oviposition. Our findings are consistent with the notion that differences in oviposition times represent a mechanism for *L. sericata* to avoid potential competition. Competition by *C. rufifacies* differs since *C. rufifacies*, in the event of a limited food supply, will prey on other maggot species. In replacement series experiments, *C. rufifacies* killed all *P. regina* in mixed treatments, representing an ultimate competitive advantage. In the United States, these two species do not often overlap because of differences in seasonal distribution. However, with climate change, phenological separation may grow less distinct. Surprisingly, in replacement series experiments with *C. rufifacies* and *L. sericata*, no competitive interactions were observed. In other studies, *L. sericata* has been shown to form clusters away from predaceous maggots, allowing improved survival, which may account for the absence of predation by *C. rufifacies*. Finally, this study shows that replacement series models are useful in measuring competition, supporting the notion that interspecific competition between necrophagous insect species may have driven life history traits of those species.

Key words: blow fly, forensic entomology, interspecific competition, replacement series model

The most common method of estimating the time of death, or, more specifically, the postmortem interval (PMI: the time between discovery of a decomposing corpse and when death occurred), is by aging blow fly maggots on the body. This PMI estimate is possible because insects develop with a predictable, temperature-dependent pattern from egg to adult, and with appropriate data, the interval between oviposition on a corpse and the observed stage of insect development can be estimated. Because they arrive most rapidly on a dead body, blow flies (Diptera: Calliphoridae) are the primary indicator species used in PMI estimates. However, calliphorid species differ in seasonal phenology and in their usual time of oviposition (relative to time of death). A longstanding reason offered for these differences among calliphorids, as well as successional patterns on carrion among insects generally, is that current traits result from interspecific competition on a spatially and temporally limited resource—carrion. Unfortunately, relatively limited direct evidence supports this contention.

A few studies have detailed the influence of interspecific competition on blow fly species and the effects of larval crowding. Regarding intraspecific competition, the classic studies of Nicholson (1954) well illustrate the influence of intraspecific competition on calliphorids, specifically *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Indeed, Nicholson's arguments for density-dependent population regulation are based on experiments with calliphorids. Beyond mortality, intraspecific competition can influence body size. For example, adults of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), when placed in high densities, are typically smaller than those at lower densities (Martinez-Sanchez et al. 2007).

Regarding interspecific competition, data are more limited. Smith and Wall (1997) examined the asymmetric competition between *Calliphora vicina* (Robineau-Desvoidy) (Diptera: Calliphoridae) and *L. sericata*. They found in mixed colonies, when the number of larval density was between 150 and 300 individuals, the

number of adults that emerged was greater for *L. sericata* than *C. vicina*. When necrophagous flies of the species *Hemipyrellia ligurriensis* (Diptera: Calliphoridae) and *Boettcherisca formosensis* (Diptera: Sarcophagidae) were placed in mixed cultures, the larvae of *B. formosensis* were higher in number than *H. ligurriensis*, which were also smaller in relative size. This suggests the *B. formosensis* is a better competitor or better at exploiting the limited resource first (So and Dudgeon 1990). Prinkkila and Hanski (1995) examined interspecific competition between four species of blow fly in the genus *Lucilia*, reporting that, at intermediate densities, some species were better competitors than others, but relationships reversed at a high densities. Unfortunately, some of their observations were made without replication, making it difficult to assess their findings. Reis et al. (1999) reported that *Chrysomya putoria* (Weidmann) (Diptera: Calliphoridae) was a superior competitor to *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) at low densities and still able to outcompete *C. macellaria* at higher densities, albeit not as efficiently. When *Chrysomya albiceps* (Weidmann) (Diptera: Calliphoridae) was placed into containers with *L. sericata*, nearly all the *L. sericata* died from predation except in containers where the ratio was 25 *L. sericata* per 1 *C. albiceps* (Kheirallah et al. 2007). Finally, Wells and Greenberg (1992a) examined competition between *Chrysomya rufifacies* and *C. macellaria*. They found when the oviposition of *C. rufifacies* was reduced, the numbers of *C. macellaria* significantly increased, showing that *C. rufifacies* has a negative impact on *C. macellaria*.

These studies illustrate some of the complications in evaluating competitive relationships among species. Evaluating and comparing results is difficult because of variable temperatures, variable densities, variable measures of competitive response, and limited replication (even no replication in some instances). A further challenge arises in distinguishing competition from mutualism. It has long been known that excretion of digestive enzymes by multiple individuals may make larval feeding easier, and it is thought that this phenomenon explains why mechanisms exist for oviposition attraction among females of some species (Smith 1986). However, it is not clear if this is a strictly intraspecific or interspecific interaction. Recently, Komo et al. (2019) conducted behavioral assays with *C. vicina*, *Calliphora vomitoria* (Linnaeus) (Diptera: Calliphoridae), and *L. sericata*, and concluded that ‘heterospecific aggregation behavior may be a resource-management strategy of blow flies to face carrion-based selection pressure’. However, our interpretation of the findings in Komo et al. (2019) is that they document differences in competitive abilities among species much more than they support mutualism.

The theoretical challenge in understanding how mutualisms in resource use by blow flies could potentially evolve is to reconcile that possibility against the competitive exclusion principle, which argues, in essence, that overlapping species requirements will lead to competition and ultimately speciation. Indeed, forms of this argument have been used to explain differences in life histories, especially succession, of necrophilous insects on carrion. We believe that the replacement series model used by Harper (1977), Higley and Pedigo (1991), Novak et al. (1993), and Oberg et al. (1996) might be a useful approach for characterizing blow fly larval competition because this has been used to study competition in other species of plants and animals. Specifically, the requirements Novak et al. (1993) proposed for using replacement series experiments with insects (namely, competition for a limited resource in a spatially limited area) apply to most necrophorus insects, given the temporal and physical limitations in resource availability represented by carrion. Consequently, we used replacement series experiments to examine intraspecific

competition among three species of forensically important blow flies: *L. sericata*, *C. rufifacies*, and *Phormia regina* (Meigen).

Here, we demonstrate the utility of replacement series experiments as a method for quantifying competition among blow fly species. Additionally, by including a consideration of competition by the invasive *C. rufifacies* versus native competitors, we evaluate the potential for species replacement through species-specific differences in larval competitive abilities.

Materials and Methods

Study Species

Phormia regina, the black blow fly, is common and widespread throughout the United States and has a Holarctic distribution. This species is most prevalent on carrion throughout the cooler months of the year in the United States (Byrd and Allen 2001, Hall 1948, Greenberg 1971, Tabor et al. 2005). However, it has been found during the summer months in Michigan especially when the weather remains cool in the evenings (Babcock et al. 2020), and a study by Byrd and Allen 2001 found no hatch in larvae above 40°C proving evidence for cooler weather preferences. *Phormia regina* has been thought to arrive later or delay oviposition upon a corpse instead of immediate oviposition as occurs with *L. sericata* (Norris 1959). However, Anderson (2011) found that *P. regina* was an initial colonizer in indoor carrion as compared to a delayed colonizer in outdoor carrion where *L. sericata*, *C. vicina*, and *Lucilia illustris* were present. Joy et al. (2006) found depending on the year, and thus the temperature, *P. regina* was an initial colonizer appearing at the same time as *Lucilia coeruleiviridis*, but earlier in the year with a higher temperature.

Lucilia sericata (Meigen) is one of the most common and widespread species of blow fly in the United States. The adults of this species are found in open fields, in sunny weather (Greenberg 1971), and are usually one of the first species to arrive at a carcass and begin oviposition (Byrd and Castner 2010).

Finally, *C. rufifacies* (Macquart), the hairy maggot blow fly, is native to Australia, but was introduced to the continental United States in the 1980s (Baumgartner 1986) and favors warm weather (Norris 1959). It was first discovered in Texas in 1982 (Richard and Ahrens 1983). Since then, it has been found throughout the United States with records from Alabama (Wells 2000), Arizona (Baumgartner 1986), Arkansas (Meek et al. 1998), California (Greenberg 1988), Colorado (De Jong and Chadwick 1997), Florida (Mertins 1991), Tennessee (Shahid et al. 2000), Louisiana (Martin et al. 1996), Nebraska (Figarola and Skoda 1998), North Carolina (Tomberlin et al. 2006), South Carolina (Cammack and Nelder 2010), Wisconsin (Marche II 2013), Oklahoma (Ahadzadeh et al. 2014), Michigan (Shahid et al. 2000), Kentucky (Shahid et al. 2000), Indiana (personal observation by Amber MacInnis), South Dakota (personal observation by Amber MacInnis), and West Virginia (Joy and D’Avanzo 2007). Wells and Greenberg (1994) demonstrated preference of *C. rufifacies* for larger carcasses of rabbit and goat, as compared to rat carcasses. Unlike many other blow flies which rapidly find and use carrion, *C. rufifacies* may delay host finding or oviposition (Norris 1959). However, O’Flynn and Morrehouse (1979), Goff et al. (1988), Zhu et al. 2006, and Sukontason et al. (2001) found that *C. rufifacies* was primary colonizer in Australia, Hawaii, China, and Thailand. The larvae of *C. rufifacies* can be predators on other species of Diptera, which reduces the numbers of other species present on carrion (Wells and Greenberg 1992b). Even with sufficient food, larvae of *C. rufifacies* have been shown to consume the

larvae of other species (Reid 2012, Rosati 2014). Even the presence of *C. rufifacies* can maggots of other species to wander earlier creating smaller, less fit adults (Shiao and Yeh 2008). Larvae can prey upon smooth-bodied maggots, wrapping their bodies around their prey and using their mouth hooks to pierce and kill their prey (Norris 1959). As adults, *C. rufifacies* are easily recognized by their metallic green and blue bodies with the black posterior margins on the first couple abdominal segments and pale genal dilations (Whitworth 2006). Among the unique characteristics of *C. rufifacies* is the fact that females exhibit monogeny, or laying eggs of only one sex, which appears to be controlled by the mother's chromosomes (Ullerich and Schottke 2006, Roy and Siddons 1939). Females lay the same sex of offspring in successive egg batches (Roy and Siddons 1939).

Fly Colonies and Egg Collection

Two species of blow flies, *P. regina* and *L. sericata*, were received as eggs from Dr. Amanda Roe at College of Saint Mary in Omaha, NE (Roe and Higley 2015). Pupae of *C. rufifacies* were received from Dr. Jeff Wells at Florida International University in Miami, FL. The colony of *C. rufifacies* was initially gathered from Homestead, FL in 2017 and reared in a laboratory. The number of generations was not counted. Colonies were kept in mesh cages approximately 46 cm³ (Bioquip products, California) and maintained at 22°C on a 12:12 photoperiod using a lamp. The flies were given water through the use of a quail waterer and cotton, and sugar as a food source. At least 5 d prior to egg collection, adult flies were given beef liver as a protein meal to help develop the female ovaries.

During egg collection, the flies were provided beef liver inside of a five-ounce paper cup, half-covered with aluminum foil, and allowed to oviposit for approximately 18 h before removing the eggs. Eggs were used from multiple clusters to ensure variation, which also ensures that both genders of *C. rufifacies* would be represented (as a female will only lay one sex of eggs). Eggs were placed inside a small glass petri dish and covered with a moist paper towel before putting the cover over the petri dish. The covered dish was placed inside a sandwich bag and placed in the growth chamber at 27°C with a 12:12 photoperiod until egg eclosion.

Growth Chambers

The growth chambers used were *DigiTherm* 38-liter Heating/Cooling Incubators to allow for temperature regulation within 0.1°C of a constant temperature and a 12:12 photoperiod until egg eclosion (Roe and Higley 2015). The incubators have internal lighting and a recirculating air system to ensure air flow.

Experimental Design

The experimental design used was a randomized block with ten replicates. Each replicate was set up as a replacement series with five treatments, or ratios, of larvae: 1:0, 3:1, 1:1, 1:3, and 0:1. Each treatment was placed in a plastic 7 × 7 × 10 cm box with approximately 2.5 cm of vermiculite in the bottom. There were three different competition pairings. The first pairing was *P. regina*/*C. rufifacies*, the second was *P. regina*/*L. sericata*, and the third was *L. sericata*/*C. rufifacies*.

For each box, one dead, immature mouse (popularly called a fuzzy) had the chest and abdomen sliced open, and the maggots were placed inside. It was then laid, incision-side up in the box. Each mouse weighed approximately 5–7 g. A total of 20 maggots were placed inside each mouse. Newly hatched maggots were transferred using a moistened paintbrush. The ratios were as follows: 20:0, 15:5, 10:10, 5:15, and 0:20. The mice were chosen as a natural food source and to help minimize mold and desiccation.

The boxes were placed in a growth chamber at 25°C until adult emergence. After the adults emerged, they were collected and placed in ethanol for storage. Each adult was identified to the species level using a modified version of Keys to the Genera and Species of Blow Flies (Diptera: Calliphoridae) of America north of Mexico (Whitworth 2006) and a Leica Stereo microscope. The total numbers for each box were then recorded. A fine mesh sieve was used to sort through the contents of each container to look for any maggots that had migrated away from the food source and died.

For each of the pairings, the relative crowding coefficient (RCC) and modified relative crowding coefficient (RCCM) were calculated. The formula used to calculate the RCC value based on Harper (1977) is:

$$RCC = \frac{\left(\frac{A_{1:1}}{B_{1:1}} \right)}{\left(\frac{A_{1:0}}{B_{0:1}} \right)}$$

The RCCM proposed in Novak et al. (1993) is:

$$RCCM = \frac{\frac{1}{3} \left[\left(\frac{1}{3} \times \frac{A_{3:1}}{B_{3:1}} \right) + \left(\frac{A_{1:1}}{B_{1:1}} \right) + \left(3 \times \frac{A_{1:3}}{B_{1:3}} \right) \right]}{3 \left(\frac{A_{1:0}}{B_{0:1}} \right)}$$

Both the RCC and RCCM values provide evidence of competition. If the calculated value were 1, it would indicate that competition between species (interspecific competition) was identical to competition within species (intraspecific competition). However, when the calculated RCC or RCCM varies from 1, it indicates competitive differences between species. A *t*-test was calculated on each RCC and RCCM to determine whether it was significantly different from 1. Prior to the *t*-test, the data were tested for normality and it met the requirements for the *t*-test. To assess the significance of competition, if the *P*-value was below 0.05, there was a significant difference from 1 indicating competition. The modified form of RCC was chosen because this takes into account variation throughout all the ratios. In the RCC calculations, only the two pure cultures and the even mixture ratios are used, which leaves some two ratios of data that are not used in the calculations.

Results

Tables 1–3 show the number of eclosed adults for each of the treatment groups. Any replicate with under 50% survival in either control (treatment 1 or 5) was removed from the calculations and considered a failed replicate. This value was decided a priori. Additionally, most failed replicates did not occur in treatment 5 (*C. rufifacies*). Since the cause of survival could not be determined to be competition among *C. rufifacies* themselves, or some other external factor, the replicates were not included in the analysis.

Table 1. The mean number of eclosed adults and standard error for the replacement series of *P. regina* and *L. sericata* shown by treatment for all 10 replicates

Trt	Ratio	Replicates 1–10			
		<i>P. regina</i>		<i>L. sericata</i>	
		Mean	SE	Mean	SE
1	1:0	14.3	2.81	0.0	0.00
2	3:1	13.3	2.05	3.8	1.07
3	1:1	7.7	1.70	6.0	2.38
4	1:3	5.0	2.24	8.8	3.13
5	0:1	0.0	0.00	16.2	2.36

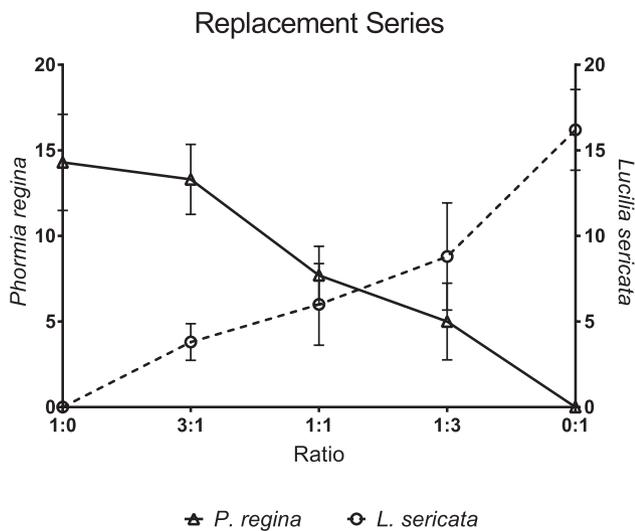


Fig. 1. Replacement diagram for reared adult survivors of *Phormia regina* and *Lucilia sericata*.

Table 2. The mean number of eclosed adults and standard error for the replacement series of *L. sericata* and *C. rufifacies* shown by treatment for replicates 1–6. Replicates 7–10 were excluded due to low survival in the controls

Trt	Ratio	Replicates 1–6			
		<i>L. sericata</i>		<i>C. rufifacies</i>	
		Mean	SE	Mean	SE
1	1:0	7.9	6.41	0.0	0.00
2	3:1	6.2	3.13	3.2	1.86
3	1:1	4.0	2.00	5.5	2.06
4	1:3	1.7	0.94	9.3	2.92
5	0:1	0.0	0.00	10.6	2.50

Table 3. The mean number of eclosed adults and standard error for the replacement series of *P. regina* and *C. rufifacies* shown by treatment for replicates 1–7, 8, and 10. Replicates 7 and 9 were excluded due to low survival in the controls

Trt	Ratio	Replicates 1–6, 8, 10			
		<i>P. regina</i>		<i>C. rufifacies</i>	
		Means	SE	Mean	SE
1	1:0	12.5	4.69	0.0	0.00
2	3:1	0.1	0.30	3.0	1.26
3	1:1	0.0	0.00	7.0	2.35
4	1:3	0.0	0.00	10.0	2.32
5	0:1	0.0	0.00	12.0	3.87

Phormia regina versus *L. sericata*

Figure 1 shows a replacement diagram using adult eclosion for numbers of *P. regina* and *L. sericata*. This diagram represents a model II replacement diagram (Oberg et al. 1996) and indicates there is competition between the two species with *P. regina* being the superior larval competitor. The calculated RCC and RCCM values are shown in Table 4. The calculated RCC of 1.56 ± 0.231 ($P > |t| = 0.039$, $df = 9$) and an RCCM value of 1.83 ± 0.517 ($P > |t| = 0.14$, $df = 9$) support the interpretation from Fig. 1. Specifically, in mixed treatments, *P. regina* was a superior competitor as compared to *L. sericata*.

Chrysomya rufifacies Versus *P. regina*

Because in the mixed treatment groups, there were no *P. regina* survivors, the RCC and RCCM values were not calculated.

Lucilia sericata Versus *C. rufifacies*

Figure 2 shows a replacement series diagram for the number of eclosed adults from *L. sericata* and *C. rufifacies*. This figure is a model I replacement diagram (Oberg et al. 1996). Figure 2 indicates that *C. rufifacies* is a slightly better competitor than *L. sericata*. The calculated RCC and RCCM values are shown in Table 4. The calculated RCC value of 2.91 ± 1.190 ($P > |t| = 0.17$, $df = 5$) and RCCM value of 2.17 ± 0.723 ($P > |t| = 0.17$, $df = 5$) show that both are not significantly different from 1, so there is no evidence of interspecific competition between the *L. sericata* and *C. rufifacies* even though the intersection of the graph appears slightly shifted to the left. The levels of intraspecific competition and interspecific competition are approximately equal.

Discussion

In these experiments, the available resource (mice of 5–7 g) is limiting for 20 blow fly larvae (Roe and Higley 2015). While this choice might seem to be merely an experimental necessity, carrion available to blow flies can be a limited food source. With vertebrate carrion sometimes a large portion of the flesh is removed by vertebrate scavengers leaving the insects to feed on the ‘scraps’ left behind (Beasley et al. 2015). This is not always the case. In some instances, large maggots consume most of the carcass. In both instances, there will be competition among resources whether it is between a smaller maggot population due to low amounts of food from vertebrate scavenging, or large larval masses. Consequently, we expect the competitive relationships seen here mirror those that can and do occur naturally.

There was a significant difference in the competitive abilities of *P. regina* and *L. sericata*; however, there did not appear to be competitive differences between *C. rufifacies* and *L. sericata*. Because no *Phormia* remained in mixed treatments between *P. regina* and *C. rufifacies*, we can conclude that *C. rufifacies* is a superior competitor, if we interpret the killing of *P. regina* by *C. rufifacies* as an ultimate type of competitive interaction. A further indicator of potential competitive advantage is that *C. rufifacies* develop more quickly than *P. regina*, requiring only 180.6 ADD at 25°C (Byrd and Butler 1997) to complete their life cycle compared to *P. regina* which requires 215 ADD at 26.7°C (Kamal 1958). Because *C. rufifacies* hatch before *P. regina*, if eggs of both species are laid at the same time, *C. rufifacies* will be able to establish themselves on the carrion and begin to use the limited resource first. Also, since *P. regina* are smaller and further behind in development, they make easy prey for larger *C. rufifacies* maggots.

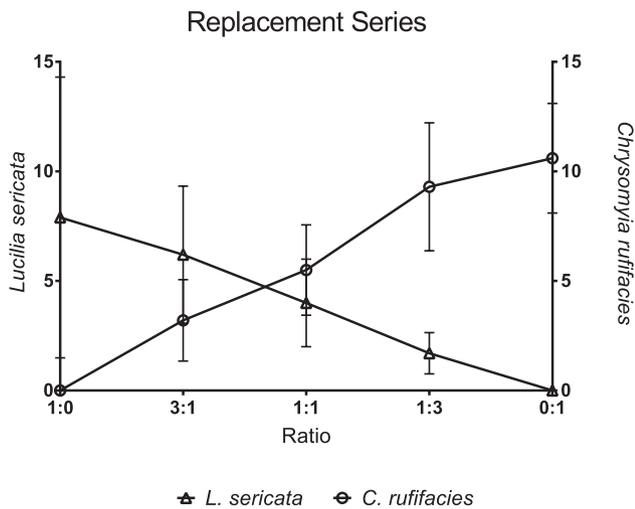
Phormia regina was able to outcompete *L. sericata*. From Fig. 1, the intersection of the graph was shifted right, indicating *P. regina* was the superior competitor. Similarly, the RCC value indicates significant differences within interspecific competition. Interestingly, Roe and Higley (2015) report that *L. sericata* requires 221.2 ADD when reared at 25°C, which is slightly slower than that of *P. regina* which requires 215 ADD. This slight difference in development rate might give *P. regina* an advantage in feeding, which could magnify through time. While this competition is not as drastic as that between *P. regina* and *C. rufifacies*, differences in development rates might be a factor in the evolution of the succession pattern of these two species. Specifically, *L. sericata* is known to arrive early to carrion and begins laying eggs almost immediately given ideal conditions. In contrast, *P. regina* delays oviposition on carrion by up to

Table 4. The average RCC and RCCM along with the standard error for each pairing: *P. regina* vs. *L. sericata*, *L. sericata* vs. *C. rufifacies*, and *P. regina* vs. *C. rufifacies*. A significant value indicates the value differed significantly from 1

<i>P. regina</i> vs <i>L. sericata</i>				<i>L. sericata</i> vs <i>C. rufifacies</i>				<i>P. regina</i> vs <i>C. rufifacies</i>			
RCC		RCCM		RCC		RCCM		RCC		RCCM	
Mean*	Std error*	Mean	Std error	Mean	Std error	Mean	Std error	Mean†	Std error†	Mean†	Std error†
1.56	0.231	1.83	0.517	2.91	1.189	2.17	0.723	—	—	—	—

*Significant value ($P < 0.05$).

†The RCC and RCCM could not be determined.

**Fig. 2.** Replacement diagram for reared adult survivors of *Lucilia sericata* and *Chrysomya rufifacies*.

24 h, even under ideal conditions. Differences in competition between ancestors of these species might have selected *L. sericata* to earlier arrival times on carrion in order to exploit the resource and avoid interspecific competition.

However, if development time was the only factor at play in determining competition, we should have seen the ability of *C. rufifacies* to outcompete *L. sericata*, which did not occur. Although Fig. 2 clearly shows a shifted intersection of the two lines to the left of center (indicating *C. rufifacies* was the superior competitor), the lack of significance in the t -test of RCC or RCCM values versus 1, leads to the conclusion that any competitive differences between *L. sericata* and *C. rufifacies* are minor at most. Because there were very few of the excluded replicates that had under 50% survival for *C. rufifacies*, it is likely that the inclusion of the data would not change the results.

Fuller (1934) and Waterhouse (1947) described a repulsive effect of *C. rufifacies* on maggots from the genus *Lucilia*, in which *Lucilia* larvae move away from *C. rufifacies* and form a mass. If we consider the mass as the maggot equivalent to herding, as in animal species such as cattle and fish, then presumable such clusters are an aid in defense from predators. In this case, and individuals maggot in a mass as less likely to be preyed upon since there are many others around.

Because the competitive exclusion principle states that no two species can coexist and occupy the same niche, competition cannot be sustained indefinitely. With blow flies, species demonstrate differences in arrival times and active times of the year. Because *L. sericata* prefers the warmer summer months and *P. regina*, the colder autumn and winter in the south, the species do not coexist often, but there

are times of overlap. However, *P. regina* has been observed to delay oviposition. This might be one way they combat the competitive nature of carrion feeding.

The observed competitive differences between *P. regina* and *C. rufifacies* could become problematic in the future. *P. regina* is a spring and fall fly avoiding the hotter and colder times of the year, and *C. rufifacies* is a summer fly. Currently, there is little overlap between the two species of flies, but depending on the area of the country these species can overlap in distribution. However, given global warming, *C. rufifacies* will likely overlap more with *P. regina* in the future. Cammack and Nelder (2010) recorded active adults at 9°C in November and development of larvae occurring below 10°C. This shows that while traditionally the species is thought to prefer warmer months (Norris 1959), the tolerable temperatures are lower, allowing for the distribution to expand. As seen in this experiment, *C. rufifacies* are predators on *P. regina*. If interactions between *P. regina* and *C. rufifacies* are correspondingly severe in natural settings, either *P. regina* would have to evolve to deal with this competition or become locally extinct. For example, selection saving more cold-tolerant *P. regina* could reduce or eliminate seasonal overlap with *C. rufifacies*. Alternatively, selection for greater delays in oviposition by *P. regina*, might also provide a means to avoid *C. rufifacies*, although it would leave *P. regina* open to competition from other later occurring species. Irrespective of the possible evolution of *P. regina*, our results imply that as seasons are extended with global warming, or as it continues to spread north even in the cooler months, *C. rufifacies* seems likely to become more common, possibly displacing *P. regina* in much of its range.

While this work focused on competition, that does not mean there are no benefits to the species interacting. However, based on Figs 1 and 2, neither show lines with a bowed up trend. In the case of some kind of benefit from an interaction with another species, the lines would be bowed up showing increased survival. It is possible that multiple species allows for different bacterial fauna which could break down the food source better. In this case, it is not evident, so the effects might be minimal. With a larger study or different methods, it might be possible to examine this effect. This work shows that experimental examinations of competition among blow fly species offers a fruitful approach for considering life history differences among species and potential interactions among blow flies with changing ranges and environmental conditions. In these studies, both species of maggots were placed on the rearing medium at the same time. However, delaying the addition of maggots in mixed treatments could correspond to delayed oviposition as seen with *P. regina* and *C. rufifacies* as compared to *L. sericata*. Also, delayed infestation studies offer the potential to characterize the role maggot age/size plays in competitive relationship, especially regarding predatory behavior by *C. rufifacies*. Another possible option would be to alter environmental conditions, especially rearing temperature. For

example, because *P. regina* is adapted to cooler temperatures, it may be possible for the competitive advantage of *C. rufifacies* over *P. regina* we observed to disappear in a colder climate.

While this study was designed with small maggot populations in a laboratory, examining competition with large maggot masses in a field setting would obviously be of value. Large maggot masses generate more metabolic heat than small masses, and the behaviors of larvae in such masses can be different than in smaller groups. Additionally, there may be interactions that occur with multiple species that makes one less likely to be preyed upon or more likely. Unfortunately, the technical challenges in conducting such experiments are formidable, especially initially quantifying maggots, timing oviposition, and collecting individuals after larval development. Nevertheless, results here demonstrate that replacement series experiments offer a valuable method for quantifying the competitive differences among blow fly species and for making testable predictions about species interactions in the field.

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