MECHANISMS AND BENEFITS OF HETEROSPECIFIC AGGREGATION IN NECROPHAGOUS LARVAE

By

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A DISSERTATION

submitted to the UNIVERSITY OF LILLE for the degree of

DOCTOR OF SCIENCE

Publicly presented and defended on 29/11/2019, in Lille

at the Faculty of Medicine (Pôle Formation), in front of the defence commission

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It is not the strongest of the species that survives, nor the most intelligent that survives.

*It is the one that is **most adaptable to change**.*

Leon C. Megginson

*The ant is a **collectively intelligent** and individually stupid animal;*

man is the opposite.

Karl von Frisch

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Résumé

Mécanismes et bénéfices de l'agrégation hétérospécifique chez les larves nécrophages

Le nécrobiome définit la communauté impliquée dans le processus de décomposition de la biomasse morte (la nécromasse). Cette communauté comprend de nombreux invertébrés et microorganismes différents qui se rassemblent sur des sites spécifiques de la nécromasse. Les mouches à viande jouent un rôle important en tant que premiers organismes décomposeurs, dont les larves (asticots) forment de grandes masses sur les cadavres. Ces regroupements spatial et temporel peuvent être constitués soit d'une espèce (agrégations conspécifiques), soit de plusieurs espèces différentes plus ou moins proches phylogénétiquement (agrégations hétérospécifiques). Cette dernière est supposée aboutir à une relation mutuellement bénéfique, c'est-à-dire entraînant des avantages partagés ne pouvant être obtenus par la seule présence de congénères.

Cette thèse s'intéresse aux agrégations hétérospécifiques de trois espèces de Diptères Calliphoridae communes : *Calliphora vomitoria*, *C. vicina* et *Lucilia sericata*. Premièrement, je démontre dans un premier temps que l'agrégation de ces larves résulte de comportements actifs, et notamment de mécanismes de choix en faveur des groupes hétérospécifiques. Deuxièmement, je montre que dans de telles agrégations hétérospécifiques, aucune des deux espèces n'a de désavantages immédiats par rapport à une agrégation conspécifique de même densité en termes de survie, de développement et de taille. Au contraire, des avantages sont obtenus dans au moins un caractère lié à la fitness à des températures en dehors de la gamme optimale de température spécifique à l'espèce. Il est intéressant de noter qu'au sein d'agrégations larvaires hétérospécifiques, l'espèce qui se trouve à une température sous-optimale adapte son développement lent à celui des espèces mieux adaptées. Troisièmement,

j'observe que *C. vicina* avec un développement larvaire rapide pendant la phase d'alimentation se développe plus lentement dans la phase post-alimentation et nymphale (par opposition à *L. sericata*). Quatrièmement, je démontre qu'un développement accéléré est compensé différemment par les deux espèces. En fait, j'ai observé une concurrence asymétrique, *C. vicina* étant le concurrent le plus faible. Par exemple, un développement larvaire rapide a entraîné une augmentation de la mortalité pré-adulte et une réduction de la taille des puparia de *C. vicina* à des températures sous-optimales. En revanche, un développement rapide n'a eu aucune conséquence négative sur la taille et la survie de *L. sericata*. Cette plasticité développementale peut expliquer le succès évolutif des larves de *L. sericata*, qui sont présentes dans plusieurs écosystèmes à travers le monde et qui dominent l'écosystème des cadavres frais. Sur cette base, je postule que l'agrégation hétérospécifique des larves de diptères nécrophages est un mécanisme adaptatif facilitant leur succès pré-reproductif dans un environnement particulièrement compétitif et *in fine* leur fitness.

En conclusion, j'ai démontré dans cette thèse des avantages des agrégations hétérospécifiques pour les larves en termes de taille, de survie ou de développement, et surtout, un compromis entre ces caractères. De plus, la pression sélective agit différemment sur les caractères en fonction, parmi d’autres facteurs, de l'espèce, de la composition du groupe et de la température. Enfin, ces résultats n’ont pas seulement un intérêt écologique, mais également applicatif. L'entomologie médico-légale utilise en effet ces larves pour dater le décès. Les conclusions de cette thèse indiquent que la taille et la composition des groupes larvaires peuvent constituer une source d’erreur pour le calcul de l’intervalle post-mortem minimum.

Abstract

Mechanisms and benefits of heterospecific aggregation in necrophagous larvae

The necrobiome defines the community that is involved in the decomposition process of dead biomass (*i.e.*, necromass). This community includes many different invertebrates and microorganisms that gather on specific sites of the necromass. Blowflies take on an important function as the primary and first digesters, whose larvae form large maggot-masses. Such spatial and temporal groupings can either consist of one species (*i.e.*, conspecific aggregations) or of several different species more or less phylogenetically related (*i.e.*, heterospecific aggregations). The latter is supposed to lead to a mutually beneficial relationship entailing benefits that are not apparent in conspecific aggregations.

This thesis focuses on heterospecific aggregations of three common Calliphoridae (Diptera) species: *Calliphora vomitoria*, *C. vicina* and *Lucilia sericata*. First, I demonstrate that the aggregation of larvae results from active behaviours and, in particular, from choice mechanisms favouring heterospecific groups. Second, I show that in such heterospecific aggregations none of the two species has short-term costs compared to a conspecific group of the same density regarding survival, larval development rate and puparium surface area. On the contrary, benefits are achieved in at least one fitness-related trait at temperatures outside the species-specific optimal range. Interestingly, within heterospecific larval aggregations, the species that finds itself at a suboptimal temperature adapts its slow development rate to that of the better-adapted species. Third, I illustrate that *C. vicina* with a fast larval development during the feeding phase developed slower in the postfeeding and intra-puparial phase (in contrast to *L. sericata*). Fourth, I demonstrate that an accelerated development is compensated differently by the two species. In fact, I observed an asymmetric competition with *C. vicina*

being the weaker competitor. For example, fast larval development led to increased preadult mortality and small *C. vicina* pupae at suboptimal temperatures. In contrast, fast development had no negative consequences on size and survival for *L. sericata*. This developmental plasticity may explain the evolutionary success of *L. sericata* larvae, which are present in several ecosystems worldwide and dominate the fresh-carrion ecosystem. On this basis, I postulate that heterospecific aggregation in necrophagous Diptera larvae is an adaptive mechanism that increases their pre-reproductive success and, ultimately, their fitness in a particularly competitive environment.

In conclusion, I established benefits for larvae in heterospecific aggregations in terms of their surface area, survival or developmental rate, and most importantly, a trade-off between these traits. Moreover, selection pressure acts differently on the traits depending, among other factors, on species, group composition and temperature. Finally, these results are not only of ecological importance, but also have consequences in the field of forensic entomology. Indeed, these larvae are used to estimate the time of death. Therefore, the present findings indicate that the density and composition of larval groups can be a source of error in estimating the minimum post-mortem interval.

Zusammenfassung

Mechanismen und Vorteile der interspezifischen Aggregation nekrophager Larven

Das Nekrobiom definiert die Gemeinschaft, die am Verwesungsprozess von abgestorbener Biomasse (d.h. Nekromasse) beteiligt ist. Diese Gemeinschaft umfasst viele verschiedene wirbellose Tiere und Mikroorganismen, die sich an bestimmten Stellen der Nekromasse sammeln. Dabei übernehmen Schmeißfliegen eine wichtige Funktion als Hauptzersetzer und Erstbesiedler, deren Larven große Madenmassen bilden. Solche räumlichen und zeitlichen Gruppierungen können entweder aus einer Art (d.h. intraspezifische Aggregation) oder aus mehreren verschiedenen Arten bestehen, die mehr oder weniger phylogenetisch verwandt sind (d.h. interspezifische Aggregation). Letzteres soll zu einer für beide Arten vorteilhaften Beziehung führen, deren Vorteile nicht alleine durch die Anwesenheit von Artgenossen erreicht werden können.

Diese Thesis konzentriert sich auf interspezifische Aggregationen von drei Calliphoridae (Diptera) Arten: *Calliphora vomitoria*, *C. vicina* und *Lucilia sericata*. Zunächst zeige ich, dass ein Aggregieren von Larven auf ein aktives Verhalten und Auswahlmechanismen zurückzuführen ist, die interspezifische Gemeinschaften bevorzugen. Als Zweites zeige ich, dass in solchen interspezifischen Aggregationen keine der beiden Arten (verglichen mit einer intraspezifischen Gruppe der gleichen Dichte) kurzfristige Kosten hinsichtlich Überlebensrate, Larvenentwicklungsrate und Puppengröße davonträgt. Im Gegenteil, in mindestens einem dieser fitnessbedingten Merkmale wurden Vorteile erzielt, wenn sich die Larven bei Temperaturen außerhalb ihres artspezifischen Optimalbereichs befanden. Interessanterweise passt die Art, die sich in interspezifischen Aggregationen in einem suboptimalen Temperaturbereich befindet, ihre langsame Entwicklungsrate an die der besser

angepassten Art an. Drittens veranschauliche ich, dass sich *C. vicina* (im Gegensatz zu *L. sericata*) mit einer anfänglich schnellen Larvenentwicklung (d.h. während der Nahrungsaufnahme) in den darauffolgenden Entwicklungsphasen (d.h. nach dem Abwandern vom Nahrungssubstrat) langsamer entwickelte. Viertens demonstriere ich, dass eine beschleunigte Larvenentwicklung von den beiden Arten unterschiedlich kompensiert wird. In diesem asymmetrischen Wettbewerb war *C. vicina* der schwächere Mitstreiter. So führte beispielsweise eine schnelle Larvenentwicklung bei für *C. vicina* suboptimalen Temperaturen zu einer erhöhten Mortalität und kleineren Puppen. Im Gegensatz dazu hatte eine schnelle Entwicklung keine negativen Auswirkungen auf Größe und Überleben von *L. sericata*. Diese Entwicklungsplastizität kann den evolutionären Erfolg von *L. sericata* Larven erklären, die in mehreren Ökosystemen weltweit vorkommen und das Ökosystem einer Nekromasse dominieren. Auf dieser Grundlage postuliere ich, dass die interspezifische Aggregation bei nekrophagen Diptera-Larven ein adaptiver Mechanismus ist, der ihren präreproduktiven Erfolg und letztlich ihre Fitness in einem besonders kompetitiven Umfeld erhöht.

Zusammenfassend habe ich Vorteile für Larven in interspezifischen Aggregationen in Bezug auf ihre Größe, Entwicklung oder ihr Überleben – und vor allem einen Kompromiss zwischen diesen Merkmalen – festgestellt. Darüber hinaus wirkt sich der Selektionsdruck je nach Fliegenart, Gruppenzusammensetzung und Temperatur unterschiedlich auf diese Merkmale aus. Abschließend sind diese Ergebnisse nicht nur von ökologischer Bedeutung, sondern haben auch Auswirkungen auf den Bereich der forensischen Entomologie. Tatsächlich werden diese Larven verwendet, um den Zeitpunkt des Todes einzuschätzen. Daher zeigen die vorliegenden Ergebnisse, dass die Gruppendichte und Zusammensetzung eine Fehlerquelle bei der Schätzung des minimalen postmortalen Intervalls darstellen kann.

Acknowledgments

I would like to thank everyone who supported me on my way up here and helped in some way with the creation of this doctoral thesis.

In particular, I would like to thank Damien Charabidzé. This thesis could not have been completed without your advice and assistance.

Cindy, Quentin et Damien, merci d'avoir rendu mon séjour à Lille si agréable, de m'avoir toujours aidé au laboratoire et surtout dans les méandres de la bureaucratie Française !
Vous êtes une équipe formidable. Vos idées, vos suggestions jusqu'à la dernière minute m'ont permis de présenter ma thèse telle qu'elle est maintenant.
Vous allez me manquer tous les trois.
Bonne soirée, à demain ; vous me manquez déjà.

Equally, I am so grateful to my family! Thank you for always believing in me and teaching me from the beginning that *Everything is Figureoutable*, even if others wanted to tell me the opposite.

Mama, Papa, Barbara, Tristan und Oma, ich danke euch von ganzem Herzen für eure moralische und finanzielle Unterstützung, und ganz besonders für den kurzfristigen Überraschungsbesuch und all die langgeplanten Ausflüge nach Lille und Umgebung, bei denen ich neue Ideen und Kraft für diese Arbeit tanken konnte.

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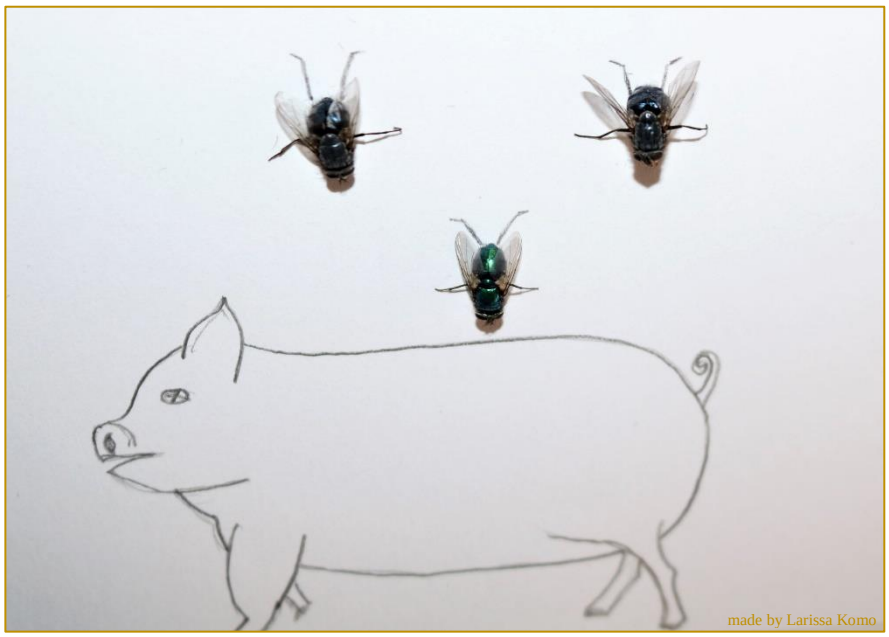
1 Introduction



Carcasses represent a highly concentrated island of fertility for its surroundings, act as a habitat for many species, and thus contribute to biodiversity (Carter *et al.*, 2007). These include necrophagous arthropods and other scavengers that are involved in the decomposition process and carrion nutrient transfer (Pechal *et al.*, 2014). For example, some fly larvae use carcasses as a food source and shelter, grouping together to form large larval masses (Tomberlin *et al.*, 2017). In this thesis, I analyse and discuss the reasons why necrophagous larvae of different species aggregate at the same time in the same spot of a carcass. In addition, trade-offs between development and the influence of temperature have been investigated in the light of adaptive ecology principles. Finally, questions that concern species-specific strategies are addressed. In short, this work contributes to uncover complex relationships between blowfly larvae in carrion ecosystems. These questions have forensic applications but also ecological and evolutionary significance. Therefore, I show how evolution has favoured heterospecific associations in the face of harsh environmental conditions and of how far maggots are able to adapt their development to such conditions.

1.1. Carrion ecology

Necromass and Necrobiome

Necromass is dead biomass and is an important habitat for many different organisms (Benbow *et al.*, 2019). The present work will concentrate on vertebrate carrions, which include human corpses, and put aside ecosystems of dung, decaying leaves or wood. The necrobiome defines the community of saprophytic species (i.e., those that are living on dead organic matter) that are involved in the decomposition process of necromass (Benbow *et al.*, 2013; Cammack *et al.*, 2015). This community flourishes shortly after the physiological and immunological functions of the host have ceased: the final consequence is a recycling process that transforms the remaining nutrient and energy pulses into new life (Benbow *et al.*, 2019). Thereby, all

three life domains are involved: Archaea, Bacteria and Eukarya (i.e., all animals, plants and fungi) (Figure 1) (Benbow *et al.*, 2015b; Polis *et al.*, 1997; Swift *et al.*, 1979).

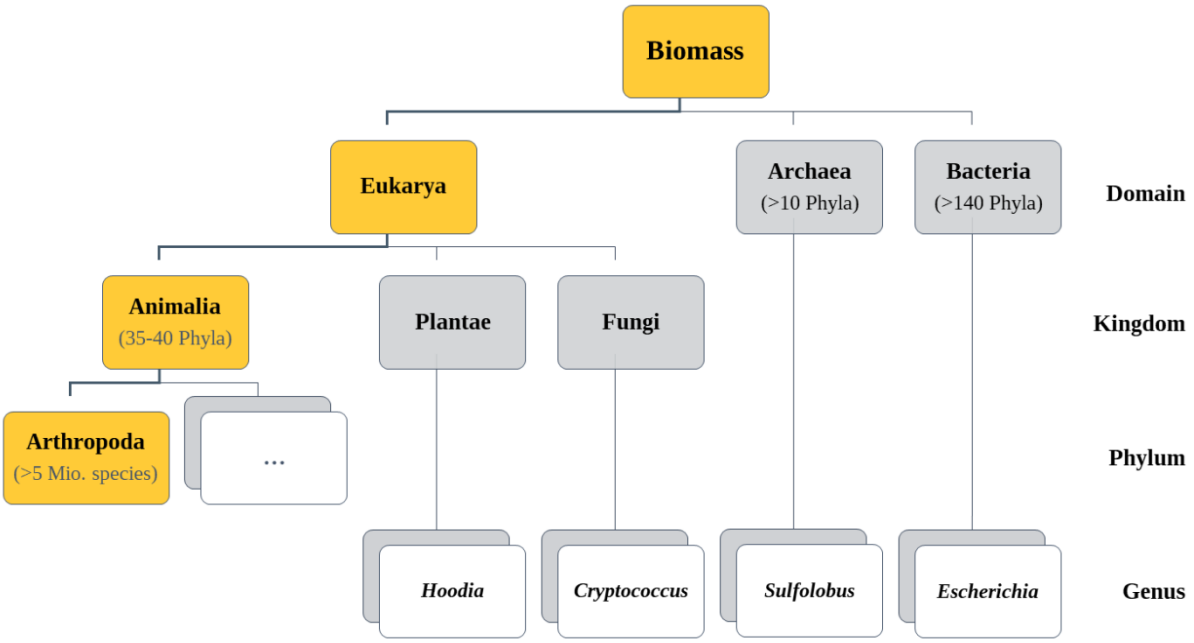


Figure 1 Classification part 1

Summary of phylogenetic relationships for biomass, which includes all three life domains: Archaea (e.g., *Sulfolobus*, a species known from geysers in Yellowstone Park), Bacteria (e.g., *Escherichia coli*, a species well known from the lower intestine of warm-blooded organisms) and Eukarya. Within the domain Eukarya we find besides *Homo sapiens* all other animals, plants such as *Hoodia gordonii* (a flower that smells like rotten flesh and thus mainly attracts necrophagous flies) and fungi such as *Cryptococcus neoformans*. The kingdom Animalia includes, among other Phyla, arthropods, which potentially comprise more than 5 million species on earth. The presumed numbers of species and Phyla were taken from an online database (GBIF.org, 2017). Figure 4 will continue the phylogenetic tree focusing on Diptera.

Carrion ecosystem

Vertebrates consist of soft and hard tissues, which are called carrion after the death of the animal. Hard tissues such as bones, hairs and teeth resist the decomposition processes and can therefore be found even after a long period has passed since the animal's death (Janaway *et*

al., 2009). The ecosystem carrion becomes anoxic and the same enzymes that contributed to the metabolic function in the living animal start to decompose the soft tissues. Additional enzymes can be transferred by insects and microorganisms (primarily bacteria) (Carter *et al.*, 2010). The majority of microorganisms migrate from the host's intestinal tract to the lymphatic system and transform macromolecules, such as proteins and lipids, into simpler compounds such as acids and gases. These gases (e.g., volatile organic compounds) are responsible for the typical odours that are associated with putrefaction and bloating of carcasses (Forbes and Carter, 2015). They attract carrion insects, who have evolved strategies to detect and quickly locate necromass (Janzen, 1977; Wall and Warnes, 1994; Dekeirsschieter *et al.*, 2009; Johansen *et al.*, 2014).

Decomposing carrion can be described as ephemeral resource that presents a valuable food source for many different invertebrates and microorganisms that gather on particular spots of the necromass (Doube, 1987; Forbes and Carter, 2015). These spots are mostly characterised by high humidity, little hair and low illuminance (Figure 2) (Archer and Elgar, 2003; Charabidze *et al.*, 2015). Moreover, these invertebrates work against time, since its food-source dissolves bit by bit. Within 7 days, insects and microbes can consume 85% of a 50 kg carcass (Spicka *et al.*, 2011). The remaining soft tissue dries out quickly and becomes a leathery texture, which is difficult or even impossible for most larvae and scavengers to digest. The fact that flies and their larvae avoid dry tissue is demonstrated by the rapid decrease in oviposition shortly after the host's death, when it begins to dry out (Archer and Elgar, 2003). On the contrary, some necrophagous mites (Acari) and beetles (especially from the family Dermestidae, Silphidae, Nitidulidae, Ptinidae and Cleridae) are interested in such dry remains (Merritt and De Jong, 2015).

Figure 2 Necromass
A 4-day-old pig carcass with first carrion feeders such as blowflies (Diptera: Calliphoridae) and their larvae represents an example of necromass and the visible necrobiome.

Carrion feeders & breeders

The mentioned interactions among decomposer organisms take very different forms, ranging from interkingdom competition to niche partitioning or cross-domain interactions and symbioses (Goodbrod and Goff, 1990; Denno and Cothran, 1975; Benbow *et al.*, 2019; Kaltenpoth and Steiger, 2014). Within eukaryotes, multiple different species of invertebrates and some vertebrates, such as vultures, crows, wild boars, dogs, foxes or hedgehogs, feed on carrion (DeVault *et al.*, 2003). However, in European (sub)urban areas, the decisive role is clearly played by invertebrates, such as flies, beetles, moths, ants, wasps and mites (Campobasso *et al.*, 2009; Lewis *et al.*, 2006). Of these, carrion flies take on an important function as the primary and first digesters of necromass (Payne, 1965).

The occurrence of necrophagous insects represents a chronological succession pattern and is relatively predictable (Figure 3) (Lefebvre and Gaudry, 2009; Matuszewski *et al.*, 2010b). In fact, these insects are capable to detect differences in carcass-age based on the volatile profile of decomposition odours (Hoermann *et al.*, 2012). As each insect species is attracted by a certain profile, the attractive power of a carcass varies over time (Johansen *et al.*, 2014). For example, a dry carcass already becomes olfactory less attractive for blowflies (Diptera:

Calliphoridae) due to the chemical changes (Wardle, 1927). The present work will focus on these blowflies, which receive much forensic and ecological attention because of their typically frequent and very early occurrence in succession as well as their worldwide distribution (Szpila, 2017; Mañas-Jordá *et al.*, 2018).

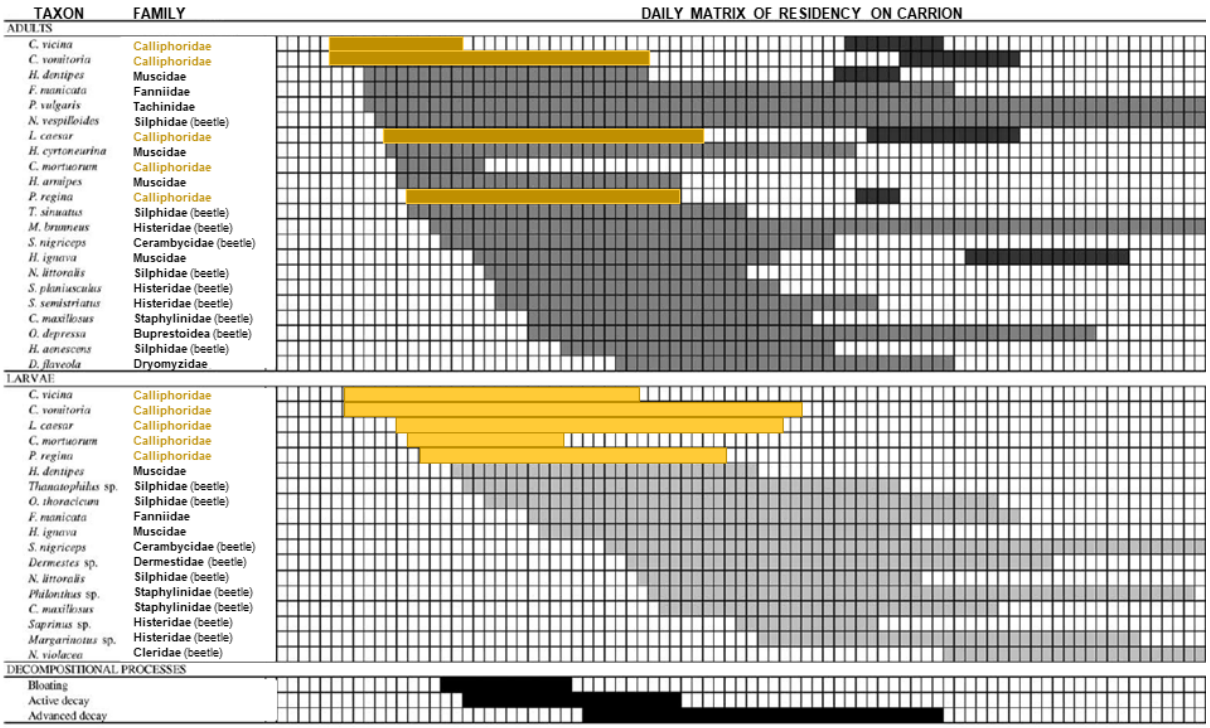


Figure 3 Insect succession model

This diagram, drawn after Matuszewski *et al.* (2011), shows the succession of fly and beetle adults (dark grey and gold) and their larvae (light grey and gold) on pig carrion in forests of central Europe in spring. Dark grey bars in the adult section mark tenerals (i.e., flies shortly after moulting, which represent the second generation of flies). However, no recolonization was observed (i.e., second generation of larvae). Species of the family Calliphoridae are highlighted in gold.

1.2. Blowflies

Phylogenetic relationships

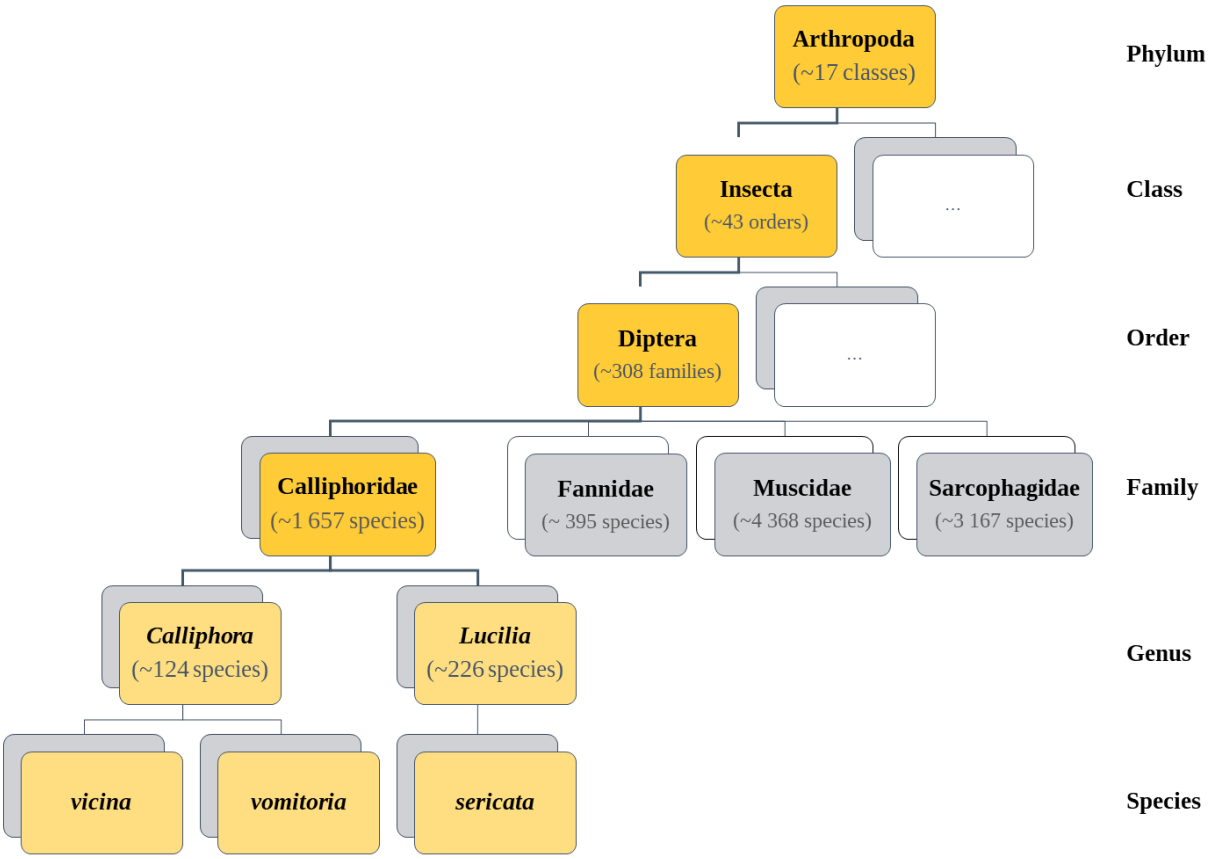


Figure 4 Classification part 2

Figure 1 is continued with a summary of phylogenetic relationships among several Diptera families. Fanniidae, Sarcophagidae, Muscidae and Calliphoridae are among the fly families described as normal carrion fauna (Merritt and De Jong, 2015). The presumed number of species, families etc. were taken from an online database (GBIF.org, 2017).

Flies (Diptera) belong to one of the most species-rich and anatomically diverse orders of insects (Gerhardt and Hribar, 2019). There are already around 167 000 species and at least 132 families known worldwide (status 2019, GBIF.org, 2017), and the trend is rising constantly, as new species are still being described and named. Although 50 - 60% of insects in the necrobiome are flies, only a relatively small number of Diptera families can be found on carrion (Matuszewski *et al.*, 2010b). Around 70 different Diptera species, which belong to

nearly 40 families, visited a 8 kg piglet carcass over one year (study performed in Portugal, Castro *et al.*, 2012). Among all these species, between 36% (in spring and summer) and 64% (in autumn) belonged to Calliphoridae, which utilised the carcass as mating and oviposition site or nutrition. Besides Calliphoridae, which generally constitute the majority of necrophagous flies on carrion throughout the world, Fanniidae, Sarcophagidae and Muscidae also occur frequently (Mañas-Jordá *et al.*, 2018; Grassberger and Frank, 2004) (Figure 4).

With approximately 1 600 different species, Calliphoridae contains clearly more species than any mammal family (e.g., cats with 210 felid species), but also clearly less than its sister family Muscidae, which counts over 4 000 species. Within all European Calliphoridae species, at least 15 species are necrophagous and colonise fresh vertebrate carcasses and necrotic tissues (Szpila, 2010). Among these necrophagous calliphorids in Europe, *Calliphora vomitoria* (Linnaeus, 1758) (Figure 5), *Calliphora vicina* Robineau-Desvoidy, 1830 and *Lucilia sericata* (Meigen, 1826) (Figure 6) dominate on carrion according to season and habitat (Martinez-Sanchez *et al.*, 2000; Matuszewski *et al.*, 2010a, 2010b; Smith and Wall, 1997). Although these three species show different behaviours and habitat preferences (i.e., sunny, warm in the city centre (e.g., *L. sericata*) or shady, mild in the forest (e.g., *C. vomitoria*)), they often aggregate actively and feed simultaneously in the same area on a carcass (Bonacci *et al.*, 2010, personal observation; Figure 7).

Figure 5 *Calliphora vomitoria*
Note the blue thorax and the dark face with the orange hairs of this blue-bottle fly. The image was taken using a Leica DMS1000 microscope.

Figure 6 *Calliphora vicina* and *Lucilia sericata*
Note the orange face with the dark hairs for the blue blowfly *C. vicina* and the white face with the black hairs of the green blowfly *L. sericata*. The image was taken using a Leica DMS1000 microscope.

Life cycle of flies and physiology of larvae

Once female blowflies have located a carcass, they lay egg batches in sheltered places close to fleshes that are easy to digest (e.g., nostrils and eyes; Byrd and Castner, 2001) or where already several other egg batches were laid (Serra *et al.* 2010) (Figure 7). Each batch usually contains between 100 and 300 eggs from which small larvae hatch (Smith 1986). This marks the beginning of a new life cycle (Figure 8).

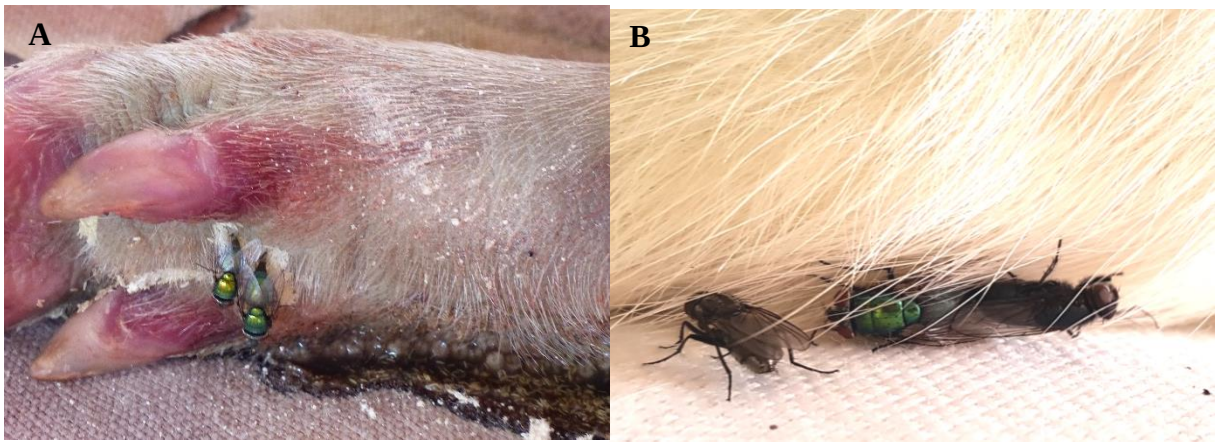


Figure 7 Oviposition sites
Blowflies (Diptera: Calliphoridae) represent in general the first insects that colonise a carcass **A** Two *Lucilia* sp. flies with their ovipositor extended to lay eggs on the foot of a pig carcass, where other egg batches have already been laid. In addition, there are already 3rd-stage larvae under the foot and hind leg. **B** Two clearly different fly species (one of the genus *Lucilia* and one *Protophormia*) have settled for oviposition on the same spot of a rat carcass.

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 constitute active (i.e., moving) stages. However, while flies can fly up to 9 km/h and cover a flying distance of several km, larvae crawl only up to 0.017 km/h (i.e., 28 cm/min) and up to 30 m away from their food source if necessary (Bomphrey *et al.*, 2009; Braack, 1981; Charabidze *et al.*, 2008; Green, 1951). Thus, flies select the carcass for their eggs and the larvae their aggregation site at the carcass.

Each necrophagous larva completes three larval instars, between which the larva sheds its skin (a process called ecdysis, representing a transition to a new physiological stage), until finally the last outer skin shrinks and hardens to a puparium (Castner, 2001; Gunn, 2009). To enter the next development stage, threshold size and weight must be met: consequently, a sufficient food intake during the feeding larval stages is mandatory (Hightower *et al.*, 1972; Shaaya and Levenbook, 1982). Once peakfeeding is reached, necrophagous maggots usually

wander away from their food source for pupariation. This movement represents a behavioural stage called *postfeeding*. Inside the puparium, the insect completes its metamorphosis, until finally the adult fly ecloses. Whether this profound transformation will be completed successfully depends on both the initial developmental success of the larva and the effect of temperature (Denlinger, 1994; Mohr, 2012).

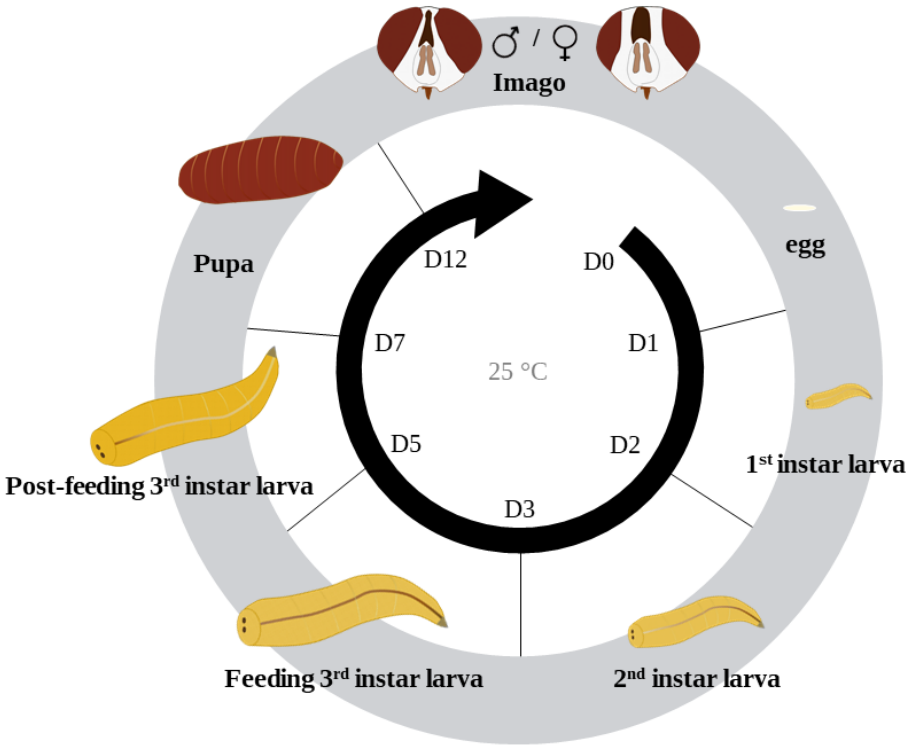


Figure 8 Life cycle of the blowfly *Lucilia sericata* at 25 °C

The life cycle shown here illustrates the development times of *L. sericata* at constant 25 °C and is therefore not applicable to other species. For example, *C. vicina* needs about 4 days longer to complete the cycle once. First instar *L. sericata* larvae hatch at 25 °C at the latest one day after a female fly laid eggs (D1). Another day later, these larvae shed their skin and continue to grow; the 2nd larval stage has begun (D2). Before entering the 3rd larval stage, they shed their skin a second time (D3). As soon as the full-grown larvae have ingested sufficient food, they leave the food source and search of a pupariation site (postfeeding stage). At a suitable site, which is characterized by the presence of a dry shelter, the outer skin shrinks and hardens to a puparium. Inside the puparium, the pupa transforms into a fly through the process of metamorphosis. The cycle can begin a new when the eclosed fly becomes fertile.

Since larvae have no mandibles to mechanically reduce the food, food items must be liquefied first. Therefore, maggots secrete a rich soup of digestive enzymes onto the substrate and then absorb the hydrolysed food products (Hobson, 1931; Nigam *et al.*, 2006; Hobson, 1932). In the case of *L. sericata, C. vicina, C. vomitoria* and many other necrophagous insects, these enzymes include lipases, amylases and proteases (especially tryptase and peptidase) (Wigglesworth, 1972). In addition to enzymes, the secretions and excretions also contain antibiotics and, most likely, chemical cues that can be detected by both their conspecifics and heterospecifics in order to find other larval groups or flee from predator larvae (Flores *et al.*, 2017). Such cues are important to understand the larval aggregation behaviour and the findings of the aggregation study in the present thesis. Consequently, a more detailed explanation of the various functions of secretion/excretion compounds follows in the discussion of the development study (3.3. *Mechanisms of mutualism*).

The feeding behaviour is also under the control of local temperature. Larvae demonstrated thermotaxis by crawling away from unfavourable temperatures and by faster feeding and locomotion rates at favourable temperatures leading to increased food intake (*1.3.2 Differences in temperature*) (Hückesfeld *et al.*, 2011; Aubernon *et al.*, 2016; Charabidze *et al.*, 2013; Podhorna *et al.*, 2018).

1.3. Abiotic and biotic interactions

Development rates and succession of necrophagous insects are determined by interactions with abiotic (e.g., temperature, humidity and soil pH) and biotic factors (e.g., bacterial load, group density and composition as well as food digestibility, moisture, freshness and type) (Benbow *et al.*, 2015a). Some of these interactions are particularly relevant for this thesis: ambient temperature, species composition and larval density have been deliberately changed and compared in the present development studies. In addition, scientists repeatedly encounter the problem that different nutrient media have been used in different publications (Clark *et al.*, 2006; El-Moaty and Kheirallah, 2013). Accordingly, fundamental information on the influence of food characteristics, temperature and group density on larvae is explained in more detail hereafter.

Differences in food

The concentration of sucrose, protein, carbohydrate and cholesterol as well as the osmolarity of an ingested diet are important characteristics that can influence the larval development (Green *et al.*, 2003). Proteins and fats are critically important nutrients in biological processes such as growth, moulting and ovarian development (Thyssen *et al.*, 2014). Studies have shown that larval growth rates of the blowfly *C. vicina* were significantly faster on human muscle tissue, brain, lung or minced pork compared to pork loin, intestine or liver (tissues with low fat contents) (Table 1, Bernhardt *et al.*, 2017; Clark *et al.*, 2006; El-Moaty and Kheirallah, 2013; Kaneshrajah and Turner, 2004). Larval crude fat content is strongly affected by nutrient concentration (as shown for the black soldier fly *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae)). If their diet contained of high fat concentrations, their crude fat content was significantly higher than on low fat diets (Barragan-Fonseca *et al.*, 2018). Thereby, crude fat content of calliphorid larvae increased with continuous feeding (from 11% to 30% when entering the third larval stage) and accounted for a significant proportion of their

dry weight (Wigglesworth, 1972). This fat content does not even differ between larvae of different sizes as long as they are of the same species and in the same development stage (Saunders, 1997). Differences can also be observed between the host species: larvae of *L. sericata* grew significantly faster on pork than on cow tissue (Clark *et al.* 2006). The nutrients that are finally responsible for a faster development only become apparent in experiments using artificial substrates. For example, larvae reared on a blood–yeast agar mix (which is a common substrate in behavioural and microbiological studies with larvae) showed a greater pupation rate but also smaller puparia than those reared on decomposed, fresh or powdered beef liver (Le Zheng *et al.*, 2017; Barnes and Gennard, 2013; Boulay *et al.*, 2013).

Table 1 Influence of food medium on larval development speed

A golden cross stands for relatively fast larval growth, while a grey minus stands for relatively slow larval growth. For example, larvae reared on pork or beef lung grew faster than their conspecifics on pork, beef or human liver. Empty cells show food mediums that were not investigated by these studies.

Study	Kaneshraja and Turner 2004	Clark *et al.* 2006	El-Moaty and Kheirallah 2013	Bernhardt *et al.* 2017
Fly species Host species	C. vicina pork	L. sericata pork, beef	L. sericata beef	C. vicina pork, human
Lung	+	+	+	
Heart	+	+		
Brain	+		+	
Kidney	+			
Human muscle				+
Minced pork				+
Pork loin				−
Intestine			−	
Liver	−	−		−

Differences in temperature

In the development study of the present thesis, the individuals experienced a constant temperature that varied between experiment series. Constant temperatures have slightly but fundamentally different consequences on larval development speed than fluctuating temperatures (Donovan *et al.*, 2006). Although fluctuating temperatures are more natural, data derived from such experiments are ambiguous (Greenberg, 1991; Warren and Anderson, 2013; Niederegger *et al.*, 2010).

Importantly, larval development rates change according to temperature, resulting in an asymmetric, sigmoid-like curve shapes (Figure 9) (Greenberg and Kunich, 2002). Although the preferential temperature and liveable temperature range differ between species, the growth curve according to temperature shows such an asymmetrical s-shape for all blowfly species (Figure 9). Generally speaking, the development speed is low to zero at cold temperatures, increases linearly at medium range temperatures and slows down at warm temperatures until a lethal threshold is reached (Higley and Haskell, 2001; Charabidze and Hedouin, 2019). This lethal threshold can easily be reached inside large larval aggregations with thousands of individuals (the so-called maggot-mass effect, Figure 10) (Charabidze *et al.*, 2011; Slone and Gruner, 2007). Since the temperature can rise inside such maggot-masses by several degrees compared to the ambient temperature and thus reach up to 50 °C, larvae are confronted with a constant trade-off between development in aggregations and at their preferential temperature (Aubernon *et al.*, 2019; Podhorna *et al.*, 2018). For example, among the three tested species, *L. sericata* larvae aggregated at 33.3 ±1.52 °C and never below 20 °C if they were given a choice on a linear thermal gradient (Aubernon *et al.*, 2016). In contrast, no *C. vicina* aggregations were found at more than 28 °C, but these larvae aggregated mainly at 22.4 ±1.55 °C. In between, *C. vomitoria* was reported with a preferential temperature of 29.6

±1.63 °C. The extent to which heterospecific conditions can contribute to an extended temperature range with comparatively fast development was investigated in this thesis.

Finally, microorganisms show an increased proliferation in warm compared to cold environments, which accelerates microbial degradation of necromass, but also leads to nutrient loss and accumulation of toxic metabolites, which affects the growth and fitness of carrion feeders (Forbes and Carter, 2015; Shukla *et al.*, 2018).

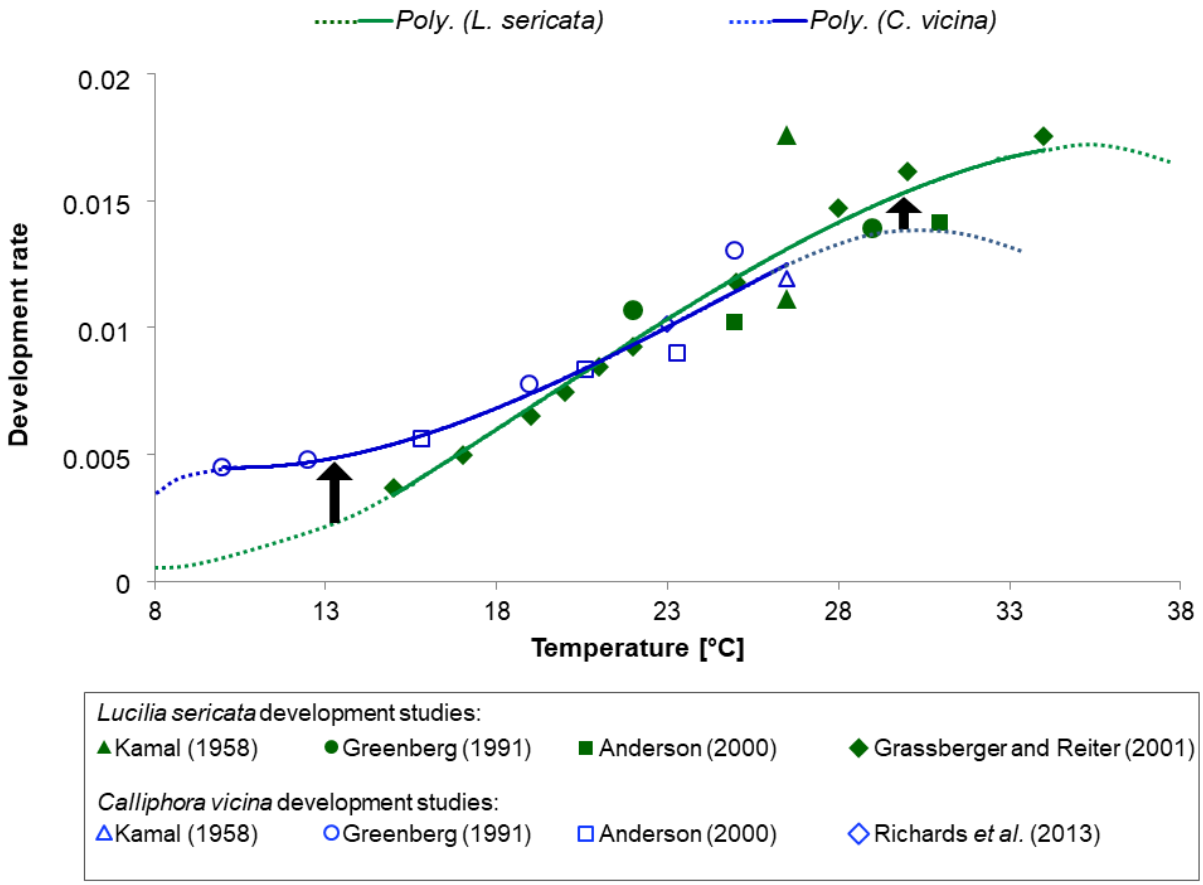


Figure 9 Temperature-dependent development rates

Development rates of third instar larvae of *L. sericata* and *C. vicina* in conspecific groups according to temperature (Greenberg, 1991; Richards *et al.*, 2013; Kamal, 1958; Anderson, 2000). The two development curves cross at approximately 21 °C. Below this point, *C. vicina* is the species with the comparatively faster development rate, above 21 °C, it is *L. sericata*.

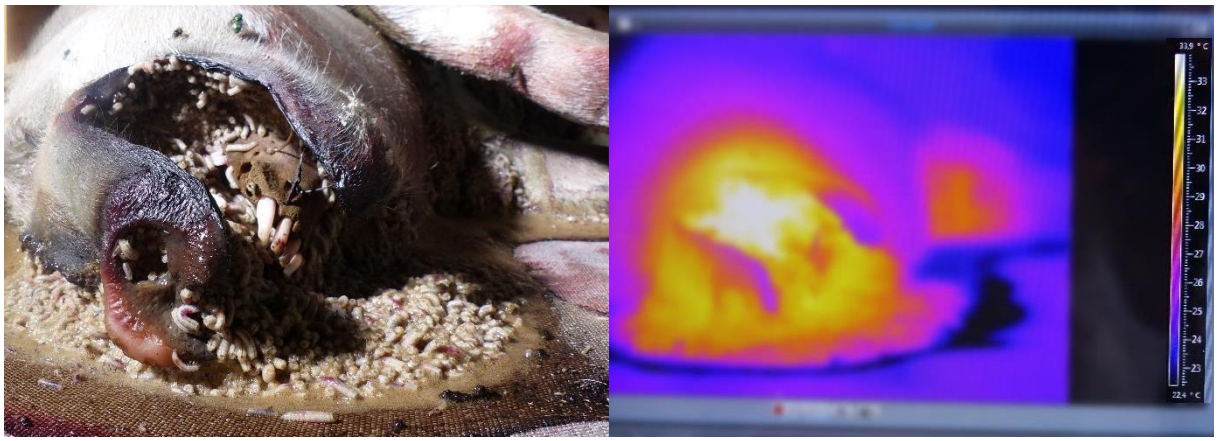


Figure 10 Maggot-mass effect
The maggot mass in the oral cavity of the dead pig reaches a temperature of almost 34 °C (white), which is 10 °C higher than the ambient temperature (purple). While also the snout is colonized by larvae and thus radiates warmth, the upper and lower jaw bones form colder spots. Note the smaller larval aggregation on the right foreleg (orange-red spot on the right, approx. 28.5 °C).

Larval density effect on fitness

The aforementioned larval aggregations fall under the general definition of an assemblage of organisms resulting in a higher density of individuals than in the surrounding area (Jeanson *et al.*, 2005; Scott *et al.*, 2003). Such an assemblage can be found in a common zone that is characterized by a preferred temperature or the presence of another preferred larvae species. If the assemblage arises through autonomous movements of these larvae towards the common zone as a reaction to social attraction among individuals, active aggregation exists (Jeanson *et al.*, 2005; Parrish and Hamner, 1997; Fraenkel and Gunn, 1940). An active aggregation is considered the first step of sociality and is observed at all scales, from bacteria to whales, or from groups of 10 to 10 million individuals (Parrish and Edelstein-Keshet, 1999; Mizell *et al.*, 2012). Professor Warder Clyde Allee first described the positive relationship between aggregation density and any component of individual fitness in 1931. Later this phenomenon was named after him, the *Allee Effect* (Schmidt, 1957; Stephens *et al.*, 1999). This means that

a measurable fitness component of an organism (e.g., survival rate) is higher in a large group than in a small or less dense population. However, the population cannot be infinitely large: as soon as the carrying capacity is reached, the fitness benefits decrease rapidly, and costs arise (Figure 11). For blowflies on carrion, this effect depends clearly on the carrion size (Serra *et al.* 2010). A former study showed that the development rate of *L. sericata* increased steadily between 50 and 250 larvae/50 g of meat but decreased slightly at 500 larvae (Scanvion *et al.*, 2018). Other studies on calliphorids also demonstrated a density-dependent increase in competition (Prinkkilá and Hanski, 1995; Ullyett, 1950; Saunders and Bee, 1995).

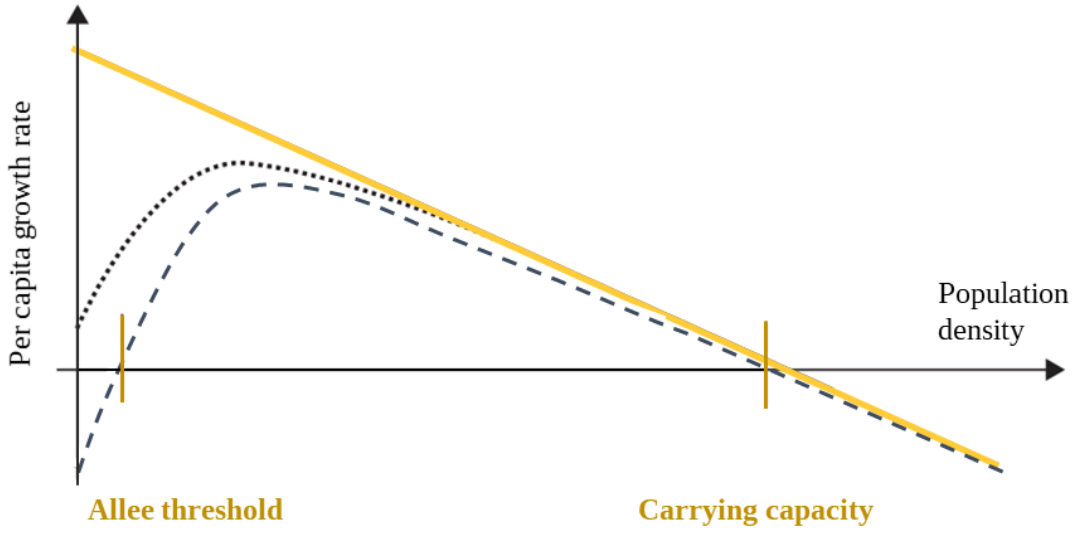


Figure 11 Negative and positive density dependence
The per capita growth rate is plotted against the population density. Compared to the classical negative density dependence (solid gold line), a weak (dotted) and strong (dashed) Allee effect highlight that both overcrowding (carrying capacity) and undercrowding (Allee threshold) can cause a decline in growth rate with a non-linear curve. Professor Allee himself wrote: 'Without for one minute forgetting or minimizing the importance of the right-hand limb of the last curve, it is for the more romantic left-hand slope that I ask your attention.' (Allee, 1938). The difference between a weak and a strong Allee effect is the presence or absence of an Allee threshold. While the growth rate of a weak effect remains positive on the left-hand slope, the rate of a strong effect will get negative and drop below the Allee threshold if the population gets too small and declines toward extinction. Slightly changed graphic after Courchamp *et al.* (2009).

1.4. Selective pressures on carrions and larval development strategies

In conclusion, necromass can be regarded as a harsh environment to develop on due to the specific conditions such as scavenging, overcrowding, patchiness, high bacterial load and temporal changes (Brown and Gaugler, 1997; Trumbo, 1997; Lewis and Shapiro-Ilan, 2002; Cornwallis *et al.*, 2017). The chemical changes associated with the decay process are driven by aggregations of organisms from many trophic levels, their conspecific and heterospecific interactions, as well as the aforementioned abiotic and biotic factors (Benbow *et al.*, 2015a). Especially on small carcasses, overcrowding can lead to conspecific and heterospecific competition (Rivers *et al.*, 2011; Denno and Cothran, 1976). Likewise, food competition with scavengers can result in a sudden food depletion and the death of larvae (Erincçlioğlu, 1996). DeVault *et al.* (2004) showed that the mean time to rodent carcass removal by scavengers was 2.58 days: larvae with longer feeding time would fear the risk of being eaten. However, predation does not only occur between blowfly larvae and scavengers, but also between different blowfly species (*e.g.*, invasive competitor species of *Chrysomya* (Diptera: Calliphoridae) predate on other Diptera larvae), highlighting again the extreme competition for the same resource (Flores *et al.*, 2017). Lastly, parasitism contributes to high mortality rates in blowflies: Frederickx and colleagues (2013) observed the death of an average of 48% pupae by hymenopteran parasitoids in the field (from 3.5% in May to 90% in September). Under these circumstances, the feeding speed and development rate of larvae decides what portion of the limited protein source they will receive, and therefore, their probability of a successful development (Levot *et al.*, 1979).

Only a few other animal species depend on this trait to a similar extent. Most likely, because of these harsh growth conditions, necrophagous larvae develop quickly and show high metabolic rates of the ingested food during their feeding stage (Hanski, 1976). Adults *L. sericata* eclose after 12 days on average when held at a constant ambient temperature of

25 °C; however, their larvae spend only 3.5 days feeding on a carrion (Grassberger and Reiter, 2001). The adult life expectancy is 3 weeks, so the larval feeding stage covers only 11% of the total life cycle (Merritt *et al.*, 2009). As a comparison, the larvae of *H. illucens*, which also grow on decaying organic material, account for 27-36% of the total life cycle of this fly (Nguyen *et al.*, 2013). Comparisons with other insects that also have long larval stages suggest that the struggle for survival may explain the fast growth rate of calliphorid larvae (Table 2).

However, while some scientists are convinced that the primary goal of a larva is quick growth (Schmolz and Lamprecht, 2000; Meekan and Fortier, 1996; Ricklefs, 1969), others have reported that excessively fast growth is counterproductive when traits such as survival and adult body size are considered (Sinervo and Doughty, 1996; Fox, 1997; Richner, 1992; Chippindale *et al.*, 1997). These costs can be both physiological (e.g., smaller adult size or lower starvation endurance (Gotthard, 2000; Fischer *et al.*, 2005)) and immunological (i.e., weakened immune system (Block and Stoks, 2008; Cotter *et al.*, 2004)). In this context, delayed development may be beneficial to receive additional nutrition and to avoid the listed disadvantages (Wessels *et al.*, 2011). Therefore, analysing the trade-off between fast and efficient larval growth can provide a better understanding of developmental strategies in behavioural ecology. This thesis deals with this topic on an experimental level in the development study (*Costs of fast development*).

Table 2 Comparison of lifespans

The average number of days that an individual needs for the whole life cycle and to reach the adult stage as well as the duration of the larval (feeding and postfeeding) and pupal stages. Also listed are the durations of the larval and pupal stages as proportions of the total time required to reach the adult stage (and relative to the estimated lifespan). As comparisons to the green blowfly *Lucilia sericata* (Grassberger and Reiter, 2001), these durations are listed for a coprophagous insect (i.e., the dung beetle *Onthophagus lecontei* Harold, 1871 (Arellano *et al.*, 2017)), which is exposed to similar predation pressures but under different environmental conditions, and other necrophagous insects that colonize cadavers later in succession (i.e., oriental latrine flies such as *Chrysomya megacephala* (Fabricius, 1794) (Siddiki and Zambare, 2017) and black soldier flies such as *Hermetia illucens* (Linnaeus, 1758) (Nguyen *et al.*, 2013)). Note that for the black soldier fly, mean values of individuals reared on liver are listed.

Species (Order: Family)	**Approx. lifespan**	**Total to adult**	**Larval stage**		**Intra-puparial stage**	
	[days]	**[days]**	**[days]**	**Prop.**	**[days]**	**Prop.**
Lucilia sericata (Diptera: Calliphoridae)	33	12	3.5	**29%** (11%)	5.2	**43%** (16%)
Chrysomya megacephala (Diptera: Calliphoridae)	28	8.8	3.3	**38%** (12%)	3.9	**44%** (14%)
Hermetia illucens (Diptera: Stratiomyidae)	82	42	22.5	**54%** (27%)	11.7	**28%** (14%)
Onthophagus lecontei (Coleoptera: Scarabaeinae)	1134	39	22	**56%** (2%)	11	**28%** (1%)

1.5. Goals and Hypothesis

In addition to regular conspecific groups, heterospecific aggregations have also been observed throughout the animal kingdom, including in birds (Forsman *et al.*, 2002; Sridhar *et al.*, 2009), fishes (Sazima *et al.*, 2007; Semeniuk and Dill, 2006) and arthropods (Sauphanor and Sureau, 1993; Bonacci *et al.*, 2011). **Such aggregations of different species raise questions of heterospecific communication processes and recognition mechanisms (i.e., how different species are able to share information), as well as of the resulting benefits, i.e., the balance between competition and mutualism** (Boulay *et al.*, 2016; Bshary and Noë, 1997; Ridley and Raihani, 2007; Griffin *et al.*, 2005; Hazlett, 1990). In contrast to cooperation, in which interaction takes place between conspecifics, mutualism presents a positive interaction between members of different species (Noë, 2001). While the existence of heterospecific aggregations is well documented in fishes, birds and mammals (Ridley and Raihani, 2007; Bshary and Noë, 1997; Moynihan, 1962; Munn, 1984), to date little is known about insects (Boulay *et al.*, 2017). **Focusing on blowfly larvae, I examined experimentally the developmental effects and relationships between gregarious maggots.** Therefore, I examined exogenous factors (e.g., social and ecological interactions) but not endogenous factors such as gene expression and kin selection.

The present work consists of two main areas: a behavioural study and a development study (Figure 12). The behavioural study (chapter 2) was conducted to detect active aggregation of different larval species and to identify species preferences between them. The development study (chapter 3) is dedicated to uncovering possible reasons for the formation of such heterospecific aggregations. **Together, these studies gain a better understanding of collective choices, aggregation dynamics and developmental consequences of heterospecific associations between blowfly species.**

Specifically, the behavioural study addressed whether different maggot species aggregate separately or whether heterospecific aggregations occur within *C. vomitoria*, *L. sericata* and *C. vicina*. The aggregation model of coexistence states that different species competing for ephemeral patches can coexist if they alternate temporally in aggregations or if they are distributed among different patches of the carrion (Presa Abos *et al.*, 2006; Hartley and Shorrocks, 2002). Regarding the successive arrival of different insect species and scavengers, this model also applies to carcasses. However, this model clearly excludes larval aggregations of different calliphorid species competing for the same patches at the same time, a phenomenon that can frequently be observed on carrion.

Following up on this idea, the development study is dedicated to test the hypothesis that heterospecific aggregations bring advantages that a conspecific group cannot reach, such as facilitating survival and possibly even increasing the fitness of individuals that grow in a heterospecific association. Therefore, this thesis focuses in the second part on trade-offs between life-history traits (i.e., fitness-related traits) within *L. sericata* and *C. vicina* at varying temperatures and group compositions.

Life-history theory addresses how the development, reproduction and aging of organisms have been shaped by natural selection (Nylin and Gotthard, 1998). Therefore, this theory deals with physiological and behavioural characteristics as well as organismal fitness by analysing life-history traits such as development rate, survival, fecundity, sex ratio, and size (Colinet *et al.*, 2005). In this context, chapter 3 (*Costs of fast development*) addresses **weather faster larval development leads to smaller puparia** (as they spend less time feeding) **and higher mortality rates** (as they cannot gather enough energy to survive the costs associated with the process of metamorphosis (Muntzer *et al.*, 2015; Ludwig *et al.*, 1964)), **or weather developmental plasticity can be regarded as costless in terms of fitness** (Buser *et al.*, 2014).

Considering the heterospecific aggregation behaviour of larvae as well as the effect of temperature and density on their development, the assumption arises that a species far from its optimal developmental temperature might benefit from the presence of another better-adapted species. Specifically, the hypothesis to be tested in chapter 3 (*Positive and negative density dependence*) is that ***L. sericata*** **benefits more from an association with** ***C. vicina*** **at low temperatures (i.e., 15 °C) and that** ***C. vicina*** **derives greater benefits from this association at higher temperatures (i.e., 28 °C).**

Concerning the effects of different growth conditions (e.g., food quality, group density or species composition), several possibilities exist of how larval development could be affected. **The hypothesis raised in this thesis is that the variability in development duration due to external effects might be higher for the larval feeding stages compared to the postfeeding stages** (*Balance between feeding and postfeeding time*). Since such deviations can have serious consequences in forensic cases when estimating the time since death, this issue is also discussed in a forensic entomology perspective and presented at the end of this thesis in the supplementary material.

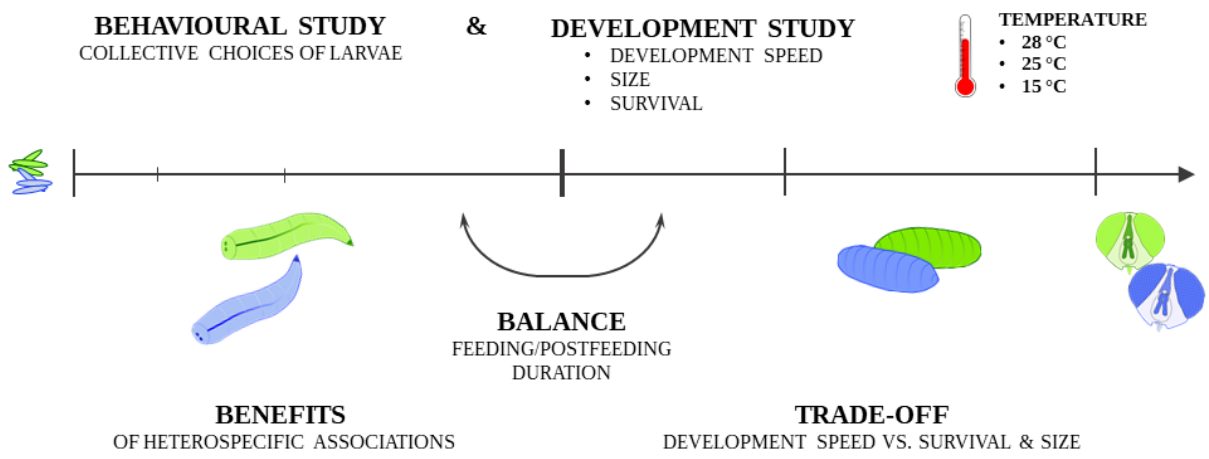
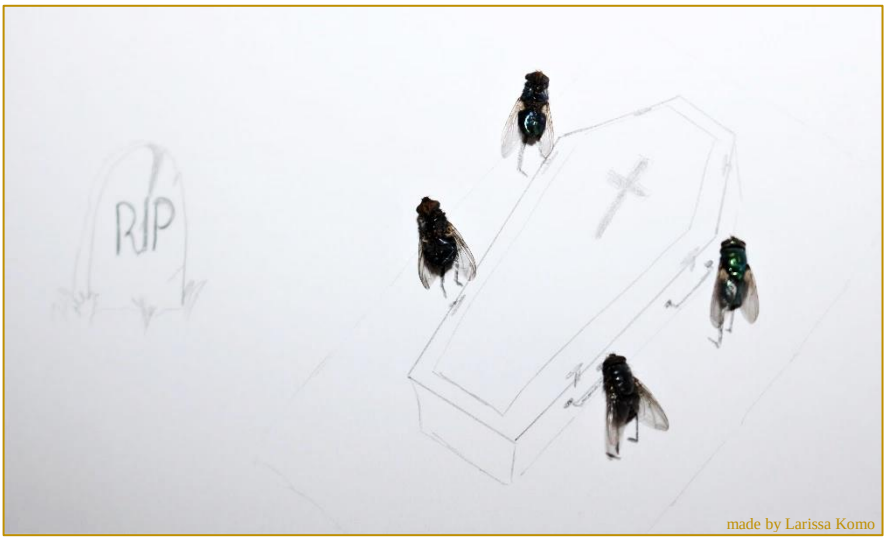


Figure 12 Topics of this thesis

This thesis consists of a behavioural and a development study. The behavioural study was conducted to detect active aggregation of different larval species and to identify species preferences between them. The development study tested three different fitness-related traits, namely development speed, puparium surface area (size) and survival, at three different temperatures. Thereby, I inspected for benefits in heterospecific aggregations, a balance between feeding and postfeeding (including pupal) duration as well as for a trade of between development speed and survival or size.

2 Behavioural study

Collective choices



2.1 Material and methods

Insect rearing

Larvae of *L. sericata*, *C. vicina* and *C. vomitoria* (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 tulle cages (50×50×50 cm) at room temperature (20±2 °C) and daylight at their natural times. Caster sugar and water *ad libitum* were offered inside the cages throughout the flies' lifetime. Pieces of pork heart were used as protein suppliers and oviposition media. For the latter purpose, they were placed in the cages for 2 hrs, guaranteeing an oviposition time with a maximum deviation of ±1 hr.

Experimental setup

The first study of this thesis was conducted to analyse the supposed collective behaviour of larvae in slightly different scenarios of binary choice tests. The decision made for or against a particular aggregation spot was evaluated after a test period of 20 hrs and compared between the three test species to determine species preferences.

This behavioural study was performed in a darkened growth chamber at 20 ±0.5 °C from March to October 2017 (ST4, POL-EKO Aparatura®, Poland). A glass Petri dish filled with a liver-agar mixture was used as the experimental arena (Pyrex®, 18.5 cm in diameter). The mixture consisted of 170 ml water, 3.1 g agar-agar (industrial agar Cat.1804 .05 CONDA) and 140 g pureed beef liver (processed by a butcher and frozen until used) (adapted from Boulay *et al.* (2016)).

Acrylic Petri dishes (6 cm in diameter) with the opening turned downwards were lightly pressed inside the liver-agar mixture on opposite poles of the glass Petri dish in order to create two spots and enable maggots to crawl underneath the dishes (Figure 13). Each of the acrylic Petri dishes contained a hole in the middle, which was covered with a fine-mesh net for

oxygenation purposes. At the beginning of the experiment, each Petri dish enclosed 50 starved, late second instar larvae of one of the three test species. Apart from the different species, the two spots were strictly identical. Three hours after placing the initial maggots under the Petri dishes, 100 new maggots (again, 50 maggots per species) were placed on the arena. This placement occurred in one line along the middle of the arena with the same distance to both spots (Figure 13).

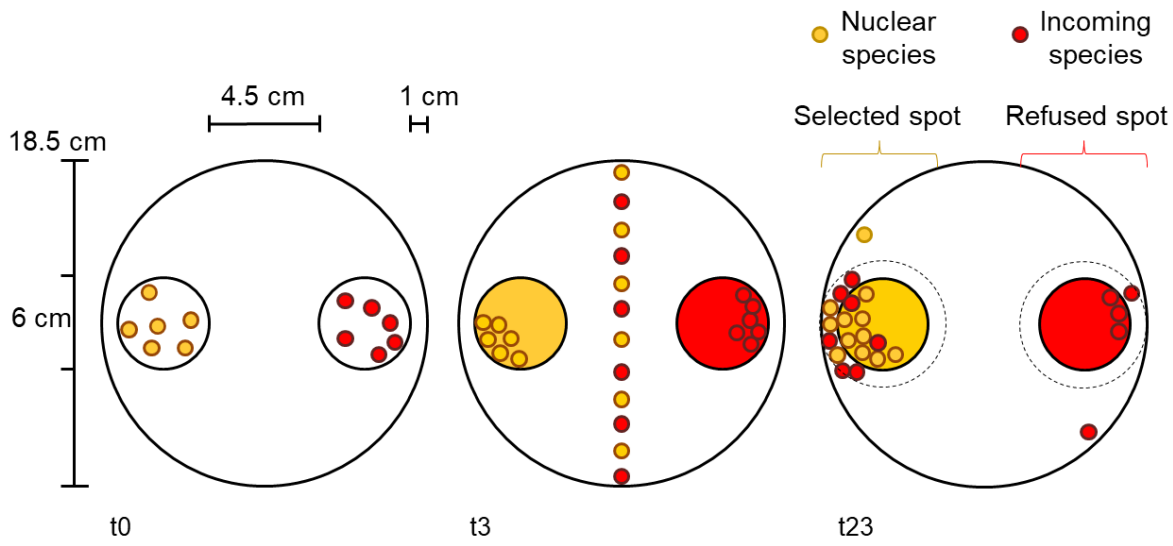


Figure 13 Binary choice setup to test species-specific attractions

At start time t0, each group of 50 maggots of a single species was placed under Petri dishes with the opening turned downwards. Three hours later (t3), a group of 100 supplementary maggots of both species was placed randomly in the middle of the arena. All individuals (including those initially placed under the Petri dishes) could move freely around the arena. After 20 additional hours (t23), all maggots were counted according to their species and location: under the Petri dishes, near them (dashed line) or in the rest of the arena. Hypothetical results are shown here by way of an example: all supplementary larvae moved together to the left spot (selected spot) where the yellow species was initially placed (the nuclear species). The red species acts, in this example, as the incoming species, which was initially placed at the so-called refused spot.

All individuals could move freely in the arena, including going in or out the Petri dishes. After another 20 hrs, all larvae were captured from (1) under the two Petri dishes, (2) directly around the Petri dishes within a radius of 1 cm and (3) in the remaining part of the arena. The spot that contained significantly more maggots than the other spot was designated the selected spot, while the opposite side was designated the refused spot. The species that was initially situated at the selected spot was considered the nuclear species, while the second one was considered the incoming species (Srinivasan *et al.*, 2010; Boulay *et al.*, 2017). In addition to these 68 replications in total[1], another four replications were performed with coloured maggots in order to distinguish between initial and later-added maggots. In these experiments, the later-added maggots (of both species) obtained a colorant via their food (Sudan III, JR Geigy, SA, Basle, Switzerland), which did not change their behaviour or developmental rate (personal observation). Two different control experiments were performed. First, using the same initial technique than for the main experiments but adding 100 larvae of only one species to the middle of the arena (testing: what is the difference between decisions made among conspecifics and those made by larvae in heterospecific groups?). Second, putting 100 larvae per species under each Petri dish and adding no new maggots (testing: do larvae crawl from their spot to the other?). In addition, another 26 replications were performed by removing the initially placed larvae after 3 hrs and adding 100 new larvae of one species to analyse the effect of cues left behind by the initially placed larvae on the aggregation behaviour of the new larvae. (Figure 41, supplementary material). Lastly, 50 larvae of two different species (also a total of 100) were added after removing the initial ones in five replications. All additional experiments can be found in the supplementary material.

[1] 25 replications *C. vicina* vs. *L. sericata*, 23 replications *C. vicina* vs. *C. vomitoria* and 20 replications *L. sericata* vs. *C. vomitoria*

Statistical analysis

The data were analysed using the R software via R studio (version 3.3.3) with the packages fifer and MASS (R Core Team, 2017)). Furthermore, Microsoft Office 2019 and XLstat version 18.07 were used (Addinsoft: Data Analysis and Statistical Solution for Microsoft Excel. Paris, France, 2016).

First, binomial tests were carried out to test for a bias towards one spot in each repetition (*i.e.*, whether one of the two spots contained more maggots than the other). Second, binomial tests were again performed concerning a bias towards one species for all three species combinations at the replicate level (*i.e.*, whether one species was chosen more frequently than the other). These analyses thus confirmed or denied the existence of both a selected spot and a nuclear species. Third, Pearson's Chi-squared tests with Bonferroni correction and Chi-squared post-hoc comparisons were conducted. For this test, the result of each replication was considered as a binary response variable to test the species preference and correlation between all three tested species (*e.g.*: which species was more often the nuclear species?). Finally, the number of maggots at the selected and refused spots was compared at the replicate level using the Wilcoxon signed rank test.

2.2 Results

In the first study of this thesis using binary choice tests, **collective choices were taken by the three blowfly larvae and heterospecific aggregation dynamics were observed.** The control trials, in which 100 larvae were initially placed either under each Petri dish or in the middle of the arena, indicated that larvae were able to move underneath the Petri dishes both outwards and inwards (*i.e.* in or out of the aggregation spots). However, most of the larvae remained under the Petri dishes where they were initially placed. On average, 81 ±22% of the larvae were still under the Petri dishes after 23 hrs, while the remaining 19 ±19% were close to them, (*i.e.*, within a radius of approximately 1 cm around the Petri dish).

During the experimental trials, **an average of 98 ±1% of the 100 later-added maggots moved to one of the pre-existing aggregations.** Among these newcomers, larvae of both species generally chose the same spot. After 23 hrs, the selected spot gathered more than 75% of all larvae (Figure 14) [2]. Overall, a significant aggregation was determined at the selected spot in 64 out of 68 replications (binominal test: $p = 0.0036$), with a balanced repartition observed in the 4 remaining replications. An average of 10 ±6 initial larvae moved from the refused spot to the selected spot (Figure 15).

[2] *C. vicina* vs. *L. sericata*: 75 ±10%, *C. vicina* vs. *C. vomitoria*: 71 ±6%, *L. sericata* vs. *C. vomitoria*: 80 ±11%

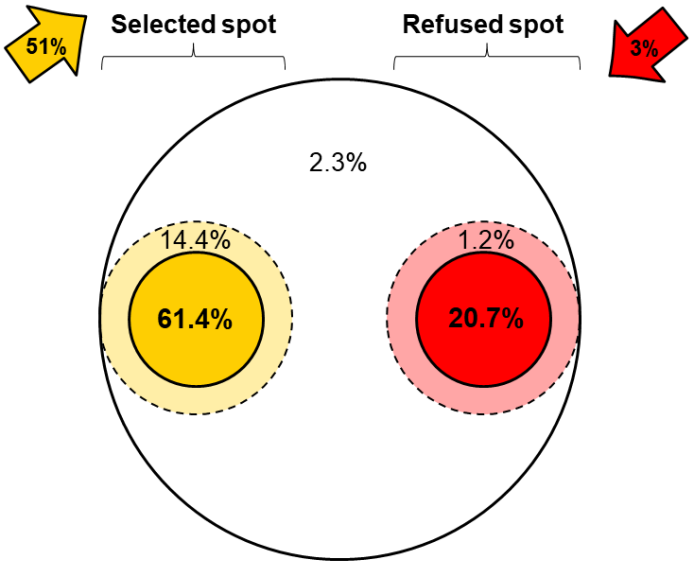


Figure 14 Average larval distribution after a total of 23 hrs

Proportions of larvae (all species together) in the selected (yellow) and refused (red) spot, within a radius of 1 cm around those spots (light yellow and light red), and in surrounding areas. After placing 100 new larvae in the middle of the arena, the number of larvae increased by approximately 51% on the selected spot and decreased by approximately 3% on the refused spot.

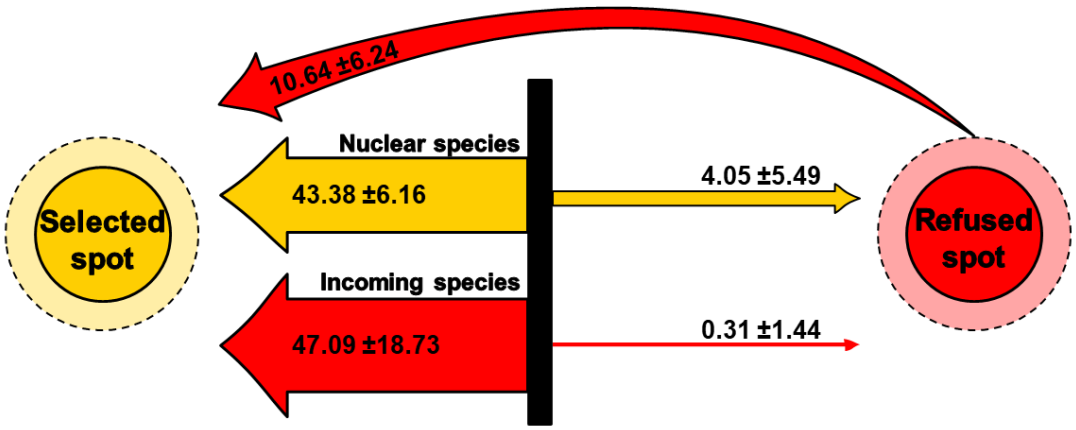


Figure 15 Larval movement in the experimental arena

Schema of the movement of larvae placed in the middle of the arena (black bar) plus the migration of the initial larvae from the refused to the selected spot. The mean number of larvae with each standard deviation is displayed.

The aggregation spot where *C. vicina* larvae were initially placed was preferred over the spots of *L. sericata* and *C. vomitoria* (Figure 16). In fact, *C. vicina* was significantly more often the nuclear species as opposed to *C. vomitoria*[3]. Among *C. vicina* and *L. sericata*, a significant preference for *C. vicina* was observed after considering all replications together and when comparing the number of larvae at the spots[4]. Regarding the combination between *L. sericata* and *C. vomitoria*, an equal choice was detected[5].

[3] Overall percentage with all species comparisons: Pearson's Chi-squared test, n = 64, df = 2, x^2 = 10.578, p = 0.005; Chi-squared post hoc comparison: $p_{vi\text{-}vom}$ = 0.007; experiments between *C. vicina* and *C. vomitoria*, binomial test with frequency being nuclear species: n = 20, p = 0.0414; Wilcoxen signed rank test with number of larvae at the two spots: n = 20, V = 162.5, $p_{vi\text{-}vom}$ = 0.0333.
[4] Chi-squared post hoc comparison: $p_{vi\text{-}s}$ = 0.0484; Wilcoxen signed rank test: n = 24, V = 227, $p_{vi\text{-}s}$ = 0.0288
[5] Chi-squared post hoc comparison: $p_{vom\text{-}s}$ = 0.506; Wilcoxen signed rank test: n = 20, V = 122, $p_{vom\text{-}s}$ = 0.5377

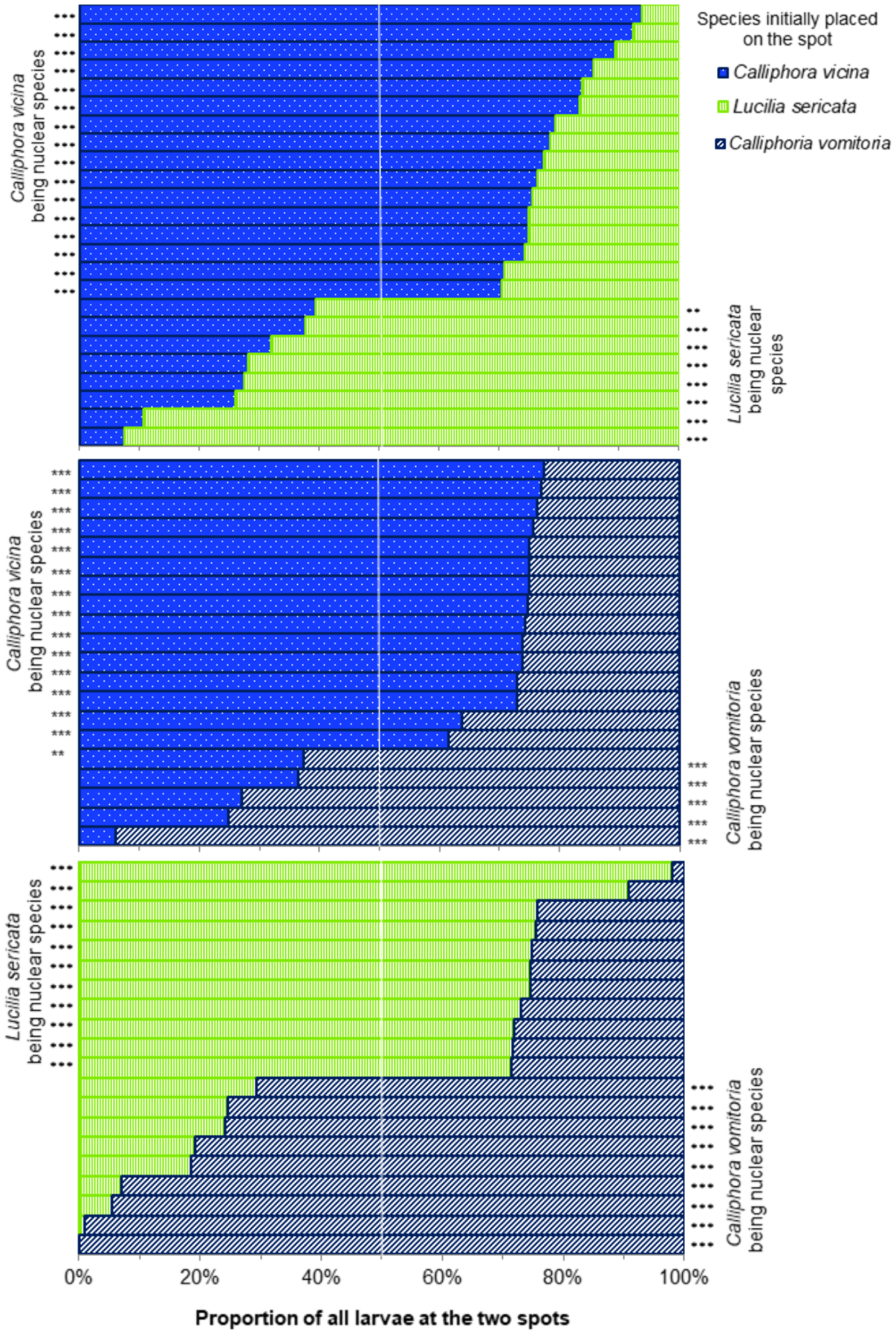

Species initially placed on the spot
Calliphora vicina
Lucilia sericata
Calliphoria vomitoria
Calliphora vicina being nuclear species
Lucilia sericata being nuclear species
Calliphora vicina being nuclear species
Calliphora vomitoria being nuclear species
Lucilia sericata being nuclear species
Calliphora vomitoria being nuclear species
0%
20%
40%
60%
80%
100%
Proportion of all larvae at the two spots

Figure 16 Nuclear species

These graphs display the proportions of all larvae found at the two spots and the frequency of being the nuclear species in direct comparisons with the test partner. For example, the first row displays that approximately 95% of all larvae (*i.e.*, about 190 larvae of both species) were finally found at the spot that initially contained 50 *C. vicina* larvae. A proportion of 25% means the spot held as many larvae as at the beginning of the experiment. A 50%-proportion means that one half of the added larvae moved to one spot and the other half moved to the opposite spot (regardless of the species). Significance of selecting one spot and the existence of a nuclear species were determined using binomial tests. Blue background with white dots refers to *C. vicina*, green stripes refer to *L. sericata* and dark blue slashes refer to *C. vomitoria*.

Summary:

- Larvae of both test species chose the same spot forming heterospecific aggregations.
- *Calliphora vicina* was mostly the nuclear species.
- The choices between *L. sericata* and *C. vomitoria* were balanced.

2.3 Discussion

Any time an animal chooses one particular behaviour from a set of possible alternatives, it has made a choice (or decision (Dill (1987)). The wording “collective choice”, mostly used for groups of primates (King and Sueur, 2011), flocks of birds (Aplin *et al.*, 2014), schools of fish (Ward *et al.*, 2008) and swarms of ants or bees (Seeley *et al.*, 2000; Pratt *et al.*, 2002), often refers to a complex grouping behaviour. For example, ants in colonies make collective foraging decisions and use recruitment pheromone trails to inform nestmates of newly discovered food sources (Krause and Ruxton, 2008; Planqué *et al.*, 2010). However, simple social rules based upon the attraction and repulsion of nearby conspecific and/or heterospecifics are often sufficient to explain collective choices (Farine *et al.*, 2014). In line with other studies on arthropods, the term “collective choice” in this thesis refers to the selection for one spot by a majority of larvae, without making any assumption about the decision process involved (Jeanson *et al.*, 2004; Couzin *et al.*, 2005; Nicolis *et al.*, 2016). However, the collective choices likely resulted from the sum of decisions made by several interacting larvae (Croquet and Schoenaers, 2016).

Using binary choice tests, aggregation experiments have shown that different species of Diptera larvae do not exploit different food spots, but form heterospecific aggregations. More specifically, our results confirm a collective and heterospecific choice for one of the two pre-existing aggregation spots. In 2016, Boulay *et al.* also observed collective choices in *L. sericata* and *C. vomitoria* larvae. However, those larvae were initially randomly spread and had the choice between two identical empty food spots. On the contrary, larvae of the present study could join a group that was already feeding on either side of the arena. Thus, later-added larvae decided against one species while moving towards the other.

In this situation, a collective choice can be taken in either one or two steps. In a two-step process, the two species of the later-added maggots first move separately to any spot. This

occurs before all larvae in one spot (the refused spot) collectively move to the other spot (the selected spot) to create one large aggregation. However, due to the observed allocation of maggots (approximately 20% of all larvae stayed at the refused spot), this two-step hypothesis seems very unlikely. In contrast, a one-step process is more probable. Under this scenario, the later-added larvae progressively moved towards one spot, which finally became the selected spot, while the initial larvae at the refused spot remained, for the most part, where they were.

Although the mechanism of heterospecific aggregation was not investigated here, previous studies suggest that larvae leave a chemical cue which is detected by other foraging larvae (Boulay *et al.*, 2013; Fouche *et al.*, 2018). A proof of this concept was recently provided by Fouché *et al.* (2018), who demonstrated that calliphorid larvae leave ground-deposited cues carrying information on group size and species composition. The more larvae in one site, the stronger the attraction/retention of this site was, and the more other larvae joined the group. Accordingly, larval aggregates probably followed a decentralized (*i.e.*, allelomimetic) process (Deneubourg and Goss, 1989; Sueur *et al.*, 2012). Interestingly, monitoring resources and sharing foraging information within group members, termed recruitment (Taylor and Jeanne, 2018), is a well-known strategy of social insects to increase their foraging effectiveness (Kirman, 1993; Seeley *et al.*, 2000; Lozada *et al.*, 2016).

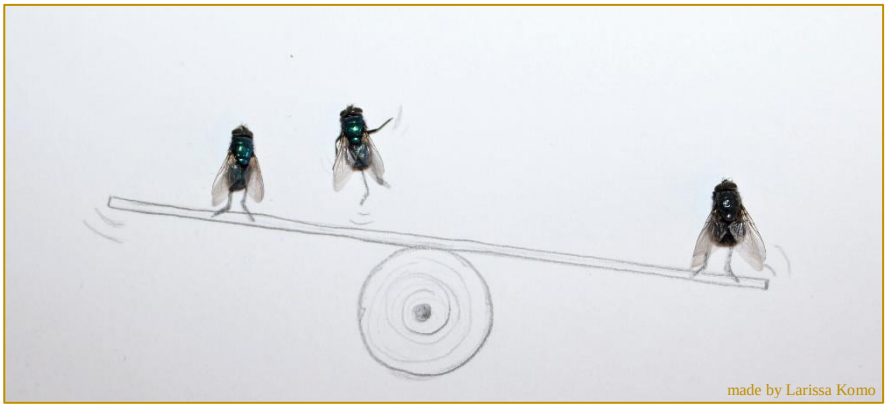
In all aggregation experiments, both aggregation spots initially contained the same number of larvae and food quantity/quality but differed in species. In this context, *C. vicina* was more often the nuclear species, highlighting the different degrees of attraction among species. Such recognition was most likely achieved through indirect communication, *i.e.*, stigmergy, a spontaneous and indirect coordination of each other's activity by modifying the environment (Beckers *et al.*, 1994; Theraulaz and Bonabeau, 1999). Aside from cues left by larvae, it is also possible that the initial larvae differentially modified the food of the spots on which they

were placed. Simply put, one food spot may become more edible than the other, causing all added larvae to join this spot. Other cues such as thigmotaxis (Rivers *et al.*, 2011; Boulay *et al.*, 2013), volatile odours (Barker *et al.*, 1988; Cobb, 1999), substrate modification by proteolytic enzymes (Pendola and Greenberg, 1975; Sandeman *et al.*, 1990) or thermal orientation (Aubernon *et al.*, 2016) could also be involved. Thus, the exact nature of the cue(s) eliciting heterospecific aggregation remains open for further investigation.

Lastly, the foraging behaviour might also be species-specific as the larval distribution patterns differed: in 2 out of 26 replications, in which *C. vomitoria* was the incoming species, the initial larvae of *C. vomitoria* moved from their already established conspecific aggregation to the heterospecific aggregate, *i.e.*, selected spot (Figure 16). This corresponds to nearly 8% of those experiments. Comparatively, *C. vicina* larvae migrated to the selected spot in 23% and *L. sericata* in 40% of the experiments in which they were the incoming species. Consequently, *C. vomitoria* larvae could either be more lethargic (and therefore remained on the rejected spot) or derive fewer benefits from heterospecific aggregations than other species. This should be used as an inspiration for future studies, as *C. vomitoria* was not further utilised in the development studies.

3 Development study

Effects in heterospecific and conspecific aggregations



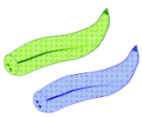
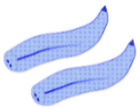
3.1 Material and methods

Cultures of *L. sericata* and *C. vicina* were reared as in the behavioural study.

Preparation and start of experiments

Based on the results of the first study (collective choices), further questions arose regarding advantages in heterospecific groups, the Allee-effect and trade-offs between fast and efficient growth. Accordingly, development quality was compared between small and big conspecific groups and a heterospecific group (Table 3). Surface areas of the puparia, survival rates and larval development rates were used as fitness-related traits (without considering reproductive performance) and were referred to as development 'quality' in accordance with the publications of Blums *et al.* (2005) and Willson and Nussey (2010). Knowing about the decisive function of temperature during insect development, the experiments were carried out at different constant temperatures (Table 4).

Table 3 Composition of *L. sericata* and *C. vicina* larvae in all series.
Five different conditions concerning species and number of individuals were tested at 15, 25 and 28 °C ±0.5 °C.

	LS_{100}	LS_{250}	$LS_{125}CV_{125}$	CV_{250}	CV_{100}
					
L. sericata	100	250	125	0	0
C. vicina	0	0	125	250	100

The setup used to monitor larval development within mono- and heterospecific groupings was adapted from Scanvion *et al.* (2018). In detail, blow fly eggs were kept in a climatic chamber

(ST4, POL-EKO Aparatura®, Poland) at 25 °C for 22 hrs (*L. sericata*) or 24 hrs (*C. vicina*) after oviposition. The previously frozen meat (50 g 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. The first instar larvae were homogeneously distributed on the prepared nutrient medium inside a small plastic box (100×75×63 mm), which was placed in a large breeding container (180×135×195 mm) covered with sand. The lids of both boxes were each provided with an air window that was sealed with a fine-mesh net (Figure 17). The inner lid of the substrate box was removed on the second (28 °C), third (25 °C) or fourth day (15 °C) after oviposition, *i.e.*, when the first larvae reached the third larval stage (the indicated temperatures refer to different test series.).

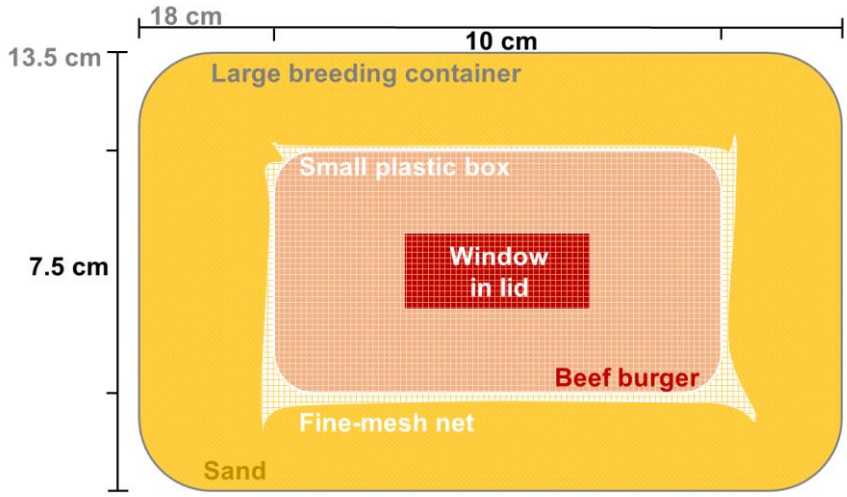


Figure 17 Breeding container, top view.

Insight into a large breeding container (180×135×195 mm) with a closed substrate box that was filled with a mixture of 50 g of minced steak and 15 ml of a 0.9% NaCl solution. On this mixture the counted first instar larvae were distributed. The lid of the substrate box and the fine mesh net were removed after some time (Table 4) when the larvae were still in the feeding stage. Postfeeding larvae were collected from the surrounding sand.

Test series and procedure

Three series of experiments were carried out at three different temperatures (denoted “cold”, “intermediate” and “hot”, Table 4). Beside the changes in temperature and the associated different test times, the experiments of these three series were carried out identically. In order to determine the proportion of postfeeding larvae, the number of migrating larvae in the sand was counted three or four times a day (Table 4) and used as a measure of the development speed. At each measuring time, the migrating larvae of each box were transferred to a new sand-covered box with a sheltered place to pupate and were raised to adulthood at 25 °C (in all series). Puparia were photographed nine (25 & 28 °C) or seventeen (15 °C) days after oviposition. Afterwards, the surface areas of all (25 °C) or a subsample of 50 ±5 puparia from each experiment (15 & 28 °C) was measured using ImageJ (version 1.43, http://rsbweb.nih.gov/ij/ (Schneider *et al.*, 2012)).

Finally, the total number of eclosed flies was counted. Neither adult body size nor female fertility were measured in these experiments. First, because previous development studies of my colleagues showed that the body size of flies did not differ significantly between different conditions (Scanvion *et al.*, 2018), and, second, because the determination of the fertility of each fly would have raise several technical problems and exceeded the time frame of this study.

In all series, the following three conditions were tested: 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 (Table 3). Six repetitions, which never ran simultaneously, were performed for each condition (*i.e.*, 5 boxes with 5 different conditions were used per test run).

Table 4 Differences between the series according to temperature.

Month and year when the three different experiment series were performed are listed with the number of persons that performed those (1 person = only the author; 2 persons = the author and Q. Scanvion (Scanvion *et al.* 2018)). At the measuring time, postfeeding larvae or eclosed flies were counted. The day when the lid of the substrate box was removed and when the puparia were photographed changed between the series due to the different development rates.

	15 °C (Cold)	25 °C (Intermediate)	28 °C (Hot)
experimental period	01/2019 – 04/2019	01/2017 – 05/2017 11/2017 – 04/2018	01/2019 – 04/2019
persons involved	1	2	1
photocycle	24 hrs dark	12:12 hrs light:dark	24 hrs dark
measuring time (during daytime)	every 4 hrs (10 a.m., 2, 6, 10 p.m.)	every 6 hrs (10 a.m., 2, 6, 10 p.m.)	every 4 hrs (10 a.m., 2, 6, 10 p.m.)
removing inner lid (after oviposition)	day 4	day 3	day 2
puparia photo shoot (after oviposition)	day 17	day 9	day 9

Development rate and statistical analysis

The relative development rate of each larva, denoted C_i, was used rather than the development time to enable direct comparisons between individuals bred under different temperatures and conditions (Table 10 in appendix). This value was calculated by the following equation (Equation 1) using the migration time of postfeeding larvae.

Equation 1

$$C_i = \frac{1}{\text{mean migration time}} * \text{migration time of larva } i$$

Relative development rates less than 1 indicate quicker development than the population average (mean value); whereas development values greater than 1 indicate slower development. For example, the relative development rate from one of the first postfeeding larvae at 28 °C was 0.894 because this larva migrated after 77 hrs in a population that migrated on average 86.175 hrs after oviposition.

The data were analysed using the R software via R studio (version 3.3.3 and 3.6) with the packages fifer, MASS, survminer, survival, pwr and ggplot2, which includes ggExtra for creating graphs (R Core Team, 2017)). Furthermore, Microsoft Office 2019 and XLstat version 18.07 were used for some charts (Addinsoft: Data Analysis and Statistical Solution for Microsoft Excel. Paris, France, 2016).

Differences in development speed and survival rate between the three conditions (*i.e.*, conspecific low- and high-density as well as heterospecific conditions) were analysed for each temperature with ANOVA and Tukey's range test (multiple comparisons of means). In addition, the Cox proportional hazard model and the Tarone-Ware test were used to compare the probability curves of feeding larvae. Moreover, the Wilcoxon signed rank test was used to

identify differences at a certain development time among the three conditions, and to compare the durations of the feeding and postfeeding phases (including intra-puparial period).

Differences in puparia surface areas and development rate between the three conditions at 15 and 28 °C were also analysed with two-way ANOVA (factors: temperature and condition, response variable: puoarial surface or development rate) and the Tukey's range test (for example, the development rates were compared by following command: TukeyHSD(aov (development rate ~ temperature * condition, data))).

Correlations between the development rates and the puparia surface areas as well as the survival rates were compared using Pearson's product-moment correlation. The Pearson correlation coefficient (r) serves as the effect size measure (0.9 = high correlation, 0.1 = weak correlation) and the power represents the probability of a type II error, *i.e.*, accepting the null hypothesis when it is in fact wrong (0.9 = high reliability, 0.1 = weak reliability of the test). Differences in puparia surface areas and survival rates between the three conditions were analysed with the Wilcoxon signed rank test. All analyses were conducted using a significance level of 0.05 and indicated the standard errors of the means.

3.2 Results

The main hypothesis of this thesis was that heterospecific aggregations bring benefits (that conspecific aggregations cannot offer), which are expressed in different fitness-related traits and vary with species and temperature. Therefore, development studies were carried out at different temperatures and with two different species.

The series “cold” and “hot” were not compared with the “intermediate” series, because they consisted of different fly populations (a usual laboratory procedure to avoid a bottleneck effect). The impact of the different fly populations was particularly apparent in the survival rate of pupae (Figure 18). Moreover, while the “intermediate” series of experiments was performed with a 12:12 hrs light:dark photoperiod (430 lumen from 9h30 to 21h30) from January 2017 to April 2018, the series “cold” and “hot” (at 15 and 28 ±0.5 °C) were carried out in the dark from January to April 2019 (Table 4). Finally, a few test experiments were conducted from October to December 2018 at 20 and 33 °C, which are only briefly described as they were not pursued further.

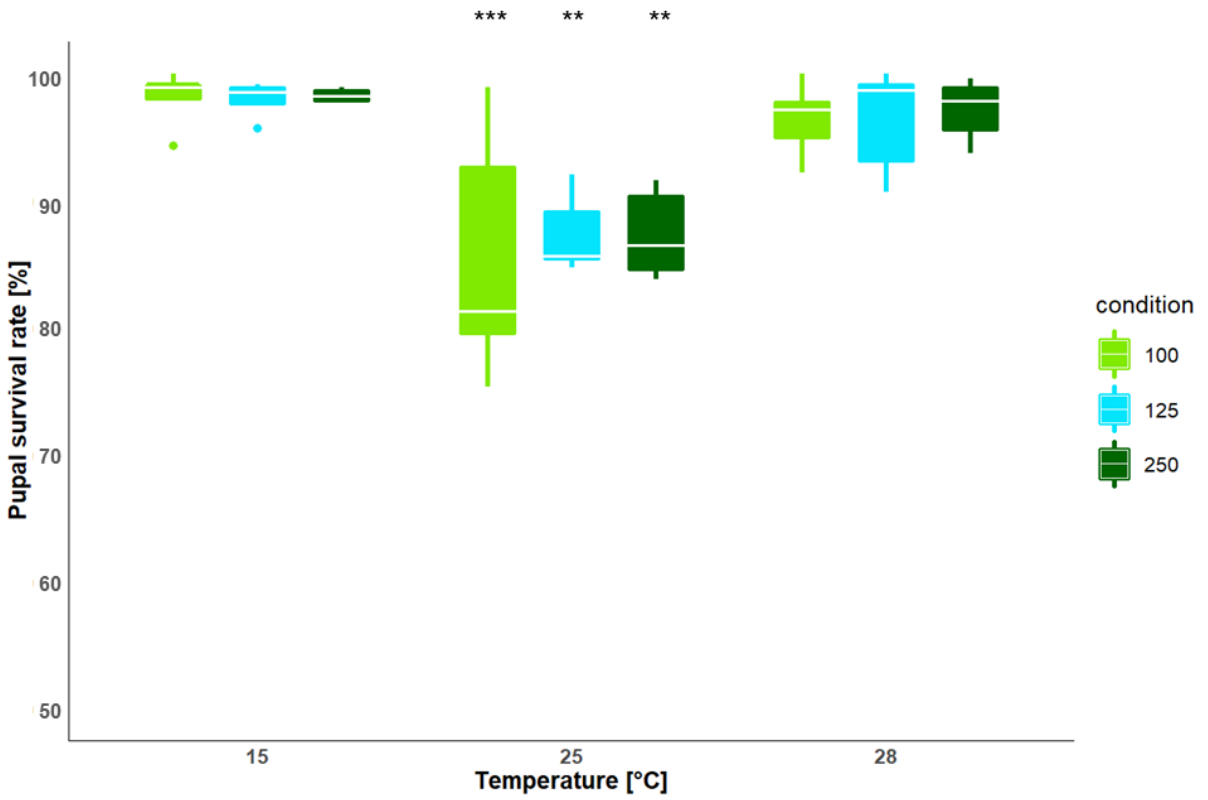


Figure 18 Survival rate of *L. sericata* pupae in three conditions at three temperatures.

The survival rate of pupae refers to the percentage of flies that eclosed from puparia in the two conspecific groups (*i.e.*, 100 and 250 larvae) and the heterospecific group (*i.e.*, 125 *L. sericata* + 125 *C. vicina*). Note that the y-axis starts at 50%. Significant differences[6] in survival between temperatures display the effects of changing fly populations (because there is no other known reason why a higher mortality rate existed at 25 °C (Evans, 1935)). Consequently, the results of the intermediate series were not compared with those of the other two series.

[6] Tukey's range test after two-way ANOVA: 25 °C, 100 larvae: $p_{28°C,100} = 0.0008$, $p_{28°C,125} = 0.0008$, $p_{28°C,250} = 0.0003$, $p_{15°C,100} = 0.0001$, $p_{15°C,125} = 0.0001$, $p_{15°C,250} = 0.0001$; 25 °C, 125 larvae: $p_{28°C,100} = 0.0092$, $p_{28°C,125} = 0.0094$, $p_{28°C,250} = 0.0038$, $p_{15°C,100} = 0.\ 001$, $p_{15°C,125} = 0.\ 0012$, $p_{15°C,250} = 0.\ 0009$; 25 °C, 250 larvae: $p_{28°C,100} = 0.0098$, $p_{28°C,125} = 0.0111$, $p_{28°C,250} = 0.004$, $p_{15°C,100} = 0.0011$, $p_{15°C,125} = 0.0013$, $p_{15°C,250} = 0.0011$

Mutual benefits at an intermediate temperature

In heterospecific groupings (*i.e.*, 125 *C. vicina* larvae mixed with 125 *L. sericata*), *C. vicina* larval development was significantly faster at 25 °C compared to that in conspecific groups of the same size (Figure 19)[7]. Conversely, *C. vicina* larvae grew only slightly faster in a large conspecific group (*i.e.*, 250 larvae) compared to a small conspecific group (*i.e.*, 100 larvae)[8]. In contrast, **the development of *L. sericata* was, on average, equal to that of 100 *L. sericata* in conspecific group and 12 hrs slower than that in equally sized conspecific groupings** (*i.e.*, 250 *L. sericata* larvae) (Figure 19). This was clearly visible during the middle of the larval stage (Figure 20; Table 5)[9]. During this period, the variance in conspecific groups of 100 larvae was greater than that in the heterospecific groups.

[7] Wilcoxon rank sum test for larval development time 72 – 84 h: $p_{72} = 0.0022$, $p_{78} = 0.0043$, $p_{84} = 0.0043$; Cox model: $p_{250\text{-}125/125} < 0.0001$

[8] Pearson's Chi-squared test: $x^2 = 48.647$, df = 8, $p < 0.0001$; Chi-squared post hoc comparison: $p_{100\text{-}125/125} < 0.0001$, $p_{250\text{-}125/125} = 0.0003$, $p_{100\text{-}250} = 0.3167$; Cox model: n = 1261, number of events = 1195, $p_{100\text{-}250} = 0.0894$

[9] Wilcoxon rank sum test between 250 *L. sericata* and 125 *L. sericata* larvae mixed with 125 *C. vicina* for development time 54 – 84 h: $p_{54} = 0.148$, p60 = 0.005, $p_{72} = 0.065$, $p_{78} = 0.041$, $p_{84} = 0.002$; Cox model: n = 1319, number of events = 1197, $p_{250\text{-}125/125} < 0.0001$

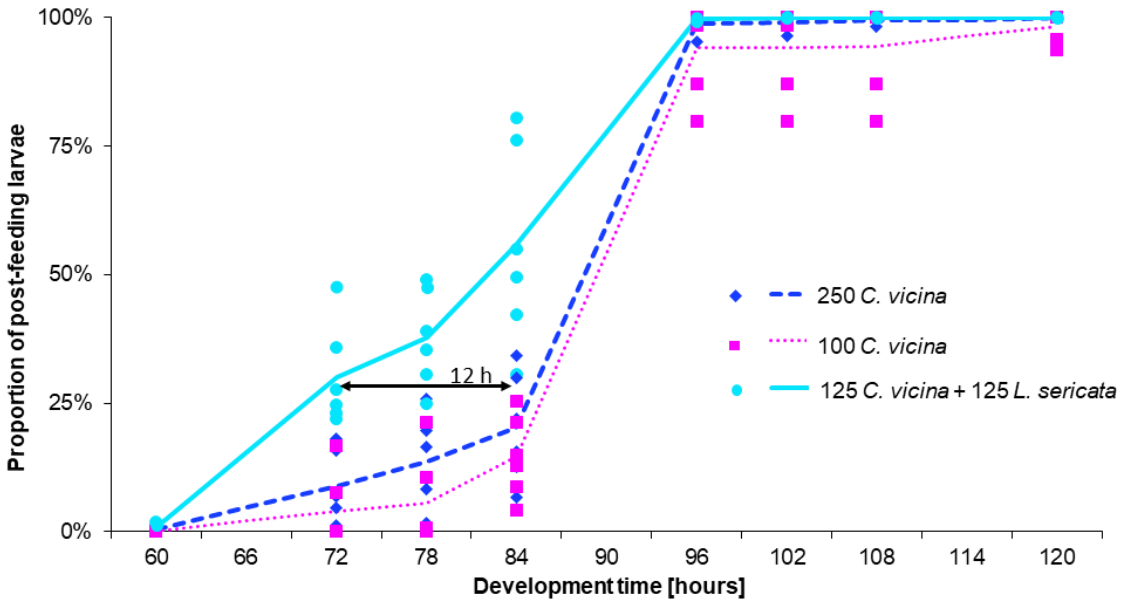


Figure 19 Development rates of *C. vicina*

Average development rate of 250 *C. vicina* larvae in conspecific group (dark blue, dashed line) compared with 100 *C. vicina* larvae (violet, dotted line) as well as 125 larvae mixed with 125 *L. sericata* larvae (turquoise, solid line). Corresponding points show each repetition. Rhombi display each experiment with 250 *C. vicina* larvae, squares with 100 *C. vicina* and circles with 125 *C. vicina* mixed with *L. sericata* larvae. The proportion of individuals corresponds to the number of postfeeding larvae normalized to the maximum of individuals found in the sand during each experiment.

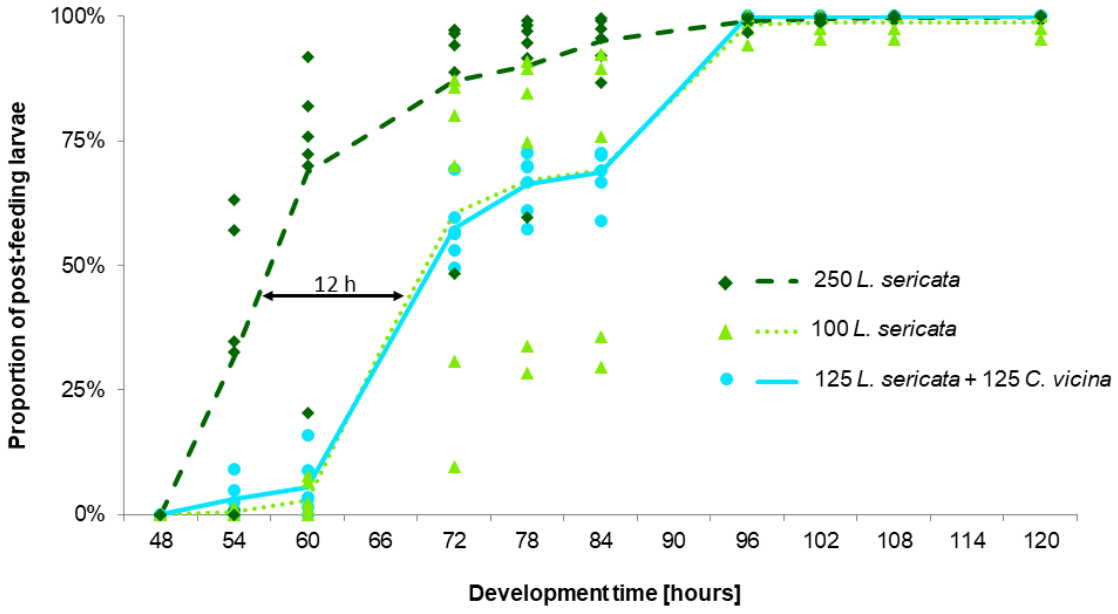


Figure 20 Development rates of *L. sericata*

Average development rate of 250 *L. sericata* larvae in conspecific group (dark green, dashed line) compared with that of 100 *L. sericata* larvae (green, dotted line) as well as 125 larvae mixed with 125 *C. vicina* larvae (turquoise, solid line). Corresponding points show each repetition. Rhombi display each experiment with 250 *L. sericata* larvae, triangles represent that with 100 *L. sericata*, and circles represent that with 125 *L. sericata* mixed with *C. vicina* larvae. The proportion of individuals corresponds to the number of postfeeding larvae normalized to the maximum of individuals found in the sand during each experiment.

Table 5 Mean number and standard deviation of *L. sericata's* postfeeding larvae

Note the higher standard deviations under conspecific conditions (100 and 250) compared to those under heterospecific condition (125+125) between 60 and 78 hrs. Values given are in percent. See Table 3 for details; hrs refer to development time since larval eclosion.

	54 hrs		60 hrs		72 hrs		78 hrs		84 hrs	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
100	0.4	3.4	3.0	32.6	60.5	28.4	66.9	29	69.2	2.3
250	31.4	26.8	68.8	24.9	87.1	19.2	90.1	15.1	95.1	5.0
125+125	3.0	3.4	5.5	5.9	57.4	6.8	66.2	5.9	68.6	5.2

Species differences were also detected concerning the mortality rate. **While *L. sericata* larvae demonstrated a slightly higher mortality rate in conspecific rather than in heterospecific conditions, the survival rate of *C. vicina* larvae remained unaffected** (Figure 21)[10]. Under the two conspecific conditions, larvae in 100 and 250 individual groups showed no significantly different mortality rates, suggesting that density had no effect on the survival rate for both *L. sericata* and *C. vicina*.

However, the number of larvae had a highly significant effect on the surface of puparia[11]. For both *L. sericata* and *C. vicina*, puparia in the 100-larvae group were significantly larger than puparia in the other groups. Moreover, **the puparia surface areas displayed significant differences between con- and heterospecific conditions.** While *L. sericata* puparia in the 250-larvae conspecific group showed a smaller surface than pupae in heterospecific conditions, the puparia of *C. vicina* had on average larger surfaces (Figure 22)[12].

[10] Wilcoxon rank sum test, *L. sericata*: $p_{250\text{-}125/125} = 0.0542$, *L. sericata*: $p_{100\text{-}125/125} = 0.1797$
[11] Wilcoxon rank sum test: *C. vicina*: $p_{100\text{-}250} < 0.0001$; *L. sericata*: $p_{100\text{-}250} < 0.0001$
[12] Wilcoxon rank sum test, *C. vicina*: $p_{100\text{-}125/125} < 0.0001$; $p_{250\text{-}125/125} = 0.0131$; *L. sericata*: $p_{100\text{-}125/125} < 0.0001$, $p_{250\text{-}125/125} < 0.0001$

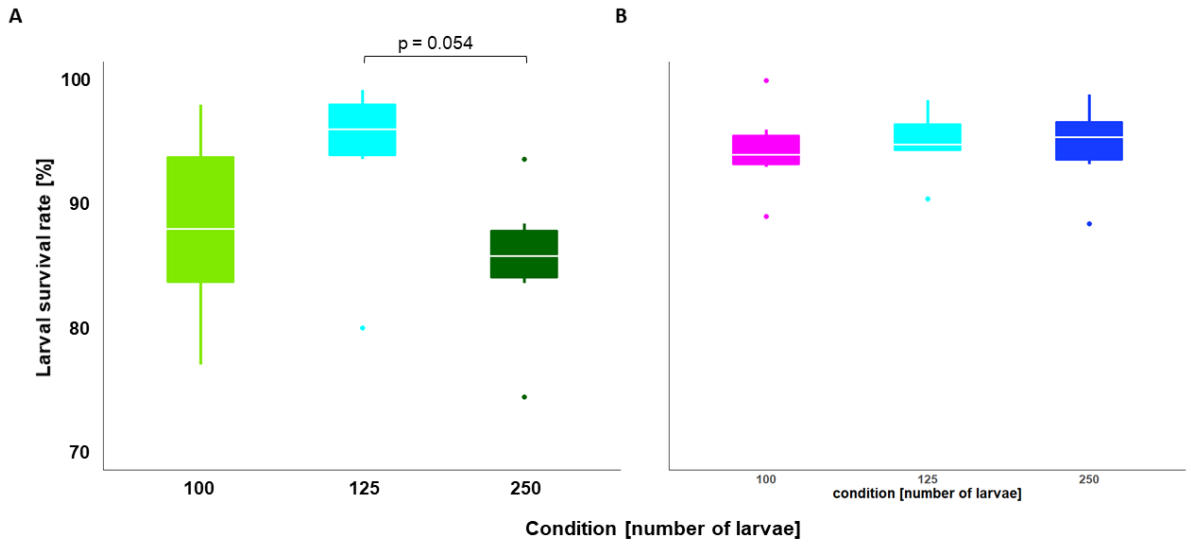


Figure 21 Survival rate of *L. sericata* (**A**) and *C. vicina* (**B**) larvae

L. sericata showed a lower (although not significant lower) mortality rate under heterospecific conditions (Wilcoxon rank sum test: $p_{250\text{-}125/125} = 0.0542$), whereas *C. vicina* larvae displayed the same low mortality rate under all three conditions.

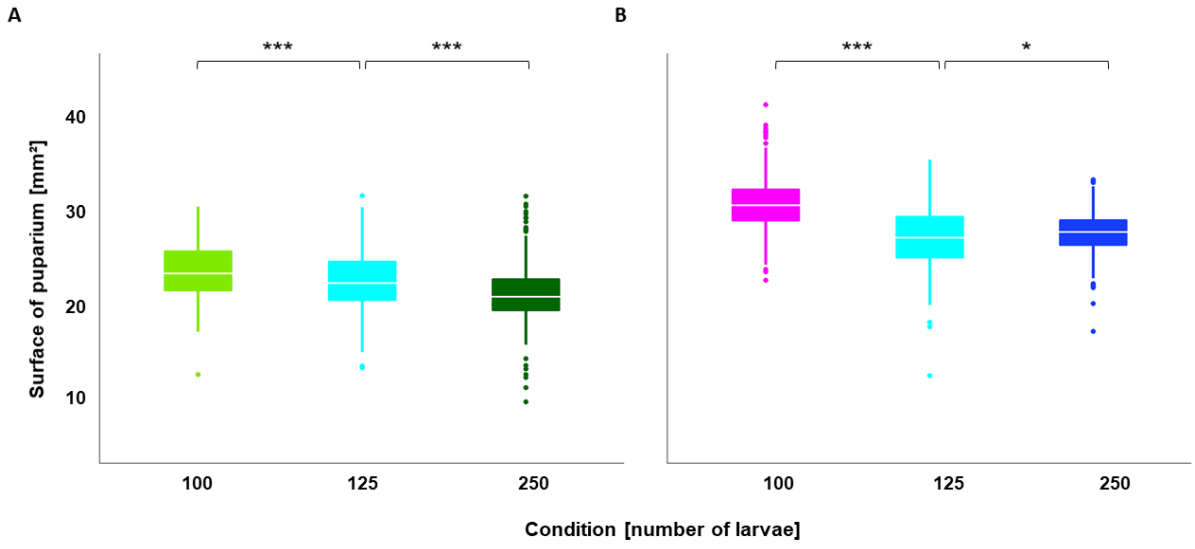


Figure 22 Surface area of *L. sericata* (**A**) and *C. vicina* (**B**) puparia

Significant differences were observed between conspecific and heterospecific conditions as well as between the two conspecific conditions with different densities (Wilcoxon rank sum test: *L. sericata* $p_{100\text{-}250} < \mathbf{0.0001}$, $p_{100\text{-}125/125} < \mathbf{0.0001}$, $p_{250\text{-}125/125} = \mathbf{0.0001}$; *C. vicina* $p_{100\text{-}250} < \mathbf{0.0001}$, $p_{100\text{-}125/125} < \mathbf{0.0001}$, $p_{250\text{-}125/125} = \mathbf{0.0131}$).

Summary

In heterospecific groups at 25 °C:

- *Lucilia sericata* showed higher survival rates and bigger individuals.
- *Calliphora vicina* showed a faster development.

Positive and negative density dependence

The aim of this part was to analyse how previously observed costs and benefits in fitness-related traits changed with ambient temperature. The associated hypothesis was that *L. sericata* benefits more from an association with *C. vicina* at low temperatures (*i.e.*, 15 °C) and that *C. vicina* derives greater benefits from this association at higher temperatures (*i.e.*, 28 °C).

First of all, experiments revealed differences in the puparia surface area, the total survival rate or the larval development speed between temperatures but also between group densities and conditions (*i.e.*, conspecific and heterospecific groups; Table 6). When comparing the mean surface area of all conditions between the cold and warm temperatures, it turned out that the **surface area of *C. vicina* decreased significantly[13] from 37 ±4.5 at 15 °C to 33 ±4.4 mm^2 at 28 °C**. In contrast, the **surface area of *L. sericata* increased slightly from 30.5 ±3.7 to 31.2 ±4.5 mm^2**. Furthermore, *L. sericata* puparia at 15 °C were significantly larger if they had developed in a heterospecific instead of a conspecific larval group (*i.e.*, 125+125 vs. 250 larvae)[14]. Such a difference was not observed at 28 °C. The probability of *C. vicina* survival was not temperature dependent but only density dependent, while the survival of *L. sericata* was neither influenced by temperature nor by species composition or group density (Table 6).

At 28 °C, *C. vicina* survived significantly better in both high-density groups than in the low-density group (Figure 23)[15]. Moreover, in heterospecific groups, fewer *C. vicina* stayed as postfeeding larvae in the food source, resulting in a lower mortality rate than that under the conspecific conditions (still apparent in the total survival; Figures 23 and 43). On average, only 1 ±0.9% *C. vicina* larva tended to remain in the substrate after the feeding stage in the heterospecific group. In contrast, in the conspecific groups at 28 °C, an average of 15.8

[13] Wilcoxen signed rank test for *C. vicina*: $p < 0.0001$
[14] ANOVA: $F = 26.96$ $p < 0.001$; Tukey HSD: $p_{high} = 0.0002$
[15] Tukey HSD: $p_{con} = 0.0345$, $p_{hetro} = 0.0096$

±15.8% (high-density group) or 22.5 ±19.1% (low-density group) of all larvae remained in the still-somewhat-moist food and were then either not able to pupate or eclose as a fly (see also supplementary material).

Table 6 Effects of group density and composition

Observation of "↑" indicates benefits, "↓" indicates costs and "=" indicates no effects. Asterisks mark significant results ($p < 0.05$). For example, the sign ↑* in the upper row of the first development column means that the heterospecific group developed significantly faster than the conspecific high-density group. Development refers to the time when 10% of the group reached the postfeeding stage; size refers to the average puparia surface area; survival refers to the total survival rate; hetero refers to the heterospecific group (125+125 larvae); low and high refer to the conspecific groups (100 and 250 larvae). See Table 13 in the appendix for all three series, *i.e.*; intermediate experiments included.

condition	**temp.**	**Development**		**Size**		**Survival**	
		C. vicina	*L. sericata*	*C. vicina*	*L. sericata*	*C. vicina*	*L. sericata*
hetero vs. high	15 °C	↑❋	↑❋	↑❋	↑❋	=	=
	28 °C	↑❋	=	=	=	=	=
hetero vs. low	15 °C	↑❋	↑❋	↓	=	↑	=
	28 °C	↑❋	=	↓*	↓*	↑❋	=
high vs. low	15 °C	↓*	↓*	↓*	↓*	↑	=
	28 °C	↓*	↓*	↓*	=	↑❋	=

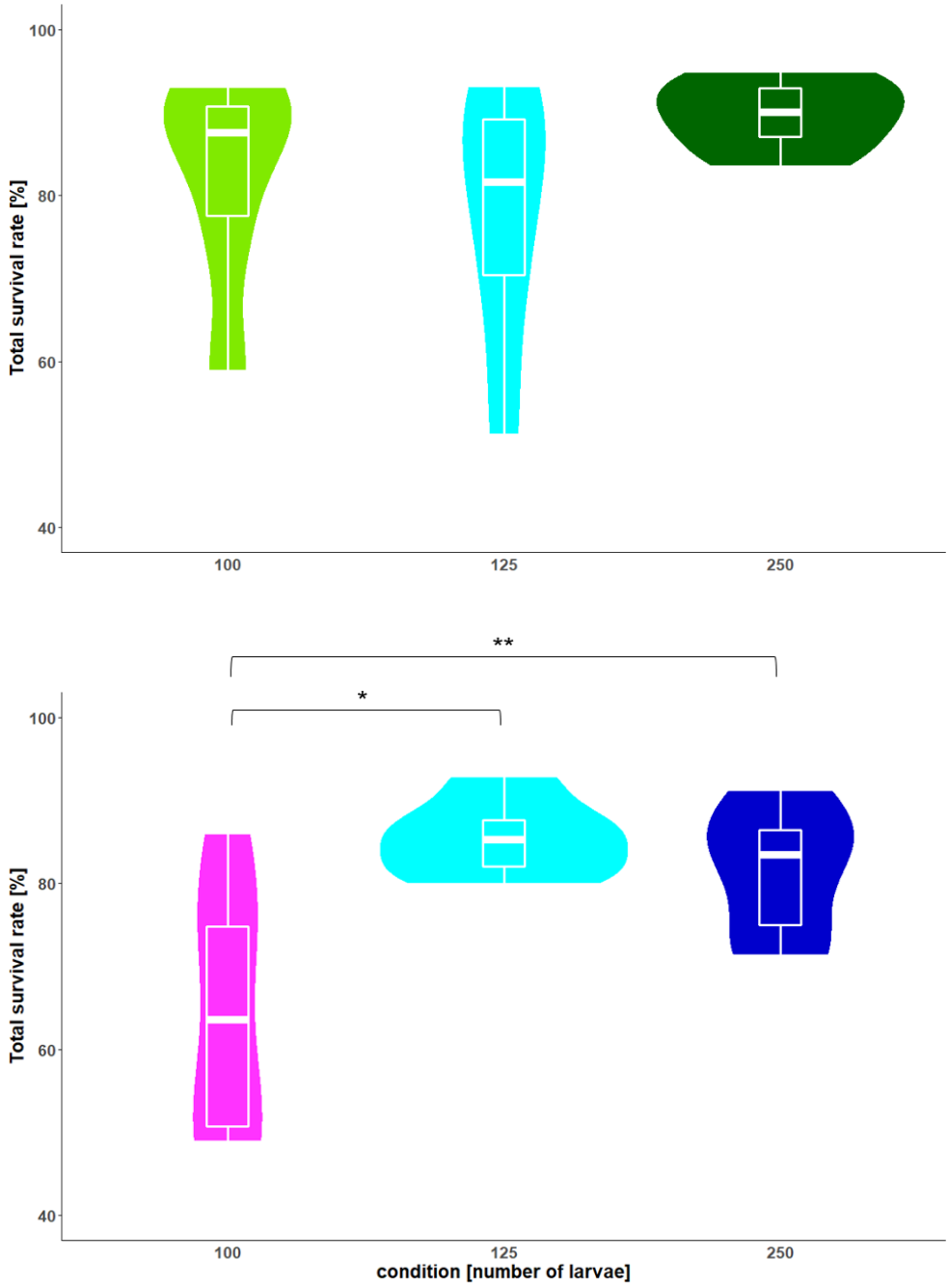


Figure 23 Total survival rate 28 °C

Survival rate of *L. sericata* **(A)** and *C. vicina* **(B)** under different conditions (low density (100 larvae), conspecific high density (250 larvae) and heterospecific (125 *C. vicina* mixed with 125 *L. sericata* larvae)). The coloured shapes around the box plots act as density curves and thus indicate the distribution of observations. Significant differences could be found for *C. vicina* between the two conspecific groups (Tukey HSD: p = **0.0345**) and between the low-density and heterospecific groups (Tukey HSD: p = **0.0096**).

The development speed until postfeeding was influenced by larval density in both species (Table 6, Figures 24 and 25)[16]. The two lower rows of Table 6 illustrate the negative density dependence: **larvae in low-density developed faster than those in conspecific high-density conditions did.** The upper rows clarify that *C. vicina* larvae developed faster in the heterospecific group than in the conspecific groups. Despite their faster larval development, individuals became larger in the low-density groups than in the high-density groups, which also applies to *L. sericata* at 15 °C.

[16] Tarone-Ware test, *C. vicina*: Chisq= 210, df = 2, $p < 0.0001$; Tukey HSD: $p_{con} < 0.0001$, $p_{hetero} = 0.0025$; *L. sericata*: Chisq= 63.2, df = 2, $p < 0.0001$; Tukey HSD: $p < 0.0001$

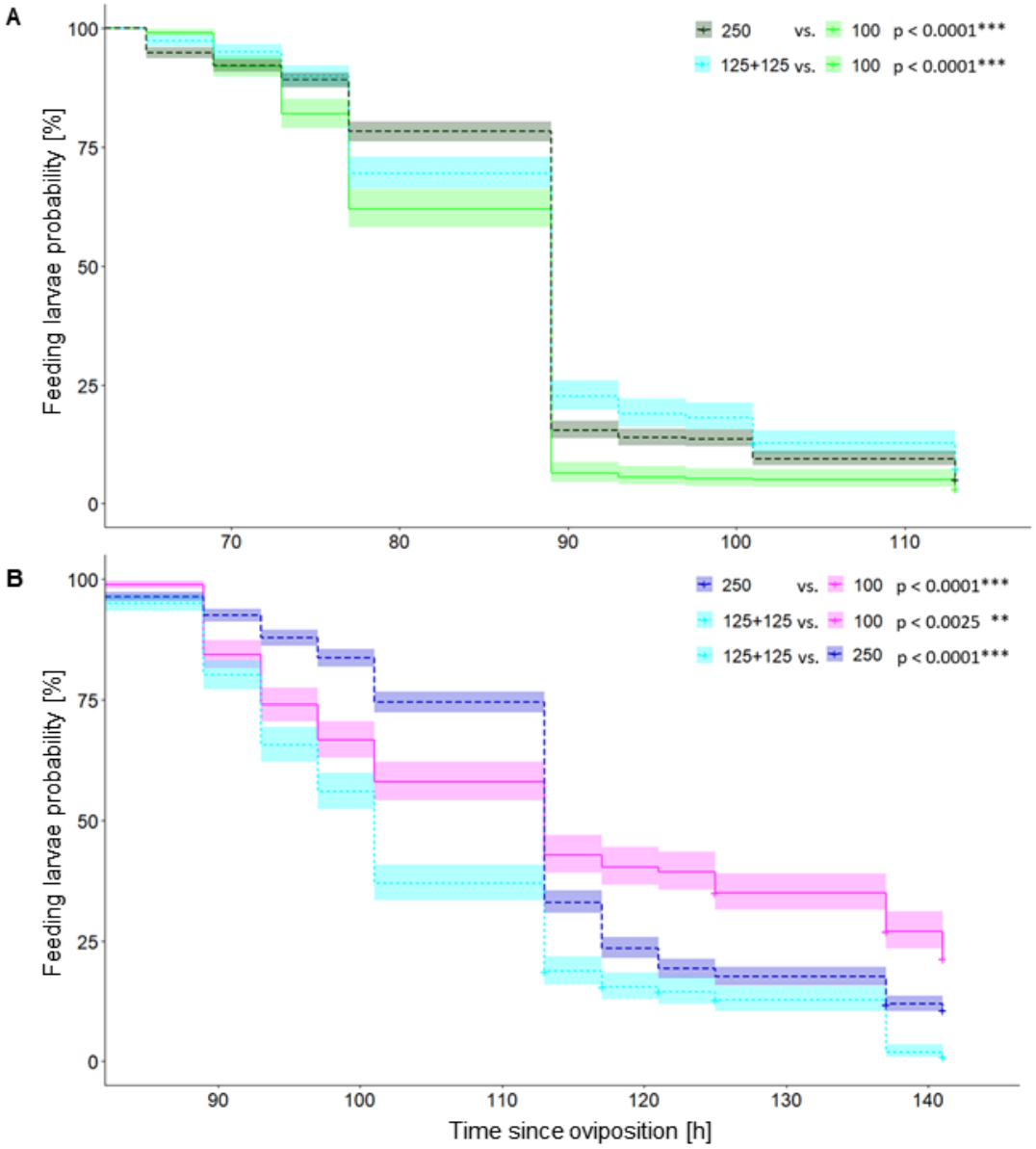


Figure 24 Survival curves of postfeeding larvae (part 1 at 28 °C)

The lower the curve of the feeding larvae probability is, the faster the larvae reached the next stage (*i.e.*, postfeeding). Standard deviations are given as light bars. Significance values were obtained from the Tarone-Ware and Tukey's HSD tests. **(A)** *L. sericata* at 28 °C: The low-density group (100) was faster than the high-density group (250). The heterospecific group (125+125) was first faster (before ~90 hrs postoviposition) and then slower than the conspecific high-density group. **(B)** *C. vicina* at 28 °C: The heterospecific group was the fastest. The low-density group was first faster (before ~110 hrs postoviposition) and then slower than the conspecific high-density group.

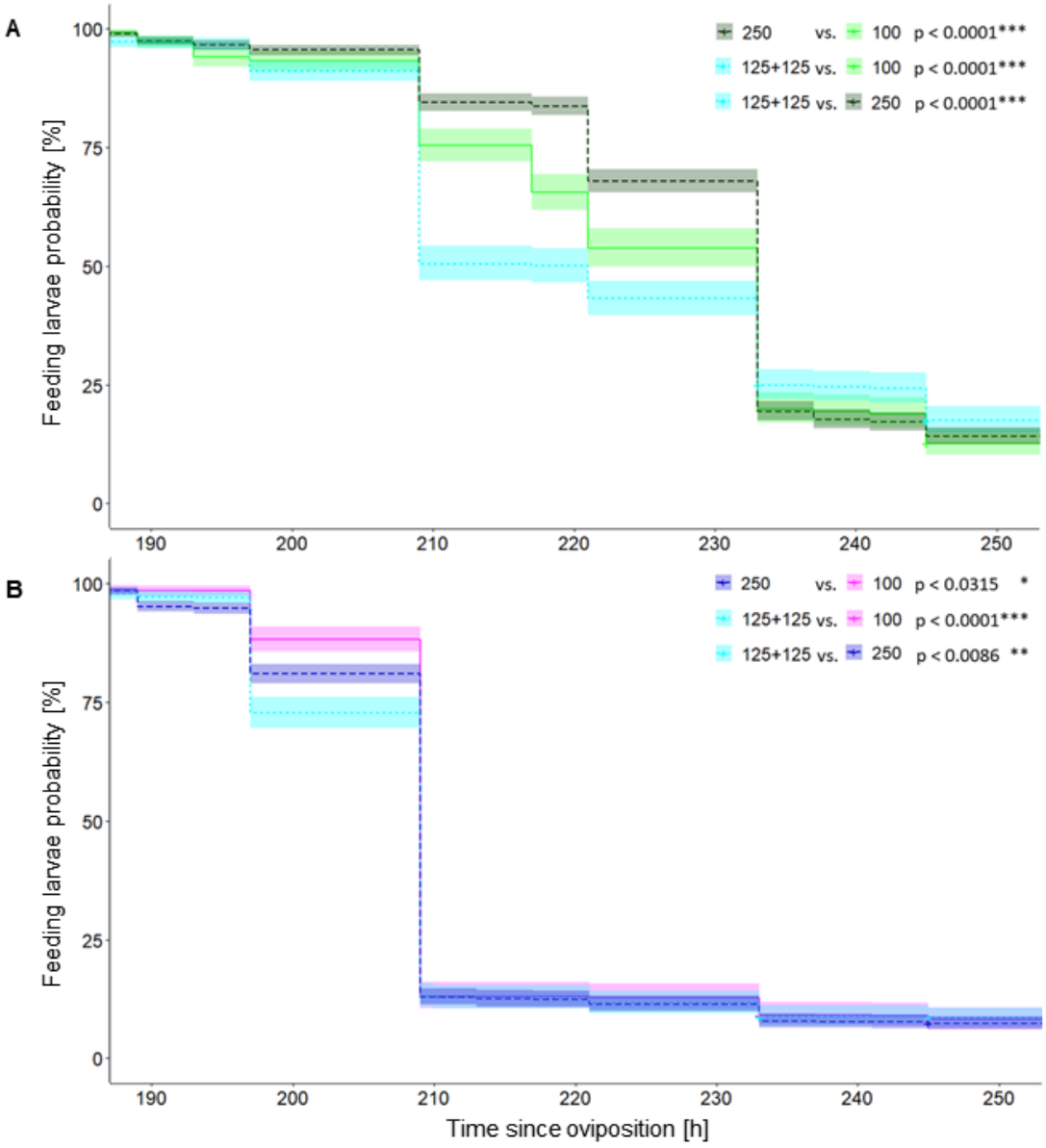


Figure 25 Survival curves of postfeeding larvae (part 2 at 15 °C)

(A) *L. sericata* at 15 °C: The low-density group was faster than the high-density group. The heterospecific group had both the fastest (before ~230 hrs postoviposition) and the slowest larvae. **(B)** *C. vicina* at 15 °C. The heterospecific group was the fastest, and the high-density group was slightly faster than the low-density group ~200 hrs after oviposition.

Concerning the effect of group composition at 28 °C, significantly earlier migration was detected for *C. vicina* in heterospecific groups (125+125 vs. 250 larvae; Figures 24 and 26)[17]. While more than 50% of the *C. vicina* larvae reached the postfeeding stage after 97 hrs (±10 hrs) in heterospecific conditions, at least 12 hrs more were needed for larvae in the equally dense conspecific group (Figure 26). All groups of *L. sericata* seem to reach the 50% threshold almost simultaneously, since this level was reached between 11 p.m. and 8 a.m., when no counting of postfeeding larvae occurred.

Finally, the following results were found at 15 °C: **in heterospecific conditions, at least 50% of the *L. sericata* larvae reached the postfeeding stage after 209 hrs (±15 hrs); at least 16 hrs more were needed for the conspecific group of the same size to reach this stage** (Figure 25)[18]. No differences between con- and heterospecific conditions were observed in *C. vicina* (Figure 26). Nevertheless, significant differences could be observed among all *C. vicina* groups, which were caused by the different migration starting times of the first 25% of all larvae[19].

[17] Tarone-Ware test: Chisq= 210, df = 2, $p < 0.0001$; Tukey HSD: $p_{high\text{-}hetero} < 0.0001$
[18] Tarone-Ware test: Chisq= 116, df = 2, $p < 0.0001$; Tukey HSD: $p_{high\text{-}hetero} < 0.0001$
[19] Tarone-Ware test: Chisq= 25.7, df = 2, $p < 0.0001$; Tukey HSD: $p_{high\text{-}hetero} = 0.0086$, $p_{low\text{-}hetero} < 0.0001$, $p_{low\text{-}high} = 0.0315$

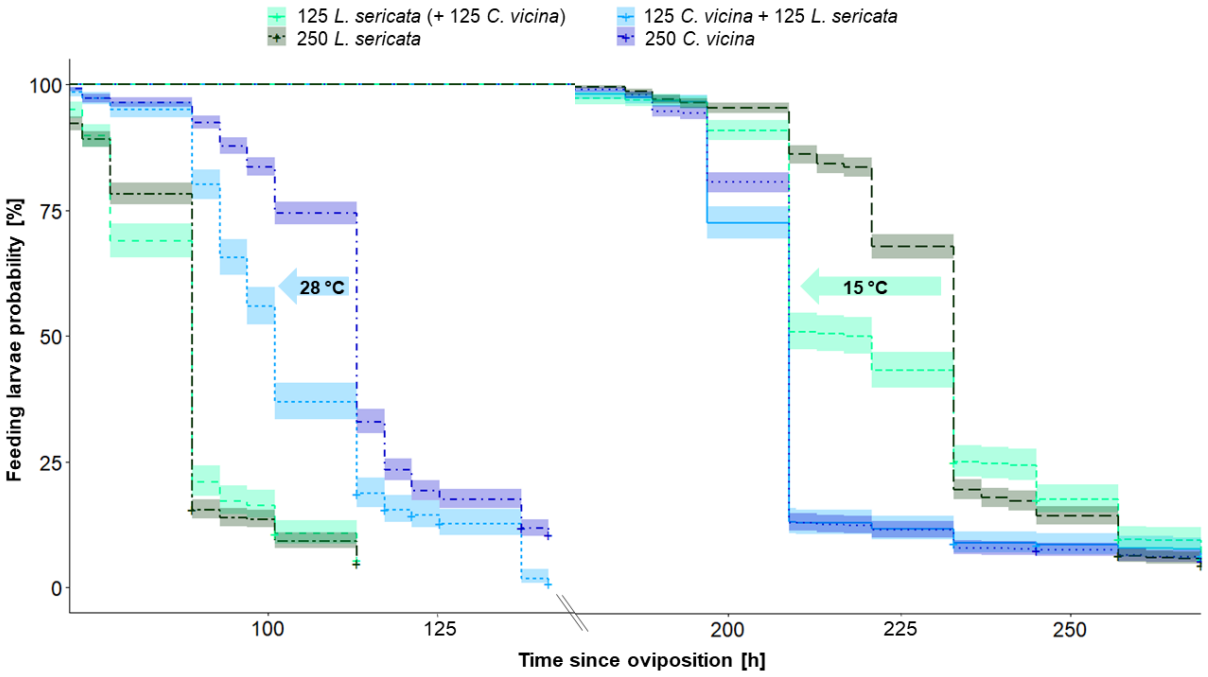


Figure 26 Development adaptation

Development speed converged to that of the other species by migrating earlier from the food source (arrow). Earlier migration in heterospecific than in conspecific groups was observed for *C. vicina* at 28 °C (approximately 12 hrs, left development curves) and for *L. sericata* at 15 °C (approximately 24 hrs), while the respective other species had comparable migration times. For all conditions, the slowest individuals migrated almost simultaneously. Standard deviations are given as light bars.

Summary

- Puparia surface areas of *C. vicina* decreased significantly and those of *L. sericata* increased slightly comparing 15 with 28 °C.
- *Calliphora vicina* benefits from higher survival rates in both high-density groups at 28 °C.
- The development rate of the slower species adapts to that of the faster one.

Balance between feeding and postfeeding time

Since most biotic and abiotic parameters (*e.g.*, food type or larval density) first impact feeding larvae, differences between the development rates during the feeding and postfeeding (including intra-puparial) phases might exist. Based on the presumption that faster growth leads to lower survival rates (Sinervo and Doughty, 1996; Fox, 1997; Richner, 1992; Arendt, 1997; Munch and Conover, 2003), I hypothesised that a blowfly can compensate for a fast development during the first phase with a slower second phase. First, the average development rates of both phases were compared at group level between the different conditions (*i.e.*, high- and low-density as well as conspecific and heterospecific groups). Second, the development rate of each larva before and after migration was compared at individual level.

To compare the average development rates between different group conditions, the conspecific high-density group was used as the control group (see Tables 11 and 12 in appendix for proportions of eclosed flies). In doing so, **the heterospecific group of *C. vicina* displayed a 12 hrs faster development in the feeding phase and a 12 hrs slower development in the postfeeding phase**, resulting in the same total development time than the control group. In contrast, the low-density group developed 6 hrs slower during the feeding phase but 18 hrs faster after larval migration; a huge catching-up resulting in a shorter total development time (Figure 27).

Figure 27 Effects of group size and composition on the development duration of *C. vicina*
Using the conspecific high-density group as control group, the heterospecific group displayed a 12 hrs faster and the low-density group a 6 hrs slower larval development until migration. During the postfeeding (including intra-puparial) phase this ratio was reversed, *i.e.*, the heterospecific group developed 12 hrs slower and the low-density group 18 hrs faster than the high-density group. Each development event (postfeeding and eclosion) is considered to have been reached when it was achieved by the first 10% of individuals.

A direct comparison of each development rate before and after larval migration confirmed that individuals with a slow development during the feeding phase developed faster in the later phases (*i.e.*, between postfeeding and eclosion) (Figure 28). However, slow larval development (*i.e.*, a rate > 1.05) was only rarely observed in high density aggregations. In contrast, **if the development during the feeding phase was faster than the median, the individual experienced a comparatively slower development in the subsequent phases.** This was especially true for larvae of the heterospecific group: no individual in the heterospecific group developed slower than the median during the feeding phase. But many individuals of this group showed slow postfeeding development (Figure 28, upper left quarter). A few exceptions to the catching-up concept were shown by individuals with an average larval development rate (*i.e.*, 1.0), but a slow development in the postfeeding phases (*i.e.*, between 1.05 and 1.2). Individuals with a slow feeding and postfeeding development did not exist (but would be found in the upper right quarter of Figure 28).

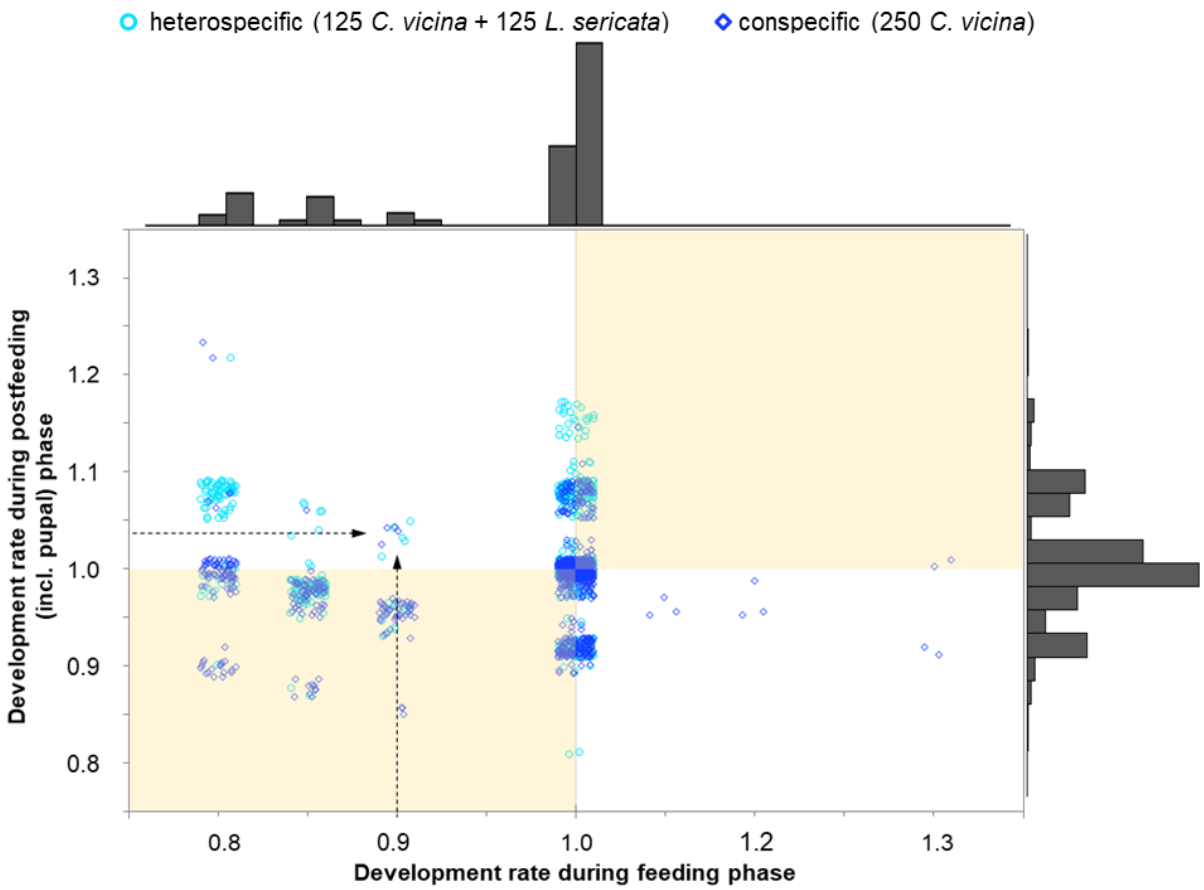


Figure 28 Trade-off between migration and eclosion time of *C. vicina*

Development rates during the feeding and postfeeding (including intra-puparial) phases of individuals in heterospecific (turquoise) and conspecific groups (blue). The middle of this graph (lower light circle) captures those individuals that grow at median values during both development phases. The upper circle represents individuals that have an average larval development rate but a longer postfeeding and intra-puparial phase. Clusters exist due to observation times. As an example, arrows point to a few individuals, with a fast larval development rate during the feeding phase and a slower rate during the postfeeding phase.

Interestingly, such catching-up effects were not observed for *L. sericata* at the group level (Figure 29). Instead, the average of the heterospecific and low-density groups developed in both development sections slower than the control group. Specifically, ***L. sericata*** **reached the postfeeding phase in the heterospecific and low-density group on average 12 hrs later than in the control group.** In contrast to *C. vicina*, no accelerated development followed until eclosion, but the time lag either remained constant (heterospecific) or increased further to 18 hrs (low-density group). At the individual level, many larvae indeed experienced a slow development time before and after migration in heterospecific conditions (Figure 30, upper right quarter). However, some *L. sericata* also showed a catching-up effect, but with significantly more slow larvae during both phases than *C. vicina*.

Figure 29 Effects of group size and composition on the development of *L. sericata*
Using the conspecific high-density group as control group, both the heterospecific and the low-density group showed a 12 hrs slower larval development until migration. During the postfeeding (including intra-puparial) phase, this time lag was maintained until eclosion in the heterospecific group and increased to 18 hrs in the low-density group (data of conspecific groups from Scanvion *et al.* (2018), 25°C). Each developmental event is considered to have been reached when it was achieved by the first 10% of individuals.

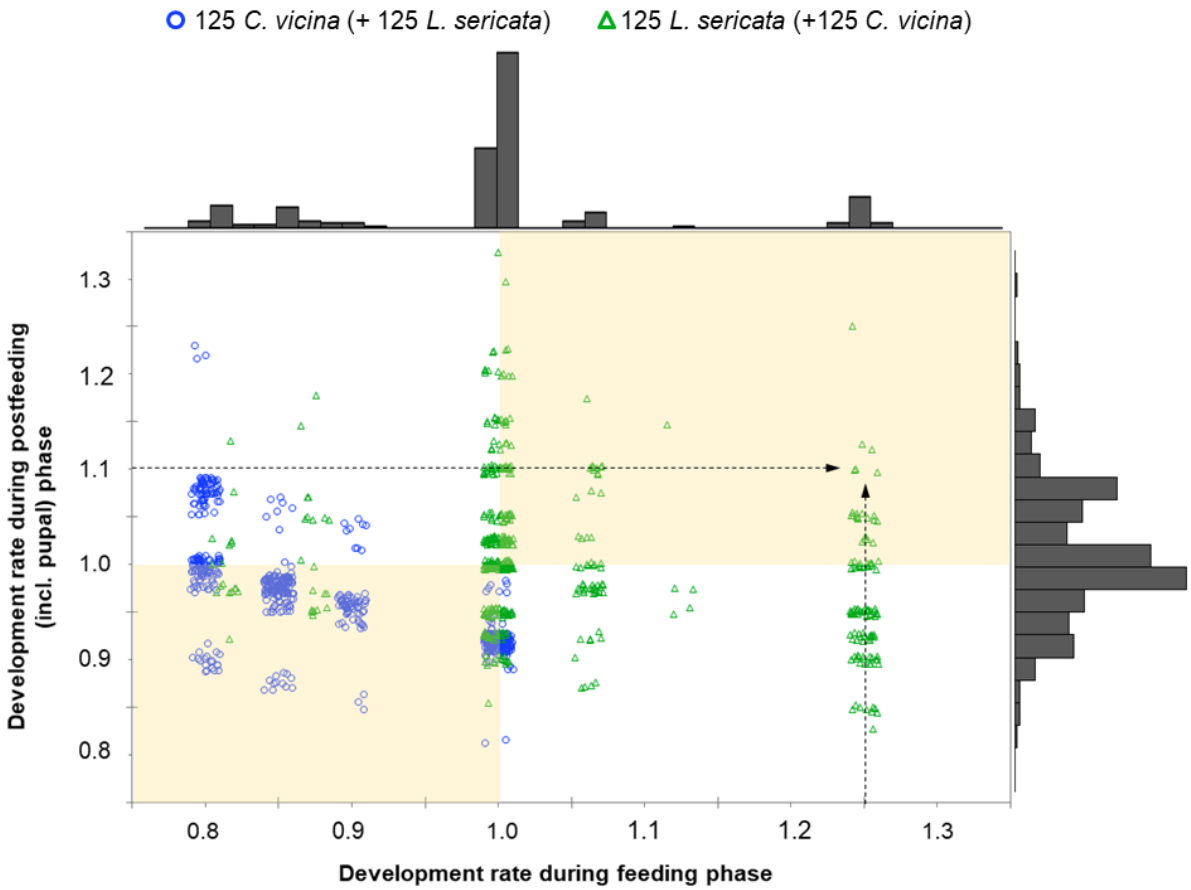


Figure 30 Trade-off in heterospecific aggregations

Displayed are development rates during the feeding and postfeeding (including intra-puparial) phases of individuals in heterospecific groups with *C. vicina* and *L. sericata*. Those with a value of 1.0 on the x-axis grow at median values during the feeding phase. Many *L. sericata* larvae show an average larval development rate but a longer postfeeding and intra-puparial phase. Clusters exist due to observation times. As an example, arrows point to a few *L. sericata* with a slow larval development rate during the feeding phase and postfeeding phase.

Summary

- *Calliphora vicina* individuals with a fast larval development during the feeding phase developed slower in the later development phases.
- Only a view *L. sericata* individuals showed a catching-up effect.

Costs of fast development

In the previous sections, differences in development rate and final surface area have been described between individuals. This plasticity exists not only between different developmental conditions, but also under constant biotic and abiotic parameters (*i.e.*, constant temperature, food availability, larval density, *etc.*) (Gosselin *et al.*, 2010; Wall *et al.*, 2001; Buser *et al.*, 2014). In this context, I hypothesized trade-offs between fitness-related traits (*e.g.*, between adult weight and larval development rate). Such a developmental strategy has already been described for the extensively examined fly *Drosophila melanogaster* (Nunney, 1996). This section focuses first on the carrion fly *L. sericata* and then on *C. vicina*, both reared at cold and hot temperatures, searching for a pattern linking development rate with size and mortality.

Most of the larvae developed successfully, with 92.7 ±11% of all *L. sericata* postfeeding larvae reaching pupal instar at 28 °C and 94.4 ±6% at 15 °C. Flies eclosed from 94.7 ±6% of all *L. sericata* puparia at 28 °C and from 98.1 ±1% in the experiments at 15 °C. **Regarding the puparia surface area, high variability was observed between the different growth conditions.** For example, significant differences existed between the conspecific groups at 15 °C (Figure 31)[20]. An interaction effect between condition and temperature existed in the comparison with puparia surface area but not with the development rate[21]. Individuals from the conspecific low-density and heterospecific group showed significantly different puparia surface areas between 15 and 28 °C[22]. Finally, **substantial variations within larvae bred under the same conditions were detected**. For example, the fastest larvae reached the postfeeding stage approximately twice as fast as the slowest individuals (Figures 32).

[20] Tukey's range test at 15 °C: n = 668, $p_{100\text{-}250} < 0.0001$
(Tukey's range test after two-way ANOVA with both temperatures, n = 1 879, $p_{250\text{-}125/125} = 0.0087$, $p_{100\text{-}125/125} = 0.0002$)
[21]Two-way ANOVA: puparia surface: n = 1 879, F = 11.031, p < 0.0001; development rate: n = 318, F = 1.801, p = 0.167
[22] Tukey's range test after two-way ANOVA, 100 larvae $p_{15\text{-}28°C} = 0.0154$; 125 larvae: $p_{15\text{-}28°C} = 0.0141$

Regarding size, the largest individuals were nearly three times the size of the smallest (Figure 31). Although the surface areas varied consistently between 15 and 40 mm^2 in all conditions, **no decisive connections were discovered when comparing puparia surface area to larval development rates** (Figure 33). In addition, there was no correlation between the development rate and the survival of larvae or pupae (Figures 34 and 35)[23]. However, a nonlinear correlation was detected for the experiments at 28 °C[24].

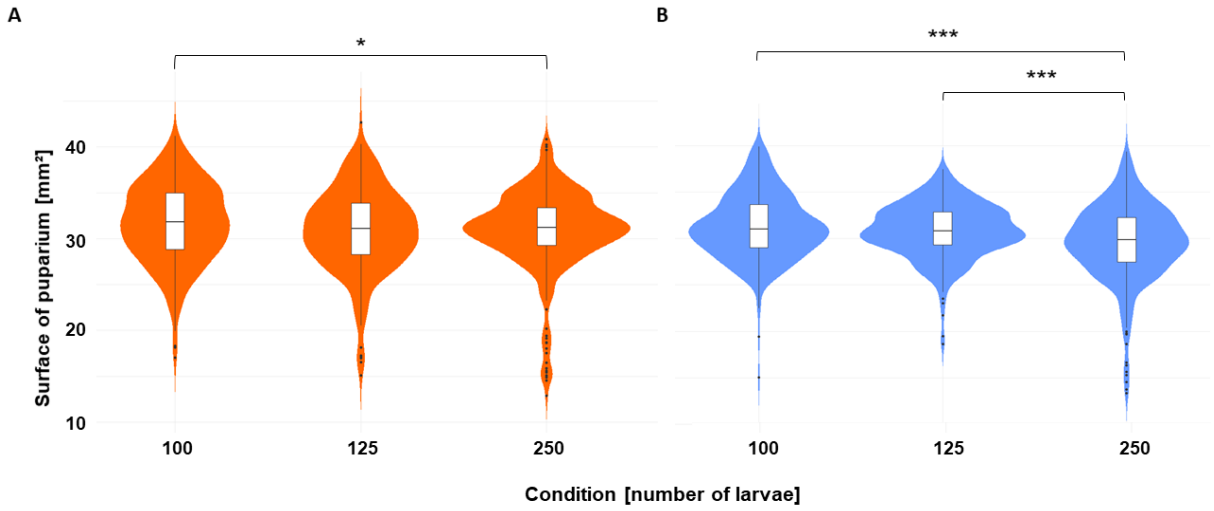


Figure 31 Size comparison of *L. sericata*

Surface of *L. sericata* puparia in mm^2 under different conditions (250 and 100 *L. sericata* larvae as well as 125 *L. sericata* mixed with 125 *C. vicina*) at 28 °C (A) and 15 °C (B). The coloured shape around each box plot (*i.e.*, the density trace) shows the distribution of observations. Significant differences were detected between the small and large conspecific group at 15 °C, between the heterospecific and small conspecific groups at 28 °C and between the heterospecific and large conspecific groups at both temperatures (ANOVA 28 °C: n = 925, F = 3.624, p = 0.027; Tukey's range test: 100 vs. 250: n = 654, p = **0.039**; 125 vs. 100: n = 584, p = 0.075; 125 vs. 250: n = 612, p = 0.991; ANOVA 15 °C: n = 954, F = 19.63 p < 0.0001; Tukey's range test: 100 vs. 250: n = 668, p < **0.0001**; 125 vs. 100: n = 596, p = 0.298; 125 vs. 250: n = 644, p < **0.0001**).

[23] Pearson correlation: survival pupae (15 °C): t = -0.017, df = 194, p = 0.987, r = -0.001, power = 0.050; survival pupae (28 °C): t = -0.936, df = 120, p = 0.351, r = -0.085, power = 0.154; survival flies (15 °C): t = 0.377, df = 194, p = 0.706, r = 0.027, power = 0.066; survival flies (28 °C): t = -0.091, df = 120, p = 0.928, r = -0.008, power = 0.051

[24] Pearson correlation: t = 7.279, df = 923, p < 0.0001, r = 0.233, power = 0.999

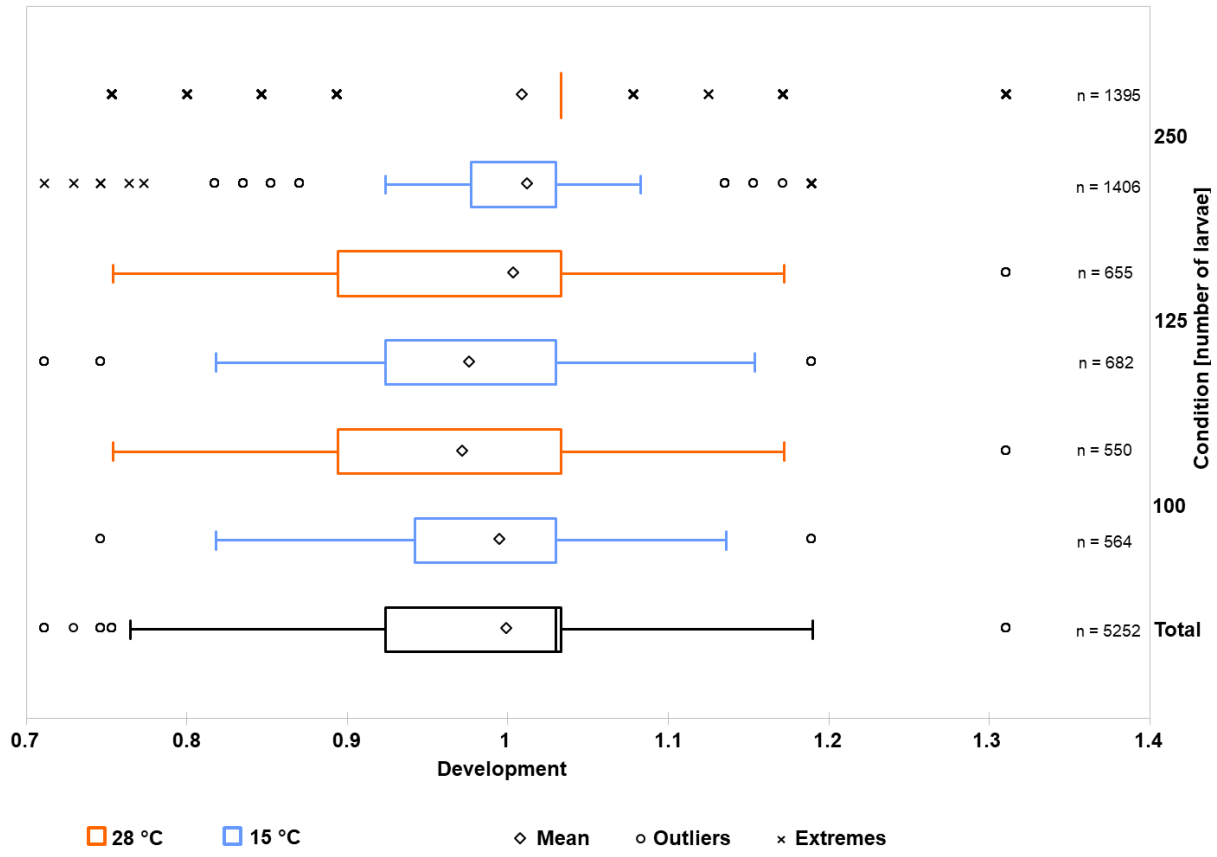


Figure 32 Comparison of development rate of *L. sericata*

Larval development rate according to condition (250 and 100 larvae in conspecific conditions as well as 125 larvae of each species in heterospecific conditions). A value of 1 on the x-axis represents the mean development rate at a given temperature (see Equation 1). Individuals with a development value under 1 were quicker than average, and those above 1 were slower. The majority of larvae in the 250 group at 28 °C showed a development rate of 1.033. The upper quartile was very small in all conditions. While at 28 °C only slight to no differences between the conditions were found, significant differences could be observed at 15 °C (ANOVA 15 °C: n = 2652, F = 50.8 p < 0.0001; Tukey's range test: 100 vs. 250: n = 1970, p < **0.0001**; 125 vs. 100: n = 1246, p < **0.0001**; 125 vs. 250: n = 2088, p < **0.0001**; ANOVA 28 °C: n = 2600, F = 22.32, p < 0.0001; Tukey's range test: 100 vs. 250: n = 1945, p < **0.0001**; 125 vs. 100: n = 1205, p < **0.0001**; 125 vs. 250: n = 2050, p = 0.565).

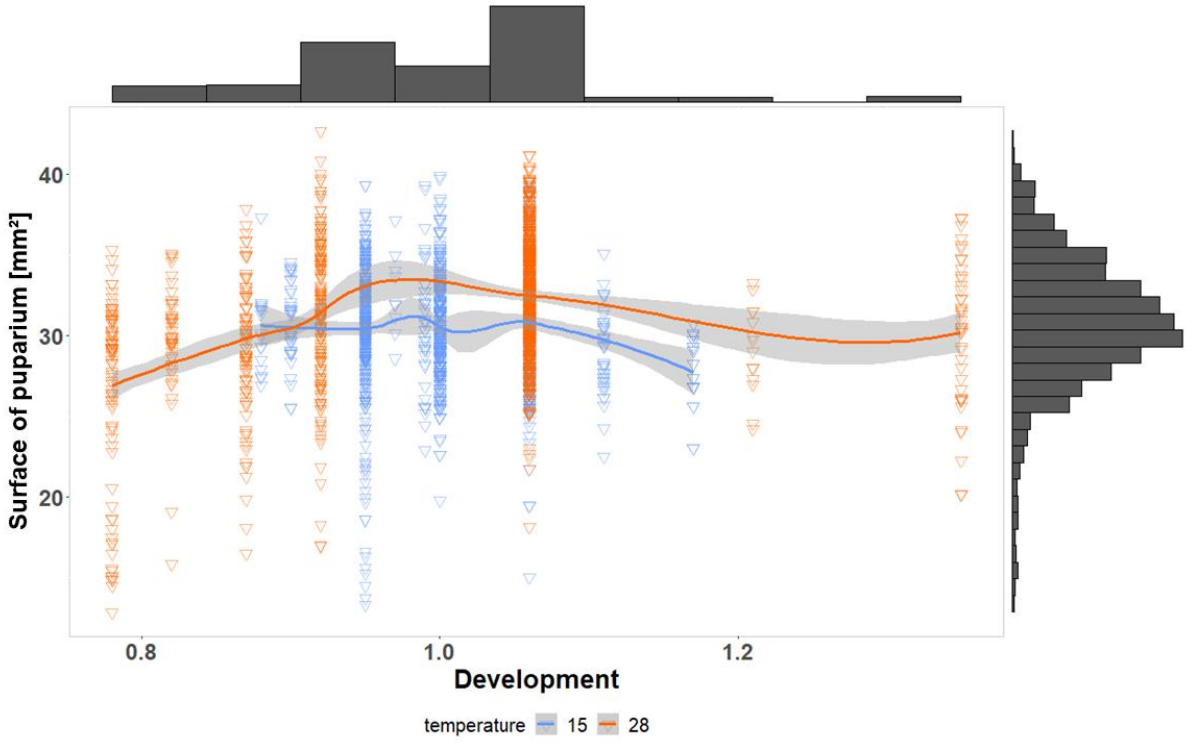


Figure 33 Correlation between size and development rate of *L. sericata*

Correlation between puparia surface area and development rate (see Equation 1) for both temperatures (15 °C in blue, 28 °C in orange). Each spot represents one individual; all developmental conditions are displayed together. A loess smoothed fit curve with a confidence region was added for both temperatures. The histograms represent the distributions of the variables on both axes. Small *L. sericata* appeared at all development rates (Pearson correlation: at 15 °C: n = 2652, t = -0.833, df = 952, p = 0.405; at 28 °C: n = 2600, t = 7.279, df = 923, p < **0.0001**, r = 0.233). Only a very slight tendency for smaller individuals having extremely fast development (<0.8) was detected.

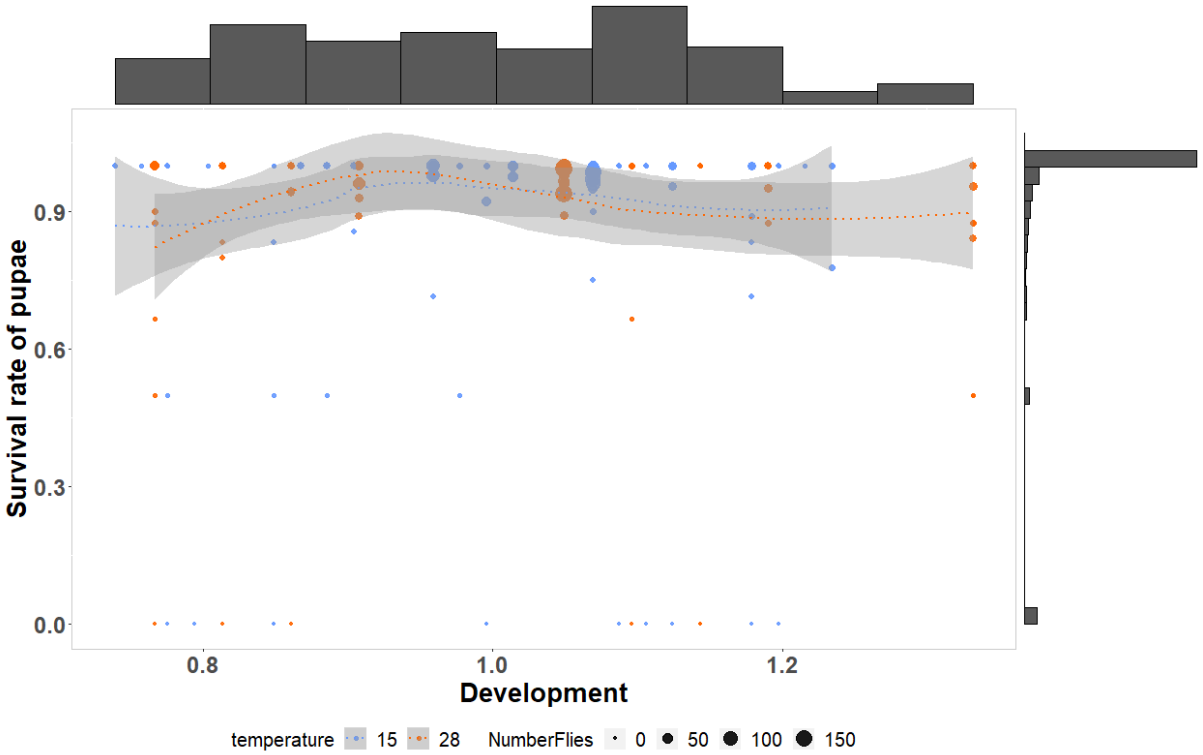


Figure 34 Correlation between pupal survival and development rate of *L. sericata*

Survival of pupae (*i.e.*, the number of successfully eclosed flies) according to development rate (see Equation 1) for both temperatures (15 °C in blue, 28 °C in orange). The surface of the bubbles represents the actual number of eclosed flies. A loess smoothed fit curve with a confidence region was added for both temperatures. The histograms represent the distributions of the variables on the x- and y-axes. Low survival rates appeared sporadically at all development times. No correlation was detected between development and survival rate (Pearson correlation: at 15 °C: n = 2652, t = 0.377, df = 194, p = 0.706, r = 0.027; at 28 °C: n = 2600, t = -0.091, df = 120, p = 0.928, r = -0.008).

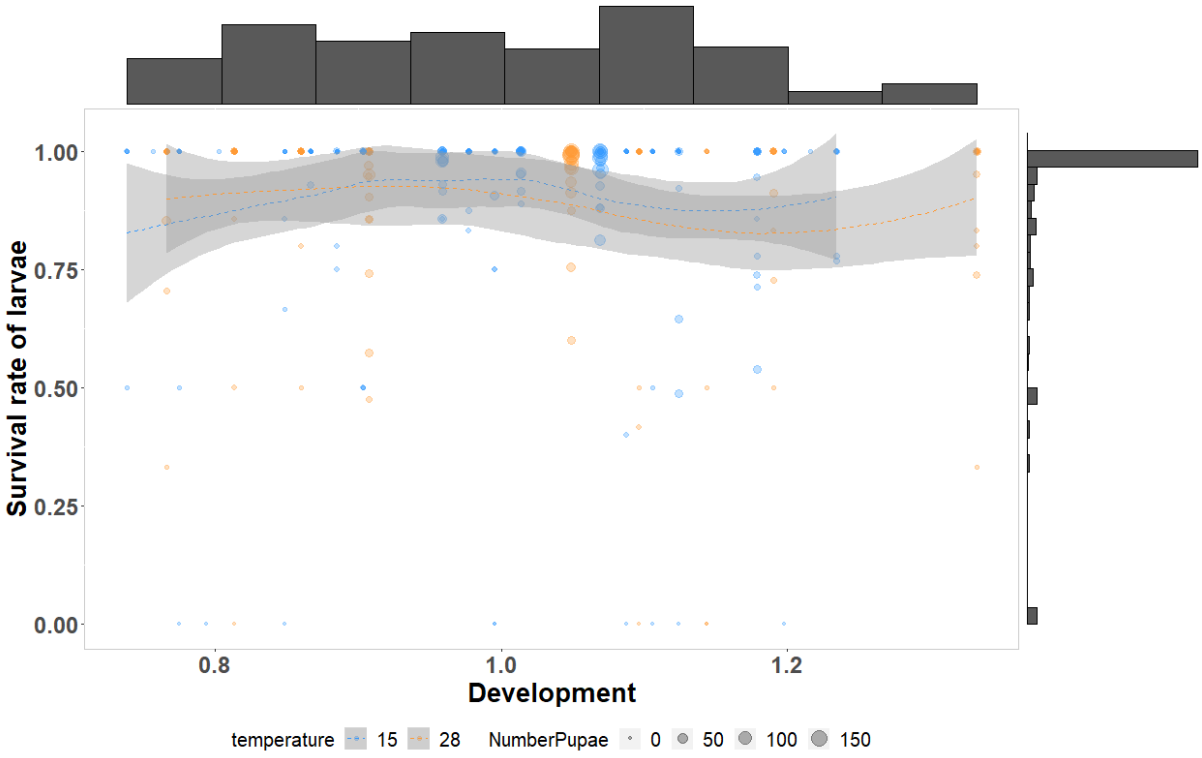


Figure 35 Correlation between larval survival and development rate of *L. sericata*

Survival rate of *L. sericata* (*i.e.*, number of successfully pupated larvae) according to development rate (see Equation 1) for both temperatures (15 °C in light blue, 28 °C in light orange). The surface of the bubbles represents the actual number of eclosed flies. A loess smoothed fit curve with a confidence region was added for both temperatures. The histograms represent the distributions of the variables on the x- and y-axes. No correlation was detected between the development and survival rates of the larvae (Pearson correlation at 15 °C: n = 2652, t = -0.017, df = 194, p = 0.987, r = -0.001; at 28 °C: 2600, t = -0.936, df = 120, p = 0.351, r = -0.085).

Calliphora vicina also successfully reached the pupal instar at both temperatures: 99.4 ±1% of the postfeeding larvae at 28 °C and 99.4 ±6% at 15 °C. Flies eclosed from 91.6 ±4% of all *C. vicina* puparia at 28 °C but only from 83.2 ±7% at 15 °C. **A significant correlation between development speed and survival rate was detected at 15 °C** (Figures 36 and 37)[25]. Comparatively low survival rates were observed at both temperatures for individuals with extremely slow or fast larval development. For example, at 28 °C, a pupal survival rate below 80% was more frequently observed for development rates below 0.7 or above 1.19 (Figure 36). However, survival rates of less than 80% were occasionally observed, even at development rates between 0.8 and 1. **A significant correlation**[26] **was discovered between puparia surface area and development speed at 15 °C but not at 28 °C**. Nevertheless, *C. vicina* with a very fast development were also at 28 °C often smaller (Figure 38). For example, larvae with development rates under 0.8 showed a different puparia surface area from the majority.

[25] Pearson correlation: at 15 °C: pupal survival: n = 2635, p = 0.0002, r = 0.36; larval survival: n = 2635, p = 0.010, r = 0.226; at 28 °C: pupal survival: n = 2413, p = 0.870, r = -0.013; larval survival: n = 2413, p = 0.299, r = 0.080

[26] Pearson correlation: at 15 °C: n = 327, p = 0.018 r = 0.076; at 28 °C: n = 282, p = 0.601, r = -0.016

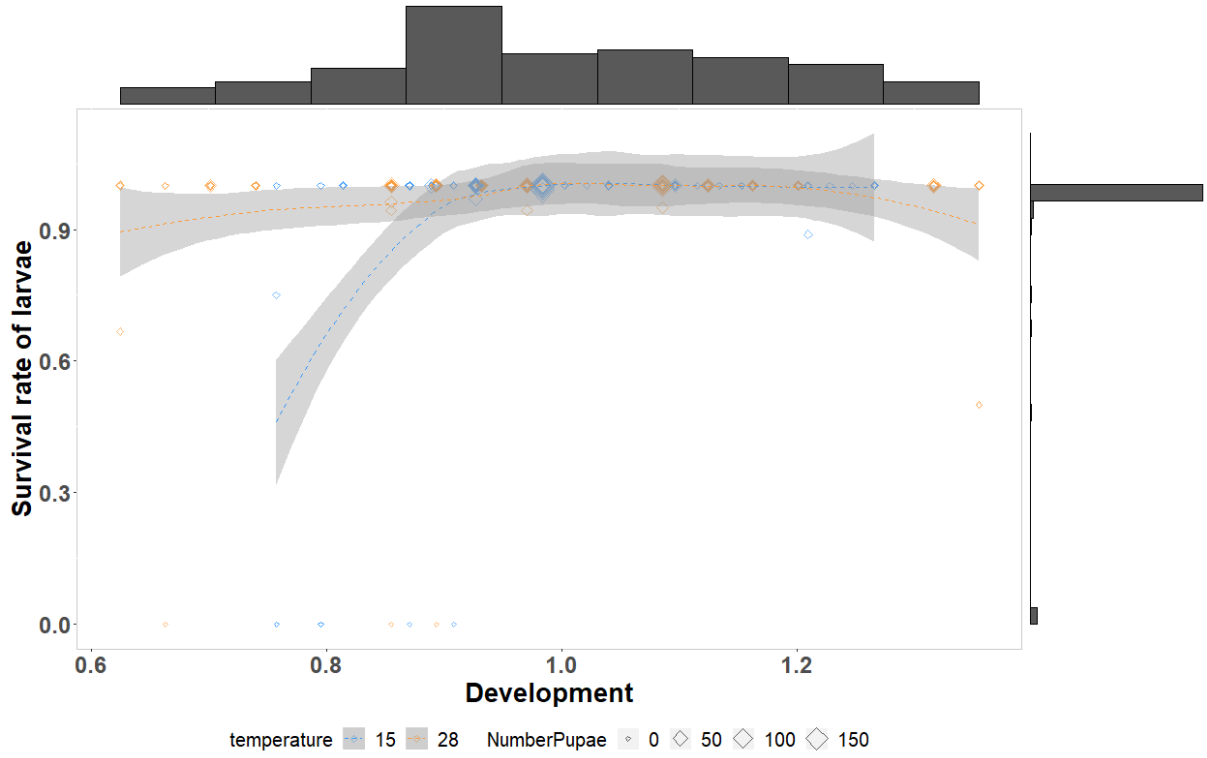


Figure 36 Correlation between larval survival and development rate of *C. vicina*

Survival rate of *C. vicina* larvae (*i.e.*, number of successfully pupated larvae) according to development rate (see Equation 1) for both temperatures (15 °C in light blue, 28 °C in light orange). The surface of the bubbles represents the actual number of eclosed flies. A loess smoothed fit curve with a confidence region was added for both temperatures. The histograms represent the distributions of the variables on the x- and y-axes. The survival rates at 15 °C showed a significant but very light linear correlation with the development rates (Pearson correlation at 15 °C: n = 2635, t = 3.897, df = 128, p = **0.0002**, r =0.36; at 28 °C: n = 2413, t = 1.042, df = 167, p = 0.299, r = 0.080). Note that for these experiments, *C. vicina* developed under the same conditions as *L. sericata* described in the main text (*Materials and methods*).

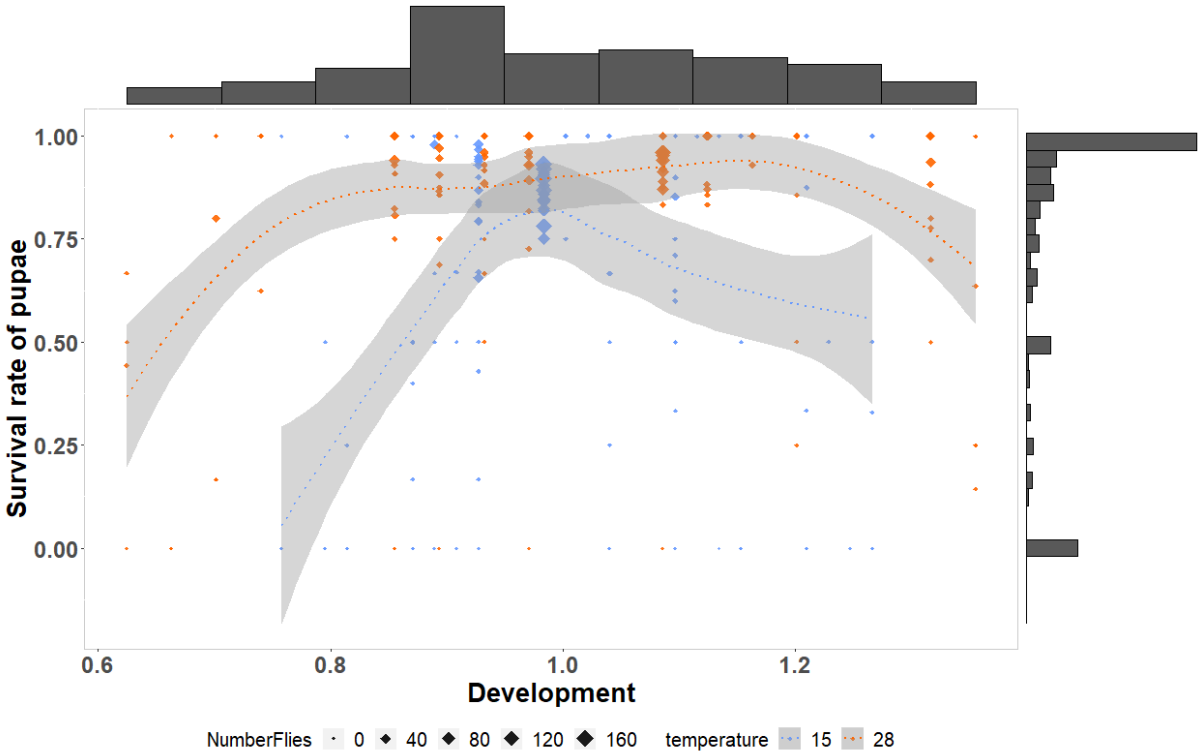


Figure 37 Correlation between pupal survival and development rate of *C. vicina*

Survival of *C. vicina* pupae (*i.e.*, the number of successfully eclosed flies) according to development rate (see Equation 1) for both temperatures (15 °C in blue, 28 °C in orange). The surface of the bubbles represents the actual number of eclosed flies. A loess smoothed fit curve with a confidence region was added for both temperatures. The histograms represent the distributions of the variables on the x- and y-axes. The survival rate lies at all development rates below 50%. However, especially at 15 °C, very quick development was associated with slightly higher mortality (Pearson correlation: at 15 °C: n = 2635, t = 2.624, df = 128, p = **0.010**, r = 0.226; at 28 °C: n = 2413, t = -0.164, df = 158, p = 0.870, r = -0.013). Although *C. vicina* prefers lower temperatures than *L. sericata*, its survival rate decreased drastically with fast development at this temperature. Note that for these experiments, *C. vicina* developed under the same conditions as *L. sericata* described above (*Materials and methods*).

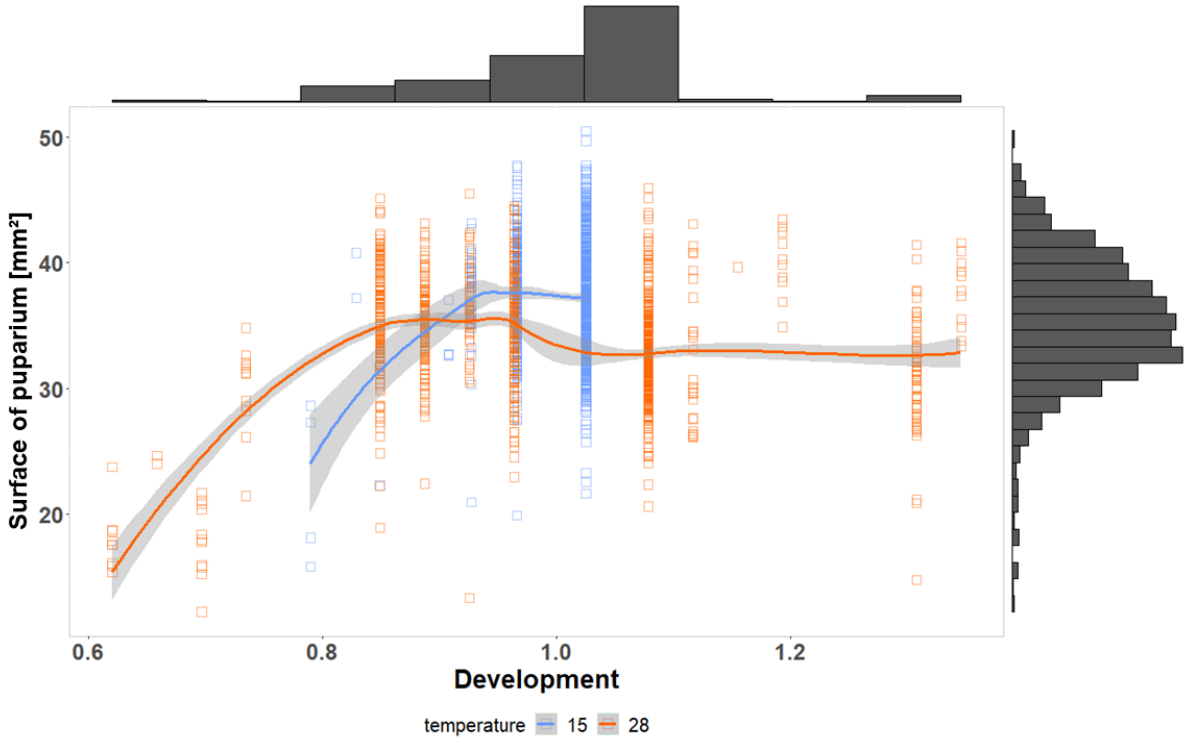


Figure 38 Correlation between size and development rate of *C. vicina*

Correlation between *C. vicina* puparia surface area and development rate (see Equation 1) for both temperatures (15 °C in blue, 28 °C in orange). Each spot represents one individual; all developmental conditions are displayed together. A loess smoothed fit curve with a confidence region was added for both temperatures. The histograms represent the distributions of the variables on the x- and y-axes. Small *C. vicina* (*i.e.*, < 25 mm^2) were found with very quick development rates (< 0.7). However, only a very slight tendency for smaller individuals to have extremely fast development (<0.8) could be detected (Pearson correlation: at 15 °C: n = 327, t = 2.370, df = 978, p = **0.018** r = 0.076; at 28 °C: n = 282, t = -0.523, df = 1041, p = 0.601, r = -0.016).

Summary

- Development speed of *L. sericata* was not correlated with size or survival.
- An interaction effect between condition and temperature existed for *L. sericata* puparia surface areas but not for development rates.
- Development speed of *C. vicina* was significantly correlated with size and survival at 15 °C.

3.3 Discussion

Following hypotheses were analysed in this thesis:

- Heterospecific aggregations bring benefits that conspecific aggregations cannot offer.
- Benefits vary with blowfly species and temperature.

  → *L. sericata* benefits from an association with *C. vicina* at low temperatures.

  → *C. vicina* benefits from this association at high temperatures.
- A trade-off between different fitness-related traits exists at cold and hot temperatures.

  → Fast larval development leads to smaller pupae and higher mortality rates.
- A fast larval development can be compensated with a slower postfeeding phase.

Self-critique

Decisive factors in the fitness of animals are the reproductive rate and the indirect influence on the development of the next generation (Ariew, 2004). However, the presented experiments have not tested this kind of fitness-related traits, because tests for next-generation effects are very time-consuming and were with the limited time and breeding space not feasible for this PhD thesis.

Another critique concerns the comparability of laboratory experiments with life in nature (Boice, 1981). For example, larvae in laboratories do not face predation pressure like necrophagous larvae in their natural environment do. Predators are a crucial factor for these larvae in balancing costs and benefits in aggregations and their population density (Courchamp *et al.*, 2009; Rivers *et al.*, 2011). If predation pressure is eliminated in the laboratory, competition for food and space will prevail. Thus, greater benefits than those measured in the present studies may exist in the field. In general, the experimental setup provided excellent larval development conditions without parasites or predators, under constant temperatures, with neither rain nor sun, and with fresh and easily edible food.

Indeed, fresh minced steaks were used in the present studies, which only begin to decompose after a few days and are easier and faster to digest than intact muscle tissues (Bernhardt *et al.*, 2017). Thus, this unrealistic but easy-to-implement food medium impedes the detection of mutualisms and interkingdom associations as described before. For this reason, the obtained results can only provide potential insights into larval development in an intensely competitive environment (Tomberlin *et al.*, 2015).

Lastly, fluctuating ambient temperatures, which are common in nature, could lead to different results. In fact, alternately cold and warm temperatures have clearly different effects on larvae, pupae and adults than the mean of these temperatures, even if the accumulated degree hours throughout a day are identical (*i.e.*, the product of the number of heating units and the time required for a specific development event to occur) (Hagstrum and Milliken, 1991; Greenberg and Kunich, 2002).

Nevertheless, laboratory studies are crucial as a starting point for further outdoor studies on carrion and for the awareness of the complexity of such an ecosystem. The preferences and advantages observed in this thesis should be even more apparent in field trials, where the ability of larval aggregations to change their microhabitat (*e.g.*, food texture, bacterial content, temperature, pH level) is even more important than on fresh minced steaks (Chotkowski and Ellen Marsden, 1999).

Benefits of aggregation

The results indicate that the two tested species benefited from growing in association with the other at 25 °C. *Calliphora vicina* reached the postfeeding stage significantly faster under heterospecific conditions without an effect on survival. In the case of *L. sericata*, this was expressed in a slightly higher larval survival rate (and a bigger size than in conspecific high-density groups). In addition, the development rate of *L. sericata* larvae showed a smaller variance in heterospecific groups, suggesting the disappearance of outsiders (*i.e.*, extremely slowly growing individuals; compare also Crooks *et al.* (2016)). However, this tendency could not be affirmed by the experiments at 15 and 28 °C. Instead, *L. sericata* and *C. vicina* larvae seem to live at 15 and 28 °C much more according to the common competition principle: the fewer individuals there are, the more resources each one has, and the better each individual will fare (Courchamp *et al.*, 2009). This negative density dependence applies, although sufficient food was available to each larva in all groups. One definition of the demographic Allee effect specifies that at least one of the individual fitness components (and the per capita reproduction) should be positively related to group size (Courchamp *et al.*, 2009). In the experiments presented here, this crucial component may not have been tested (*e.g.*, reproductive rate, next-generation fitness or robustness to fluctuating).

When blowfly larvae develop fast and quickly pupate in sheltered places, less time is spent on the carcass and the probabilities of food shortage, predation and parasitism decrease (Grassberger and Frank, 2004; Rivers *et al.*, 2011). Thus, a reduced feeding period as observed for *C. vicina* supposedly results in increased fitness. However, a reduced feed consumption can demonstrably lead to smaller animals. *Drosophila* flies (Prasad *et al.*, 2000; Chippindale *et al.*, 1997) and the water strider *Gerris buenoi* Kirkaldy, 1911 (Heteroptera: Gerridae) (Klingenberg and Spencer, 1997)), as well as fish (Henrich, 1988), birds (Galbraith, 1988) and even crop plants (Adams, 1967) demonstrated a negative correlation between

development rate and surface area. Nevertheless, *C. vicina* did not have to make a trade-off at 25 °C: larvae were faster in the heterospecific group, but not smaller or less successful (regarding survival) than in the conspecific high-density group. Since the heterospecific group of *L. sericata* benefited not from a faster development but from the other two fitness-related traits, benefits might be gained either in development speed or in surface area and survival rate. Moreover, these benefits were not associated with costs for the larvae in heterospecific (compared to the conspecific high-density) conditions at all temperatures (Table 13 in appendix); at least as far as this can be assessed at group level. Whether this observation also applies at the individual level is discussed by a direct comparison of development rate and the two other fitness-related traits of each larva (*Balance between feeding and postfeeding time*).

Mechanisms of mutualism

The developmental benefits observed for larvae bred in heterospecific groups may result from different mechanisms. One hypothesis is based on the excretion/secretion of various hydrolytic, proteolytic and lipolytic enzymes (Pendola and Greenberg, 1975; Terra and Ferreira, 1994). The sharing of such enzymes has been reported as a likely benefit of larval aggregation, and this idea is supported by recent studies (Wilson *et al.*, 2016). In 2018, Scanvion *et al.* observed an increase in survival and development rate with an increase in the number of aggregated *L. sericata* larvae and the addition of a trypsin-like enzyme. Therefore, they concluded that a pooling of exodigestive enzymes leads to faster development. In the present experiments, larvae of different species may have provided different species-specific enzymes, resulting in a better exodigestion. Hobson (1931) detected tryptases, peptidases and lipases in the midguts of *L. sericata*, but only found a weak activity of the carbohydrate-splitting enzyme amylase in the salivary glands. If another species were to produce amylase in a higher concentration, or even tryptases in the salivary glands, then aggregating with this species could be beneficial for *L. sericata*. In turn, *L. sericata* might produce more or different enzymes than *C. vicina*, resulting in the observed faster development of this species under heterospecific conditions (compared to both conspecific groups at the three tested temperatures, Table 13). However, this has not yet been explicitly confirmed (Pendola and Greenberg, 1975; Yakovlev *et al.*, 2019).

Another hypothesis, which does not necessarily exclude the first one, entails the sharing of antimicrobial compounds and the control of more microbe species that accumulate during decomposition (Barnes *et al.*, 2010; Crippen *et al.*, 2015). Barnes and colleagues (2010) demonstrated that necrophagous larvae show differences in their abilities to defend against various species of bacteria. While no differences were detected between the blowflies *L. sericata* and *C. vicina* (5 bacterial species tested), *L. sericata* was more effective in

controlling the bacterial species *Staphylococcus aureus* Rosenbach, 1884 and *Pseudomonas aeruginosa* Migula, 1900 (Barnes *et al.*, 2010). Consequently, *C. vicina* would be more efficient in reducing bacterial numbers when aggregating with *L. sericata*. Although the number of enzymes and antibiotics increase with the number of larvae, their diversity will only increase if different species simultaneously feed on the same spot. Therefore, a mix of several secretion cocktails in heterospecific aggregations should control a wider range of bacteria and suppress more microbial competitors (Shukla *et al.*, 2018; Rivers *et al.*, 2011).

Finally, a further hypothesis would be a beneficial extension of the species-specific temperature range in which larval development is possible (Aubernon *et al.*, 2016; Marchenko, 2001). Since the efficiency of enzymes, antibiotics and the excretion/secretion rate are temperature-dependent (Daniel and Danson, 2010; Atkinson and Vaughn, 2015), their benefits may differ between species according to the temperature. For example, enzymes of a species that is adapted to high temperatures (*e.g.*, *L. sericata*) might be more efficient through a higher catalytic activity at high temperatures (*e.g.*, 28 °C) than those enzymes of a cold-adapted species (Marchenko, 1988; Byrd and Castner, 2001; Ames and Turner, 2003; Hwang and Turner, 2009; Davies and Ratcliffe, 1994). Thus, *C. vicina* growing in a conspecific group at 28 °C might only have low-active enzymes at its proposal. Therefore, in heterospecific aggregations, *C. vicina* could benefit from *L. sericata*'s better heat-adapted enzymes and their increased catalytic activity at warm temperatures (Somero, 1978; Isaksen *et al.*, 2014). Organisms have indeed specialized in producing enzymes with thermal optima according to their habitation temperature (Gerday *et al.*, 1997; Wallenstein *et al.*, 2011), and modifications of the microenvironment are a common consequence of aggregations (Jordan and Tomberlin, 2017). In addition, sharing of different abilities is a known benefit of heterospecific groups (Lorenzo Figueiras and Lazzari, 1998). Indeed, differential capabilities within aggregated species can lead to fitness advantages, such as improved foraging efficiency, faster resource

acquisition and reduced predation risk (Semeniuk and Dill, 2006; Parrish and Edelstein-Keshet, 1999). For example, Forsman *et al.* (2002) evidenced earlier breeding, larger broods and heavier young in birds living in close coexistence with another bird species. Similarly, *Porcellio scaber* Latreille, 1804 woodlice most likely aggregate with *Armadillidium vulgare* Latreille, 1804 to better withstand low relative humidity and high ambient temperatures (Hassall *et al.*, 2005; Broly *et al.*, 2015). In conclusion, it is possible that the less adapted blowfly species does not compete with better-adapted species but rather benefit from them passively. This would be the first demonstration of accelerated development of the less adapted species in heterospecific aggregations due to *enzyme-sharing* and an extension of the species-specific temperature range (Riemann and Helmke, 2002; Damos and Savopoulou-Soultani, 2012).

Although no clear species-specific and temperature-dependent trend for benefits in heterospecific aggregations could be observed in the fitness-related traits tested (Table 6), the hypothesis that the development rate of the slower species adapts to that of the faster species was confirmed (Figures 26 and 39). Specifically, *C. vicina* larvae migrated earlier in heterospecific groups with *L. sericata* than in conspecific groups at 28 °C (Table 6, Figures 24 and 39). In addition, *C. vicina* moved at 28 °C not only earlier but also in higher numbers, resulting in a lower mortality rate than that in conspecific conditions. However, the smaller surface area of *C. vicina* at 28 °C compared with that at 15 °C confirms that this high temperature is at the outer edge of the acceptable range for this species (Donovan *et al.*, 2006).

At 15 °C, both species benefited from heterospecific associations, expressed by relatively fast development and large pupae. Interestingly, 15 °C represents not a temperature that would be voluntarily sought by *C. vicina* (preferred temperature ~22 °C) nor by *L. sericata*.

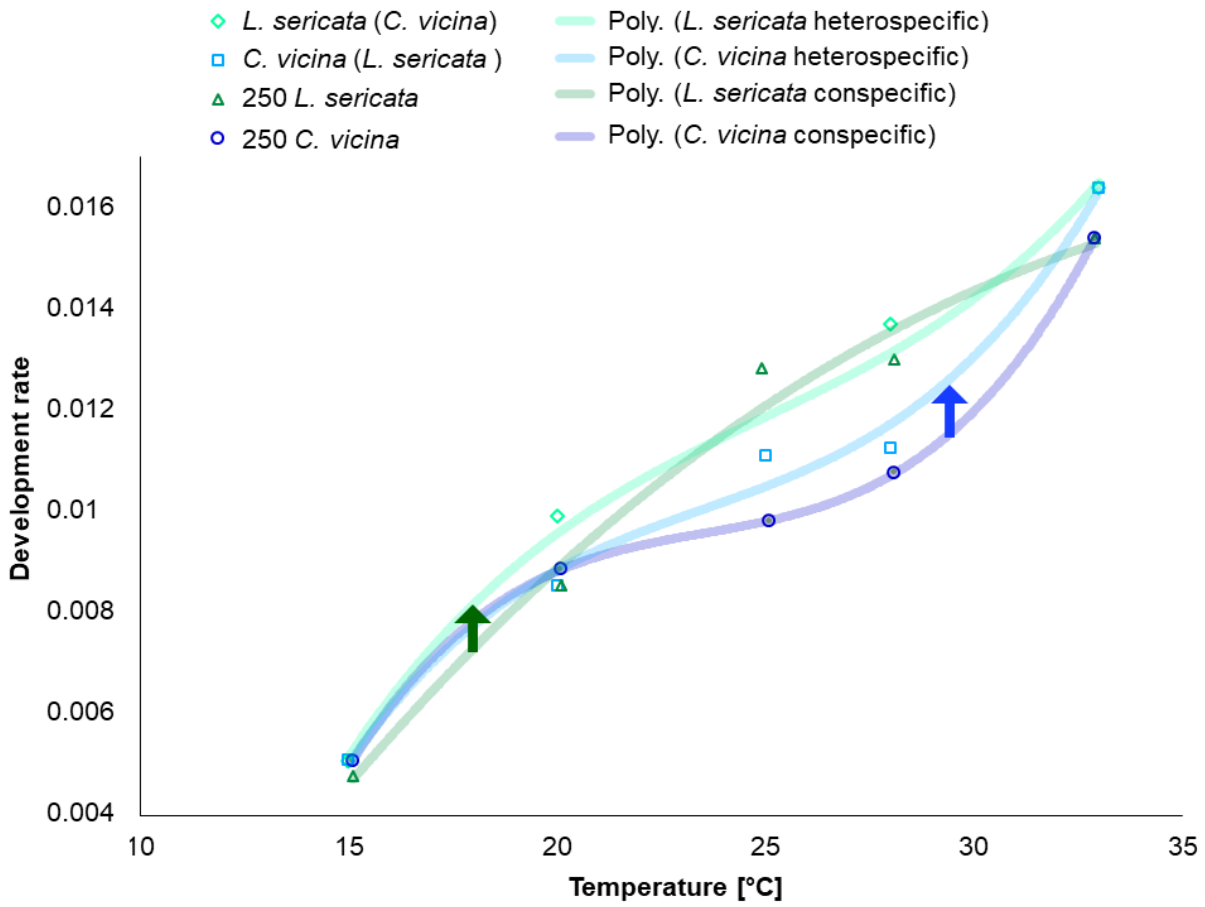


Figure 39 Adaptation of development rate in heterospecific aggregations

The cold-adapted species *C. vicina* developed faster in heterospecific groups with *L. sericata* than in conspecific groups at 25, 28 and 33 °C. The warm-adapted species *L. sericata* migrated earlier in heterospecific groups at 15 and 20 °C. Development rate refers to the time when the first 10% of larvae migrated in the pupariation medium.

In conclusion, larvae in heterospecific aggregations might benefit not only through qualitatively but also quantitatively better food digestion/ingestion than in conspecific aggregations. Thus, while more food components can be broken down by a greater diversity of enzymes, species-specific temperature-adaptation of enzymes would lead to higher excretion/secretion or catalytic rates. Both contribute to more efficient food intake and thus to faster growth.

Since temperature changes not only throughout the day but also over the year, the species that benefits most from such a collective association also changes (*i.e.*, cross-generational mutualism (Conner, 1995; Wright *et al.*, 2010)). This highlights that as also in blowfly larvae, there are complex trade-offs among fitness-related traits. The probably most decisive of these trade-offs will be analysed in the two following sections: development speed vs. surface area and mortality.

Balance between feeding and postfeeding time

In harsh environments, such as carrion, selection pressures act on the timing of larval migration and reproduction (Prasad *et al.*, 2001). Selection for faster development might derive, on the one hand, directly due to parasitism, predation pressure, *etc.*, and on the other hand, indirectly through feeding disturbances, food limitation, bacterial accumulation *etc.* (Prasad *et al.*, 2001; Arendt, 1997). While a slow development in the larval feeding phase of *L. sericata* was not always caught up later, *C. vicina* showed both types of balancing development duration. In the case of an initially fast development, *C. vicina* took longer for the subsequent development processes, and in the case of an initially slow development, individuals passed through the later development phases quicker. Thus, *C. vicina* was neither able to maintain an initially very fast development rate over the entire duration of its development, nor to sustain a permanently slow development. The latter reinforces the harshness of carrion, whose quality decreases with the decomposition process (Burkepile *et al.*, 2006). Conversely, the impossibility of a continuously fast development strengthens the assumption that fast development entails costs and risks that are only to a certain extent counterbalanced by the resulting benefits (Blanckenhorn, 1998). The shorter the feeding period, the less energy can be stored, and the fewer resources remain available for later phases (Khelifa *et al.*, 2019).

Instead of investing more resources in fast development, *C. vicina* could invest in size and biological functions (such as immunity) to increase their lifespan (Blanckenhorn, 1998). Newmann (1992) already assumed that adaptive plasticity develops when phenotypes with the highest fitness vary predictably with the environment. Whether developmental plasticity can be considered as costless in regard to survival and size is debated in the next section.

Costs of fast development

Large body size has been referred to as a beneficial trait when comparing between individuals and growth conditions (Nee *et al.*, 1991; Santos *et al.*, 1994; Miller *et al.*, 1988; Blanckenhorn *et al.*, 2018). However, the final adult size must be regarded as a trade-off between increased larval fitness-related traits and delayed reproduction due to prolonged larval periods (Sibly *et al.*, 1991). Furthermore, slow-growing larvae are exposed to resource depletion, parasites and predators for longer periods; in contrast, fast larval development quickly shelters individuals at their pupariation site, reducing the risks incurred by frail larvae and giving them a selective advantage (Brundage *et al.*, 2014; Greenberg, 1990; Roberts, 1933).

Some previous studies on Calliphoridae suggested that a critical weight was required to enter pupariation (Ribeiro and Zuben, 2010). However, the high survival rate of postfeeding larvae observed during the presented studies indicates that most individuals, even those with the fastest development (*i.e.*, early migration from the food source), achieved sufficient weight to complete their development. Thus, fast growth did not lead to premature wandering, poor larval condition, or increased mortality risk. In fact, neither the surface area nor the survival of *L. sericata* larvae or pupae decreased dramatically at a high larval development rate (C_i <1). In contrast, very slow development had no significant advantages in terms of surface area or survival rate. Although fast growth is certainly not the only way for necrophagous larvae to grow effectively (Ghosh *et al.*, 2013), it is probably the most pertinent one for larvae growing under harsh conditions. In brief, these data support the idea of a developmental plasticity that is divided into two levels for *L. sericata*. At the population level, larvae can grow faster with no impact on their fitness. At the individual level, some larvae can achieve their development faster than the mean without decreasing their surface area or survival chance.

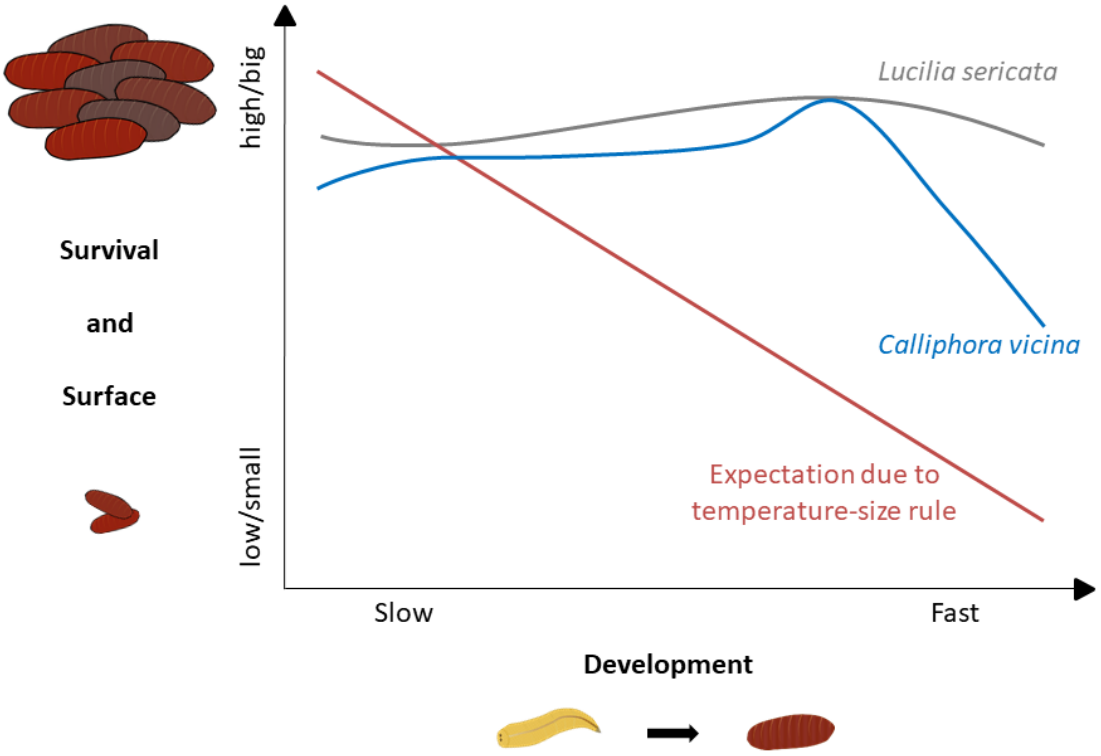


Figure 40 Trade-off between larval development speed and puparia surface or survival
While *L. sericata* (Diptera: Calliphoridae) showed no trade-offs between larval development speed and puparia surface area or survival at 15 and 28 °C, *C. vicina* was on average smaller with lower survival chances when its larval development was extremely fast. In contrast to *L. sericata*, *C. vicina* also showed temperature-related differences in the survival rate (not shown in the graph).

However, this does not have to apply to all Calliphoridae species (Figure 40) (Williams and Richardson, 1984). *C. vicina* showed smaller individuals with a very fast development, and higher mortality rates with extremely slow or fast development (Figures 36-38). Especially at 15 °C, *C. vicina* clearly showed more difficulties than *L. sericata* when compensating for very fast or slow development. Since *L. sericata* was exposed to the same conditions, *C. vicina* must be more sensitive to variations and low temperatures. In fact, other studies have also observed a high sensitivity to temperatures outside the optimal range of *C. vicina* (Vinogradova, 2009; Aubernon *et al.*, 2016). In addition, fast or very slow larval development had a significantly stronger effect on the survival rate of pupae than directly on larvae. Simply

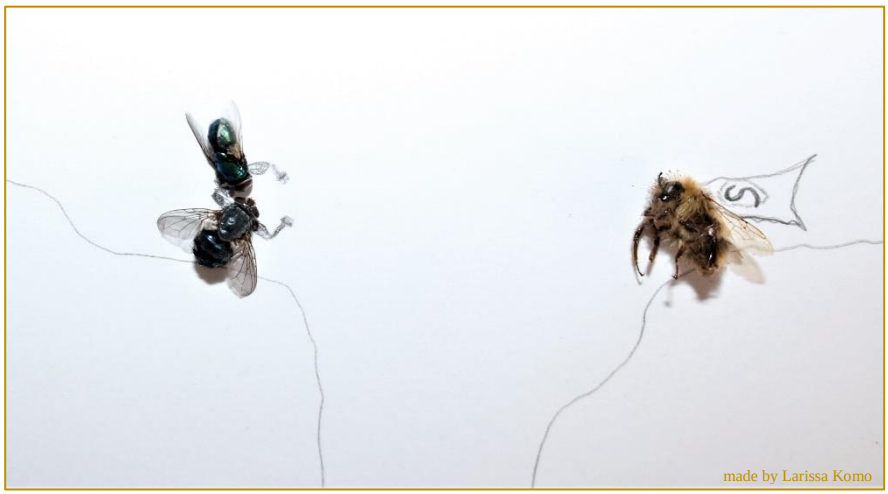
put, *C. vicina* must pay tribute during metamorphosis to a development speed that differs from the average. Therefore, an above- or below-average development speed might have even more far-reaching consequences and a negative effect on the fitness of their offspring. Consequently, it can be speculated that there are also hidden disadvantages to the very quick development of *L. sericata*. Increases in growth efficiency could be traded off against long-term reproductive success and other fitness components (Nunney, 1996; Fischer *et al.*, 2005).

In other animals, the assumption that high growth rates are costly has repeatedly been discussed (Sinervo and Doughty, 1996; Fox, 1997; Richner, 1992; Arendt, 1997; Munch and Conover, 2003). Metcalfe and Monaghan (2001) hypothesized that although all types of catch-up growth (*i.e.*, accelerating previously retarded growth as conditions improve) can bring quick, short-term benefits, they are also associated with a variety of costs that are often not apparent until much later in adult life or in the next generation. Examples of these costs include lower reproductive output or small offspring size (Sinervo and Doughty, 1996; Henderson *et al.*, 1988), lower starvation endurance, less energy reserves and reduced ability to respond to environmental stress (*e.g.*, Stockhoff, 1991; Gotthard *et al.*, 1994; Chippindale *et al.*, 1997), higher weight losses (*e.g.*, Fischer *et al.*, 2005), reduced immunity (*e.g.*, Cotter *et al.*, 2004), lower adult longevity (*e.g.*, Sevenster and van Alphen, 1993) and higher vulnerability to predation (*e.g.*, Stoks *et al.*, 2005). Just as the quick short-term benefits vary between species, the costs will also vary among them.

Therefore, the long-term costs are likely less decisive for the global fitness of *L. sericata* than a longer stay on the carcass during the larval feeding phase. However, *L. sericata* could suffer serious long-term consequences precisely because there were no direct consequences of the fast larval development. According to this theory, *C. vicina* would not experience any serious long-term effects, because the weak individuals already died during the larval or intra-

puparial stages of the first generation. However, no empirical data exist to support this theory. Only Wall (1993) found a considerably lower reproductive output for *L. sericata* (independent of the development rate) than that reported for other Calliphoridae species (Spradbery and Vogt, 1993; Thomas and Chen, 1990). In contrast, Davies (2006) found at a direct comparison that *L. sericata* was throughout its adult lifespan significantly more fecund than *C. vicina*. While each *C. vicina* female laid approximately 400 eggs during their lifespan, 1400 eggs were laid per *L. sericata* female. Nevertheless, different focuses on short-term or long-term advantages between *C. vicina* and *L. sericata* are most probably due to the very common heterospecific association of these two species in nature (Brandmayr *et al.*, 2010, personal observation), representing a different type of niche division that allows simultaneous aggregation on a carcass. Indeed, the coexistence of necrophagous blowfly species is in the classical sense characterized by niche separation according to their seasonal, successional and circadian as well as geographical and ecological occurrence on carrion (Denno and Cothran, 1975; Merritt and De Jong, 2015; Matuszewski *et al.*, 2011). The observations of this thesis suggest that not only the chronological occurrence of flies constitutes a niche, but also the alternating and development phase-specific investment in fitness-related traits between different blowfly species that aggregate in the same area at the same time. This proposal is supported by the contrasting survival chances between *C. vicina* and *L. sericata* in each development phase (supplementary material), and has already been postulated in a similar way for biofilms, in which temporal changes as well as differences in behavioural and photophysiological traits have been observed between different algae species (Underwood *et al.*, 2005).

4 Conclusion



4.1. Adaptive ecology and novelties of this thesis

In the first part of this work, I observed necrophagous larvae of different species creating heterospecific aggregations. For these non-random clusterings, species preferences were observed, letting *C. vicina* appear more attractive for *L. sericata* and *C. vomitoria* than conspecifics. In the second part, I demonstrated that such heterospecific aggregations provide benefits that are not apparent in conspecific groupings, highlighting a balance between heterospecific competition and mutualism. I also observed that benefits in fitness-related traits were gained as a function of abiotic factors, especially temperature. **Indeed, I evidenced that temperature not only affects development time, size and survival, but most importantly, the trade-off between these traits.**

This thesis showed for the first time that **within heterospecific larval aggregations, the species that finds itself at a suboptimal temperature adapts its development rate to that of the better-adapted species**. Specifically, the rather cold-adapted species developed at high temperatures faster in heterospecific aggregations with a warm-adapted species than in conspecific aggregations, and the other way around. Thus, while, *C. vicina* and *L. sericata* benefited from a faster development at 15 °C, only *C. vicina* developed faster in heterospecific aggregations at 25 and 28 °C. However, *L. sericata* benefited from higher survival rates and bigger pupae at 25 °C, highlighting that a heterospecific aggregation did not lead to costs for this species in the fitness-related traits studied (compared to a group of equal density). In addition, I concluded that an individually **fast development could be regarded as costless in terms of *L. sericata's* pre-reproductive success** that may explain the versatility of this species (Davies, 2006). However, if a nutrient deficiency was caused by too fast larval development, profound and permanent effects on the adult individual and even on its offspring can be expected (*e.g.*, reduced immunity and lower adult longevity) (Sevenster

and van Alphen, 1993, Cotter *et al.*, 2004). The highly flexible behavioural responses of *L. sericata* 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).

In contrast, *C. vicina* larvae complied with the classical assumption that fast larval growth leads to increased preadult mortality (Block and Stoks, 2008). At temperatures outside its optimal range, this cold-adapted species showed a smaller size and lower survival rates. Thus, ***C. vicina* clearly followed a different developmental strategy than *L. sericata***, in which larvae compensated an initially too fast or too slow development in the later development stages, most likely to avoid long-term consequences (Metcalfe and Monaghan, 2001). These findings are in line 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 the warm-adapted *L. sericata* (Meyer and Schaub, 1973). Thus, in contrast to the assumption of Smith and Wall (1997), my results showed that ***C. vicina* is the weaker competitor against *L. sericata* as soon as the abiotic (and biotic) conditions do not meet their restricted needs**. In fact, although *C. vicina* generally benefits from a larger size than *L. sericata*, this difference was almost eliminated at 28 °C (in all conditions). Therefore, *C. vicina* might have worse adaptation possibilities than *L. sericata* in nature, because carrion is not a stable ecosystem with moderate temperatures (Korolev and Brygadyrenko, 2014; Benbow *et al.*, 2015a; Charabidze and Hedouin, 2019). Nevertheless, according to Darwin and the *struggle of existence*, a specialisation in a narrow temperature range and disadvantages as experienced by *C. vicina* must be accompanied by advantages to be maintained in the course of evolution (Michod, 2000). Consequently, my observations suggest the existence of long-term benefits for *C. vicina* that were not analysed in my studies (*e.g.*, a

higher reproductive output) (Thomas and Chen, 1990; Spradbery and Vogt, 1993; Benbow *et al.*, 2015b).

Finally, behavioural adaptations were observed during the development experiments, which provide a further indication that **maggot-masses could be considered as a superorganism**, *i.e.*, a group of single individuals who together possess a functional organisation and undergo adaptations as a whole (Wilson and Sober, 1989; Gardner and Grafen, 2009). The larger the group, the more pronounced the characteristics of a superorganism are (Moritz and Southwick, 1992). These characteristics include, for example, strategies against pathogens and predators (*e.g.*, nest hygiene or sting of bees), food conservation (*e.g.*, honey of bees), chemical communication (*e.g.*, trail-laying by ants), self-organisation (*e.g.*, interactions between nestmates), as well as many group members (*i.e.*, a large colony) who together function as one cooperative unit (Wilson, 1962; Moritz and Southwick, 1992; Detrain and Deneubourg, 2006; Hölldobler *et al.*, 2009). Maggot-masses can consist of several thousand larvae (Charabidze *et al.*, 2011; Rivers *et al.*, 2011), which follow anti-predator strategies (Reigada and Godoy, 2012), discriminate between different communication cues (Fouche *et al.*, 2018) and make collective decisions (Boulay *et al.*, 2016). In this superorganism concept, each larva would be a cell, their flies the genitals, and their antibiotics and enzymes the equivalent of an immune system (Hölldobler *et al.*, 2009). Moreover, I observed in my development study that *C. vicina* larvae reared at hot temperatures survived better in heterospecific groups because they followed *L. sericata* into the pupation substrate and did not died as postfeeding larvae or pupae in the food. Conversely, young *L. sericata* larvae spent more time feeding in presence of *C. vicina* and died less often by migrating too early. Therefore, I suggest that **necrophagous larvae of different (non-predatory) species may act together as a functional organisation, in which each species can adapt its behaviour and development speed, gaining fitness relevant advantages that they do not obtain in**

conspecific aggregations. Thus, although no division of labour exists in blowflies, they could passively function as a superorganism by actively forming heterospecific aggregations, and thereby combining their species-specific behaviour, antibiotics and enzymes that are apparently adapted to a specific temperature range (Barnes *et al.*, 2010; Atkinson and Vaughn, 2015). To prove this hypothesis, the next step would be to conduct field studies with heterospecific aggregations of blowfly larvae on necromass. Thereby, a cooperative unit may be evidenced by showing that adaptations occur at the group and not at the individual or gene level and demonstrating a balance between costs and benefits in the long-term fitness of these blowflies.

4.2. Publications

This thesis contains only the most important results of my work: readers interested in more detailed data, analysis or discussion can refer to my publications and data deposition in Dryad (Komo *et al.*, 2019b). For example, the results presented as *mutual benefits at an intermediate temperature* were published in this year's issue 4 of the journal *Behavioral Ecology* with the title “Facing death together: heterospecific aggregations of blowfly larvae evince mutual benefits” (Komo *et al.*, 2019a). The subject of *positive and negative density dependence* was submitted to the journal *Insect Science* titled “Benefits of heterospecific aggregation on necromass: influence of temperature, group density and composition on fitness-related traits”. The findings about *costs of fast development* have been reviewed in the journal *Physiological Entomology*, and the improved manuscript with the title “Quickie well done: no evidence of physiological costs in the development race of Lucilia sericata necrophagous larvae” has been resubmitted at the beginning of September.

A proposed approach for forensic entomologists was submitted to the *International Journal of Legal Medicine* (supplementary material). However, the short note was rejected, claiming that the article would not contribute to a better understanding of the PMI assessment. The non-forensic part of this manuscript was merged with the comparative *balance between feeding and postfeeding time* focusing on the ecological phenomenon of the catching-up effect. This new short note will be submitted to the Journal *Insect Physiology* soon.

5 Supplementary Material

1.1. Additional behavioural experiments

The preference for aggregating at the *C. vicina* spot was still apparent even when the larvae themselves were no longer present (Figure 41), but only in a small number of replications (5 experiments, Table 7). In all three experiments in which 50 larvae of both species were added and a decision occurred, the aggregation took place at the former *C. vicina* spot. In the two other experiments, larvae of both species aggregated together in the periphery, and thus in a neutral area. These preliminary results suggest that a collective choice for one of the two spots is rarer when no larval aggregation is still present, especially if two species were added that could already mutually benefit from each other.

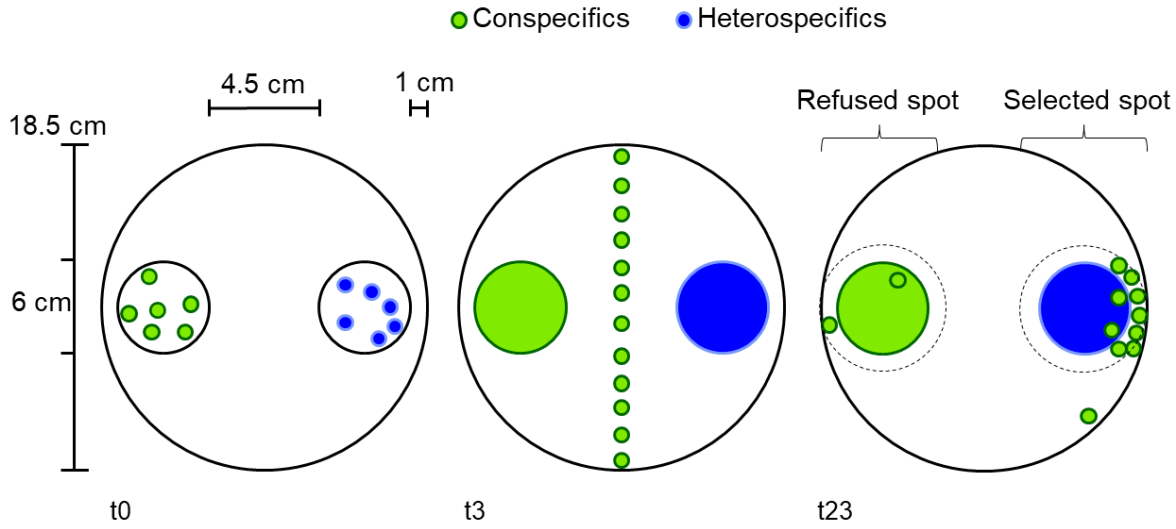


Figure 41 Experiments testing deposited cues

Experimental setup to test species-specific attractions of deposited clues without the presence of aggregated maggots, for which the initially placed larvae were removed. At start time t0, each group of 50 maggots of a single species was placed under Petri dishes. Three hours later (t3), all larvae were removed and a group of 100 supplementary maggots of one of the two species (conspecifics) was placed in the middle of the arena. This was followed by the same procedure as for the other experiments (Figure 12). Hypothetical results are shown here by way of an example: significant more larvae selected the spot marked by their heterospecifics than their conspecifics.

Table 7 Removing initially placed larvae and adding both species

Results of the additional test in which the initial larvae were removed before 50 new larvae of both species were added. The initially placed species are listed as well as the total number of larvae (of both species) in the periphery, in and closely around the selected and refused spot at the end of each experiment. The last column gives at a glance the decision to which species the added larvae migrated (binominal tests). If the largest aggregation occurred in the periphery of the arena, "no decision" was noted.

Added species	Larval count in periphery	Larval count at refused spot	Larval count at selected spot	Larvae moved to
C. vomitoria/C. vicina	4	0	96	***C. vicina***
L. sericata/C. vicina	1	8	91	***C. vicina***
L. sericata/C. vicina	9	7	84	***C. vicina***
L. sericata/C. vicina	74	2	24	No decision
L. sericata/C. vicina	57	19	24	No decision

Table 8 Removing initially placed larvae and adding one species

Results of the additional test in which the initial larvae were removed before 100 new larvae of the same species were added. The species of the later added larvae and the initially placed larvae at the selected spot are listed as well as the total number of larvae in and closely around the selected and refused spot at the end of each experiment. The last column gives at a glance whether the added larvae have migrated to their conspecifics or heterospecifics. If neither of the two aggregations was significantly larger than the other, "no decision" was noted.

Added species	Species at selected spot	Larval count at refused spot	Larval count at selected spot	Larvae moved to
C. vicina	*L. sericata*	24	71	**heterospecific**
C. vicina	*C. vicina*	10	90	conspecific
C. vomitoria	-	37	50	*No decision*
C. vomitoria	*L. sericata*	0	100	**heterospecific**
C. vomitoria	*L. sericata*	0	96	**heterospecific**
C. vomitoria	*L. sericata*	9	75	**heterospecific**
C. vomitoria	*L. sericata*	0	97	**heterospecific**
C. vomitoria	*L. sericata*	0	100	**heterospecific**
C. vomitoria	*L. sericata*	1	98	**heterospecific**
C. vomitoria	*C. vomitoria*	3	86	conspecific
C. vomitoria	*C. vomitoria*	1	96	conspecific
C. vomitoria	*C. vomitoria*	18	82	conspecific
C. vomitoria	*C. vomitoria*	1	97	conspecific
C. vomitoria	*C. vicina*	13	84	**heterospecific**
L. sericata	*L. sericata*	0	100	conspecific
L. sericata	*L. sericata*	2	98	conspecific
L. sericata	*L. sericata*	3	92	conspecific
L. sericata	*L. sericata*	2	96	conspecific
L. sericata	*L. sericata*	1	99	conspecific
L. sericata	*C. vomitoria*	0	100	**heterospecific**
L. sericata	*C. vomitoria*	0	98	**heterospecific**
L. sericata	*C. vomitoria*	31	72	**heterospecific**
L. sericata	*C. vomitoria*	24	68	**heterospecific**
L. sericata	*C. vomitoria*	10	89	**heterospecific**
L. sericata	*C. vicina*	5	85	**heterospecific**
L. sericata	*C. vicina*	28	72	**heterospecific**

By adding 100 larvae of a single species, the spot of *C. vicina* was chosen in 4 out of 6 experiments (Table 8). No preference was observed between *L. sericata* and *C. vomitoria*. For example, *L. sericata* chose the spot that had previously been colonized by its conspecifics and the spot of *C. vomitoria* each five times. Overall, the spot marked by heterospecifics was chosen in 15 of 25 experiments, demonstrating a slight trend but no significant preference for the spot marked by heterospecifics (binominal test: $p = 0.4244$). The same applies to the control experiments in which 100 larvae of one species were added (but initial larvae were not removed): the heterospecific spot was chosen in 6 of 9 replications (binominal test: $p = 0.508$). In fact, no experiments have been carried out between the species pairs that are of particular importance considering the main experiments, *i.e.*, those pairs that include *C. vicina*. This was due to a shortage of *C. vicina* larvae and explains the discontinuation of this experiment series. Nevertheless, the spot of *C. vomitoria* was chosen and not that of *L. sericata* in 7 out of 9 replications (Table 9; binominal test: $p = 0.180$). Moreover, the same larval distribution than in the main experiments were found in these control experiments, *i.e.*, approximately 75% of all larvae in the selected and 25% in the refused spot.

Table 9 Adding only one species

Results of the additional binary choice test in which only one of the two species (*L. sericata* or *C. vomitoria*) was added but the initially larvae stayed on their places. The species of the later added larvae and the initially placed larvae at the selected spot are listed as well as the total number of larvae (of both species) in and closely around the selected and refused spot at the end of each experiment. The last column gives at a glance whether the added larvae have migrated to their conspecifics or heterospecifics.

Added species	Species at selected spot	Larval count at refused spot	Larval count at selected spot	Larvae moved to
L. sericata	*L. sericata*	52	125	conspecifics
L. sericata	*C. vomitoria*	50	143	**heterospecifics**
L. sericata	*C. vomitoria*	48	151	**heterospecifics**
L. sericata	*C. vomitoria*	59	138	**heterospecifics**
L. sericata	*C. vomitoria*	48	135	**heterospecifics**
L. sericata	*C. vomitoria*	47	135	**heterospecifics**
C. vomitoria	*L. sericata*	50	120	**heterospecifics**
C. vomitoria	*C. vomitoria*	44	127	conspecifics
C. vomitoria	*C. vomitoria*	50	140	conspecifics

1.2. Deeper look at the species-specific mortality

Divergent needs of *L. sericata* and *C. vicina* might be the reason why the development studies observed an improvement in different fitness components. For example, *L. sericata* increased in size and survival, and *C. vicina* accelerated their development when reared in heterospecific cultures at 25 °C. As a further example, *L. sericata* did not face a higher mortality rate or smaller size when developing fast at hot or cold temperatures in contrast to *C. vicina*. Influence of the group composition, temperature and genetics on the development time was also reflected in the allocation of species-specific benefits during the distinct development phases. In fact, besides the physiological and behavioural differences between *L. sericata* and *C. vicina* mentioned in the main part of this thesis, different survival probabilities were also observed for each developmental stage (*i.e.*, early and late feeding, postfeeding and intra-puparial). Specifically, early feeding larvae of *C. vicina* (*i.e.*, 1st and 2nd instars) showed a low mortality rate but *L. sericata* a high mortality rate due to an early abandonment of the food. In contrast, late feeding larvae (*i.e.*, 3rd instar) showed a low mortality rate among *L. sericata* but a high mortality rate among *C. vicina*, as larvae tended to stay in the substrate box till pupariation (especially at 28 °C; Figures 42 and 43). The postfeeding *C. vicina* larvae that were placed from the food remains into a sand covered box (*i.e.*, they were forced to migrate), hatched as flies, the others that were left in the substrate box, died as postfeeding larva or pupa. This clarifies that these larvae would not have died anyway because of a deficit, but only because of the non-migration.

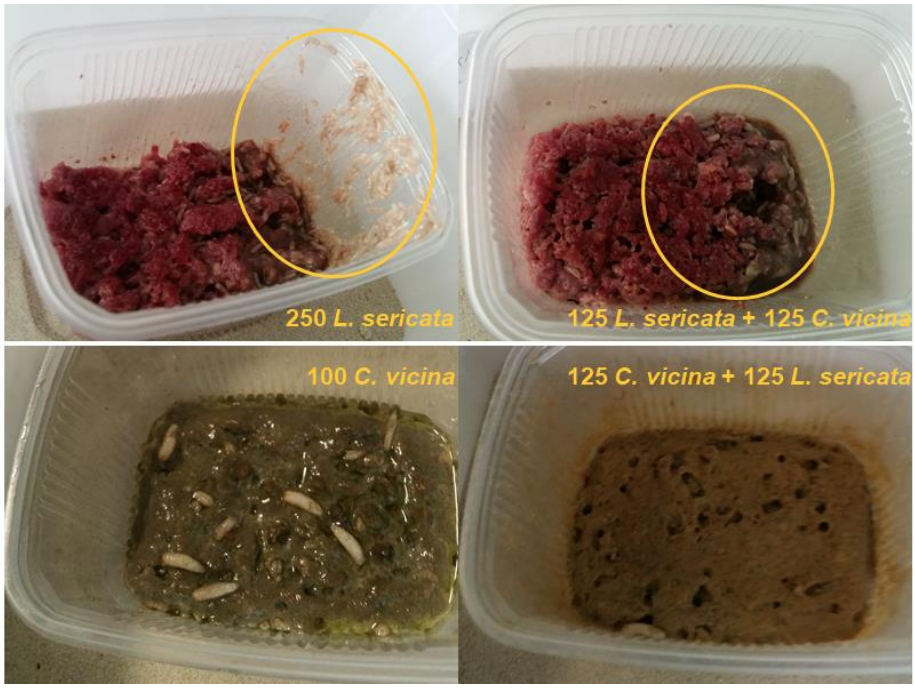


Figure 42 Influence of another species on larval behaviour

2nd instar larvae of *L. sericata* used to crawl up the substrate box. Some of those also got in the sand and failed to pupate. However, in heterospecific conditions such a behaviour was rarely seen. Postfeeding larvae of *C. vicina* in the low-density group stayed often inside the substrate box and continued secreting/excreting giving the substrate an oily consistency. In contrast, nearly all larvae of the heterospecific group went outside the box during the postfeeding phase. Both observations confirm the benefits of heterospecific aggregations as they decrease the mortality rate of both species, only during different live stages.

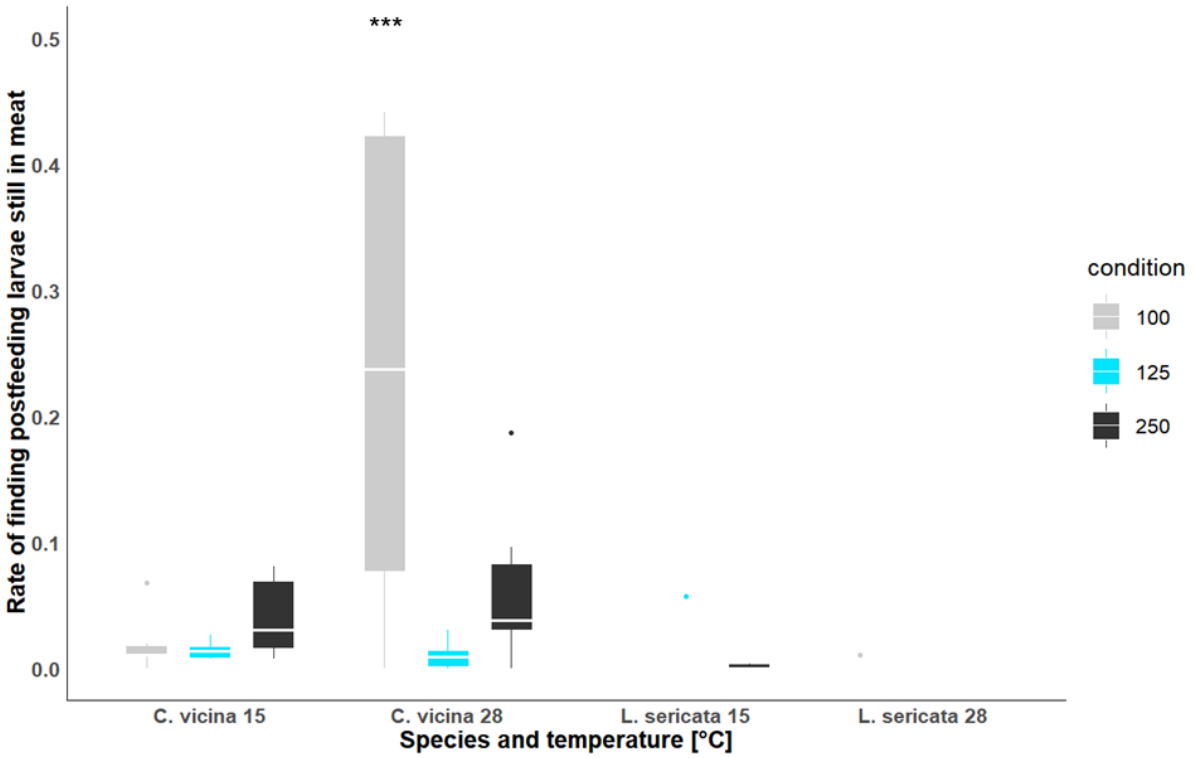


Figure 43 Postfeeding larvae stayed in the meat

Significantly more *C. vicina* larvae of the low-density group at 28 °C did not leave the substrate box after peakfeeding compared to all other conditions (Tukey's range test: $p < 0.0001$).

Regarding the development stages after migration, *L. sericata* often remained in the postfeeding stage for an extremely long time before pupating or did not pupate at all. An observation that could not be made for *C. vicina*. If *C. vicina* larvae reached the postfeeding phase, they also pupated with a probability of approximately 99.5%. However, *C. vicina* showed a high mortality rate during the intra-puparial stage, especially in small groups at 15 °C, whereas pupae of *L. sericata* showed a low mortality rate (Figure 44).

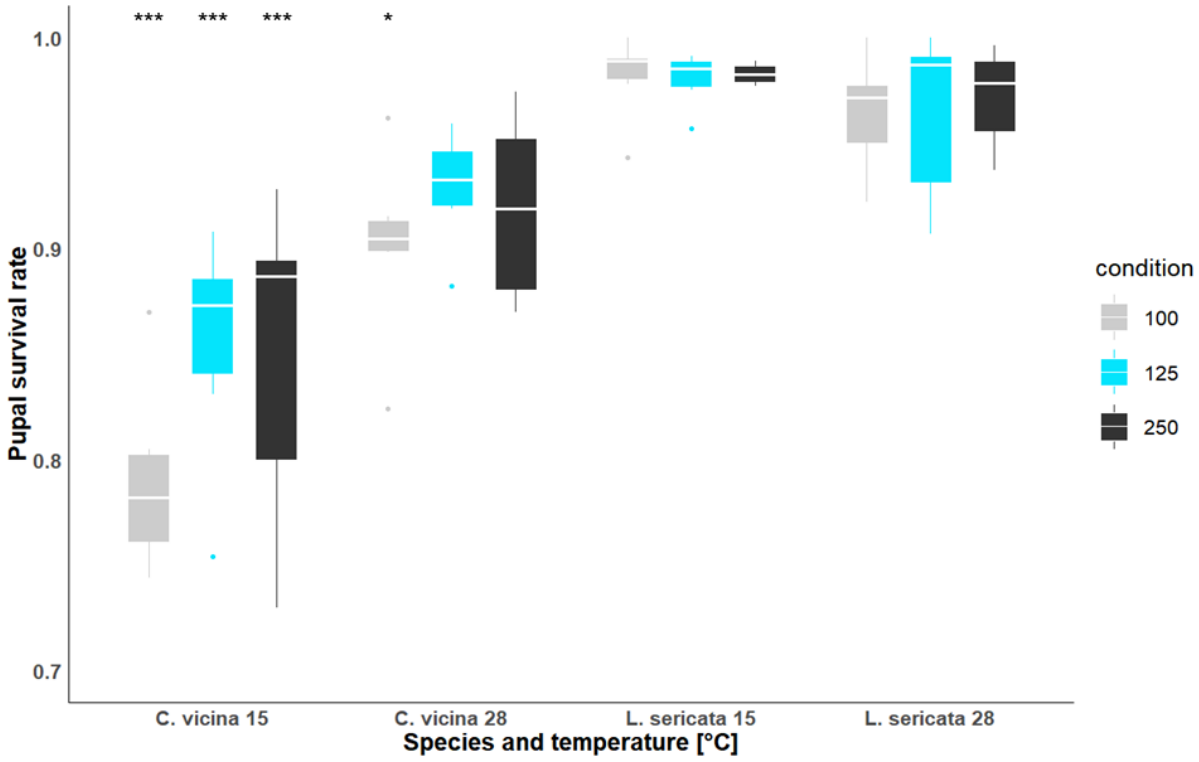


Figure 44 Survival rate of pupae at 15 and 28 °C

Significantly[27] more pupae of *C. vicina* died in comparisons with *L. sericata*, especially at 15 °C. Note that the y-axis starts at 70%.

[27] TukeyHSD: $p_{15°C,100} < 0.0001$, $p_{15°C,125} < 0.0001$, $p_{15°C,250} < 0.0001$; 100 *C. vicina* 28 °C vs. 100 *L. sericata* 15 °C: p = 0.0476, 100 *C. vicina* 28 °C v.s. 250 *L. sericata* 15 °C: p =0.0416

1.3. Forensic entomology perspective

Principle of minimum postmortem interval estimation

The presented results have direct implication in forensic entomology, as the beginning of a new development event or larval size is used to estimate the minimum time since death (PMI_{min}). In this context, larvae are sampled from the crime scene and their presumed oviposition time is calculated (Figure 45) (Adams and Hall, 2003; Amendt *et al.*, 2007; Amendt *et al.*, 2011). As blowflies colonize an accessible corpse shortly after death, age of the oldest larvae can almost exactly predict the time of death. However, this also means that even minor differences in the time sequence or size can have a major influence on the age determination of the individuals and, therefore, on the PMI_{min} estimation. Consequently, forensic scientists depend on the accuracy of the correlation between larval age and time sequence or size (see isomegalen and isomorphen diagram in Grassberger and Reiter (2001)).

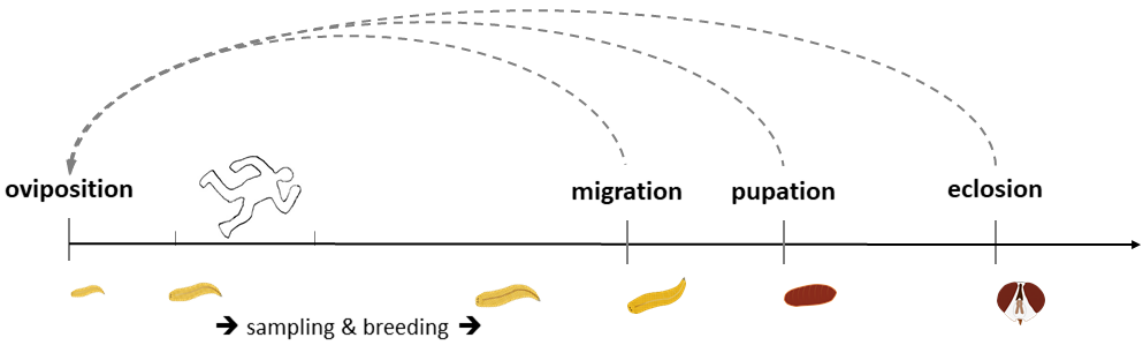


Figure 45 Development events for age estimation

Determining the age of sampled necrophagous larvae on a corpse is one common method in forensic entomology to estimate the minimum postmortem interval. Applied approaches are measuring the size of larvae and determining the beginning of a new development event (*i.e.*, shedding, migration, pupariation or eclosion). Thereby, the time of oviposition can be estimated, which is in general correlated with the time since death. The body outline marks the time of discovery of the corpse, and therefore, the sampling time of larvae.

Since biotic parameters impact development times of blowflies, forensic entomologists would in fact need to consider such biotic parameters to estimate an accurate PMI_{min}. However, these parameters are mostly unknown and impossible to assess. It seems utopian to determine *a posteriori* upon what flesh or in what kind of group a larva fed during its development. Nevertheless, the biotic parameters should not be overlooked, but rather the fact that these parameters are expressed to varying degrees in the development stages should be exploited. Accordingly, a development event must be found to which the influence of biotic parameters has the smallest impact and with which therefore, the PMI_{min} estimation would be most precise.

The age of a larva can be estimated from any developmental stage (therefore, larval weight or length can also be measured but varies enormously according to the biotic and abiotic conditions (Hadjer-Kounouz and Louadi, 2017)). However, a stage is a period of time and not a point in time and will thus result in a rough PMI_{min} estimation with a broad possible timeframe for egg-laying. The shedding of larvae is a very difficult to observe event and will therefore result in a rough PMI estimation with a broad possible time span for oviposition. The pupariation time (*e.g.*, used by Byrd and Butler (1996, 1997), Yanmanee *et al.* (2016) and Niederegger *et al.* (2013)) also suffers a clear disadvantage: the decision whether an animal is already a prepupa or still a dormant postfeeding larvae is based on a subjective assessment (Ashburner, 1989; Zdarek, 1985; Rabossi *et al.*, 1992). Moreover, the search of hidden prepupae distracts and artificially delays their pupariation process, and thus, possibly affecting their age determination. Indeed, Robinson *et al.* (2018) observed a delayed pupariation on several common indoor surfaces, whereas the start of migration was independent of ground surfaces. Furthermore, the migration of larvae and also the eclosion of flies are points in time that are easy to observe, for example using video-recording devices (Wang *et al.*, 1997; Gibson *et al.*, 2014), and could represent valuable events for development-time calculations.

A meta-analysis of migration and eclosion time

These two development events were compared in terms of accuracy and precision. Therefore, published data have been collected from studies that meet the following criteria: first, examining necrophagous larvae feeding under different conditions (*e.g.*, differing in food freshness, food type or group size); second, specifying the development time until the end of the feeding stage (*i.e.*, when larvae migrated away from the food source or reached their maximum length) and until the eclosion of flies (Table 14 in appendix)[28]. Taking the data from these publications into account, the total period of migration was always shorter than the period of eclosion, from at least one hour and up to 87 h. This migration time span was significantly different to the eclosion time span (paired t-test: t = -3.679, df = 9, p = 0.0051, mean of the differences = 17.667; Table 14). Consequently, the event of migration appears more precise and reliable than eclosion for PMI_{min} calculation.

To highlight the idea of using the migration time, data from the intermediate series of this thesis were used as well as two other studies that focus on the development of *L. sericata* at 25 °C (Grassberger and Reiter (2001) and Scanvion *et al.* (2018); Figure 46). Starting from the time of oviposition, the time until 10% of the larvae had migrated and 10% of the flies had eclosed are reported. Based on the resulting time spans, the estimated time of oviposition can now be inferred retroactively, as it would be done for a PMI_{min} estimation. Counting back from the time of eclosion, the possible period of oviposition varies about 72h. However, counting back from migration, this time span is reduced to 48h only. Accordingly, the PMI_{min}

[28] Since these studies collected development times under at least two different conditions, the time of migration and eclosion of at least two groups are known resulting in a time span. At least for the studies in which sample intervals were given (*i.e.*, Greenberg (1991), Grassberger & Reiter (2001), Tarone & Foran (2006), El-Moaty *et al.* (2013), Richards *et al.* (2013), Bernhardt *et al.* (2017), Scanvion *et al.* (2018), Komo *et al.*(2019a)), it can be confirmed that the postfeeding larvae were not controlled more frequently (*i.e.* at shorter intervals) than the pupae for eclosed flies.

can be reported with a deviation of ±24 h by the time of migration versus ±36 h by the time of eclosion. Indeed, using the time specifications of Grassberger & Reiter (2001) as control values, the temporal deviations of the other studies are significantly smaller for the time of migration than for the eclosion (paired t-test: $t = 5.3235$, $df = 17$, $p < 0.0001$, mean of the differences = -41.3; Figure 46). Using the data from *C. vicina* in the intermediate series, the impression was the same (Table 14 in appendix).

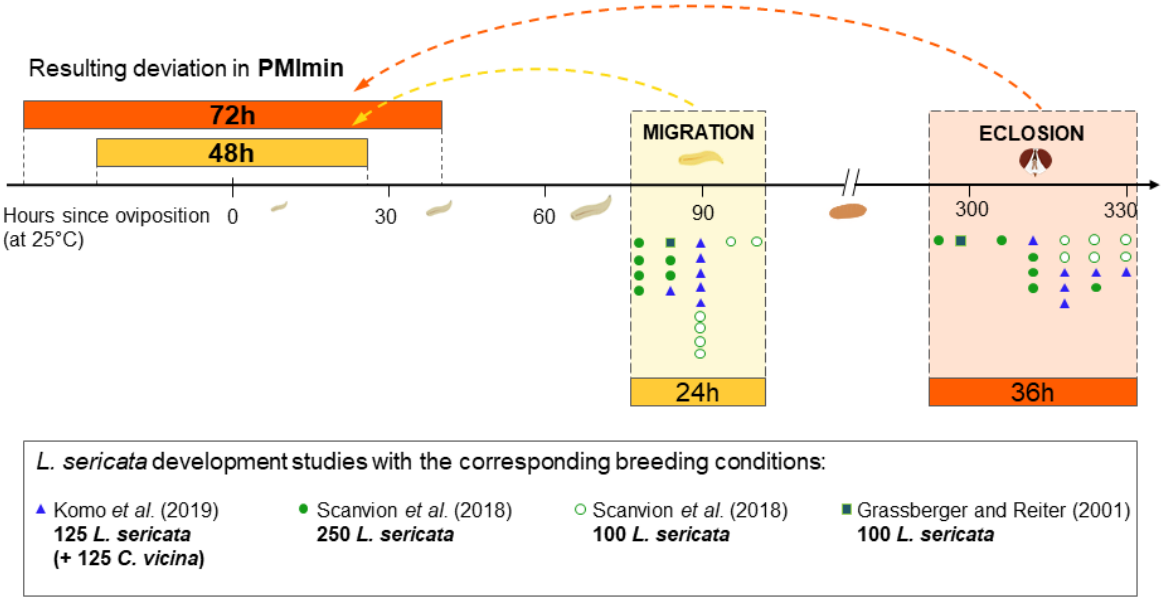


Figure 46 PMI_{min} estimation

This timeline demonstrates that backward estimations can be made more precisely by using the time of larval migration instead of fly eclosion. Each mark (*i.e.*, triangle, circle and square) represents one replication of the mentioned studies. The times for migrating and eclosion can be specified in this example very precisely since the time of oviposition is known to the hour. Therefore, from a scientists' point of view, there is a 24-h interval in which the first 10% of individuals migrate. However, for minimum postmortem interval (PMI_{min}) estimations, the time of oviposition can only be narrowed down by the oldest animals at the crime scene of which the development conditions (*i.e.*, biotic parameters) are unknown. Accordingly, the 24-h interval must be calculated in both directions from the assumed zero point (*i.e.*, oviposition), resulting in a specification of plus/minus 24 h. This leads to an overall 48 h interval for migrating larvae and, for the same reason, to a 72-h interval for eclosing flies.

As Byrd and Butler already mentioned in 1997, the migrating event is an important and often overlooked landmark for estimating the age of Calliphoridae. To this day, some developmental studies dedicated to forensic entomology still report only development times to pupariation or adult eclosion, without mentioning the days till the migration of postfeeding larvae (Ireland and Turner, 2006; Niederegger *et al.*, 2013; Yanmanee *et al.*, 2016; Flores *et al.*, 2014). Even if biotic parameters (*e.g.*, group composition) impinge on individuals only during the feeding stages, they ultimately also affect the metamorphosis duration. In this context, this thesis demonstrated that the time of migration displays a smaller variability than the time of eclosion, allowing a more precise and accurate PMI_{min} estimation. Moreover, breeding individuals to adulthood is time consuming and can lead to potential errors, for instance, due to temperature fluctuations or a parasite infestation of the pupae. In conclusion, the use of migration time rather than fly eclosion is suggested for backward development-time calculations, and the inclusion of postfeeding data in future studies.

Critique

This proposed approach for forensic entomologists was submitted to the *International Journal of Legal Medicine*. However, it was rejected by the reviewers (1) expressing concerns regarding the validity of findings and conclusions given; (2) claiming that the article would not contribute to a better understanding of the PMI assessment but rather confuse and open “a field of research where there is no need to analyses something”; and (3) because the assertion of migration and eclosion times would be incomparable, as they would not be developmental events of the same kind. These reasons are understandable, but from the authors' point of view not completely justified and are therefore discussed below.

Obviously, if a forensic scientist only got dead flies for PMI estimation, they must be used; there is no other choice. The comparison presented in the meta-analysis refers to the scenario

in which the forensic scientist receives young living larvae from a crime scene and theoretically has the free choice of up to which stage he/she continues to rear these larvae to determine their age. For this case, the reviewer assumes that the worldwide rule is rearing to the closest development event for which reference development data are available. Nevertheless, many laboratories worldwide (personal observation), including the one in Lille, rear sampled larvae until fly eclosion. Thus, even if samples come as third instar larvae in the laboratory (the most likely stage to be sampled on a corpse as it represents the longest feeding stage), they will be reared until adults. First, because the event of ecdysis is difficult to observe, second, because it would require an almost hourly control of larvae, and third, because several species can't be identified at larval stage. Therefore, even if larvae do theoretically not need to be reared until eclosion (because it probably enlarges the error of age estimation) it is in fact practiced.

Variations between development events might indeed accumulate through development and thus being larger for later than for earlier events. However, first, catching-up effects exist as observed for many *C. vicina* and some *L. sericata* individuals in this thesis. Second, a variation of 12 h is approximately 14% taking a migration time of 85 h and only 4% taking an eclosion time of 300 h. However, for the PMI estimation not the percentage shares are decisive, but the information of hours or days. Third, if variation accumulates actually over time, PMI estimations based on later events will always be more variable. For this reason, the short note submitted should draw attention to this problem and encourage forensic scientists (who are accustomed to estimate the age of samples based on eclosion time) to rethink their approach and adapt their technique to any criminal case. This indeed includes that the migration time is not the best solution for every case in which young larvae have been sampled, but that relevant developmental stages are passed through before. However, those

are more time-consuming and difficult to determine, thus making the age estimation less accurate.

Moreover, differences in PMI assessments, in the rearing of flies and in the experimental design of development studies require consistent standards and a general guideline that are followed by all forensic laboratories worldwide (Lutz and Amendt, 2019). Indeed, published data are only to a limited extend comparable as Table 14 (appendix) shows by comprising missing data due to the lack of information about the used sample intervals. As far as indicated, the control times of the experiments were as frequent for migration as for eclosion. In addition, the starting point of migration has in some studies be defined as the point when (1) all, (2) the first 50%, (3) the first 10% or (4) the very first larva either began to leave the food source or was found in the pupariation substrate (that also varied between studies). This illustrates again the insufficient plasticity and repeatability in these works.

Another claim of the reviewer was the use of range to display the variation in time as it is largely affected by outliers. However, the times given are the times when the first 10% of a group had reached the appropriate development event, which give less weight to the extremely fast and slow individuals. Moreover, the diversity of development times is exactly the problem that forensic experts are constantly facing and that should be highlighted in this manuscript. A distribution of times around a central value is exactly what was not supposed to be demonstrated in this manuscript. In contrast, the length of the presence period of new postfeeding larvae or flies is decisive, because a shorter presence period narrows the width of PMI estimates (Matuszewski *et al.*, 2010b). In forensic entomological reports, the standard error is a standard deviation measure that must be specified at the end of PMI estimations, but is a generally taken time laps, such as ±24 h, instead of the case-related sampling distribution.

References

Allee, W.C. (1938), *The social life of animals*, 1st ed., W.W. Norton & Company, inc, New York.

Adams, M.W. (1967), "Basis of yield component compensation in crop plants with special reference to the field bean, *Phaseolus vulgaris*", *Crop Science*, Vol. 7 No. 5, pp. 505-510.

Adams, Z.J.O. and Hall, M.J.R. (2003), "Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length", *Forensic Science International*, Vol. 138 No. 1-3, pp. 50–61.

Amendt, J., Campobasso, C.P., Gaudry, E., Reiter, C., LeBlanc, H.N. and Hall, M.J.R. (2007), "Best practice in forensic entomology--standards and guidelines", *International journal of legal medicine*, Vol. 121 No. 2, pp. 90–104.

Amendt, J., Richards, C.S., Campobasso, C.P., Zehner, R. and Hall, M.J.R. (2011), "Forensic entomology: applications and limitations", *Forensic science, medicine, and pathology*, Vol. 7 No. 4, pp. 379–392.

Ames, C. and Turner, B. (2003), "Low temperature episodes in development of blowflies: implications for postmortem interval estimation", *Medical and Veterinary Entomology*, Vol. 17 No. 2, pp. 178–186.

Anderson, G.S. (2000), "Minimum and maximum development rates of some forensically important Calliphoridae (Diptera)", *Journal of Forensic Sciences*, Vol. 45 No. 4, 824-832.

Aplin, L.M., Farine, D.R., Mann, R.P. and Sheldon, B.C. (2014), "Individual-level personality influences social foraging and collective behaviour in wild birds", *Proceedings. Biological sciences*, Vol. 281 No. 1789, p. 20141016.

Archer, M.S. and Elgar, M.A. (2003), "Female breeding-site preferences and larval feeding strategies of carrion-breeding Calliphoridae and Sarcophagidae (Diptera): a quantitative analysis", *Australian Journal of Zoology*, Vol. 51 No. 2, pp. 165-174.

Arellano, L., Castillo-Guevara, C., Huerta, C., Germán-García, A. and Lara, C. (2017), "Nesting biology and life history of the dung beetle *Onthophagus lecontei* (Coleoptera: Scarabaeinae)", *Animal Biology*, Vol. 67 No. 1, pp. 41–52.

Arendt, J. d. (1997), “Adaptive intrinsic growth rates: an integration across taxa”, *The Quarterly Review of Biology*, Vol. 72 No. 2, pp. 149–177.

Ariew, A. (2004), “The confusions of fitness”, *The British Journal for the Philosophy of Science*, Vol. 55 No. 2, pp. 347–363.

Arnaldos, I., Romera, E., García, M.D. and 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*, Vol. 114 No. 3, pp. 156–162.

Ashburner, M. (1989), *Drosophila: A laboratory handbook*, Cold Spring Harbor Laboratory Pr, Cold Spring Harbor, N.Y.

Atkinson, C.L. and Vaughn, C.C. (2015), “Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function”, *Freshwater Biology*, Vol. 60 No. 3, pp. 563–574.

Aubernon, C., Boulay, J., Hédouin, V. and Charabidzé, D. (2016), “Thermoregulation in gregarious dipteran larvae: evidence of species-specific temperature selection”, *Entomologia Experimentalis et Applicata*, Vol. 160 No. 2, pp. 101–108.

Aubernon, C., Hedouin, V. and Charabidze, D. (2019), “The maggot, the ethologist and the forensic entomologist: Sociality and thermoregulation in necrophagous larvae”, *Journal of advanced research*, Vol. 16, pp. 67–73.

Barker, J.S.F., Vacek, D.C. and East, P.D. (1988), “Attraction of larvae of *Drosophila-Buzzatii* and *Drosophila-Aldrichi* to yeast species isolated from their natural-environment”, *Australian Journal of Zoology*, Vol. 36 No. 1, p. 53-63.

Barnes, K.M. and Gennard, D.E. (2013), “Rearing bacteria and maggots concurrently: a protocol using *Lucilia sericata* (Diptera: Calliphoridae) as a model species”, *Applied Entomology and Zoology*, Vol. 48 No. 3, pp. 247–253.

Barnes, K.M., Gennard, D.E. and Dixon, R.A. (2010), “An assessment of the antibacterial activity in larval excretion/secretion of four species of insects recorded in association with corpses, using *Lucilia sericata* Meigen as the marker species”, *Bulletin of entomological research*, Vol. 100 No. 6, pp. 635–640.

Barragan-Fonseca, K.B., Dicke, M. and van Loon, J.J.A. (2018), “Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black

soldier fly larvae (*Hermetia illucens*)", *Entomologia Experimentalis et Applicata*, Vol. 166 No. 9, pp. 761–770.

Beckers, R., Holland, O.E. and Deneubourg, J.L. (1994), "From local actions to global tasks: Stigmergy and collective robotics", in Cruse, H., Dean J., Ritter, H. (Eds.), *Prerational Intelligence: Adaptive Behavior and Intelligent Systems Without Symbols and Logic, Volume 1, Volume 2 Prerational Intelligence: Interdisciplinary Perspectives on the Behavior of Natural and Artificial Systems, Volume 3*, Studies in Cognitive Systems, Vol. 26. Springer, Dordrecht, pp. 181–189.

Benbow, M.E., Barton, P.S., Ulyshen, M.D., Beasley, J.C., DeVault, T.L., Strickland, M.S., Tomberlin, J.K., Jordan, H.R. and Pechal, J.L. (2019), "Necrobiome framework for bridging decomposition ecology of autotrophically and heterotrophically derived organic matter", *Ecological Monographs*, Vol. 89 No. 1, e01331.

Benbow, M.E., Lewis, A.J., Tomberlin, J.K. and Pechal, J.L. (2013), "Seasonal necrophagous insect community assembly during vertebrate carrion decomposition", *Journal of medical entomology*, Vol. 50 No. 2, pp. 440–450.

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

Benbow, M.E., Tomberlin, J.K. and Tarone, A.M. (Eds.) (2015b), *Carrion ecology, evolution, and their applications*, CRC Press, Hoboken.

Bernhardt, V., Schomerus, C., Verhoff, M.A. and Amendt, J. (2017), "Of pigs and men-comparing the development of *Calliphora vicina* (Diptera: Calliphoridae) on human and porcine tissue", *International journal of legal medicine*, Vol. 131 No. 3, pp. 847–853.

Blanckenhorn, W.U. (1998), "Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly", *Evolution; international journal of organic evolution*, Vol. 52 No. 5, pp. 1394–1407.

Blanckenhorn, W.U., Bauerfeind, S.S., Berger, D., Davidowitz, G., Fox, C.W., Guillaume, F., Nakamura, S., Nishimura, K., Sasaki, H., Stillwell, R.C., Tachi, T. and Schäfer, M.A. (2018), "Life history traits, but not body size, vary systematically along latitudinal gradients on three continents in the widespread yellow dung fly", *Ecography*, Vol. 41 No. 12, pp. 2080–2091.

Block, M. de and Stoks, R. (2008), “Short-term larval food stress and associated compensatory growth reduce adult immune function in a damselfly”, *Ecological Entomology*, Vol. 33 No. 6, pp. 796–801.

Blums, P., Nichols, J.D., Hines, J.E., Lindberg, M.S. and Mednis, A. (2005), “Individual quality, survival variation and patterns of phenotypic selection on body condition and timing of nesting in birds”, *Oecologia*, Vol. 143 No. 3, pp. 365–376.

Boice, R. (1981), “Behavioral comparability of wild and domesticated rats”, *Behavior Genetics*, Vol. 11 No. 5, pp. 545–553.

Bomphrey, R.J., Walker, S.M. and 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*, Vol. 4 No. 11, e7852.

Bonacci, T., Brandmayr, P. and Zetto Brandmayr, T. (2011), “Predator feeding choice on conspicuous and non-conspicuous carabid beetles: first results”, *ZooKeys*, No. 100, pp. 171–179.

Boulay, J., Aubernon, C., Ruxton, G.D., Hédouin, V., Deneubourg, J.-L. and Charabidzé, D. (2017), “Mixed-species aggregations in arthropods”, *Insect science*, Vol. 26 No. 1, pp. 2–19.

Boulay, J., Deneubourg, J.-L., Hédouin, V. and Charabidzé, D. (2016), “Interspecific shared collective decision-making in two forensically important species”, *Proceedings of the Royal Society B: Biological Sciences*, Vol. 283 No. 1824, pp. 1–9.

Boulay, J., Devigne, C., Gosset, D. and Charabidze, D. (2013), “Evidence of active aggregation behaviour in *Lucilia sericata* larvae and possible implication of a conspecific mark”, *Animal Behaviour*, Vol. 85 No. 6, pp. 1191–1197.

Braack, L.E.O. (1981), “Visitation patterns of principal species of the insect-complex at carcasses in the Kruger National Park”, *Koedoe*, Vol. 24 No. 1, pp. 33–49.

Brandmayr, P., Greco, S., Brandmayr Brandmayr, T.Z., Bonacci, T., Tersaruolo, C. and Vercillo, V. (2010), “A preliminary investigation of insect succession on carrion in Calabria (southern Italy)”, *Terrestrial Arthropod Reviews*, Vol. 3 No. 2, pp. 97–110.

Broly, P., Devigne, C. and Deneubourg, J.-L. (2015), “Body shape in terrestrial isopods: A morphological mechanism to resist desiccation?”, *Journal of morphology*, Vol. 276 No. 11, pp. 1283–1289.

Brown, I.M. and Gaugler, R. (1997), “Temperature and humidity influence emergence and survival of entomopathogenic nematodes”, *Nematologica*, Vol. 43 No. 5, pp. 363–375.

Brundage, A., Benbow, M.E. and Tomberlin, J.K. (2014), “Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species”, *Ecological Entomology*, Vol. 39 No. 5, pp. 539–547.

Bshary, R. and Noë, R. (1997), “Red colobus and Diana monkeys provide mutual protection against predators”, *Animal Behaviour*, Vol. 54 No. 6, pp. 1461–1474.

Burkepile, D.E., Parker, J.D., Woodson, C.B., Mills, H.J., Kubanek, J., Sobecky, P.A. and Hay, M.E. (2006), “Chemically mediated competition between microbes and animals: microbes as consumers in food webs”, *Ecology*, Vol. 87 No. 11, pp. 2821–2831.

Buser, C.C., Ward, P.I. and Bussière, L.F. (2014), “Adaptive maternal plasticity in response to perceptions of larval competition”, *Functional Ecology*, Vol. 28 No. 3, pp. 669–681.

Byrd, J.H. and Butler, J.F. (1996), “Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development”, *Journal of medical entomology*, Vol. 33 No. 6, pp. 901–905.

Byrd, J.H. and Butler, J.F. (1997), “Effects of temperature on *Chrysomya rufifacies* (Diptera: Calliphoridae) development”, *Journal of medical entomology*, Vol. 34 No. 3, pp. 353–358.

Byrd, J.H. and Castner, J.L. (Eds.) (2001), *Forensic entomology: The utility of arthropods in legal investigations*, CRC Press, Boca Raton.

Cammack, J.A., Pimsler, M.L., Crippen, T.L. and Tomberlin, J.K. (2015), “Chemical ecology of vertebrate carrion”, in Benbow, M.E., Tomberlin, J.K. and Tarone, A.M. (Eds.), *Carrion Ecology, Evolution, and Their Applications*, CRC Press, Hoboken, pp. 187–212.

Campobasso, C.P., Marchetti, D., Introna, F. and Colonna, M.F. (2009), “Postmortem artifacts made by ants and the effect of ant activity on decompositional rates”, *The American journal of forensic medicine and pathology*, Vol. 30 No. 1, pp. 84–87.

Carter, D.O., Yellowlees, D. and Tibbett, M. (2007), “Cadaver decomposition in terrestrial ecosystems”, *Die Naturwissenschaften*, Vol. 94 No. 1, pp. 12–24.

Carter, D.O., Yellowlees, D. and Tibbett, M. (2010), “Moisture can be the dominant environmental parameter governing cadaver decomposition in soil”, *Forensic science international*, Vol. 200 No. 1-3, pp. 60–66.

Castner, J.L. (2001), “General entomology and arthropod biology”, in Byrd, J.H. and Castner, J.L. (Eds.), *Forensic entomology: The utility of arthropods in legal investigations*, CRC Press, Boca Raton, pp. 17–41.

Castro, P.C., Serrano, A., Martins Da Silva, P. and García, M.D. (2012), “Carrion flies of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal”, *Medical and veterinary entomology*, Vol. 26 No. 4, pp. 417–431.

Charabidze, D., Bourel, B. and Gosset, D. (2011), “Larval-mass effect: Characterisation of heat emission by necrophageous blowflies (Diptera: Calliphoridae) larval aggregates”, *Forensic science international*, Vol. 211 No. 1-3, pp. 61–66.

Charabidze, D., Bourel, B., Leblanc, H., Hedouin, V. and 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*, Vol. 54 No. 3, pp. 529–533.

Charabidze, D., Depeme, A., Devigne, C. and Hedouin, V. (2015), “Do necrophagous blowflies (Diptera: Calliphoridae) lay their eggs in wounds?: Experimental data and implications for forensic entomology”, *Forensic science international*, Vol. 253, pp. 71–75.

Charabidze, D. and Hedouin, V. (2019), “Temperature: the weak point of forensic entomology”, *International journal of legal medicine*, Vol. 133 No. 2, pp. 633–639.

Charabidze, D., Hedouin, V. and Gosset, D. (2013), “Discontinuous foraging behavior of necrophagous *Lucilia sericata* (Meigen 1826) (Diptera Calliphoridae) larvae”, *Journal of insect physiology*, Vol. 59 No. 3, pp. 325–331.

Chippindale, A.K., Alipaz, J.A., Chen, H.-W. and Rose, M.R. (1997), “Experimental evolution of accelerated development in Drosophila. 1. Developmental speed and larval survival”, *Evolution*, Vol. 51 No. 5, pp. 1536–1551.

Chotkowski, M.A. and Ellen Marsden, J. (1999), “Round goby and mottled sculpin predation on lake trout eggs and fry: Field predictions from laboratory experiments”, *Journal of Great Lakes Research*, Vol. 25 No. 1, pp. 26–35.

Clark, K., Evans, L. and Wall, R. (2006), “Growth rates of the blowfly, *Lucilia sericata*, on different body tissues”, *Forensic science international*, Vol. 156 No. 2-3, pp. 145–149.

Cobb, M. (1999), “What and how do maggots smell?”, *Biological Reviews*, Vol. 74 No. 4, pp. 425–459

Colinet, H., Salin, C., Boivin, G. and Hance, T. (2005), “Host age and fitness-related traits in a koinobiont aphid parasitoid”, *Ecological Entomology*, Vol. 30 No. 4, pp. 473–479.

Conner, R.C. (1995), “The benefits of mutualism: a conceptual framework”, *Biological Reviews*, Vol. 70 No. 3, pp. 427–457.

Cornwallis, C.K., Botero, C.A., Rubenstein, D.R., Downing, P.A., West, S.A. and Griffin, A.S. (2017), “Cooperation facilitates the colonization of harsh environments”, *Nature Ecology & Evolution*, Vol. 1 No. 3, p. 57.

Cotter, S.C., Kruuk, L.E.B. and Wilson, K. (2004), “Costs of resistance: genetic correlations and potential trade-offs in an insect immune system”, *Journal of evolutionary biology*, Vol. 17 No. 2, pp. 421–429.

Courchamp, F., Berec, L. and Gascoigne, J. (2009), *Allee effects in ecology and conservation*, *Oxford biology*, 1. paperback ed., Oxford Univ. Press, Oxford.

Couzin, I.D., Krause, J., Franks, N.R. and Levin, S.A. (2005), “Effective leadership and decision-making in animal groups on the move”, *Nature*, Vol. 433 No. 7025, pp. 513–516.

Crippen, T.L., Benbow, M.E. and Pechal, J.L. (2015), “Microbial interactions during carrion decomposition”, in Benbow, M.E., Tomberlin, J.K. and Tarone, A.M. (Eds.), *Carrion Ecology, Evolution, and Their Applications*, CRC Press, Hoboken, pp. 31–64.

Crooks, E.R., Bulling, M.T. and Barnes, K.M. (2016), “Microbial effects on the development of forensically important blow fly species”, *Forensic science international*, Vol. 266, pp. 185–190.

Croquet, A. and Schoenaers, F. (2016), “Managing the ‘Overall integrated security policy’ at the local level: An analysis of inter-institutional dialogue”, in Hondeghem, A., Rousseaux, X. and Schoenaers, F. (Eds.), *Modernisation of the criminal justice chain and the judicial system: new insights on trust, cooperation and human capital*,*ius gentium*, Vol. 50, 1st ed. 2016, Springer International Publishing, Cham, *s.l.*, pp. 131–144.

Damos, P. and Savopoulou-Soultani, M. (2012), “Temperature-driven models for insect development and vital thermal requirements”, *Psyche: A Journal of Entomology*, Vol. 2012 No. 1-2, pp. 1–13.

Daniel, R.M. and Danson, M.J. (2010), “A new understanding of how temperature affects the catalytic activity of enzymes”, *Trends in biochemical sciences*, Vol. 35 No. 10, pp. 584–591.

Davies, L. (2006), “Lifetime reproductive output of *Calliphora vicina* and *Lucilia sericata* in outdoor caged and field populations; flight vs. egg production?”, *Medical and veterinary entomology*, Vol. 20 No. 4, pp. 453–458.

Davies, L. and Ratcliffe, G.G. (1994), “Development rates of some pre-adult stages in blowflies with reference to low temperatures”, *Medical and Veterinary Entomology*, Vol. 8 No. 3, pp. 245–254.

Dekeirsschieter, J., Verheggen, F.J., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G. and Haubruge, E. (2009), “Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes”, Forensic science international, Vol. 189 No. 1-3, pp. 46–53.

Deneubourg, J.L. and Goss, S. (1989), “Collective patterns and decision-making”, *Ethology Ecology & Evolution*, Vol. 1 No. 4, pp. 295–311.

Denlinger, D.L. (1994), “Metamorphosis behavior of flies”, *Annual review of entomology*, Vol. 39, pp. 243–266.

Denno, R.F. and Cothran, W.R. (1975), “Niche relationships of a guild of necrophagous flies”, *Annals of the Entomological Society of America*, Vol. 68 No. 4, pp. 741–754.

Denno, R.F. and Cothran, W.R. (1976), “Competitive interactions and ecological strategies of sarcophagid and calliphorid flies inhabiting rabbit carrion”, *Annals of the Entomological Society of America*, Vol. 69 No. 1, pp. 109–113.

Detrain, C. and Deneubourg, J.-L. (2006), “Self-organized structures in a superorganism: do ants “behave” like molecules?”, *Physics of Life Reviews*, Vol. 3 No. 3, pp. 162–187.

DeVault, T.L., Brisbin, J.I.L. and Rhodes, J.O.E. (2004), “Factors influencing the acquisition of rodent carrion by vertebrate scavengers and decomposers”, *Canadian Journal of Zoology*, Vol. 82 No. 3, pp. 502–509.

DeVault, T.L., Rhodes, J.O.E. and Shivik, J.A. (2003), “Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems”, *Oikos*, Vol. 102 No. 2, pp. 225–234.

Dill, L.M. (1987), “Animal decision making and its ecological consequences: the future of aquatic ecology and behaviour”, *Canadian Journal of Zoology*, Vol. 65 No. 4, pp. 803–811.

Donovan, S.E., Hall, M.J.R., Turner, B.D. and Moncrieff, C.B. (2006), “Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures”, *Medical and Veterinary Entomology*, Vol. 20 No. 1, pp. 106–114.

Doube, B.M. (1987), “Spatial and temporal organization in communities associated with dung pats and carcasses”, in Gee, J.H.R. and Giller, P.S. (Eds.), *Organization of communities past and present, Symposium of the British Ecological Society*, Blackwell Scientific Publ, Oxford, pp. 253–280.

El-Moaty, Z.A. and Kheirallah, A.E.M. (2013), “Developmental variation of the blow fly *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) by different substrate tissue types”, *Journal of Asia-Pacific Entomology*, Vol. 16 No. 3, pp. 297–300.

Erincçlioğlu, Z. (1996), *Blowflies, The naturalists' handbooks*, Vol. 23, Great Britain: Richmond Publishing Co. Ltd, Slough.

Evans, A.C. (1935), “Studies on the influence of the environment on the sheep blow-fly, *Lucilia sericata* Meig”, *Parasitology*, Vol. 27 No. 2, pp. 291–298.

Farine, D.R., Aplin, L.M., Garroway, C.J., Mann, R.P. and Sheldon, B.C. (2014), “Collective decision making and social interaction rules in mixed-species flocks of songbirds”, *Animal Behaviour*, Vol. 95 No. 100, pp. 173–182.

Fischer, K., Zeilstra, I., Hetz, S.K. and Fiedler, K. (2005), “Physiological costs of growing fast: does accelerated growth reduce pay-off in adult fitness?”, *Evolutionary Ecology*, Vol. 18 No. 4, pp. 343–353.

Flores, M., Crippen, T.L., Longnecker, M. and Tomberlin, J.K. (2017), “Nonconsumptive effects of predatory *Chrysomya rufifacies* (Diptera: Calliphoridae) larval cues on larval *Cochliomyia macellaria* (Diptera: Calliphoridae) growth and development”, *Journal of medical entomology*, Vol. 54 No. 5, pp. 1167–1174.

Flores, M., Longnecker, M. and Tomberlin, J.K. (2014), “Effects of temperature and tissue type on *Chrysomya rufifacies* (Diptera: Calliphoridae) (Macquart) development”, *Forensic Science International*, Vol. 245, pp. 24–29.

Forbes, S.L. and Carter, D.O. (2015), “Carrion ecology, evolution, and their applications”, in Benbow, M.E., Tomberlin, J.K. and Tarone, A.M. (Eds.), *Carrion Ecology, Evolution, and Their Applications*, CRC Press, Hoboken, pp. 13–30.

Forsman, J.T., Seppänen, J.-T. and Mönkkönen, M. (2002), “Positive fitness consequences of interspecific interaction with a potential competitor”, *Proceedings. Biological sciences*, Vol. 269 No. 1500, pp. 1619–1623.

Fouche, Q., Hedouin, V. and Charabidze, D. (2018), “Communication in necrophagous Diptera larvae: interspecific effect of cues left behind by maggots and implications in their aggregation”, *Scientific reports*, Vol. 8 No. 1, p. 2844.

Fox, C.W. (1997), “The ecology of body size in a seed beetle, *Stator limbatus*: persistence of environmental variation across generations?”, *Evolution*, Vol. 51 No. 3, pp. 1005–1010.

Fraenkel, G.S. and Gunn, D.L. (1940), *The orientation of animals: Kineses, taxes and compass reactions, Monographs on animal biology*, Oxford Univ. Pr, London u.a.

Frederickx, C., Dekeirsschieter, J., Verheggen, F.J. and Haubruge, E. (2013), “The community of Hymenoptera parasitizing necrophagous Diptera in an urban biotope”, *Journal of insect science (Online)*, Vol. 13 No. 1, p. 32.

Galbraith, H. (1988), “Effects of egg size and composition on the size, quality and survival of lapwing *Vanellus vanellus* chicks”, *Journal of Zoology*, Vol. 214 No. 3, pp. 383–398.

Gardner, A. and Grafen, A. (2009), “Capturing the superorganism: a formal theory of group adaptation”, *Journal of evolutionary biology*, Vol. 22 No. 4, pp. 659–671.

GBIF.org (2017), “GBIF Home Page. GBIF Backbone Taxonomy”, available at: https://www.gbif.org.

Gerday, C., Aittaleb, M., Arpigny, J.L., Baise, E., Chessa, J.-P., Garsoux, G., Petrescu, I. and Feller, G. (1997), “Psychrophilic enzymes: a thermodynamic challenge”, *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, Vol. 1342 No. 2, pp. 119–131.

Gerhardt, R.R. and Hribar, L.J. (2019), “Flies (Diptera)”, in *Medical and Veterinary Entomology*, Elsevier, pp. 171–190.

Ghosh, S.M., Testa, N.D. and Shingleton, A.W. (2013), “Temperature-size rule is mediated by thermal plasticity of critical size in *Drosophila melanogaster*”, *Proceedings. Biological sciences*, Vol. 280 No. 1760, 20130174.

Gibson, J.F., Slatosky, A.D., Malfi, R.L., Roulston, T. and Davis, S.E. (2014), “Eclosion of *Physocephala tibialis* (say)(Diptera: Cnopidae) from a *Bombus* (Apidae: Hymenoptera) host: A video record”, *The Journal of the Entomological Society of Ontario*, Vol. 145.

Goodbrod, J.R. and Goff, M.L. (1990), “Effects of larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture”, *Journal of medical entomology*, Vol. 27 No. 3, pp. 338–343.

Gosselin, M., Charabidze, D., Frippiat, C., Bourel, B. and Gosset, D. (2010), “Development time variability: adaptation of Régnière’s method to the intrinsic variability of Belgian *Lucilia sericata* (Diptera, Calliphoridae) population”, *Journal of Forensic Research*, Vol. 01 No. 109.

Gotthard, K. (2000), “Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly”, *Journal of Animal Ecology*, Vol. 69 No. 5, pp. 896–902.

Gotthard, K., Nylin, S. and Wiklund, C. (1994), “Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*”, *Oecologia*, Vol. 99 No. 3-4, pp. 281–289.

Grassberger, M. and Frank, C. (2004), “Initial study of arthropod succession on pig carrion in a central European urban habitat”, *Journal of medical entomology*, Vol. 41 No. 3, pp. 511–523.

Grassberger, M. and Reiter, C. (2001), “Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram”, *Forensic science international*, Vol. 120 No. 1-2, pp. 32–36.

Green, A.A. (1951), “The control of blowflies infesting slaughter-houses I. Field observations of the habits of blowflies”, *Annals of Applied Biology*, Vol. 38 No. 2, pp. 475–494.

Green, P.W.C., Simmonds, M.S.J. and Blaney, W.M. (2003), “Diet nutriment and rearing density affect the growth of black blowfly larvae, *Phormia regina* (Diptera: Calliphoridae).”, *European Journal of Entomology*, Vol. 100 No. 1, pp. 39–42.

Greenberg, B. (1990), “Behavior of postfeeding larvae of some Calliphoridae and a Muscid (Diptera)”, *Annals of the Entomological Society of America*, Vol. 83 No. 6, pp. 1210–1214.

Greenberg, B. (1991), “Flies as forensic indicators”, *Journal of medical entomology*, Vol. 28 No. 5, pp. 565–577.

Greenberg, B. and Kunich, J.C. (2002), *Entomology and the law: Flies as forensic indicators*, First paperback version, Cambridge University Press, Cambridge, New York.

Griffin, A.S., Savani, R.S., Hausmanis, K. and Lefebvre, L. (2005), “Mixed-species aggregations in birds: zenaida doves, *Zenaida aurita*, respond to the alarm calls of carib grackles, *Quiscalus lugubris*”, *Animal Behaviour*, Vol. 70 No. 3, pp. 507–515.

Gunn, A. (2009), *Essential forensic biology*, 2nd ed., Wiley - Blackwell, Chichester, West Sussex.

Hadjer-Kounouz, S. and Louadi, K. (2017), “Development of *Calliphora vicina* (Robineau-Desvoid) (Diptera: Calliphoridae) under different biotic and abiotic conditions”, *Journal of Entomology and Zoology studies*, Vol. 5 No. 1, pp. 683–691.

Hagstrum, D.W. and Milliken, G.A. (1991), “Modeling differences in insect developmental times between constant and fluctuating temperatures”, *Annals of the Entomological Society of America*, Vol. 84 No. 4, pp. 369–379.

Hanski, I. (1976), “Assimilation by *Lucilia illustris* (Diptera) larvae in constant and changing temperatures”, *Oikos*, Vol. 27 No. 2, p. 288.

Hartley, S. and Shorrocks, B. (2002), “A general framework for the aggregation model of coexistence”, *Journal of Animal Ecology*, Vol. 71 No. 4, pp. 651–662.

Hassall, M., Tuck, J.M. and James, R. (2005), “Effects of density and spatial heterogeneity on foraging behaviour and fitness correlates of *Armadillidium vulgare* (Isopoda Oniscidea)”, *Ethology Ecology & Evolution*, Vol. 17 No. 3, pp. 233–247.

Hazlett, B.A. (1990), “Source and nature of disturbance-chemical system in crayfish”, *Journal of chemical ecology*, Vol. 16 No. 7, pp. 2263–2275.

Henderson, P.A., Holmes, R.H.A. and Bamber, R.N. (1988), “Size-selective overwintering mortality in the sand smelt, *Atherina boyeri* Risso, and its role in population regulation”, *Journal of Fish Biology*, Vol. 33 No. 2, pp. 221–233.

Henrich, S. (1988), “Variation in offspring sizes of the poeciliid fish *Heterandria formosa* in relation to fitness”, *Oikos*, Vol. 51 No. 1, p. 13.

Hightower, B.G., Spates, G.E. and Garcia, J.J. (1972), “Growth and critical size at pupation for larvae of the screwworm developing in fresh wounds”, *Journal of Economic Entomology*, Vol. 65 No. 5, pp. 1349–1352.

Higley, L.G. and Haskell, N.H. (2001), “Insect development and forensic entomology.”, in Byrd, J.H. and Castner, J.L. (Eds.), *Forensic entomology: The utility of arthropods in legal investigations*, CRC Press, Boca Raton, pp. 287–302.

Hobson, R.P. (1931), “Studies on the nutrition of blow-fly larvae. I. Structure and function of the alimentary tract”, *The Journal of Experimental Biology*, Vol. 8 No. 2, pp. 109–123.

Hobson, R.P. (1932), “Studies on the nutrition of blow-fly larvae. III. The liquefaction of muscle”, *The Journal of Experimental Biology*, Vol. 9 No. 4, pp. 359–365.

Hölldobler, B., Nelson, M.C. and Wilson, E.O. (2009), *The superorganism: The beauty, elegance, and strangeness of insect societies*, 1st ed., W.W. Norton, New York.

Hoermann, C. von, Ruther, J. and Ayasse, M. (2012), “The attraction of virgin female hide beetles (*Dermestes maculatus*) to cadavers by a combination of decomposition odour and male sex pheromones”, *Frontiers in zoology*, Vol. 9 No. 18.

Hückesfeld, S., Niederegger, S., Schlegel, P., Heinzel, H.-G. and Spiess, R. (2011), “Feel the heat: The effect of temperature on development, behavior and central pattern generation in 3rd instar *Calliphora vicina* larvae”, *Journal of insect physiology*, Vol. 57 No. 1, pp. 136–146.

Hwang, C.C. and Turner, B.D. (2009), “Small-scaled geographical variation in life-history traits of the blowfly *Calliphora vicina* between rural and urban populations”, *Entomologia Experimentalis et Applicata*, Vol. 132 No. 3, pp. 218–224.

Ireland, S. and Turner, B. (2006), “The effects of larval crowding and food type on the size and development of the blowfly, *Calliphora vomitoria*”, *Forensic Science International*, Vol. 159 No. 2-3, pp. 175–181.

Isaksen, G.V., Åqvist, J. and Brandsdal, B.O. (2014), “Protein surface softness is the origin of enzyme cold-adaptation of trypsin”, *PLoS computational biology*, Vol. 10 No. 8, e1003813.

Janaway, R.C., Percival, S.L. and Wilson, A.S. (2009), “Decomposition of human remains”, in Percival, S.L. (Ed.), *Microbiology and Aging*, Vol. 1, Humana Press, Totowa, NJ, pp. 313–334.

Janzen, D.H. (1977), “Why fruits rot, seeds mold, and meat spoils”, *The American Naturalist*, Vol. 111 No. 980, pp. 691–713.

Jeanson, R., Deneubourg, J.-L., Grimal, A. and Theraulaz, G. (2004), “Modulation of individual behavior and collective decision-making during aggregation site selection by the ant *Messor barbarus*”, *Behavioral Ecology and Sociobiology*, Vol. 55 No. 4, pp. 388–394.

Jeanson, R., Rivault, C., Deneubourg, J.-L., Blanco, S., Fournier, R., Jost, C. and Theraulaz, G. (2005), “Self-organized aggregation in cockroaches”, *Animal Behaviour*, Vol. 69 No. 1, pp. 169–180.

Johansen, H., Solum, M., Knudsen, G.K., Hågvar, E.B., Norli, H.R. and Aak, A. (2014), “Blow fly responses to semiochemicals produced by decaying carcasses”, *Medical and veterinary entomology*, Vol. 28 No. 1, pp. 26–34.

Jordan, H.R. and Tomberlin, J.K. (2017), “Abiotic and biotic factors regulating inter-kingdom engagement between insects and microbe activity on vertebrate remains”, *Insects*, Vol. 8 No. 2., p. 54

Kaltenpoth, M. and Steiger, S. (2014), “Unearthing carrion beetles' microbiome: characterization of bacterial and fungal hindgut communities across the Silphidae”, *Molecular ecology*, Vol. 23 No. 6, pp. 1251–1267.

Kamal, A.S. (1958), “Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera)1 I. Bionomics”, *Annals of the Entomological Society of America*, Vol. 51 No. 3, pp. 261–271.

Kaneshrajah, G. and Turner, B. (2004), “*Calliphora vicina* larvae grow at different rates on different body tissues”, *International journal of legal medicine*, Vol. 118 No. 4, pp. 242–244.

Khelifa, R., Zebsa, R., Amari, H., Mellal, M.K. and Mahdjoub, H. (2019), “Field estimates of fitness costs of the pace-of-life in an endangered damselfly”, *Journal of evolutionary biology*, Vol. 32 No. 9, pp. 943–954.

King, A.J. and Sueur, C. (2011), “Where next? Group coordination and collective decision making by primates”, *International Journal of Primatology*, Vol. 32 No. 6, pp. 1245–1267.

Kirman, A. (1993), “Ants, rationality, and recruitment”, *The Quarterly Journal of Economics*, Vol. 108 No. 1, pp. 137–156.

Klingenberg, C.P. and Spencer, J. (1997), “On the role of body size for life-history evolution”, *Ecological Entomology*, Vol. 22 No. 1, pp. 55–68.

Komo, L., Scanvion, Q., Hedouin, V. and Charabidze, D. (2019a), “Facing death together: heterospecific aggregations of blowfly larvae evince mutual benefits”, *Behavioral Ecology*, Vol. 30 No. 4, pp. 1113–1122.

Komo, L., Scanvion, Q., Hédouin, V. and Charabidze, D. (2019b), “Data from: Facing death together: heterospecific aggregations of blowfly larvae evince mutual benefits. Behavioral Ecology. Dryad Digital Repository”, dryad.tb214n0.

Korolev, O.V. and Brygadyrenko, V.V. (2014), “Influence of individual variation in the trophic spectra of *Pterostichus melanarius* (Coleoptera, Carabidae) on the adaptation possibilities of its population”, *Folia oecologica*, Vol. 41 No. 1, pp. 34–43.

Krause, J. and Ruxton, G.D. (2008), *Living in groups, Oxford series in ecology and evolution*, Repr, Oxford University Press, Oxford.

Le Zheng, Crippen, T.L., Dabney, A., Gordy, A. and Tomberlin, J.K. (2017), “Evaluation of sterilized artificial diets for mass rearing the *Lucilia sericata* (Diptera: Calliphoridae)”, *Journal of medical entomology*, Vol. 54 No. 5, pp. 1122–1128.

Lefebvre, F. and Gaudry, E. (2009), “Forensic entomology: a new hypothesis for the chronological succession pattern of necrophagous insect on human corpses”, *Annales de la Société entomologique de France (N.S.)*, Vol. 45 No. 3, pp. 377–392.

Levot, G.W., Brown, K.R. and Shipp, E. (1979), “Larval growth of some calliphorid and sarcophagid Diptera”, *Bulletin of Entomological Research*, Vol. 69 No. 03, p. 469-475.

Lewis, E.E., Campbell, J., Griffin, C., Kaya, H. and Peters, A. (2006), “Behavioral ecology of entomopathogenic nematodes. Third International Symposium on Entomopathogenic Nematodes and Symbiotic Bacteria”, *Biological Control*, Vol. 38 No. 1, pp. 66–79.

Lewis, E.E. and Shapiro-Ilan, D.I. (2002), “Host cadavers protect entomopathogenic nematodes during freezing”, *Journal of Invertebrate Pathology*, Vol. 81 No. 1, pp. 25–32.

Lorenzo Figueiras, A.N. and Lazzari, C.R. (1998), “Aggregation behaviour and interspecific responses in three species of Triatominae”, *Memorias do Instituto Oswaldo Cruz*, Vol. 93 No. 1, pp. 133–137.

Lozada, M., Adamo, P. d', Buteler, M. and Kuperman, M.N. (2016), “Social learning in *Vespula Germanica* wasps: Do they use collective foraging strategies?”, *PloS one*, Vol. 11 No. 3, e0152080.

Ludwig, D., Crowe, P.A. and Hassmer, M.M. (1964), “Free fat and glycogen during metamorphosis of *Musca domestica* L.”, *Journal of the New York Entomological Society*, Vol. 72 No. 1, pp. 23–28.

Lutz, L. and Amendt, J. (2019), “Insect breeding in forensic laboratories - A survey”, paper presented at 16th meeting of the European Assoiation of Forensic Entomology, Bordeaux.

Mañas-Jordá, S., León-Cortés, J.L., García-García, M.D., Caballero, U. and Infante, F. (2018), “Dipteran diversity and ecological succession on dead pigs in contrasting mountain habitats of chiapas, Mexico”, *Journal of medical entomology*, Vol. 55 No. 1, pp. 59–68.

Marchenko, M.I. (1988), “Medico-legal relevance of cadaver entomofauna for the determination of the time since death”, *Acta medicinae legalis et socialis*, Vol. 38 No. 1, pp. 257–302.

Marchenko, M.I. (2001), “Medicolegal relevance of cadaver entomofauna for the determination of the time of death”, *Forensic science international*, Vol. 120 No. 1-2, pp. 89–109.

Martinez-Sanchez, A., Rojo, S. and Marcos-Garcia, M.A. (2000), “Annual and spatial activity of dung flies and carrion in a Mediterranean holm-oak pasture ecosystem”, *Medical and Veterinary Entomology*, Vol. 14 No. 1, pp. 56–63.

Matuszewski, S., Bajerlein, D., Konwerski, S. and Szpila, K. (2010a), “Insect succession and carrion decomposition in selected forests of Central Europe. Part 1: Pattern and rate of decomposition”, *Forensic science international*, Vol. 194 No. 1-3, pp. 85–93.

Matuszewski, S., Bajerlein, D., Konwerski, S. and Szpila, K. (2010b), “Insect succession and carrion decomposition in selected forests of Central Europe. Part 2: Composition and

residency patterns of carrion fauna", *Forensic science international*, Vol. 195 No. 1-3, pp. 42–51.

Matuszewski, S., Bajerlein, D., Konwerski, S. and Szpila, K. (2011), "Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna", *Forensic science international*, Vol. 207 No. 1-3, pp. 150–163.

Meekan, M.G. and Fortier, L. (1996), "Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf", *Marine Ecology Progress Series*, Vol. 137, pp. 25–37.

Merritt, R.W., Courtney, G.W. and Keiper, J.B. (2009), "Diptera", in *Encyclopedia of Insects*, Elsevier, pp. 284–297.

Merritt, R.W. and De Jong, G.D. (2015), "Arthropod communitiesin terrestrial environments", in Benbow, M.E., Tomberlin, J.K. and Tarone, A.M. (Eds.), *Carrion Ecology, Evolution, and Their Applications*, CRC Press, Hoboken, pp. 65–91.

Metcalfe, N.B. and Monaghan, P. (2001), "Compensation for a bad start: grow now, pay later?", *Trends in Ecology & Evolution*, Vol. 16 No. 5, pp. 254–260.

Meyer, S.G.E. and Schaub, G. (1973), "Der respiratorische Stoffwechsel von Calliphoriden Larven in Beziehung zu Temperaturadaptation und Regulation", *Journal of Insect Physiology*, Vol. 19 No. 11, pp. 2183–2198.

Michod, R.E. (2000), *Darwinian dynamics: Evolutionary transitions in fitness and individuality*, Princeton University Press, Princeton, N.J.

Miller, T.J., Crowder, L.B., Rice, J.A. and Marschall, E.A. (1988), "Larval size and recruitment mechanisms in fishes: Toward a conceptual framework", *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. 45 No. 9, pp. 1657–1670.

Mizell, R.F., Andersen, P.C., Brodbeck, B.V. and Hunter, W.B. (2012), "Congener response reduces risks from bottom-up and top-down forces: Behavioral parsimony by a xylophage", *American Entomologist*, Vol. 58 No. 2, pp. 106–115.

Mohr, R.M. (2012), "Female blow fly (Diptera: Calliphoridae) arrival patterns and consequences for larval development on ephemeral resources", Dissertation, Texas A&M University, 2012.

Moritz, R.F.A. and Southwick, E.E. (1992), *Bees as superorganisms: An evolutionary reality*, Springer Berlin Heidelberg, Berlin, Heidelberg.

Moynihan, M. (1962), *The organization and probable evolution of some mixed species flocks of neotropical birds*, *Publication / Smithsonian Institution*, Vol. 4473, Smithsonian Inst, Washington, DC.

Munch, S.B. and Conover, D.O. (2003), "Rapid growth results in increased susceptibility to predation in *Menidia menidia*", *Evolution*, Vol. 57 No. 9, pp. 2119–2127.

Munn, C.A. (1984), "The behavioral ecology of mixed-species bird flocks in Amazonian Peru", Dissertation, Princeton Univ., Phil., Ann Arbor, 1984.

Muntzer, A., Montagne, C., Ellse, L. and Wall, R. (2015), "Temperature-dependent lipid metabolism in the blow fly *Lucilia sericata*", *Medical and veterinary entomology*, Vol. 29 No. 3, pp. 305–313.

Nee, S., Read, A.F., Greenwood, J.J.D. and Harvey, P.H. (1991), "The relationship between abundance and body size in British birds", *Nature*, Vol. 351 No. 6324, pp. 312–313.

Newman, R.A. (1992), "Adaptive plasticity in Amphibian metamorphosis", *BioScience*, Vol. 42 No. 9, pp. 671–678.

Nguyen, T.T.X., Tomberlin, J.K. and Vanlaerhoven, S. (2013), "Influence of resources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development", *Journal of medical entomology*, Vol. 50 No. 4, pp. 898–906.

Nicolis, S.C., Halloy, J. and Deneubourg, J.-L. (2016), "Transition between segregation and aggregation: the role of environmental constraints", *Scientific reports*, Vol. 6, p. 32703.

Niederegger, S., Pastuschek, J. and Mall, G. (2010), "Preliminary studies of the influence of fluctuating temperatures on the development of various forensically relevant flies", *Forensic science international*, Vol. 199 No. 1-3, pp. 72–78.

Niederegger, S., Wartenberg, N., Spiess, R. and Mall, G. (2013), "Influence of food substrates on the development of the blowflies *Calliphora vicina* and *Calliphora vomitoria* (Diptera, Calliphoridae)", *Parasitology research*, Vol. 112 No. 8, pp. 2847–2853.

Nigam, Y., Bexfield, A., Thomas, S. and Ratcliffe, N.A. (2006), "Maggot therapy: The science and implication for CAM part I-history and bacterial resistance", *Evidence-based complementary and alternative medicine eCAM*, Vol. 3 No. 2, pp. 223–227.

Noë, R. (2001), “Biological markets: partner choice as the driving force behind the evolution of mutualisms”, in Noë, R., van Hooff, J.A.R.A.M. and Hammerstein, P. (Eds.), *Economics in Nature: Social dilemmas, mate choice and biological markets*, Cambridge University Press, Cambridge, pp. 93–118.

Nunney, L. (1996), “The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: an example of a fitness trade-off”, *Evolution; international journal of organic evolution*, Vol. 50 No. 3, pp. 1193–1204.

Nylin, S. and Gotthard, K. (1998), “Plasticity in life-history traits”, *Annual review of entomology*, Vol. 43, pp. 63–83.

Parrish, J.K. and Edelstein-Keshet, L. (1999), “Complexity, pattern, and evolutionary trade-offs in animal aggregation”, *Science*, Vol. 284 No. 5411, pp. 99–101.

Parrish, J.K. and Hamner, W.M. (1997), *Animal groups in three dimensions*, Cambridge University Press, Cambridge.

Payne, J.A. (1965), “A summer carrion study of the baby pig *Sus Scrofa* Linnaeus”, *Ecology*, Vol. 46 No. 5, pp. 592–602.

Pechal, J.L., Benbow, M.E., Crippen, T.L., Tarone, A.M. and Tomberlin, J.K. (2014), “Delayed insect access alters carrion decomposition and necrophagous insect community assembly”, *Ecosphere*, Vol. 5 No. 4, 1-21.

Pendola, S. and Greenberg, B. (1975), “Substrate-specific analysis of proteolytic enzymes in the larval midgut of *Calliphora vicina*”, *Annals of the Entomological Society of America*, Vol. 68 No. 2, pp. 341–345.

Planqué, R., van den Berg, J.B. and Franks, N.R. (2010), “Recruitment strategies and colony size in ants”, *PloS one*, Vol. 5 No. 8, e11664.

Podhorna, J., Aubernon, C., Borkovcova, M., Boulay, J., Hedouin, V. and Charabidze, D. (2018), “To eat or get heat: Behavioral trade-offs between thermoregulation and feeding in gregarious necrophagous larvae”, *Insect science*, Vol. 25 No. 5, pp. 883–893.

Polis, G.A., Anderson, W.B. and Holt, R.D. (1997), “Toward an integration of landscape and food web: the dynamics of spatially subsidized food webs”, *Annual Review of Ecology and Systematics*, Vol. 28 No. 1, pp. 289–316.

Prasad, N.G., Shakarad, M., Anitha, D., Rajamani, M. and Joshi, A. (2001), “Correlated responses to selection for faster development and early reproduction in Drosophila: the evolution of larval traits”, *Evolution; international journal of organic evolution*, Vol. 55 No. 7, pp. 1363–1372.

Prasad, N.G., Shakarad, M., Gohil, V.M., Sheeba, V., Rajamani, M. and Joshi, A. (2000), “Evolution of reduced pre-adult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time”, *Genetics Research*, Vol. 73 No. 3, pp. 249–259.

Pratt, S., Mallon, E., Sumpter, D. and Franks, N. (2002), “Quorum sensing, recruitment, and collective decision-making during colony emigration by the ant *Leptothorax albipennis*”, *Behavioral Ecology and Sociobiology*, Vol. 52 No. 2, pp. 117–127.

Presa Abos, C., Lepori, F., Mckie, B.G. and Malmqvist, B. (2006), “Aggregation among resource patches can promote coexistence in stream-living shredders”, *Freshwater Biology*, Vol. 51 No. 3, pp. 545–553.

Prinkkilá, M.-L. and Hanski, I. (1995), “Complex competitive interactions in four species of *Lucilia* blowflies”, *Ecological Entomology*, Vol. 20 No. 3, pp. 261–272.

R Core Team (2017), *R: A language and environment for statistical computing*, Vienna, Austria.

Rabossi, A., Wappner, P. and Quesada-Allué, L.A. (1992), “Larva to pharate adult transformation in the medfly *Creatitis capitata* (Wiedemann) (Diptera:Tephritidae)”, *The Canadian Entomologist*, Vol. 124 No. 06, pp. 1139–1147.

Reigada, C. and Godoy, W.A.C. (2012), “Direct and indirect top-down effects of previous contact with an enemy on the feeding behavior of blowfly larvae”, *Entomologia Experimentalis et Applicata*, Vol. 142 No. 1, pp. 71–77.

Ribeiro, C.d.S. and Zuben, C.J. von (2010), “Nutritional ecology of blowflies (Diptera, Calliphoridae): estimates of critical larval weight for pupation on two different”, *Revista Brasileira de Entomologia*, Vol. 54 No. 4, pp. 661–664.

Richards, C.S., Rowlinson, C.C., Cuttiford, L., Grimsley, R. and Hall, M.J.R. (2013), “Decomposed liver has a significantly adverse affect on the development rate of the blowfly *Calliphora vicina*”, *International journal of legal medicine*, Vol. 127 No. 1, pp. 259–262.

Richner, H. (1992), “The effect of extra food on fitness in breeding carrion crows”, *Ecology*, Vol. 73 No. 1, pp. 330–335.

Ricklefs, R.E. (1969), “Natural selection and the development of mortality rates in young birds”, *Nature*, Vol. 223 No. 5209, pp. 922–925.

Ridley, A. and Raihani, N. (2007), “Facultative response to a kleptoparasite by the cooperatively breeding pied babbler”, *Behavioral Ecology*, Vol. 18 No. 2, pp. 324–330.

Riemann, F. and Helmke, E. (2002), “Symbiotic relations of sediment-agglutinating nematodes and bacteria in detrital habitats: The enzyme-sharing concept”, *Marine Ecology*, Vol. 23 No. 2, pp. 93–113.

Rivers, D.B., Thompson, C. and Brogan, R. (2011), “Physiological trade-offs of forming maggot masses by necrophagous flies on vertebrate carrion”, *Bulletin of entomological research*, Vol. 101 No. 5, pp. 599–611.

Roberts, R.A. (1933), “Biology of *Brachymeria fonscolombei* (Dufour), a hymenopterous parasite of blowfly larvae”, *Technical Bulletin*, No. 365, pp. 1–22.

Robinson, L.A., Bryson, D., Bulling, M.T., Sparks, N. and Wellard, K.S. (2018), “Post-feeding activity of *Lucilia sericata* (Diptera: Calliphoridae) on common domestic indoor surfaces and its effect on development”, *Forensic Science International*, Vol. 286, pp. 177–184.

Sandeman, R., Feehan, J., Chandler, R. and Bowles, V. (1990), “Tryptic and chymotryptic proteases released by larvae of the blowfly, *Lucilia cuprina*”, *International Journal for Parasitology*, Vol. 20 No. 8, pp. 1019–1023.

Santos, M., Fowler, K. and Partridge, L. (1994), “Gene-environment interaction for body size and larval density in *Drosophila melanogaster*: an investigation of effects on development time, thorax length and adult sex ratio”, *Heredity*, Vol. 72 No. 5, pp. 515–521.

Saunders, D.S. (1997), “Under-sized larvae from short-day adults of the blow fly, *Calliphora vicina*, side-step the diapause programme”, *Physiological Entomology*, Vol. 22 No. 3, pp. 249–255.

Saunders, D.S. and Bee, A. (1995), “Effects of larval crowding on size and fecundity of the blow fly, *Calliphora vicina* (Diptera: Calliphoridae)”, *European Journal of Entomology*, Vol. 92 No. 4, pp. 615–622.

Sauphanor, B. and Sureau, F. (1993), “Aggregation behaviour and interspecific relationships in Dermaptera”, *Oecologia*, Vol. 96 No. 3, pp. 360–364.

Sazima, C., Krajewski, J.P., Bonaldo, R.M. and Sazima, I. (2007), “Nuclear-follower foraging associations of reef fishes and other animals at an oceanic archipelago”, *Environmental Biology of Fishes*, Vol. 80 No. 4, pp. 351–361.

Scanvion, Q., Hédouin, V. and Charabidzé, D. (2018), “Collective exodigestion favours blow fly colonization and development on fresh carcasses”, *Animal Behaviour*, Vol. 141, pp. 221–232.

Schmidt, K.P. (1957), *Warder Clyde Allee 1885-1955: A Biographical Memoir*. National Academy of Sciences of the United States.

Schmolz, E. and Lamprecht, I. (2000), “Calorimetric investigations on activity states and development of holometabolous insects”, *Thermochimica Acta*, Vol. 349 No. 1-2, pp. 61–68.

Schneider, C.A., Rasband, W.S. and Eliceiri, K.W. (2012), “NIH Image to ImageJ: 25 years of image analysis”, *Nature methods*, Vol. 9 No. 7, pp. 671–675.

Scott, C., Deneubourg, J.-L., Franks, N.R., Sneyd, J., Bonabeau, E. and Theraulaz, G. (2003), *Self-organization in biological systems, Princeton studies in complexity*, 2. print. and 1. paperback print, Princeton Univ. Press, Princeton, NJ.

Seeley, T.D., Mikheyev, A.S. and Pagano, G.J. (2000), “Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability”, *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, Vol. 186 No. 9, pp. 813–819.

Semeniuk, C.A.D. and Dill, L.M. (2006), “Anti-predator benefits of mixed-species groups of cowtail stingrays (*Pastinachus sephen*) and whiprays (*Himantura uarnak*) at rest”, *Ethology*, Vol. 112 No. 1, pp. 33–43.

Sevenster, J.G. and 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*, Vol. 62 No. 4, p. 720-736.

Shaaya, E. and 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*, Vol. 28 No. 8, pp. 683–688.

Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M., Vilcinskas, A. and Vogel, H. (2018), "Microbiome-assisted carrion preservation aids larval development in a burying beetle", *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 115 No. 44, pp. 11274–11279.

Siddiki, S. and Zambare, S.P. (2017), "Studies on time duration of life stages of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) during different seasons", *Journal of Forensic Research*, Vol. 08 No. 03.

Sibly, R.M., Smith, R.H. and Moller, H. (1991), "Evolutionary demography of a bruchid beetle. IV. Genetic trade-off, stabilizing selection and a model of optimal body size", *Functional Ecology*, Vol. 5 No. 5, p. 594.

Sinervo, B. and 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*, Vol. 50 No. 3, pp. 1314–1327.

Slone, D.H. and Gruner, S.V. (2007), "Thermoregulation in larval aggregations of carrion-feeding blow flies (Diptera: Calliphoridae)", *Journal of Medical Entomology*, Vol. 44 No. 3, pp. 516–523.

Smith, K.E. and Wall, R. (1997), "The use of carrion as breeding sites by the blowfly *Lucilia sericata* and other Calliphoridae", *Medical and Veterinary Entomology*, Vol. 11 No. 1, pp. 38–44.

Somero, G.N. (1978), "Temperature adaptation of enzymes: Biological optimization through structure-function compromises", *Annual Review of Ecology and Systematics*, Vol. 9 No. 1, pp. 1–29.

Spicka, A., Johnson, R., Bushing, J., Higley, L.G. and Carter, D.O. (2011), "Carcass mass can influence rate of decomposition and release of ninhydrin-reactive nitrogen into gravesoil", *Forensic science international*, Vol. 209 No. 1-3, pp. 80–85.

Spradbery, J.P. and Vogt, W.G. (1993), "Mean life expectancy of Old World screwworm fly, *Chrysomya bezziana*, inferred from the reproductive age-structure of native females caught on swormlure-baited sticky traps", *Medical and veterinary entomology*, Vol. 7 No. 2, pp. 147–154.

Sridhar, H., Beauchamp, G. and Shanker, K. (2009), “Why do birds participate in mixed-species foraging flocks? A large-scale synthesis”, *Animal Behaviour*, Vol. 78 No. 2, pp. 337–347.

Srinivasan, U., Raza, R.H. and Quader, S. (2010), “The nuclear question: rethinking species importance in multi-species animal groups”, *The Journal of Animal Ecology*, Vol. 79 No. 5, pp. 948–954.

Stephens, P.A., Sutherland, W.J. and Freckleton, R.P. (1999), “What is the Allee effect?”, *Oikos*, Vol. 87 No. 1, p. 185.

Stockhoff, B.A. (1991), “Starvation resistance of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae): tradeoffs among growth, body size, and survival”, *Oecologia*, Vol. 88 No. 3, pp. 422–429.

Stoks, R., Block, M. de, van de Meutter, F. and Johansson, F. (2005), “Predation cost of rapid growth: behavioural coupling and physiological decoupling”, *Journal of Animal Ecology*, Vol. 74 No. 4, pp. 708–715.

Sueur, C., Deneubourg, J.-L. and Petit, O. (2012), “From social network (centralized vs. decentralized) to collective decision-making (unshared vs. shared consensus)”, *PloS one*, Vol. 7 No. 2, e32566.

Swift, M.J., Heal, O.W. and Anderson, J.M. (1979), *Decomposition in terrestrial ecosystems*, *Studies in ecology*, Vol. 5, Univ. of Calif. Pr, Berkeley.

Szpila, K. (2010), “Key for the identification of third instars of European blowflies (Diptera: Calliphoridae) of forensic importance”, in Amendt, J., Goff, M.L., Campobasso, C.P. and Grassberger, M. (Eds.), *Current Concepts in Forensic Entomology*, Vol. 43, Springer Netherlands, Dordrecht, pp. 43–56.

Szpila, K. (2017), “Annotated list of blowflies (Diptera: Calliphoridae) recorded during studies of insect succession on large carrion in Poland.”, *Dipteron*, No. 33, pp. 85–93.

Tarone, A.M. and Foran, D.R. (2006), “Components of developmental plasticity in a Michigan population of *Lucilia sericata* (Diptera: Calliphoridae)”, *Journal of medical entomology*, Vol. 43 No. 5, pp. 1023–1033.

Taylor, B.J. and Jeanne, R.L. (2018), “Gastral drumming: a nest-based food-recruitment signal in a social wasp”, *Die Naturwissenschaften*, Vol. 105 No. 3-4, p. 23.

Terra, W.R. and Ferreira, C. (1994), “Insect digestive enzymes: properties, compartmentalization and function”, *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, Vol. 109 No. 1, pp. 1–62.

Theraulaz, G. and Bonabeau, E. (1999), “A brief history of stigmergy”, *Artificial Life*, Vol. 5 No. 2, pp. 97–116.

Thomas, D.B. and Chen, A.C. (1990), “Age distribution of adult female screwworms (Diptera: Calliphoridae) captured on sentinel animals in the coastal lowlands of Guatemala”, *Journal of economic entomology*, Vol. 83 No. 4, pp. 1422–1429.

Thyssen, P.J., Souza, C.M. de, Shimamoto, P.M., Salewski, T.d.B. and Moretti, T.C. (2014), “Rates of development of immatures of three species of *Chrysomya* (Diptera: Calliphoridae) reared in different types of animal tissues: implications for estimating the postmortem interval”, *Parasitology research*, Vol. 113 No. 9, pp. 3373–3380.

Tomberlin, J.K., Benbow, M.E., Barnes, K.M. and Jordan, H.R. (2017), “Arthropod-microbe interactions on vertebrate remains: potential applications in the forensic sciences”, in David O. Carter, Jeffery K. Tomberlin, M. Eric Benbow and Jessica L. (Eds.), *Forensic microbiology*, *Forensic science in focus*, Wiley, Hoboken, NJ, pp. 274–311.

Tomberlin, J.K., Sanford, M.R., Pimsler, M.L. and VanLaerhoven, S.L. (2015), “Vertebrate carrion as a model for conducting behavior research”, in Benbow, M.E., Tomberlin, J.K. and 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”, *Archives of Insect Biochemistry and Physiology*, Vol. 35 No. 4, pp. 479–490.

Ullyett, G.C. (1950), “Competition for food and allied phenomena in sheep-blowfly populations”, *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, Vol. 234 No. 610, pp. 77–174.

Underwood, G.J.C., Perkins, R.G., Consalvey, M.C., Hanlon, A.R.M., Oxborough, K., Baker, N.R. and Paterson, D.M. (2005), “Patterns in microphytobenthic primary productivity: Species-specific variation in migratory rhythms and photosynthetic efficiency in mixed-species biofilms”, *Limnology and Oceanography*, Vol. 50 No. 3, pp. 755–767.

Vinogradova, E.B. (2009), “Effect of food and temperature on the reproduction of the blowfly, *Calliphora vicina* R.-D. (Diptera, Calliphoridae), a popular model object in biological research”, *Entomological Review*, Vol. 89 No. 2, pp. 137–142.

Wall, R. (1993), “The reproductive output of the blowfly *Lucilia sericata*”, *Journal of Insect Physiology*, Vol. 39 No. 9, pp. 743–750.

Wall, R., Pitts, K.M. and Smith, K.E. (2001), “Pre-adult mortality in the blowfly *Lucilia sericata*”, *Medical and Veterinary Entomology*, Vol. 15 No. 3, pp. 328–334.

Wall, R. and Warnes, M.L. (1994), “Responses of the sheep blowfly *Lucilia sericata* to carrion odour and carbon dioxide”, *Entomologia Experimentalis et Applicata*, Vol. 73 No. 3, pp. 239–246.

Wallenstein, M., Allison, S.D., Ernakovich, J., Steinweg, J.M. and Sinsabaugh, R. (2011), “Controls on the temperature sensitivity of soil enzymes: A key driver of in situ enzyme activity rates”, in Shukla, G. and Varma, A. (Eds.), *Soil Enzymology*, *Soil Biology*, Vol. 22, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 245–258.

Wang, J.W., Sylwester, A.W., Reed, D., Wu, D.-A.J., Soll, D.R. and 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*, Vol. 11 No. 3-4, pp. 231–254.

Ward, A.J.W., Sumpter, D.J.T., Couzin, I.D., Hart, P.J.B. and Krause, J. (2008), “Quorum decision-making facilitates information transfer in fish shoals”, *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 105 No. 19, pp. 6948–6953.

Wardle, R.A. (1927), “The seasonal frequency of Calliphorine blowflies in Great Britain”, *The Journal of hygiene*, Vol. 26 No. 4, pp. 441–464.

Warren, J.-A. and Anderson, G.S. (2013), “Effect of fluctuating temperatures on the development of a forensically important blow fly, *Protophormia terraenovae* (Diptera: Calliphoridae)”, *Environmental entomology*, Vol. 42 No. 1, pp. 167–172.

Wessels, F.J., Kristal, R., Netter, F., Hatle, J.D. and Hahn, D.A. (2011), “Does it pay to delay? Flesh flies show adaptive plasticity in reproductive timing”, *Oecologia*, Vol. 165 No. 2, pp. 311–320.

Wigglesworth, V.B. (1972), *The Principles of Insect Physiology*, Seventh Edition, Springer Netherlands, Dordrecht.

Williams, H. and Richardson, A.M.M. (1984), "Growth energetics in relation to temperature for larvae of four species of necrophagous flies (Diptera: Calliphoridae)", *Austral Ecology*, Vol. 9 No. 2, pp. 141–152.

Wilson, E.O. (1962), "Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith) 1. The Organization of Mass-Foraging", *Animal Behaviour*, Vol. 10 No. 1-2, pp. 134–147.

Wilson, D.S. and Sober, E. (1989), "Reviving the superorganism", *Journal of Theoretical Biology*, Vol. 136 No. 3, pp. 337–356.

Wilson, A.J. and Nussey, D.H. (2010), "What is individual quality? An evolutionary perspective", *Trends in Ecology & Evolution*, Vol. 25 No. 4, pp. 207–214.

Wilson, M.R., Nigam, Y., Jung, W., Knight, J. and Pritchard, D.I. (2016), "The impacts of larval density and protease inhibition on feeding in medicinal larvae of the greenbottle fly *Lucilia sericata*", *Medical and veterinary entomology*, Vol. 30 No. 1, pp. 1–7.

Wright, J., McDonald, P.G., te Marvelde, L., Kazem, A.J.N. and Bishop, C.M. (2010), "Helping effort increases with relatedness in bell miners, but 'unrelated' helpers of both sexes still provide substantial care", *Proceedings. Biological sciences*, Vol. 277 No. 1680, pp. 437–445.

Yakovlev, A.Y., Kruglikova, A.A. and Chernysh, S.I. (2019), "Calliphoridae flies in medical biotechnology", *Entomological Review*, Vol. 99 No. 3, pp. 292–301.

Yanmanee, S., Husemann, M., Benbow, M.E. and Suwannapong, G. (2016), "Larval development rates of *Chrysomya rufifacies* Macquart, 1842 (Diptera: Calliphoridae) within its native range in South-East Asia", *Forensic Science International*, Vol. 266, pp. 63–67.

Zdarek, J. (1985), "Regulation of pupariation in flies", in Kerkut, G.A. (Ed.), *Endocrinology II: Volume 8*, Elsevier Science, Burlington, pp. 301–333.

Appendix

Table 10 Development numbers for statistics

Number of *L. sericata* larvae under each larval composition (i.e., conspecific groups with 100 and 250 individuals as well as heterospecific groups with 125 *L. sericata* mixed with 125 *C. vicina*) and in total for the datasets 'Development' (all individuals) and 'Size' (only the individuals whose surface area were measured). In addition, the average migration times and the values used for the cumulative development rate equation are listed.

	Dataset Development		**Dataset Size**	
	15 °C	28 °C	15 °C	28 °C
$\mathbf{n_{100}}$	564	550	310	313
$\mathbf{n_{125}}$	682	655	286	271
$\mathbf{n_{250}}$	1 406	1 395	358	341
$\mathbf{n_{total}}$	2 652	2 600	954	925
Mean migration time	226.135 h [± 17.82 h]	86.175 h [± 9.80 h]	219.651 h [± 13.17 h]	83.664 h [± 10.89 h]
$\frac{\mathbf{1}}{\mathbf{Mean\ migration\ time}}$	0.004422	0.011604	0.004553	0.011953

Table 11 Proportion of eclosed flies of *L. sericata*

Eclosed flies of *L. sericata* in the three conditions: mean value from the six replicates with standard error.

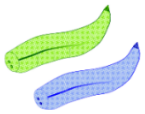
Development time from oviposition [hours]	100 *L. sericata* 	250 *L. sericata* 	125 *L. s* (+125 *C. vi*) 
294	0.0 ± 0.0%	2.0 ± 4.3%	0.0 ± 0.0%
300	0.0 ± 0.0%	7.4 ± 13.0%	0.3 ± 0.4%
312	1.6 ± 1.6%	29.0 ± 20.7%	5.6 ± 3.0%
318	7.0 ± 7.3%	52.9 ± 27.0%	13.1 ± 8.7%
324	14.5 ± 10.6%	69.9 ± 22.9%	23.7 ± 11.3%
336	42.4 ± 19.1%	80.6 ± 22.0%	46.3 ± 13.9%
342	69.2 ± 18.9%	87.7 ± 18.5%	64.9 ± 12.7%
348	78.7 ± 16.3%	92.4 ± 13.0%	77.0 ± 12.0%
360	93.0 ± 2.6%	95.6 ± 6.6%	87.2 ± 7.6%
366	97.8 ± 0.6%	98.1 ± 2.3%	91.8 ± 5.9%
372	98.8 ± 1.1%	98.6 ± 1.4%	96.0 ± 5.3%
384	99.5 ± 0.8%	99.3 ± 0.3%	98.4 ± 2.1%
390	99.5 ± 0.8%	99.4 ± 0.4%	99.3 ± 0.5%
396	100 ± 0.0%	100 ± 0.0%	99.5 ± 0.6%
408	100 ± 0.0%	100 ± 0.0%	99.7 ± 0.5%

Table 12 Proportion of eclosed flies of *C. vicina*

Eclosed flies of *C. vicina* in the three conditions: mean value from the six replicates with standard error.

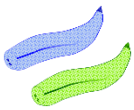
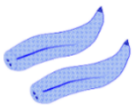
Development time from oviposition [hours]	125 *C. vi* (+125 *L. s*) 	250 *C. vicina* 	100 *C. vicina* 
360	0.0 ± 0.0%	0.5 ± 0.9%	0.0 ± 0.0%
366	1.1 ± 1.8%	3.7 ± 3.1%	0.2 ± 0.4%
372	1.1 ± 1.8%	4.1 ± 3.1%	9.6 ± 6.4%
384	5.4 ± 3.5%	9.4 ± 4.6%	24.3 ± 5.1%
390	31.5 ± 16.3%	33.5 ± 12.6%	32.5 ± 3.7%
396	32.2 ± 16.4%	34.6 ± 13.3%	39.5 ± 4.6%
408	38.3 ± 17.4%	42.7 ± 10.4%	48.8 ± 2.9%
414	74.2 ± 29.1%	89.3 ± 11.9%	61.5 ± 7.6%
420	75.5 ± 29.4%	91.3 ± 12.3%	79.1 ± 6.4%
432	80.5 ± 23.0%	93.8 ± 7.7%	87.8 ± 4.7%
438	94.6 ± 9.1%	99.4 ± 0.6%	92.2 ± 2.3%
444	96.0 ± 8.8%	99.7 ± 0.5%	97.7 ± 1.5%
456	97.7 ± 5.7%	100 ± 0.0%	100 ± 0.0%
462	100 ± 0.0%	100 ± 0.0%	100 ± 0.0%

Table 13 Effects of group density and composition at all temperatures

Observation of "↑" indicates benefits, "↓" indicates costs and "=" indicates no effects. Asterisks mark significant results ($p < 0.05$). Interestingly, no disadvantages could be observed between the heterospecific and the conspecific high-density groups (hetero vs. high). Note further that at each temperature tested, a faster development of *C. vicina* led to smaller individuals in heterospecific groups compared to the conspecific low-density groups (hetero vs. low). Development refers to the time when 10 % of the group reached the postfeeding stage; size refers to the average puparia surface area; survival refers to the total survival rate; hetero refers to the heterospecific group (125+125 larvae); low and high refer to the conspecific groups (100 and 250 larvae).

condition	**temp.**	**Development**		**Size**		**Survival**	
		C. vicina	*L. sericata*	*C. vicina*	*L. sericata*	*C. vicina*	*L. sericata*
hetero vs. high	15 °C	↑*	↑*	↑*	↑*	=	=
	25 °C	↑*	↓*	↑	↑*	=	↑
	28 °C	↑*	=	=	=	=	=
hetero vs. low	15 °C	↑*	↑*	↓	=	↑	=
	25 °C	↑*	=	↓*	↓*	=	↑
	28 °C	↑*	=	↓*	↓*	↑*	=
high vs. low	15 °C	↓*	↓*	↓*	↓*	↑	=
	25 °C	=	↑*	↓*	↓*	=	=
	28 °C	↓*	↓*	↓*	=	↑*	=

Table 14 Biotic conditions affecting development stages

Blowflies' developmental studies focusing on different biotic conditions (e.g., food freshness, food type or group size) that provide data about the end of the feeding stage (i.e., migration) and the end of nymphosis (i.e., eclosion) are reported. The times given depend on the studies' definition when migration and eclosion are reached for one group (i.e., 10 or 50 % of all group individuals). The columns for migration and eclosion indicate both the minimum and maximum time that resulted from the different tested conditions as well as the corresponding variation (i.e., difference) and sample intervals (in gold). The last column shows how many hours the eclosion time span is longer than the migration time span. The differences for migrating are significantly shorter than for eclosing (paired t-test: t = -3.679, df = 9, p = 0.0051).

	species	**temp. [° C]**	**migration [h]**			**eclosion [h]**			**diff. [h]**
Bernhardt et al. (2017)	*C. vicina*	25	113-121	8	8-16	385-394	9	8-16	+1
Komo et al. (2019a)	*C. vicina*	25	90-114	24	6-12	372-408	36	6-12	+12
Komo et al. (2019a), Scanvion et al. (2018)	*L. sericata*	25	78-102	24	6-12	294-330	36	6-12	+12
Richards et al. (2013)	*C. vicina*	23	70-112	42	-	397-455	58	-	+16
El-Moaty et al. (2013)[a]	*L. sericata*	25	70-106	35	6	275-330	55	(5)	+20
Greenberg (1991)	*L. sericata*	22-29	72-94	22	-	296-345	49	-	+27
Greenberg (1991)	*Phormia regina*	22-29	70-95	25	-	279-337	58	-	+33
Grassberger & Reiter (2001)	*L. sericata*	21-25	85-118	33	6	297-379	82	(6)	+49
Greenberg (1991)	*C. vicina*	19-25	77-129	52	-	460-583	123	-	+71
Tarone & Foran (2006)	*L. sericata*	25	99-142	43	(24)	335-465	130	-	+87

[a] The eclosion times from El-Moaty et al. (2013) are derived from the published diagram.

Truth in science *can be defined as the working hypothesis best suited to open the way to the next better one.*

Konrad Lorenz