Plasticity of senescence in the antler fly (*Protopiophila litigata*)

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It is indeed remarkable that after a seemingly miraculous feat of morphogenesis a complex metazoan should be unable to perform the much simpler task of merely maintaining what is already formed.

— George C. Williams, "Pleiotropy, natural selection, and the evolution of senescence"

[Spears:] How far do you have to hike in the woods to find a moose antler?

[Angell:] A long way, let me tell you.

— Tom Spears, "Science of spring: When I see an antler fly"

There is nothing better than to know that you don't know.

— Lao Tzu, *Tao Te Ching* 71 (trans. Charles Muller)

Abstract

As most multicellular organisms age, they undergo senescence: a progressive physiological deterioration that leads to declines in survival, reproduction, and performance in late life. Although senescence was once thought to be a phenomenon peculiar to captive animals and humans, field data have demonstrated age-related performance declines in a variety of taxa. Nevertheless, the ecology and evolution of senescence is not fully understood. The bulk of our knowledge about senescence in wild populations comes from studies of long-lived vertebrates, while short-lived invertebrates are often studied in the lab. Male antler flies (*Protopiophila litigata*; Diptera: Piophilidae) are an emerging insect model for studying senescence in nature, as they have short lifespans and high site-fidelity, facilitating collection of longitudinal data, and they can be easily reared and manipulated in the lab. This species is an ideal model to connect our lab-invertebrate- and field-vertebrate-based insights into aging biology.

The developmental environment can have an especially large impact on life-history plasticity, including plasticity in senescence. This is because a developing organism makes “decisions” affecting phenotypes such as body size, sexual investment, metabolic rate, etc., which in turn can influence longevity and senescence. In my dissertation, I investigate how the early life environment, including larval diet and parental effects, plastically alters longevity and senescence in antler flies, primarily in the field in Algonquin Provincial Park.

First, I quantified the effect of experimentally manipulated larval nutrient concentration on both early-life (growth and development) and late-life traits (reproduction, survival, senescence). Rich larval diet decreased development time, and although fast developers grew large and had low initial mortality in the field (and high average lifespan), they aged rapidly and had low mating rate. Due to these contrasting effects, diet and development time did not predict lifetime mating success, suggesting trade-offs among fitness components and alternative strategies in low condition males.

Only male antler flies can be tracked in the field, so nothing is known about aging in females. In my second study, I compared longevity and aging of female and male antler flies in the lab. Theory suggests that males may age faster and die sooner than females, but empirical data are highly variable. Furthermore, the sexes may respond differently to variation in nutrition, so I reared flies on different larval diets based on the design of my first chapter. The sexes did not differ in senescence or longevity in the lab, and diet had a negligible effect. Large-bodied flies of both sexes senesced slower, in contrast to previous field data, highlighting plastic differences in senescence between wild and captive populations.

In my third study, I quantified parental age effects on male antler flies. Offspring quality often changes with parental age, due to accumulation of germline mutations and/or changes in nongenetic maternal or paternal effects. To investigate whether and how parental age influences performance in wild insects, I mated lab-reared young and old females and males to one another in all combinations, and tracked their male offspring's performance in the wild. Old fathers had long-lived sons, while maternal age had no effect on offspring survival in the field. Parental age did not affect mating success. Thus, the one parental age effect I observed was in fact positive, not negative.

In my final study, I looked at how natural differences in larval diet, rather than artificial lab diets, influenced survival, mating, and senescence in wild male antler flies. Antlers become depleted of resources from year to year, as multiple generations of larvae feed within them. I collected larvae that grew inside nine different shed moose antlers, and tracked them in the field as adults. Males from high quality antlers (those that were more attractive to adult flies) completed metamorphosis more quickly, but did not differ in body size, longevity or lifetime mating success. However, large flies tended to live longer and have higher mating success.

In conclusion, my dissertation research expands our understanding of plasticity in life history and senescence, particularly in insects, which are enormously abundant but understudied in this area. I quantified, for the first time to my knowledge, the effects of juvenile diet and parental age on longevity, mating success, and senescence in a wild insect.

Résumé

Parce que la plupart des organismes multicellulaires vieillissent, ils subissent une senescence : une détérioration physiologique progressive qui entraine un déclin de la survie, de la reproduction et des performances en fin de vie. Bien que la senescence fût pensée comme un phénomène propre aux humains et aux animaux en captivité, les données en milieu naturel ont démontré que les performances relatives à l'âge déclinaient dans de nombreux taxons. Cependant, l'écologie et l'évolution de la senescence ne sont pas comprises dans leur entièreté. L'essentiel de nos connaissances sur la senescence dans les populations en milieu sauvage provient d'études sur les vertébrés à longue durée de vie, alors que les invertébrés à courte durée de vie sont plus souvent étudiés en laboratoire.

Les mouche de bois de cervidés mâles (*Protopiophila litigata*; Diptera: Piophilidae) sont un modèle d'insecte émergeant pour étudier la senescence en milieu naturel, car ils ont une courte durée de vie et une haute fidélité au site, ce qui facilite la collection de donnée longitudinal et ainsi rend l'élevage et la manipulation facile en laboratoire. Cette espèce est un modèle idéal pour connecter les connaissances des invertébrés en laboratoire et des vertébrés en milieu naturel et ainsi mieux comprendre la biologie du vieillissement.

L'environnement du développement peut avoir un impact particulièrement large sur la plasticité de l'histoire de vie, comprenant la plasticité de la senescence. C'est pourquoi un organisme en développement prend des « décisions » qui affecte son phénotype comme sa taille corporelle, son investissement sexuel, son taux métabolique, etc ; qui par la suite affecte sa longévité et sa senescence. Dans ma dissertation, j'examine comment l'environnement précoce dans la vie ; incluant le régime alimentaire larvaire et les effets parentaux ; altère plastiquement la longévité et la senescence chez les mouches de bois de cervidés, principalement sur le terrain dans le parc provincial Algonquin.

Premièrement, j'ai quantifié l'effet d'une manipulation expérimentale sur la concentration des nutriments larvaires au sein de la vie précoce (développement et croissance) et de la vie tardive

(reproduction, survie, senescence). Le régime alimentaire larvaire riche réduit le temps de développement, et bien que les larves avec un développement rapide aient grandi plus large avec une faible mortalité initiale au milieu sauvage (et avec une grande longévité en moyenne), ils ont vieilli plus rapidement et ont un taux de reproduction plus faible. En raison de ces effets contradictoires, le régime alimentaire et le temps de développement ne permettent pas de prédire le succès reproducteur à vie, suggérant des compromis entre les composants de la valeur adaptative et des stratégies alternatives pour les mâles en mauvaise condition.

Seuls les mouches de bois de cervidés mâles peuvent être suivi dans la nature, donc rien n'est connu sur le vieillissement des femelles. Dans ma deuxième étude, j'ai comparé la longévité et le vieillissement des mouche de bois de cervidés femelles et mâles en laboratoire. La théorie suggère que les mâles devraient vieillir plus rapidement et mourir plus tôt que les femelles, mais les données empiriques sont très variables. En outre, les sexes pourraient répondre différemment aux variations nutritionnelles, j'ai donc élevé des mouches dans différent régimes alimentaire basés sur le même protocole que le premier chapitre. Les sexes ne diffèrent pas dans leurs senescence ou longévité en laboratoire, et le régime alimentaire a eu un effet négligeable. La sénescence des mouches avec de corps plus larges dans les deux sexes a été plus lente, en contradiction avec le précédant chapitre en milieu sauvage, ce qui met en évidence les différences plastiques de la senescence entre les populations captives et sauvages.

Dans ma troisième étude, j'ai quantifié les effets de l'âge parental sur les mouches de bois de cervidés mâles. La qualité de la progéniture change souvent avec l'âge parental, due à l'accumulation de mutations germinales et/ou aux changement des effets non-génétique maternel ou paternel. Pour déterminer si et comment l'âge parental influence les performances des insectes sauvage, j'ai accouplé des femelles et mâles jeunes et vieux élevés en laboratoire entre eux selon toutes les combinaisons possibles et ainsi mesurer les performances de la progéniture mâle en milieux naturel. Les pères âgés ont des fils avec une longue durée de vie, alors que l'âge maternel n'a aucun effet sur la survie des descendants en milieu sauvage. L'âge parental n'a pas d'effet sur le succès reproducteur. Ainsi, le seul effet de l'âge parental que j'ai observé était un effet positif et non négatif.

Dans ma dernière étude, j'ai examiné comment les différences naturelles dans le régime alimentaire larvaire, plutôt qu'artificiel en laboratoire, influençaient la survie, la reproduction et la senescence chez les mouches de bois de cervidés mâles. Les bois s'épuisent en ressource d'année en année, car plusieurs générations de larves s'y nourrissent. J'ai collecté des larves qui se sont développé à l'intérieur de neufs différents bois d'élan, et suivit sur le terrain jusqu'au stade adulte. Les mâles provenant de bois de haute qualité (ceux qui étaient plus attractif pour les mouches adultes) ont achevé leur métamorphose plus rapidement, mais ne présentent aucune différence dans leur taille corporelle, longévité ou succès reproducteur à vie. Cependant les larges mouches ont tendance à vivre plus longtemps et à avoir de meilleurs taux de succès reproducteur.

En conclusion, ma recherche étend notre compréhension de la plasticité dans l'histoire de vie et la senescence, particulièrement chez les insectes, qui sont largement abondant mais sous-étudié dans ce domaine. J'ai quantifié, pour la première fois à ma connaissance, les effets du régime alimentaire chez les jeunes et les effets de l'âge parental sur la longévité, le succès reproducteur et la senescence chez un insecte sauvage.

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I really am profoundly fortunate to be here, submitting my PhD thesis. I have spent my four and a half years in Ontario playing with bugs in the woods, and, incredibly, that will be enough to build a doctorate upon. I recognize that a lot of things out of my control have contributed to my successful journey so far.

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Indigenous Affirmation

Ni manàdjiyànànig Màmìwininì Anishinàbeg, ogog kà nàgadawàbandadjig iyo akì eko weshkad. Ako nongom ega wìkàd kì mìgiwewàdj.

Ni manàdjiyànànig kakina Anishinàbeg ondaje kaye ogog kakina eniyagizidjig enigokamigàg Kanadàng eji ondàpinangig endàwàdjin Odàwàng.

Ninisidawinawànànig kenawendamòdjig kije kikenindamàwin; weshkinìgidjig kaye kejeyàdizidjig.

Nigijeweninmànànig ogog kà nìgànì sòngideyedjig; weshkad, nongom; kaye àyànikàdj.

We pay respect to the Algonquin people, who are the traditional guardians of this land. We acknowledge their longstanding relationship with this territory, which remains unceded.

We pay respect to all Indigenous people in this region, from all nations across Canada, who call Ottawa home.

We acknowledge the traditional knowledge keepers, both young and old.

And we honour their courageous leaders: past, present, and future.

Preface

The work presented in Chapter 2 is a slightly modified version of the following published article:

> Angell, C. S., M. J. Oudin, N. Rode, B. Mautz, R. Bonduriansky, and H. D. Rundle. 2020. Development time mediates the effect of larval diet on ageing and mating success of male antler flies in the wild. Proceedings of the Royal Society B 287:20201876.

The experimental design of Chapter 2 was developed by Mathieu Oudin, Howard Rundle, and Russell Bonduriansky and data were collected by Mathieu Oudin and Brian Mautz. I performed statistical analysis of the data (with help from Nicolas Rode) and took the lead in interpretation and writing (with input from all co-authors).

For Chapters 3, 4, and 5, I took the lead role in study design, analysis, interpretation, and writing (with input from Howard Rundle), and collected the data with help from Rebecca Janacek (Chapter 4), Tracey Yu (Chapter 5), and Tia Chen (Chapter 5).

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Chapter 1: General Introduction

Senescence is the progressive physiological deterioration of an organism with age, leading to within-individual declines in survival, reproduction, and performance in late life. This process is widespread, but not ubiquitous, across multicellular life (Jones et al. 2014), despite its apparent cost to individual fitness. In my thesis, I distinguish between "aging," which refers to any changes in phenotypes as an organism becomes older, and reserve "senescence" for deleterious effects of age-related declines in organismal function (Williams 1957). Although senescence was once thought to be a phenomenon peculiar to captive animals and humans, data from natural systems have demonstrated age-related declines in a variety of taxa (Nussey et al. 2013; Lemaître and Gaillard 2017; Zajitschek et al. 2020). Senescence is costly to individual fitness, so the relative rarity of non-senescence among multicellular organisms demands an evolutionary explanation. Although the physiological mechanisms of senescence are not well resolved (Hughes and Reynolds 2005), there are two widely accepted, non-exclusive hypotheses for its evolution. Both rely on the assumption that even a "theoretically immortal" organism will eventually die due to extrinsic factors (starvation, disease, predation, etc.), so selection is relaxed on traits expressed later in life.

The mutation accumulation hypothesis proposes that late-acting deleterious alleles are rendered nearly neutral by this relaxation of selection, allowing them to persist in populations (Medawar 1952). The antagonistic pleiotropy hypothesis suggests that alleles causing declines late in life may in fact have positive fitness effects on average, and proliferate in populations, if they also increase early life performance (Williams 1957). A related idea is the disposable soma hypothesis, which proposes an energetic conflict between reproductive investment and somatic maintenance, leading to widespread survival—reproduction trade-offs (Kirkwood and Holliday 1979). If organisms maximize their fitness when they allocate less energy to maintenance than would be required to repair somatic damage, then selection will favor individuals that allow senescent deterioration to accumulate. Both mutation accumulation and antagonistic pleiotropy probably act on most populations to some extent, and there is empirical support for each mechanism (Hughes and Reynolds 2005). Although laboratory studies have not always demonstrated trade-offs between early- and late-life function (Maklakov et al. 2015), studies of populations in nature, where senescent phenotypes have evolved, generally do (Lemaître et al. 2015).

Life-history traits, including senescence, vary among individuals in natural populations. Some of this variation is surely genetic, as evidenced by the discovery of "aging genes" in the laboratory (e.g., Maklakov et al. 2017) and the ability of senescence to evolve under selection (Hillesheim and Stearns 1992; Stearns et al. 2000; Stojković and Savković 2011), but life-history traits generally are especially plastic compared to other traits (Price and Schluter 1991). In particular, conditions during the early stages of life, while an organism develops, can influence their life-history traits throughout adulthood. This can include variation in both prenatal (e.g., in the egg or during gestation) and post-natal environments. For many traits, a high-quality early-life environment (e.g., abundant resources or low competition) can improve adult performance and fitness (Lindström 1999; Nussey et al. 2007; Tigreros 2013), but the evidence for such a beneficial effect on senescence is more mixed (Cooper and Kruuk 2018; Spagopoulou et al. 2020). Another possible source of life-history plasticity are maternal and paternal effects, in which aspects of the developmental environment or offspring physiology are determined by parental genotype and/or phenotype (Mousseau and Dingle 1991; Crean and Bonduriansky 2014). Parents of low condition or old age may pass on costs to their offspring (Moorad and Nussey 2016; Bonduriansky and Crean 2018), including shorter longevity and faster senescence, but once again, the empirical evidence is mixed (Schroeder et al. 2015; Heinze et al. 2018; Ivimey-Cook and Moorad 2020).

Most studies of the ecology and evolution of senescence have been conducted either on relatively long-lived vertebrates in the wild (e.g., Promislow 1991; Robinson et al. 2006; Hayward et al. 2015), or on short-lived invertebrates in the laboratory (e.g., Stearns et al. 2000; Chen and Maklakov

2012; Archer et al. 2017; Hooper et al. 2017; van den Heuvel et al. 2017). The former requires long-term monitoring to collect longitudinal data, which is challenging, while the latter generally feature very different environments than nature, hampering biological interpretation. Although it was traditionally assumed that insects do not appreciably senesce in the wild, a number of more recent studies have quantified aging in insects under natural or semi-natural conditions, including flies (Bonduriansky and Brassil 2002, 2005; Kawasaki et al. 2008; Mautz et al. 2019), crickets (Zajitschek et al. 2009a,b; Rodríguez-Muñoz et al. 2019a,b), bees (Dukas 2008), odonates (Sherratt et al. 2010, 2011), and lepidopterans (Carroll and Sherratt 2017). These studies showcase the valuable opportunity provided by insects' short lifespans to explore the evolutionary ecology of senescence (Zajitschek et al. 2020). In this thesis, I use the antler fly *Protopiophila litigata* to investigate the causes and consequences of senescence in the wild.

Protopiophila litigata, the antler fly, is a small fly species in the skipper fly family Piophilidae (Bonduriansky 1995). Adult males of this species defend territories on the surfaces of discarded antlers from moose and deer (Bonduriansky and Brooks 1999). While females move freely among the antlers, on which they feed, mate, and oviposit, males usually remain on a given antler for their entire adult lives, allowing longitudinal data to be collected on components of a marked individual's fitness (Bonduriansky and Brassil 2002, 2005; Mautz et al. 2019). Their high site fidelity and short adult lifespan (under 32 days) make male antler flies an ideal field system for measuring senescence in the wild.

This thesis presents the results of four studies of aging in antler flies. In the first study (Chapter 2), I analyzed the results of a field experiment testing the effects of (artificial) variation in larval nutrient concentration on adult survival, mating success, and senescence of male antler flies in the field. In the second (Chapter 3), I carried out a laboratory experiment to characterize senescence in female antler flies, which was previously totally unknown, and to quantify the extent of sex differences in longevity and aging. I also manipulated larval diet in both sexes, as in the previous chapter, to test whether the effect of larval nutrition was sex-specific. In the third study (Chapter 4), I investigated parental age effects in antler flies by experimentally manipulating both maternal and paternal age in a 2×2 factorial design and tracking their male offspring's survival and mating success in the field. Finally, in the fourth study (Chapter 5), I collected wild larvae that developed in nine different shed moose antlers to test whether natural variation in larval environmental quality affected adult performance in the same way as in Chapter 2.

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Chapter 2: Development time mediates the effect of larval diet on ageing and mating success of male antler flies in the wild

Note

This chapter is a slightly modified version of the following published article:

> Angell, C. S., M. J. Oudin, N. Rode, B. Mautz, R. Bonduriansky, and H. D. Rundle. 2020. Development time mediates the effect of larval diet on ageing and mating success of male antler flies in the wild. Proceedings of the Royal Society B 287:20201876.

The experimental design of Chapter 2 was developed by Mathieu Oudin, Howard Rundle, and Russell Bonduriansky and data were collected by Mathieu Oudin and Brian Mautz. I performed statistical analysis of the data (with help from Nicolas Rode) and took the lead in interpretation and writing (with input from all co-authors).

Introduction

Early-life resource availability can be a critical contributor to variation in individual performance. This is because organisms must make developmental "decisions" in early life, such as the relative allocation of resources toward energy reserves (which can be mobilized later for metabolic processes) versus body structure (which cannot), which can have long-lasting fitness effects (Gurney et al. 2003; Dmitriew 2011). A high-quality developmental environment is generally predicted to confer lasting benefits on individual performance (Lindström 1999); this is known as the "silver-spoon" effect (Grafen 1988). For instance, high quality environments in early life can lead to increased survival (Kelly et al. 2014; Griffin et al. 2018), fecundity (Haywood and Perrins 1992), mating success

(Tigreros 2013; Kleinteich et al. 2015; Plesnar-Bielak et al. 2017), sperm quality and quantity (Tigreros 2013; Vega-Trejo et al. 2016; Macartney et al. 2018), and immune function (Birkhead et al. 1999; Peters et al. 2019) in adulthood, compared to individuals from poor environments. However, late-life traits such as senescence—the progressive, intrinsic deterioration of organisms with age which leads to increased mortality and decreased reproductive performance—do not necessarily follow the same silver-spoon pattern as life-history traits expressed during development and early adulthood.

In many cases, senescence rates are affected by energetic and physiological trade-offs with traits expressed in early life. Much of the research on trade-offs between early- and late-life performance has focused on the costs of reproductive investment (Robinson et al. 2006; Lemaître et al. 2014; Travers et al. 2015; Adler et al. 2016; Rodríguez-Muñoz et al. 2019). As future survival is uncertain, individuals with abundant access to resources may allocate highly to early-life performance, leading to more rapid declines with age (Hunt et al. 2004; Adler et al. 2016; Hooper et al. 2017, 2018; Spagopoulou et al. 2020). Likewise, but less extensively studied, juvenile growth and development may also influence senescence, and are likely to depend on early-life environmental quality. There is a long theoretical tradition linking rapid growth and development to earlier or faster senescence (Pearl 1928; Williams 1957; Monaghan et al. 2009). Faster growth also requires greater energy expenditure, leaving fewer resources available for subsequent somatic maintenance (Dmitriew 2011; Lee et al. 2011). Some empirical studies have indeed found negative phenotypic (Hooper et al. 2017) or genetic correlations (Lind et al. 2017) between development rate and lifespan, although not all show this pattern (Pijpe et al. 2006; Tigreros 2013). Conversely, individuals with high resource acquisition may experience relaxed trade-offs (van Noordwijk and de Jong 1986) and enjoy high physiological performance throughout their lifespan. Thus, the ultimate effect of early-life environmental quality on senescence is unclear. Two recent meta-analyses failed to detect consistent silver-spoon effects across taxa on longevity or actuarial senescence, and only a small effect on reproductive senescence (English and Uller 2016; Cooper and

Kruuk 2018). Nevertheless, some studies have reported significant increases in lifespan and reduced senescence for individuals that experienced high quality developmental environments (Nussey et al. 2007; Kleinteich et al. 2015; Griffin et al. 2018).

While studies of insect life histories and senescence in captivity are common (e.g. (Stearns et al. 2000; Moore and Moore 2001; Zajitschek et al. 2009)), studies of senescence in wild populations have focused mainly on vertebrates (Lemaître et al. 2015; Lemaître and Gaillard 2017). Patterns of survival and performance can differ markedly between wild and captive animals, including insects (Kawasaki et al. 2008; Hämäläinen et al. 2014; Mautz et al. 2019), and it is important to verify lab-based inferences under natural conditions. However, collecting longitudinal data on small, short-lived invertebrates poses significant logistical challenges, and studies of senescence in insects remain scarce, despite the abundance and diversity of these organisms (Zajitschek et al. 2020). A few field studies have detected trade-offs linking body size and reproductive effort to senescence rates in insects (Bonduriansky and Brassil 2005; Rodríguez-Muñoz et al. 2019), but additional longitudinal studies are needed to understand the causes and fitness consequences of life history variation in wild insects.

To determine the impact of early-life environmental quality on senescence in survival and mating success of an insect under natural conditions, we manipulated diet quality of antler fly larvae (*Protopiophila litigata*; Diptera: Piophilidae) raised in the lab. We then marked males individually, released them at antlers stationed in a natural forest environment, and monitored their survivorship and mating success in the wild. Antler flies are small (~2 mm) necrophagous flies that oviposit exclusively on shed moose and deer antlers (Bonduriansky 1995). Males defend territories in large aggregations on the antler surface (Bonduriansky and Brooks 1999), and their high site fidelity and short adult lifespan make them well suited for studies of senescence in the wild because marked males can be released (in the absence of any enclosure) and their subsequent mating success and lifespan observed under entirely natural conditions. Previous studies have demonstrated significant increases in mortality rate (i.e.

“actuarial senescence”) and decreases in mating rate (i.e. “reproductive senescence”) with age in wild male antler flies (Bonduriansky and Brassil 2002, 2005; Mautz et al. 2019). However, the effect of larval environment on such senescence remains unknown. In this study, we measured development time, body size, mating rate, and longevity to determine the impact of early-life resource availability on both early- and late-life traits. This allowed us to assess whether a nutrient-rich early-life environment causes a “silver spoon” reduction in senescence, or whether it leads to an increase in senescence rates through physiological or energetic trade-offs with growth, development rate, or reproduction.

Material and Methods

Experimental procedure

Flies and culture techniques

An outbred laboratory stock population of *Protopiophila litigata* was created from a large sample (>500) of adult flies collected in the spring and early summer of 2012 at the Wildlife Research Station, Algonquin Park, Ontario, Canada. The population was maintained at the University of Ottawa with non-overlapping generations at 23°C, 60% relative humidity and under a 17:7 L:D photoperiod. The maintenance protocol is described in detail in reference (Oudin et al. 2015). In brief, adult flies are kept in acrylic cages, from which eggs are collected each generation via an oviposition dish placed in each cage. Oviposition dishes contain a layer of 2.5 g of ground beef covered by foam sponge moistened with variable amounts of a 20% w/v ground beef solution (Oudin et al. 2015) up to three times/week to maintain moisture. Larvae feed and develop within these dishes, after which they emerge to pupate in a layer of coco peat (Nutri+, India).

Diet manipulation

Our experiment involved a manipulation of the larval diet to create four treatments (A, B, C, D) that differed in the ratio of ground beef to plant fibre within the oviposition dishes. The A diet used only regular ground beef, the same as the stock population, while diets B, C and D, consisted of 9:1, 8:1, and 7:1 mixtures of ground beef:powdered inulin fibre (Exact, Canada), respectively. All four diets were prepared by homogenising the ground beef, with or without added fibre, using a standard household food blender. Preparations were stored in a freezer at -20°C prior to use. During larval development, all diets also received 1.5 ml of ground beef solution three times per week.

Our experiment used flies that had been reared for one generation on one of these four diets. To obtain these flies, we collected adults from the stock population and randomly placed them in five cages containing 125 individuals of each sex, with access to abundant sugar and water. We replaced dead flies daily to ensure constant sex ratio and density. An oviposition dish containing a sponge was added to each cage for 48 h, after which it was removed and replaced with a new one. Once the oviposition dishes were removed from the cage, each sponge was placed on 2.5 g of one of the four larval diets (ground beef with different levels of fibre or without fibre). Oviposition dishes were collected after each of nine consecutive 48 h laying periods beginning on May 2nd, 2013, creating nine temporal blocks of offspring. As there were five parental cages, one diet treatment within each block was applied to two oviposition dishes, and the treatments were rotated among cages across blocks. Larval diet treatments were not applied until after the oviposition dishes were removed, preventing females from adjusting their egg laying in relation to diet quality. After application of the diet treatment, oviposition dishes were individually relocated to separate 250 ml mason jars with 10 g of dry coco peat layering the base and a mesh cap. These were incubated as described above for the stock population.

Field relocation and observation

On May 28th, 2013, all nine larval blocks were relocated to the Wildlife Research Station, Algonquin Provincial Park, Ontario, Canada. All containers sat on a bench in an uninsulated wood cabin with no environmental controls, and hence individuals were exposed to variable temperature, humidity and photoperiod, similar to what would be experienced in the wild. Emerging males were removed daily and individually held in a vial to allow their cuticles to sclerotize. Each male was placed in a holding chamber (Bonduriansky and Brooks 1997) and photographed in dorsal view using a Canon A640 PowerShot digital camera mounted on a dissecting microscope with an ocular micrometer. From these images, wing length was measured from the tegula to the distal tip of the M vein using ImageJ v1.47 (Schneider et al. 2012). In this species, wing length is positively correlated with thorax length (Figure S1; Pearson correlation, $r = 0.645$; $p < 0.001$) and this measurement is highly repeatable ($R = 0.99$; Oudin et al. 2015). An individual numeric code was painted on each male's thorax using enamel paint (The Testor Corporation, USA) and a paintbrush with a trimmed tip (Bonduriansky and Brooks 1997). Males were immediately released within 1 m of one of two discarded moose antlers (A and B) that were set up on separate 0.8 m high wooden stands in the forest and separated by approximately 50 m distance. Antlers can only support flies for a few years after they are dropped, so supply is limited and subsequent monitoring is also labor-intensive; two antlers was therefore the most that was feasible. We released 179 males on the larger antler A and 41 males on the smaller antler B (Table S2.1). Dispersal among antlers is generally low in this species (Bonduriansky 1996), and only 12 individuals were detected moved between antlers during the course of the study. Fewer than ten marked males dispersed to a third antler within 50 m, monitored as part of a separate study, and these were returned to antler A or B.

Antlers were surveyed every two hours from 09:00 to 19:00 for 42 consecutive days starting June 11th, 2013. Only the 11:00 observation on July 3rd was missed. During each observation, the identity and mating status (i.e. mating or not) of all marked males was recorded on each antler. The total number of flies and total number of mating pairs (involving marked and/or unmarked males) was also recorded at each observation. Individuals were excluded from the analysis if they failed to survive at least two days after marking, as they may have been injured during the measuring and marking process (Bonduriansky and Brassil 2005). Our analyses included 161 males tracked over 251 observation periods (7.04 ± 7.12 SD observations per male on average).

Statistical analyses

All analyses were performed in R v 3.6.3 (R Core Team 2020).

Effect of diet on development time and wing length

We first assessed the impact of our diet treatment on egg-to-adult development time and adult body size. To test for the effect of larval diet on development time, we used a linear model (LM) that included effects of diet treatment and larval block as categorical variables. To test for the effects of larval diet treatment on wing length (our proxy for body size), we used a LM that included diet treatment and larval block, as well as a second LM containing diet treatment, development time (a continuous variable), their interaction, and larval block. We performed type III *F*-tests using the R package *car* (Fox and Weisberg 2011).

Adult performance and senescence

Development time (number of days between egg laying and adult emergence) varied among diet treatments (see Results), but there was also substantial independent variation within treatment levels such that we were able to discriminate the respective effects of diet and development time on male performance and actuarial and reproductive senescence. These analyses included additional confounding variables that could potentially affect male survival and mating success (see below for details). Continuous variables were scaled to mean of zero and standard deviation of one prior to analysis (Schielzeth 2010). Model selection was carried out using a backward and forward stepwise likelihood ratio test (LRT) procedure, in which a global model was simplified (or a minimal model was complexified) until the model was not significantly improved by removing (or adding) any further terms, based on LRT (Crawley 2013). If the two selected models differed, a LRT was used to compare them, and the significance of all terms was assessed using LRTs relative to the final model (i.e., the minimal adequate model, including block).

Actuarial senescence

The effects of diet treatment, development time, and body size on male actuarial senescence were analyzed using parametric survival models, implemented in the R packages *survival* (Therneau 2015) and *flexsurv* (Jackson 2016). We chose this approach over semi-parametric Cox proportional hazards regression because Cox models only test for differences in overall mortality rate, but cannot detect differences in aging rates among groups. We used an interval-censored survival model (Gómez et al. 2009) in which we assumed death occurred between the age of last observation and the following day. To account for potential confounding effects, our model also included antler (coded as a continuous variable representing the proportion of observations for a given individual that occurred on antler A relative to antler B, to account for males that moved between antlers), average population density, average sex ratio, and average mating rate (all as experienced over the lifetime of a given individual) as covariates. A fixed effect of larval block was included in all models (i.e., was not allowed to drop during model selection). To avoid overfitting given the modest size of this dataset (*n* = 33-47 individuals in each diet treatment), we did not test interactions.

We performed survival model selection in three sequential steps. First, we used the R package *MuMIn* (Bartoń 2016) to select the survival distribution that best fit the data based on the corrected Akaike Information Criterion (AICc; Hurvich and Tsai 1989). Second, we performed LRT model selection on the shape parameter, and then third we performed stepwise LRT model selection on the scale parameter. For distribution selection (i.e. step 1), we used the *survival* package to fit models with exponential, Weibull, Gaussian, logistic, log-normal, log-logistic, and extreme value distributions, and used the *flexsurv* package to fit the two-parameter Gompertz and three-parameter Weibull models (see Supporting Information). The Weibull distribution consistently provided the best fit to our data independent of effects on scale (Table S2.2). The scale parameter (λ) of the Weibull model represents the time at which ~63% of the individuals are dead, while the shape (α) describes the change in the age-specific mortality rate, which can remain constant ($\alpha = 1$) or can increase ($\alpha > 1$) or decrease ($\alpha < 1$) with age (Crawley 1993).

Next, we performed LRT model selection on the Weibull shape parameter (i.e. step 2). The *survival* package allows only a single factor to be fit to the shape parameter, and any number of factors and covariates to be fit to the scale parameter of the Weibull regression. Therefore, development time and wing length, being continuous variables of particular interest, were each binned into two levels corresponding to individuals above vs. below the median value across the whole dataset, allowing us to test their effects, alongside diet treatment, as potential predictors of the shape of actuarial senescence. We then compared models that included either diet, binned development time, binned wing length effects, or a single intercept (i.e. no effect), on the shape parameter (α) using LRT. Models included all single term effects described above (without interactions) on scale. As development time caused the greatest improvement in the model (see Results), we allowed shape values to vary between levels of binned development time for subsequent analyses. Finally, we performed forward and backward stepwise model selection on the scale parameter, considering all variables described above (i.e. step 3).

Both selection processes converged on the same minimal adequate model.

Mating rate and reproductive senescence

To test whether larval diet treatment affected male mating rate and/or reproductive senescence, we used generalized linear mixed-effects models (GLMM) using the R package *lme4* (Bates et al. 2015). Mating rate, quantified as the probability of observing a male mating during an observation period, was analyzed using a binomial error distribution with a logit link function. Mating in antler flies lasts 137 ± 52 min (Bonduriansky and Brooks 1998a), and a given male was never observed mating in two consecutive observations (separated by 2 h). We tested for the effects of diet, development time, and wing length on mating rate, as well as the effect of age and its interaction with each of these variables to test for effects on senescence. We also included potential confounding variables in all our models. Lifespan, antler fly density, and sex ratio (the latter two estimated at the time of observation) were included as covariates, while antler, hour of day, and larval block were included as categorical fixed effects (block was included in all models and not permitted to drop during model selection). We included observation (nested within day) and male identity as random effects in all models to account for non-independence among males during a particular observation and for repeated measures of the same male across observations respectively. Observation periods with zero flies present on an antler were excluded from the analysis, as sex ratio cannot be calculated for these periods, but results were qualitatively similar when they were included (Table S2.10, S2.11). The initial model for backward selection contained all terms listed above. Forward selection from an initial model containing the two random effects (observation and male identity) and a fixed effect of block, converged on the same minimal adequate model.

Lifetime mating success

Because males are generally mate-limited, lifetime mating success (LMS) is a major component of male fitness. LMS depends both on an individual's longevity and their mating rate throughout life. To investigate the effects of diet, development time, and body size on male LMS (the total number of matings observed for each male), we used a generalized linear model with a negative binomial distribution and a log link function, implemented with the "glm.nb" function in the R package *MASS* (Venables and Ripley 2002).The initial model for backward selection contained the following terms: diet treatment, development time, wing length, antler, lifetime average density, and lifetime average sex ratio, and larval block (as above, block was not permitted to drop during model selection). Forward selection from an initial model containing only a fixed effect of block converged on the same minimal adequate model.

Analyses of residual development time and residual wing length

Given collinearity among diet treatment, development time, and wing length (see Results), we performed additional analyses using residual values as a conservative approach to inferring independent effects (Graham 2003). We calculated residual development time from a one-way ANOVA among diets —thereby representing only within-diet treatment variation in development time—and residual wing length from a regression against development time—representing the effect of body size independent of development time. We then performed model selection for survival, mating rate, and LMS as above, using residual development time and residual wing length instead of the 'raw' variables. An effect of residual development time and/or residual wing length would infer the importance of that variable even when diet or development time respectively is allowed to account for all shared variation.

Results

Effect of diet on development time and wing length

Egg-to-adult development time increased with decreasing diet quality ($F_{3,149} = 23.0$, $p < 0.001$, Fig. 2.1a), with a 28% increase in mean time between highest- and lowest-quality diets, but there was also substantial variation within each diet. Larval diet treatment did not significantly influence male wing length when considered alone ($F_{3,149} = 0.431$, $p = 0.731$). When considering development time and diet treatment together, wing length was negatively related to development time ($F_{1,145} = 13.4$, $p < 0.001$; Fig. 2.1b), diet quality still did not affect wing length ($F_{3,145} = 1.26$, $p = 0.289$), and there was no interaction between diet and development time on wing length ($F_{3,145} = 1.52$, $p = 0.212$).

Actuarial senescence

A Weibull survival distribution was a consistently best fit to the data (Table S2.2) and an effect of binned development time on the Weibull shape parameter significantly improved the fit compared to an intercept-only model (LRT: $\chi^2_1 = 6.01$, $p = 0.014$). Effects on the shape parameter of diet (LRT: $\chi^2_3 = 0.733$, $p = 0.865$) and wing length (LRT: $\chi^2_1 = 2.92$, $p = 0.087$) did not improve fit (see also AICc values in Table S2.2). We therefore included an effect of binned development time on shape in subsequent analyses of scale.

For the scale parameter, both forward and backward model selection converged on a common model that included significant effects on scale of development time (LRT: $\chi^2_1 = 11.5$, $p < 0.001$) and wing length (LRT: $\chi^2_1 = 3.85$, $p = 0.0498$), but did not include diet treatment (LRT: $\chi^2_3 = 3.71$, $p = 0.294$). There was also no significant effect of antler, sex ratio, density, or average mating rate on the scale of actuarial senescence (Table S2.3a). The development time effects reflected a higher initial mortality rate of slow compared to fast developers, and a steady increase in mortality rate with age for fast developers compared to a convex, decelerating mortality curve in slow developers (Fig. 2.2a; Table S2.4; shape parameter $\alpha = 2.47$ vs. 1.75 for males with a development time below or above the median, respectively). The net outcome of these contrasting effects on shape and scale is that fast developing males tended to live longer (median lifespan, pooling across diets: 11 days [95% CI: 4.0–20.3]) than slow developers (8 days [95% CI: 2.0–20.8]). There was also a small, but significant, trend for larger flies to experience lower mortality and increased lifespan (Fig. 2.2b).

Mating rate and reproductive senescence

Males that developed more slowly had significantly higher mating rates (LRT: $\chi^2_1 = 11.5$, $p < 0.001$; Fig. 2.3; Table S2.5), but diet treatment did not significantly affect average mating rates (LRT: $\chi^2_3 = 2.65$, $p = 0.449$) when accounting for the effect of development time. In addition, mating rate was higher at high density and on antler B, but there was no significant relationship between mating rate and wing length, longevity, hour of day, or block (Table S2.3b). Mating rate was not affected by age (LRT: $\chi^2_1 = 1.74$, $p = 0.187$), nor did age interact with either diet treatment, development time, or wing length (all $p > 0.05$). Therefore, we do not detect reproductive senescence in our data. If an age term is added to the final GLMM, the estimate of its effect on mating success is negative, as would be expected for reproductive senescence, but it is non-significant (reduced model + age: β [logit scale] = -0.112 ± 0.086 SE).

Lifetime mating success

Diet treatment did not affect LMS, nor did development time or wing length (all $p > 0.05$; Table S2.3c). LMS was significantly affected by the average fly density (LRT: $\chi^2_1 = 7.11$, $p = 0.008$) and the average sex ratio experienced over a male's life (LRT: $\chi^2_1 = 19.6$, $p < 0.001$), such that males that experienced higher density and less male-biased sex ratios tended to have higher LMS (Table S2.6). LMS did not differ among blocks or between antlers (Table S2.3c).

Analyses of residual development time and residual wing length

Our supplementary analysis using residual development time and residual wing length allowed diet treatment to account for all shared variation with development time. Consequently, residual development time represented only development time variation within diet treatment levels, and residual wing length reflected only size variation that was independent of development time. As expected, the previously non-significant effect of larval diet became significant when it was allowed to explain all shared variation with development time, with decreasing nutrient concentration being associated with both higher mortality (Table S2.7a; Table S2.8) and greater average mating rate (Table S2.7b; Table S2.9). However, the previously significant effects of development time persisted such that males with shorter residual development time had reduced mortality (Table S2.8) and had lower average mating rates (Table S2.9), consistent with the main analyses. Also consistent with the main analyses, residual wing length had a small effect on survival (Table S2.7a; Table S2.8), but not mating success (Table S2.7b; Table S2.9). There was again no effect of diet treatment on the shape of actuarial senescence; unlike in the main analysis, however, the effect of residual development time on shape was no longer significant, although it approached so ($p = 0.07$; Table S2.7a). Again, none of the variables of interest influenced LMS (Table S2.7c).

Discussion

In this study, we manipulated diet quality of larval antler flies, *Protopiophila litigata*, to investigate whether adult performance and lifespan would be improved by high larval diet quality under natural conditions, consistent with the silver spoon hypothesis (Grafen 1988; Lindström 1999), or whether they would decline due to trade-offs with increased allocation toward growth, development rate, or reproduction. Our results revealed complex effects of larval diet: males experiencing a richer diet developed faster, and fast-developing males tended to reach greater adult sizes and lived longer.

However, fast developers also tended to have a lower average mating rate than slow developers such that the lifetime mating success of slow vs. fast developers did not differ significantly. When accounting for the effect of development time, larval diet itself did not explain significant variation in adult body size, survival, or mating rate. Furthermore, after accounting for development time, we found no significant effects of body size on survival or mating rate, nor significant trade-offs between mating rate and longevity.

Early-life diet did not have a consistent "silver spoon" effect on all adult traits in male antler flies: fast development, caused at least in part by variation in diet quality among (and/or within) treatments, was associated with extended adult lifespan and larger size, but also more intense senescence and lower average mating rate. As a result, fast-developing males had similar LMS to slow developers, although they may ultimately have had somewhat higher fitness due to potential differences in postcopulatory performance (see below). Other studies have similarly reported complex phenotypic effects of early life environmental quality: rich larval diets can lead to increased reproductive effort and a shortened lifespan and/or accelerated senescence (Hunt et al. 2004; Adler et al. 2016; Hooper et al. 2017; Spagopoulou et al. 2020), although we observed the opposite effect on lifespan and reproduction as previous studies. Given the complex influence of early-life conditions reported in this and other studies, it is not surprising that two recent meta-analyses failed to detect consistent silver spoon effects on lifespan or actuarial senescence in laboratory or wild populations (English and Uller 2016; Cooper and Kruuk 2018).

We did not detect strong evidence of trade-offs between early and late life performance in our antler flies. Fast development was associated with longer lifespan, not shorter, and there was no significant relationship between longevity and average mating rate. Furthermore, body size, which depends on allocation toward growth in the larval stage, was not significantly associated with survival, mating success, or senescence rate. This positive correlation of life-history traits suggests high variation in resource acquisition and/or genetic quality among individuals (van Noordwijk and de Jong 1986). Nevertheless, development time had opposing effects on average mating rate and survival, which could arise from an underlying survival–reproduction trade-off. This would be consistent with a previous study of this species that reported a significantly higher average mating rate in short-lived males (Bonduriansky and Brassil 2005). Although it can be difficult to detect trade-offs in nature, studies of wild vertebrates have often identified trade-offs between early and late life (Lemaître et al. 2015). However, wild field crickets (*Gryllus campestris*) experience no apparent trade-offs between early reproduction and survival, and only a modest effect of early reproduction on senescence in calling activity (Rodríguez-Muñoz et al. 2019).

Decreasing diet quality tended to increase development time and decrease body size, but there was substantial variation in development time within each diet treatment, and in body size for a given development time, allowing the effects of these variables to be partitioned. Nevertheless, to ensure that the effect of development time in our analyses did not simply represent differences among diets, we also performed an alternative analysis using residual development time and residual wing length, representing the effects of these variables independent of larval diet and development time, respectively Using this more conservative approach, development time remained a significant predictor of the scale of actuarial senescence, and of average mating rate, alongside larval diet which was now, unsurprisingly, also significant (Table S2.7a-b). Taken together, these results suggest that not only does intrinsic variation in development time covary with adult life history traits, development time also mediates the plastic effects of larval diet quality on adult performance and ageing. Alternatively, an unmeasured variable highly correlated with development time could mediate the relationship between diet and life history traits across life stages. Regardless, we find that development time is closely linked to variation in adult performance.

Development time had a complex effect on actuarial senescence. Rapid larval development was associated with a higher Weibull scale parameter, reflecting a lower initial mortality rate (Fig. 2.2; Table S2.4). However, as indicated by their higher Weibull shape parameter, males that developed quickly also senesced more rapidly, while the age-specific mortality of slow developers plateaued at later ages (Fig. 2.2; Table S2.4). The co-occurrence of rapid development and rapid aging is consistent with physiological trade-offs between early- and late-life performance (Pearl 1928; Williams 1957; Lind et al. 2017). However, this did not translate into a survival cost, as the median lifespan of fast developers was greater than that of slow developers. Furthermore, only 37% of males survived beyond 12 days, the point at which age-specific mortality for fast developers exceeded that of slow developers (Fig. 2.2). Accordingly, the majority of fast-developing males never experienced senescence-related mortality costs, and most that did were at higher risk of death for only a small a portion of their lives. These results highlight the distinction between lifespan and senescence *per se*. All else being equal, faster senescing individuals will have a shorter lifespan on average, but longevity is also influenced by the baseline mortality rate and timing of onset of senescence. Therefore, variation in lifespan among groups may not simply reflect variation in senescence rate, and can differ in direction, as in our study. Researchers wanting to make inferences about senescence must be sure to measure changes in performance through time, rather than relying on lifespan (and *vice versa*).

Slow-developing male antler flies had a higher average mating rate than fast developers (Fig. 2.3). This result is surprising, especially since slow developers were smaller on average and large male antler flies are more successful in territorial combat (Bonduriansky and Brooks 1999) and are preferred by females (Bonduriansky and Brooks 1998b). Furthermore, a previous study of male mating success in antler flies found that larger males had a high daily mating rate (Bonduriansky and Brassil 2005). Notably, since slow developers also lived shorter on average, there was no net effect of development time on LMS. The high average mating rate of these slower developing, males may represent an alternative mating strategy which either compensates for, or contributes to, their short lifespan. In yellow dung flies, for example, small males which cannot compete on dung successfully mate on patches of apple pomace where male–male combat is low (Pitnick et al. 2009). Small male antler flies may similarly localize to areas of the antler where males do not defend territories, such as the underside (whichever side of the antler happens to face the ground; Bonduriansky and Brooks 1999). They may also be more willing to accept matings from less fecund females that high-quality males would reject (Bonduriansky and Brooks 1998b).

Despite their high average mating rate, slow-developing males may not have achieved equal fitness as their peers. We only recorded mating success, which does not take into account variation in female fecundity or postcopulatory effects including sperm viability, sperm competition, and female choice (Eberhard 2009). These males might be more susceptible to copulatory take-overs by rivals (Bonduriansky and Brooks 1998a), be willing to accept less fecund females (Bonduriansky and Brooks 1998b), lose paternity due to sperm expulsion by females (Bonduriansky and Brooks 1998a), or produce semen with a reduced stimulatory effect on egg production (see Bonduriansky et al. 2005). If these mechanisms of postcopulatory selection act against slow-developing males, their siring success could be lower than other males, despite similar LMS.

Our detection of actuarial senescence in male antler flies in the wild is consistent with multiple previous studies and further reinforces the existence of senescence in a short-lived insect in nature (Bonduriansky and Brassil 2002, 2005; Mautz et al. 2019). Previous studies have also reported reproductive senescence in this species (Bonduriansky and Brassil 2002, 2005; Mautz et al. 2019), but we did not find a significant decline in male mating rate with age, although the trend was negative. Reproductive declines may simply be difficult to detect at smaller sample sizes, as Mautz et al. (2019) detected clear reproductive senescence in male antler flies in one year (n = 432 males), but found only low support in the other (n = 219) in which sample size was similar to the current study.

Wing length had a small effect on male actuarial senescence (Weibull scale) and no effect on average mating rate in our results. In our study, large males tended to live longer. Similarly, Bonduriansky and Brassil (2005) found that larger male size was associated with greater longevity and mating success, but faster reproductive senescence in antler flies. Interestingly, Mautz et al. (2019) reported differing effects of body size between years: large males experienced substantially higher mortality in one year, but slightly lower mortality in the other, and slightly higher mating rate in both years. However, none of these past studies measured development time, so they could not partition the effects of development time and body size, which are correlated in antler flies (Fig. 2.2.1B; Angell and Cook 2019). Thus, the significant effects of body size on lifespan, mating success, and senescence reported by Bonduriansky and Brassil (2005) may in fact be consistent with the effects of development time reported here.

This is the first study, to our knowledge, to experimentally test for silver-spoon effects in an insect in nature (Zajitschek et al. 2020) and one of the first to investigate early–late life trade-offs in wild insects (but see Rodríguez-Muñoz et al. 2019). Overall, our findings suggest that development time is an important contributor to adult life-history traits and senescence, and that this depends on early life environmental quality. However, the phenotypic consequences of variation in development time were mixed and were consistent with a silver spoon effect on some adult traits, but not others. More research is required to elucidate the mechanism behind the paradoxical high average mating rate of otherwise apparently low-quality males and to determine whether their postcopulatory performance is similarly high. Due to the antler flies' complex phenotypic response, larval diet will likely affect fitness differently as environmental and social conditions vary through time and space. For example, living longer could be critical if female encounter rates are reduced in a particular year or location (e.g. because of bad weather). Much work remains to be done to characterize factors that influence the life-history traits and fitness of insects in nature.

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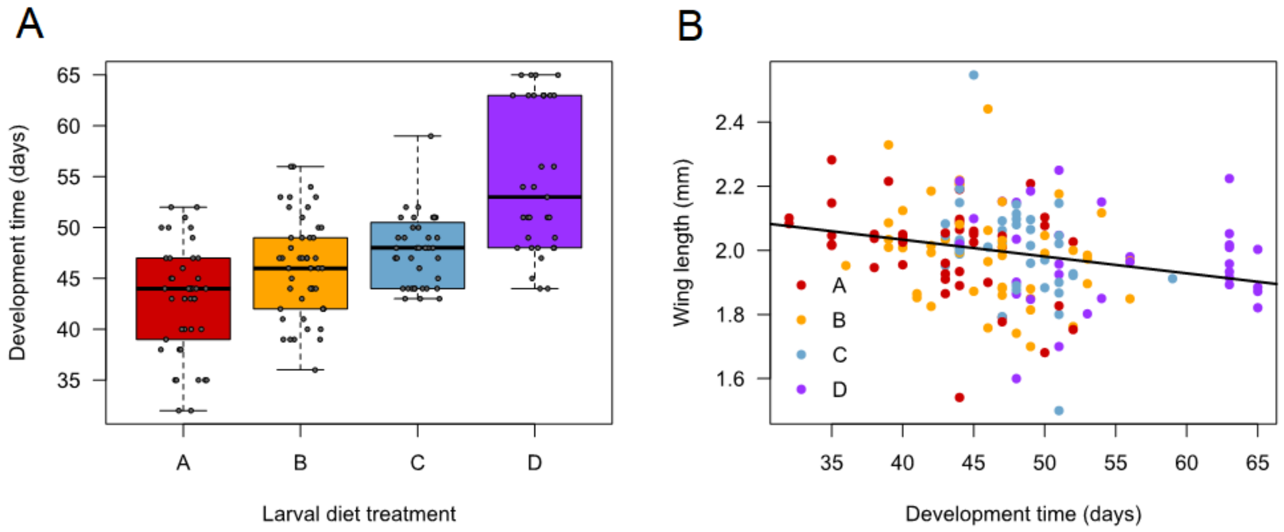

Fig. 2.1. Variation in egg-to-adult development time and wing length within and among larval diet treatments. *A*, boxplot of development time in each diet. Thick horizontal lines denote the median, boxes demarcate the first and third quartiles and whiskers indicate the minimum and maximum values. *B*, wing size as a function of developmental time across all larval diet treatments. The regression was fit on the pooled data set ($F_{1,159} = 9.39$, $p = 0.003$ for this simplified regression), as there was no significant difference in intercept or slope among diets. Diet treatments: A (100% ground beef); B (9:1 ratio of ground beef:fibre); C (8:1 ratio of ground beef:fibre); D (7:1 ratio of ground beef:fibre).

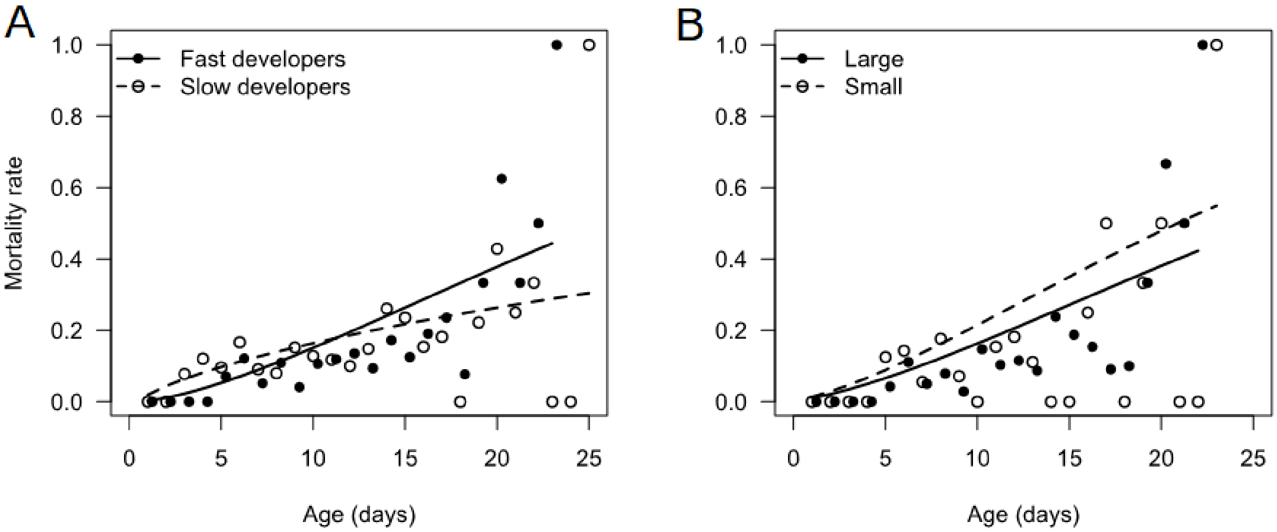

Fig. 2.2. The effect of *A*, egg-to-adult development time and *B*, wing length (body size) on actuarial senescence (daily mortality rate) in male *P. litigata*. The effect of development time and wing length on the scale parameter were analyzed as continuous variables, but are plotted as mortality curves for males above or below the median trait value. Symbols are observed daily mortality rates for the two groups, while the lines represent fitted mortality curves based on the best supported Weibull survival model (weighted means across blocks). Due to the shape effect of development time, panel *B* shows mortality rates for fast developers only.

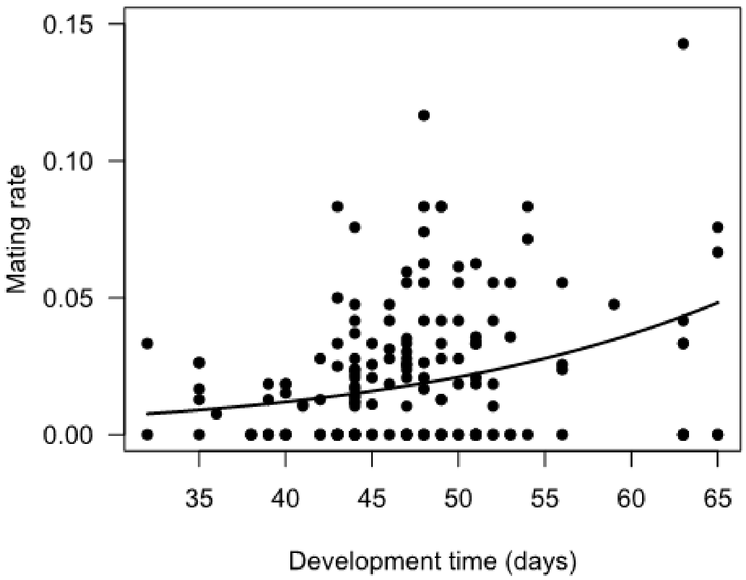

Fig. 2.3. Relationship between egg-to-adult development time and average mating rate in male antler flies. Points represent lifetime average mating rate for each male and the line represents predicted values from the minimal adequate GLMM (weighted mean across antlers and blocks).

Chapter 2: Supplementary information

Survival distribution selection

As the first step of our actuarial senescence analysis, we tested which of the survival distributions supported by the R packages *survival* (Therneau 2015) and *flexsurv* (Jackson 2016) provided the best fit to our data. We tested the exponential, two-parameter Weibull, Gaussian, logistic, log-logistic, log-normal, and extreme value distributions in *survival* and we tested Gompertz and three-parameter Weibull distributions in flexsurv. The *survival* package allows fitting only a single factor to the shape parameter of the two- or three-parameter distributions (i.e. all except exponential), and any number of continuous and/or categorical variables to the scale parameter. Development time and wing length, being continuous variables of particular interest, were therefore each binned into two levels corresponding to individuals above vs. below the median value across the whole dataset, allowing us to test their effects, alongside diet treatment, as potential predictors of the shape of actuarial senescence.

We compared the various survival distributions with AICc (Hurvitch and Tsai 1989) using the R package *MuMIn* (Bartoń 2016), considering models with ΔAICc < 2 to be equally well supported (Burnham and Anderson 2002). For each distribution, we fit a full model including the effects of all our variables (without interactions) on the scale parameter. We also included the effects of either diet, development time, or wing length on the shape parameter of two- and three-parameter distributions. The two-parameter Weibull distribution provided the best fit to our data regardless of the shape variable (ΔAICc > 5; Table S2.2) and was therefore used in subsequent model selection.

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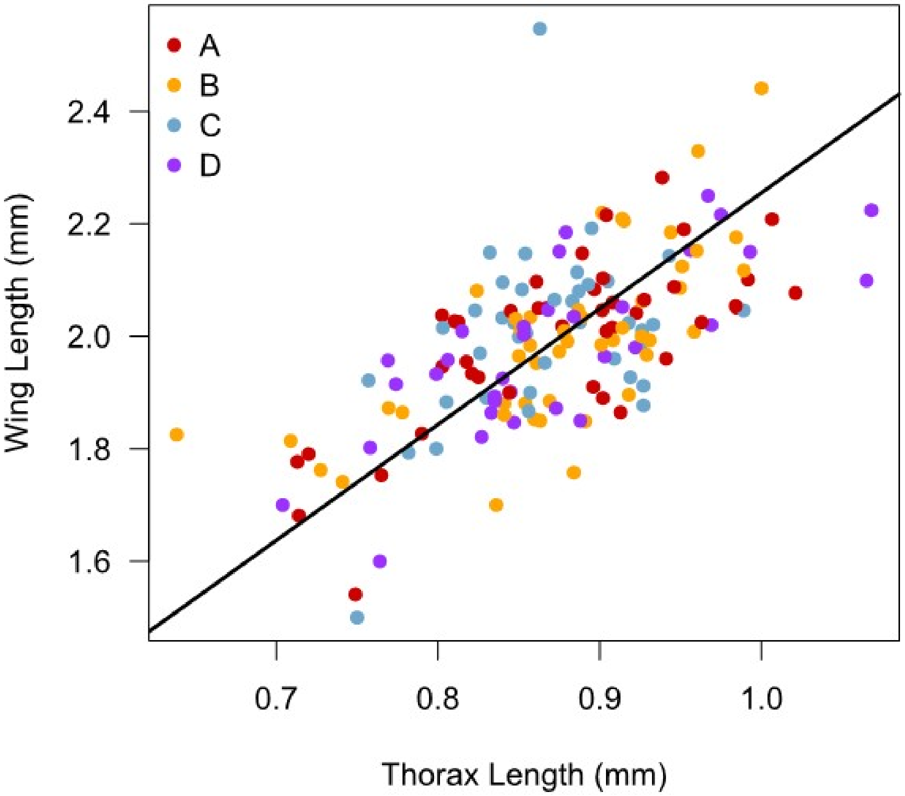

Fig. S2.1. Relationship between wing length (mm) and thorax length (mm) in male antler flies across four larval diet treatments (colors). Treatments did not differ significantly in slope or intercept, so the overall reduced major axis fit is represented by the black line ($r = 0.645$, $p < 0.001$).

Table S2.1. Number of males from each treatment released on antlers A and B in each block.

Antler A					Antler B				
Treatment	Block	Flies Released	Total (Treatment)	Total (Antler)	Treatment	Block	Flies Released	Total (Treatment)	Total (Antler)
A	1	1	49	179	A	1	0	11	41
	2	3				2	0		
	3	12				3	1		
	4	5				4	0		
	5	13				5	4		
	6	4				6	4		
	7	4				7	2		
	8	0				8	0		
	9	7				9	0		
B	1	13	48		B	1	0	10	
	2	2				2	0		
	3	10				3	0		
	4	6				4	0		
	5	6				5	5		
	6	6				6	0		
	7	3				7	5		
	8	1				8	0		
	9	1				9	0		
C	1	7	42		C	1	0	10	
	2	11				2	0		
	3	3				3	0		
	4	4				4	8		
	5	1				5	0		
	6	0				6	0		
	7	8				7	2		
	8	7				8	0		
	9	1				9	0		
D	1	0	40		D	1	0	10	
	2	10				2	0		
	3	0				3	0		
	4	20				4	8		
	5	4				5	0		
	6	1				6	0		
	7	5				7	2		
	8	0				8	0		
	9	0				9	0		

Table S2.2. Survival distribution selection using AICc. ΔAICc values were calculated relative to the Weibull model with the same factor on the shape parameter. All models contained the following variables on the scale parameter: larval diet treatment, development time, wing length, average population density, average sex ratio, antler, average mating rate, and block. Regardless of shape variable, the two-parameter Weibull distribution provided the best fit to the data.

	Shape parameter							
	Intercept (single level)		Larval diet treatment (four levels)		Development time (two levels)		Wing length (two levels)	
	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc
Two-parameter Weibull	972.9	0	980.1	0	969.5	0	972.6	0
Three-parameter Weibull	978.2	5.3	986.5	6.4	976.3	6.8	979.1	6.5
Gompertz	984.3	11.4	989.0	8.9	985.9	16.4	986.4	13.8
Log-normal	986.6	13.7	992.5	12.4	980.6	11.1	986.1	13.5
Log-logistic	993.0	20.1	999.5	19.4	988.8	19.3	993.1	20.5
Gaussian	1003.6	30.7	1006.2	26.1	1006.0	36.5	1006.2	33.6
Extreme value	1026.7	53.8	1029.4	49.3	1028.9	59.4	1029.1	56.5
Exponential	1067.2	94.3	NA	NA	NA	NA	NA	NA

Table S2.3. Significance of all fixed effects in the main (i.e. non-residual) model selection, based on LRT relative to the final (i.e. best fit) model, or for terms present in the minimal adequate model, relative to a model lacking this term. Terms in bold were present in the minimal adequate model.

Variable	χ^2	df	*p*
A. Actuarial senescence (Weibull regression)			
Development time	**11.5**	**1**	**< 0.001**
Wing length	**3.85**	**1**	**0.0498**
Block	**15.2**	**8**	**0.055**
Shape: Development time	**6.24**	**1**	**0.013**
Larval diet treatment	3.71	3	0.294
Lifetime average sex ratio	2.91	1	0.088
Lifetime average population density	0.429	1	0.513
Lifetime average mating rate	2.50	1	0.114
Antler	3.50	1	0.061
B. Mating rate (binomial GLMM)			
Development time	**11.5**	**1**	**< 0.001**
Population density	**17.2**	**1**	**< 0.001**
Sex ratio	**5.63**	**1**	**0.018**
Antler	**23.0**	**1**	**< 0.001**
Block	**12.9**	**8**	**0.116**
Age	1.74	1	0.187
Larval diet treatment	2.65	3	0.449
Wing length	1.29	1	0.256
Longevity	0.001	1	0.977
Hour of day	8.87	5	0.114
C. Lifetime mating success (negative binomial GLM)			
Lifetime average sex ratio	**19.6**	**1**	**< 0.001**
Lifetime average population density	**7.11**	**1**	**0.008**
Block	**11.4**	**8**	**0.182**
Larval diet treatment	1.65	3	0.648
Development time	0.867	1	0.352
Wing length	1.87	1	0.172
Antler	0.198	1	0.656

Table S2.4. Parameter estimates from the final parametric Weibull survival model. Estimates for the scale parameter are on a log scale, and covariates were standardized to a mean of zero and a standard deviation of one. The reference level for “Block” was block 1.

	Estimate	SE	*z*	*p*
Scale effect (λ)				
Intercept	2.44	0.122	20.1	< 0.001
Development time	-0.19	0.055	-3.49	< 0.001
Wing length	0.083	0.043	1.95	0.051
Block 2	0.165	0.180	0.92	0.358
Block 3	-0.029	0.161	-0.18	0.857
Block 4	-0.186	0.161	-1.15	0.250
Block 5	-0.130	0.154	-0.84	0.400
Block 6	-0.249	0.209	-1.19	0.235
Block 7	-0.186	0.152	-1.22	0.221
Block 8	-0.407	0.266	-1.53	0.127
Block 9	-0.578	0.206	-2.81	0.005
Shape effect (α)				
Development time < median	2.47			
Development time ≥ median	1.75			

Table S2.5. Parameter estimates from the final mating rate binomial GLMM. Estimates are on a logit scale, and covariates were standardized to a mean of zero and a standard deviation of one. The reference level for “Antler” is antler A, and for “Block” is block 1.

Fixed effect	Estimate	SE	*z*	*p*
Intercept	-4.32	0.320	-13.5	< 0.001
Development time	0.342	0.099	3.46	< 0.001
Sex ratio (proportion male)	-0.263	0.111	-2.37	0.018
Population density (flies/antler)	0.450	0.109	4.21	< 0.001
Antler B	1.31	0.285	4.60	< 0.001
Block 2	0.035	0.382	0.09	0.928
Block 3	0.384	0.384	1.00	0.317
Block 4	-0.203	0.385	-0.53	0.598
Block 5	-0.174	0.386	-0.45	0.653
Block 6	-0.555	0.520	-1.07	0.286
Block 7	-0.498	0.401	-1.24	0.214
Block 8	-0.795	0.698	-1.14	0.255
Block 9	0.779	0.515	1.51	0.130
Random effect	**Variance**	**SD**		
Male identity	0.228	0.477		
Observation (nested within day)	0.327	0.572		

Table S2.6. Parameter estimates from the final LMS negative-binomial GLM. Estimates are on a log scale, and covariates were standardized to a mean of zero and a standard deviation of one. The reference level for “Block” is block 1.

	Estimate	SE	*z*	*p*
Intercept	0.423	0.320	1.32	0.186
Lifetime average sex ratio (proportion male)	-0.696	0.148	-4.72	< 0.001
Lifetime average population density (flies/antler)	0.429	0.147	2.92	0.004
Block 2	0.195	0.404	0.483	0.629
Block 3	0.031	0.397	0.078	0.938
Block 4	-0.195	0.376	-0.519	0.603
Block 5	-0.295	0.401	-0.735	0.462
Block 6	-0.856	0.542	-1.58	0.114
Block 7	-0.592	0.411	-1.44	0.150
Block 8	-1.08	0.711	-1.53	0.127
Block 9	0.053	0.148	-4.72	< 0.001
Dispersion parameter (θ)	2.45	0.823		

Table S2.7. Significance of all fixed effects in the residual model selection, based on LRT relative to the minimal adequate model (or to a model lacking the given term). Terms in bold are included in the minimal adequate model.

Variable	χ^2	df	p
A. Actuarial senescence (Weibull regression)			
Larval diet treatment	**12.0**	**3**	**0.007**
Residual development time	**13.6**	**1**	**< 0.001**
Residual wing length	**3.88**	**1**	**0.049**
Block	**16.2**	**8**	**0.040**
Lifetime average sex ratio	2.36	1	0.125
Lifetime average population density	0.457	1	0.499
Lifetime average mating rate	2.34	1	0.126
Antler	3.17	1	0.075
Shape: Larval diet treatment	1.66	3	0.647
Shape: Residual development time	3.28	1	0.070
Shape: Residual wing length	2.04	1	0.153
B. Mating rate (binomial GLMM)			
Larval diet treatment	**9.01**	**3**	**0.029**
Residual development time	**8.28**	**1**	**0.004**
Population density	**17.7**	**1**	**< 0.001**
Sex ratio	**5.63**	**1**	**0.018**
Antler	**24.1**	**1**	**< 0.001**
Block	**11.5**	**8**	**0.175**
Age	1.54	1	0.215
Residual wing length	0.090	1	0.342
Longevity	0.061	1	0.805
Hour of day	8.75	5	0.119
C. Lifetime mating success (negative binomial GLM)			
Lifetime average sex ratio	**19.6**	**1**	**< 0.001**
Lifetime average population density	**7.11**	**1**	**0.008**
Block	**11.4**	**8**	**0.182**
Larval diet treatment	1.65	3	0.648
Residual development time	0.357	1	0.550
Residual wing length	2.56	1	0.110
Antler	0.198	1	0.656

Table S2.8. Parameter estimates from the final parametric Weibull survival model after model selection using residual development time and residual wing length. Estimates for the scale parameter are on a log scale, and covariates were standardized to a mean of zero and a standard deviation of one. The reference level for "Larval diet treatment" was treatment A (100% beef), and for "Block" was block 1.

	Estimate	SE	*z*	*p*
Scale effect (λ)				
Intercept	2.50	0.161	15.5	< 0.001
Larval diet treatment B	0.009	0.116	0.08	0.935
Larval diet treatment C	0.027	0.147	0.18	0.855
Larval diet treatment D	-0.410	0.160	-2.56	0.011
Residual development time	-0.170	0.044	-3.84	< 0.001
Residual wing length	0.084	0.043	1.97	0.048
Block 2	0.205	0.201	1.02	0.308
Block 3	-0.019	0.180	-0.11	0.916
Block 4	-0.083	0.185	-0.45	0.654
Block 5	-0.127	0.173	-0.73	0.463
Block 6	-0.163	0.213	-0.77	0.443
Block 7	-0.286	0.180	-1.58	0.114
Block 8	-0.457	0.255	-1.79	0.073
Block 9	-0.505	0.259	-1.95	0.051
Shape effect (α)				
Intercept	2.04			

Table S2.9. Parameter estimates from the final mating rate binomial GLMM after model selection using residual development time and residual wing length. Estimates are on a logit scale, and covariates were standardized to a mean of zero and a standard deviation of one. The reference level for "Larval diet treatment" is treatment A (100% beef), "Antler" is antler A, and for "Block" is block 1.

Fixed effect	Estimate	SE	*z*	*p*
Intercept	-4.31	0.382	-11.3	< 0.001
Larval diet treatment B	-0.113	0.254	-0.444	0.657
Larval diet treatment C	0.109	0.301	0.363	0.716
Larval diet treatment D	0.761	0.329	2.31	0.028
Residual development time	0.261	0.089	2.92	0.004
Sex ratio (proportion male)	-0.263	0.111	-2.37	0.018
Population density (flies/antler)	0.468	0.109	4.28	< 0.001
Antler B	1.34	0.285	4.71	< 0.001
Block 2	-0.131	0.414	-0.317	0.751
Block 3	0.271	0.389	0.696	0.486
Block 4	-0.418	0.426	-0.980	0.327
Block 5	-0.316	0.396	-0.798	0.425
Block 6	-0.660	0.529	-1.25	0.213
Block 7	-0.628	0.418	-1.50	0.133
Block 8	-0.834	0.714	-1.17	0.243
Block 9	0.528	0.549	0.961	0.337
Random effect	Variance	SD		
Male identity	0.208	0.457		
Observation (nested within day)	0.327	0.572		

Table S2.10. Results of mating rate GLMM model selection when including observation periods with an undefined sex ratio (see Methods). Significance of all fixed effects are based on LRT relative to the best fit model (or to a model lacking the given term). Terms in bold are included in the minimal adequate model.

Variable	χ^2	df	*p*
Development time	**12.0**	**1**	**< 0.001**
Population density	**12.6**	**1**	**< 0.001**
Antler	**39.1**	**1**	**< 0.001**
Block	**12.7**	**8**	**0.123**
Age	1.27	1	0.260
Wing length	1.34	1	0.246
Longevity	0.022	1	0.882
Hour of day	7.67	5	0.176

Table S2.11. Parameter estimates from the final mating rate binomial GLMM after model selection, including observations with an undefined sex ratio. Estimates are on a logit scale, and covariates were standardized to a mean of zero and a standard deviation of one. The reference level for “Antler” is antler A, and for “Block” is block 1.

Fixed effect	Estimate	SE	*z*	*p*
Intercept	-4.38	0.321	-13.7	< 0.001
Development time	0.351	0.991	3.54	< 0.001
Population density (flies/antler)	0.372	0.103	3.59	< 0.001
Antler B	1.58	0.265	5.96	< 0.001
Block 2	0.338	0.383	0.088	0.930
Block 3	0.395	0.395	1.03	0.305
Block 4	-0.185	0.386	-0.479	0.632
Block 5	-0.156	0.387	-0.404	0.687
Block 6	-0.532	0.521	-1.02	0.307
Block 7	-0.474	0.403	-1.18	0.239
Block 8	-0.775	0.699	-1.11	0.268
Block 9	0.818	0.518	1.58	0.114
Random effect	**Variance**	**SD**		
Male identity	0.233	0.483		
Observation (nested within day)	0.362	0.602		

Chapter 3: Diet, longevity, and senescence in female and male antler flies

Introduction

Females and males often differ in their lifespan and senescence rates, but patterns across species are variable. In humans and other mammals, as well as many insects, females generally live longer than males (Owens 2002; Zajitschek et al. 2009b; Duxbury et al. 2017; Lemaître et al. 2020), but in some species, such as nematodes and some birds, the opposite is true (e.g., Chen and Maklakov 2014; Grunst et al. 2018). Sex differences in senescence and lifespan might represent sex-specific maladaptation caused by asymmetric inheritance of sex chromosomes ("unguarded X" hypothesis; Trivers 1985; Sultanova et al. 2018) and mitochondrial genomes ("mother's curse" hypothesis; Camus et al. 2012). However, empirical data do not consistently support these hypotheses (e.g., Owens and Bennett 1994; Maklakov and Lummaa 2013; Sultanova et al. 2018). On the other hand, sexual dimorphism in lifespan and senescence might be a consequence of adaptive life-history evolution under sex-specific selection (Promislow 2003; Bonduriansky et al. 2008; Maklakov and Lummaa 2013).

Under strong sexual selection, males may pursue risky or costly "live fast, die young" mating strategies that lead to higher mortality and faster somatic deterioration compared to females (Bonduriansky et al. 2008). Senescence evolves because survival probability decreases with age, so Williams (1957) predicted that more rapid senescence would evolve in the sex with higher overall mortality. Accordingly, costly male mating behaviour may not only proximately increase mortality and accelerate senescence in a given individual, but also can lead to the evolution of faster senescence in males. However, theory does not make the same prediction in all circumstances. Selection on overall condition in one or both sexes can improve whole organism performance, including increasing longevity and decreasing senescence rate (Chen and Maklakov 2012, 2014; Ronget et al. 2017). Thus, sexual selection on condition (Rowe and Houle 1996) might conversely lead to increased lifespan in males. Furthermore, in species where old males can monopolize a disproportionate number of females, they will be under strong selection to reach old ages and may evolve long lifespan (Bonduriansky et al. 2008).

Meta-analyses have found greater male-biased mortality and actuarial senescence on average in polygamous species than monogamous species, in wild mammals (Clutton-Brock and Isvaran 2007) and captive ruminants (Tidière et al. 2015). In contrast, another recent meta-analysis of wild mammals (Lemaître et al. 2020) found no effect of mating system and only a small effect of sexual size dimorphism (as a proxy for sexual selection) on longevity, and there was no effect of sexual selection on aging rates across species. Furthermore, although females generally live longer than males, but there was no consistent difference in aging rates between the sexes. Although theoretical predictions rarely distinguish between sexual dimorphism in senescence vs. lifespan, these can be largely uncoupled, as lifespan depends on additional life history parameters including baseline mortality and the timing of the onset of senescence (Péron et al. 2019). Accordingly, relatively short male lifespan is present in species where males senesce faster, slower, and similarly to females (Lemaître et al. 2020).

Life history is highly plastic to nutrition, and females and males can have different optimal diets, in terms of nutrient concentration and/or composition (Maklakov et al. 2008). Nutrition has can have large effects on lifespan and senescence; a rich diet can improve longevity in some cases (e.g., (Nussey et al. 2007; Angell et al. 2020a; Chapter 2), but can increase mortality (Mautz et al. 2019) or senescence rate (Hooper et al. 2017) in others. Importantly, such diet effects can differ between the sexes, such that a beneficial diet in one sex is neutral or deleterious in the other (Hunt et al. 2004; Maklakov et al. 2009; Duxbury and Chapman 2020). Therefore, plastic differences in life history (such as those seen in Chapter 2) may manifest differently in female antler flies than in males.

Antler flies (*Protopiophila litigata*) have been used in field studies of male aging because their high site fidelity allows them to be tracked when released in the wild (Bonduriansky and Brassil 2002, 2005; Mautz et al. 2019; Angell et al. 2020a; Chapter 2). Studies of aging in wild insects are scarce (Zajitschek et al. 2020), but they have generally found higher mortality and/or faster senescence in males than females (Kawasaki et al. 2008; Zajitschek et al. 2009a,b; Sherratt et al. 2011; Carroll and Sherratt 2017; Makai et al. 2020). Unfortunately, senescence in female antler flies is entirely uncharacterised, as females lack males' site fidelity and thus cannot be monitored in the field. Consequently, we know nothing about how senescence in females compares to that of males, nor whether environmental factors, such as nutrition, have sex-specific influences on senescence.

In this chapter, I address this gap using a laboratory experiment to characterize male and female aging I reared antler flies on four larval diets of decreasing nutrient concentration. I recorded the lifespan of males and females in the laboratory to quantify sex differences in lifespan, actuarial aging, and the effect of nutrition on these traits. Antler flies have moderate sexual size dimorphism (Bonduriansky 1995; Angell and Cook 2019), but this may not reflect the intensity of sexual selection because females are larger than males, as is common in insects. Nevertheless, because male antler flies are subject to intrasexual selection when defending territories (Bonduriansky and Brooks 1999) and intersexual selection on epicuticular compounds (contact pheromones; Angell et al. 2020b), I expected that males would live slightly shorter than females.

This study also allowed me to compare the effect of larval diet on antler flies in a controlled laboratory setting to the results of Chapter 2, which was carried out in the field. Mautz et al. (2019) found marked differences in actuarial and reproductive senescence of antler flies in captive and wild conditions, but they reported similar decreases in longevity in both environments after adult protein supplementation. Here, I manipulated larval, rather than adult, nutrition, so it is not obvious whether the effect on survival and senescence should differ between the field and the laboratory.

Methods

Experimental flies were derived from an outbred laboratory stock population originally collected in2017 in Algonquin Provincial Park, Ontario, Canada. Both adults and larvae were maintained at constant 23 °C and 70% relative humidity, with a 17h:7h light-dark cycle. I created five parental cages (125 adults of each sex per cage) from generation 8 of the stock, each containing water, sugar, and a small plastic petri dish with a sponge soaked in 1.5 ml of 20% w/v "ground beef solution" (Oudin et al. 2015) for oviposition and as a source of protein. After 24 h, I replaced the oviposition dish and placed the old sponge, now containing eggs, onto one of four larval diet treatments, creating a larval dish. Each treatment consisted of 6 g of larval food made from lean ground beef and inulin fibre homogenized with a kitchen blender, to create a gradient of nutrient concentration: 100% beef (diet A), 89% beef (diet B), 87.5% beef (diet C), and 86% beef (diet D) in a small petri dish. These diets were based a similar manipulation by Angell et al. (2020a; Chapter 2), which was associated with differences in development time, body size, survival, and mating success; however, here I provided larvae a greater amount of food than the previous study (6 g vs. 2.5 g). I made this change because in Chapter 2, I observed high within-treatment variation in development time, which I suspected was caused in part by competition among larvae and degradation of the environment where later-hatching individuals found themselves (see Houle and Rowe 2003). I increased the total amount of food to hopefully reduce variation in performance due to individual competitive ability and hatching order, but I included a new level below those of the previous study (in which the lowest diet concentration was 87.5% beef) to maintain variation in environmental quality among treatments.

Diet treatments were not applied to the offspring until after oviposition dishes were removed, preventing the parental females from altering their egg laying behaviour in response to diet quality. Diet treatment levels were rotated evenly among parental cages. Although sponges were replaced every day, I discarded the eggs I collected on days 10, 11, 13, 15, 17, and 19 rather than rearing them to adulthood, so that I reared a total of 14 sets of eggs collected from each cage over 20 days. This schedule produced many flies at the beginning of the experiment for founding new cages, while continuing to produce a lower number of new adults through the later part of the experiment to replace dead flies. Each larval dish with the applied larval diet was placed in a small glass jar with 10-15 g of cocopeat (a pupation substrate) and a mesh lid. Larval dishes were supplemented three times a week with up to 1.5 ml of beef solution to prevent desiccation.

Adult female and male offspring that emerged from the diet treatments were restrained without anaesthesia, photographed under a microscope, and individually marked on the thorax with enamel paint (The Testor Corporation, USA) following Bonduriansky and Brooks (1997). Following Angell et al. (2020a), I measured wing length (from the tegula to the tip of the M-vein) as a proxy for body size using ImageJ v1.52a (Schneider et al. 2012). After they were marked, flies were placed into one of six 2.7 L cages, each cage containing four flies of each sex from each diet treatment (32 flies per cage total). As above, each cage was provided with water, sugar, and a beef-solution saturated oviposition dish. Cages were monitored daily, and dead flies were removed and replaced with either another newly-emerged, marked fly of the same sex and treatment or, when that was not possible, a fly of the appropriate sex from the stock population. Replacing dead flies helped to maintain a relatively constant social environment (e.g., density, sex ratio, and average phenotype of the population) over an individual's lifetime, and increased the sample size somewhat.

Over the course of the experiment, 289 individuals were measured and marked (142 females and 147 males). For the survival analysis, I excluded any individuals that died within one day following marking (to minimize effects of handling-related mortality) and those that had an unknown date of death (e.g., due to escape). In addition, the incubator suffered a humidifier malfunction on the 19th day of the experiment and had to be shut down for several hours for repair. Mortality was about twice as high as normal on the following day, and so individuals that died that day were excluded. This left 246 individuals (119 females and 127 males). Nineteen individuals recorded to be alive on the final day of the experiment were right-censored.

All statistical analyses were performed in R v. 3.6.3 (R Core Team 2020). Continuous independent variables were standardized to a mean of zero and a standard deviation of one (Schielzeth 2010).

I first compared differences in development time and wing length between sexes and among treatments using linear mixed-effects models (LMMs) implemented in the R package *lme4* (Bates et al. 2015). The LMM for development time contained fixed effects of sex, larval diet treatment, the sex × diet interaction, and laying date (as a continuous variable), as well as a random effect of larval rearing jar. Larval jar was a 62-level factor representing the interaction of parental cage × laying date (eight of the 70 larval jars failed to produce any offspring and were not represented in the longevity assay). The LMM for wing length included fixed effects of sex, larval diet treatment, development time, sex × diet, sex × development time, and laying date, and a random effect of larval jar. To avoid unnecessary model complexity, I only considered a subset of possible interactions, those related to my research question: the two way interactions of sex × diet and sex × development time. I performed Type-III *F*-tests on these LMMs using "Anova" in the R package *car* (Fox and Weisberg 2011), with contrasts for categorical variables set to "contr.sum". The interactions in both LMMs were non-significant and were removed. Partial residual plots were created using the R package *visreg* (Breheny and Burchett 2017).

I then tested for differences in longevity and aging in female and male antler flies. I used the R package *survival* (Therneau 2015) to fit parametric survival regressions to my data. This package can accommodate any number of fixed factors and covariates, as well as one random factor, on the "location" parameter of the survival distribution, but only a single categorical factor on the "scale" parameter. Therefore, I converted the continuous variables of development time and wing length to factors by binning above and below the median value, because previous studies have found that these variables affect senescence rates in antler flies (Bonduriansky and Brassil 2005; Angell et al. 2020a). The *survival* package supports six different survival distributions: exponential, Weibull, Gaussian, log-normal, logistic, and log-logistic.

I performed model selection in two steps. First, I selected a survival distribution based on the corrected Akaike Information Criterion (AICc; Hurvich and Tsai 1989). All tested models contained the following location parameter variables: sex, larval diet treatment, development time, wing length, sex × diet, sex × development time, sex × wing length, and lay date (as a continuous variable) as fixed effects, and larval jar as a random effect. I chose not to fit all possible interactions. Instead, I only tested for sex-specific effects of diet, development time, and wing length on survival, because those were related to my motivating research question. A fixed effect of observational cage was not significant and was removed. A Gaussian survival model fitting separate scale parameter values between the sexes was the best fit to my data (see Results). The Gaussian distribution contains two parameters: the location parameter (μ), which represents mean lifespan and is related to the average mortality, and the scale parameter (σ), which represents the standard deviation of lifespan and is related to senescence rate (a higher σ value represents slower senescence).

Second, I selected a scale parameter factor using LRT, by comparing an “intercept” model estimating a single global scale parameter value to models estimating separate scale values depending on one of seven factors: sex, larval diet treatment, binned wing length, binned development time, sex × diet, sex × development time, and sex × wing length. Finally, I used *car* to perform type-II LRT on all variables in the selected model, as the design is not balanced and contrasts cannot be properly set for type-III tests in the *survival* package.

Results

Egg-to-adult development time did not differ between sexes ($F_{1,260.6} = 1.98$, $p = 0.161$) or among larval diet treatments ($F_{3,65.3} = 0.739$, $p = 0.532$; Fig. 3.1A), it did not change with lay date ($F_{1,46.52} = 1.00$, $p = 0.322$). Females were larger than males on average (i.e. had greater wing length; $F_{1,250.2} = 101.8$, $p < 0.001$; Fig. 3.1B), and faster-developing flies tended to be larger ($F_{1,274.3} = 21.2$, $p < 0.001$; Fig. 3.1C) as did flies laid later in the study ($F_{1,49.9} = 37.3$, $p < 0.001$; Fig. 3.1D). There was no difference in wing length among diets ($F_{3,79.2} = 0.191$, $p = 0.902$).

For the parametric survival regression, first I compared the fit of the six survival distributions supported by *survival*, using each potential scale parameter factor (Table 3.1). Regardless of the scale parameter factor, a Gaussian survival distribution was the best fit to the data (ΔAICc = 7.7–8.6 for the next best supported distribution, logistic), indicating the presence of actuarial senescence in the flies. An exponential survival distribution, representing no senescence, was a very poor fit (ΔAICc = 167.0).

The models where the scale parameter value was permitted to vary by binned wing length (LRT $\chi^2_1 = 7.16$, $p = 0.007$) and sex × binned wing length (LRT $\chi^2_3 = 9.70$, $p = 0.021$) were a significantly better fit than a model with a single global scale value (see also AICc values in Table 3.1). On the other hand, models where the scale value varied by sex (LRT $\chi^2_1 = 1.27$, $p = 0.261$), larval diet treatment (LRT $\chi^2_3 = 2.07$, $p = 0.558$), binned development time (LRT $\chi^2_1 = 0.011$, $p = 0.916$), sex × diet (LRT $\chi^2_7 = 7.87$, $p = 0.344$), and sex × development time (LRT $\chi^2_3 = 1.43$, $p = 0.699$) were not. When compared directly, the model including an interaction between sex and binned wing length (i.e., allowing the effect of wing length on senescence to differ depending on the sex of the fly) was not better than the one considering wing length alone (LRT $\chi^2_2 = 2.54$, $p = 0.281$). Consequently, I focus on the Gaussian model using binned wing length as a scale parameter factor for the remainder of the analysis.

Females had longer median lifespan than males overall (20 vs. 18 d; Fig 3.2A), but not significantly so: there was no significant main effect of sex (LRT $\chi^2_1 = 0.380$, $p = 0.538$; Fig 3.3B) on the location parameter (μ, representing mean lifespan) of the best supported Gaussian survival regression (summarized in Table 3.2). The main effects of larval diet treatment (LRT $\chi^2_3 = 7.52$, $p = 0.057$; Fig 3.2C), development time (LRT $\chi^2_1 = 0.013$, $p = 0.909$), and wing length (LRT $\chi^2_1 = 2.66$, $p = 0.103$) were also not significant, but individuals laid later tended not to live as long ($\chi^2_1 = 14.4$, $p < 0.001$).

Although neither main effect was significant, there was a significant interaction between sex and wing length (LRT $\chi^2_1 = 8.31$, $p = 0.004$), such that large flies of both sexes had greater mean lifespan, but the difference was slightly greater in females than males (Table 3.2; Fig 3.2D). The predicted difference in average lifespan between the smallest and largest females in this study was 12.6 d (13.6 vs. 26.2 d, respectively), while the difference between the smallest and largest males was 7.3 d (12.6 vs. 21.4 d, respectively). There was also a significant interaction of sex with development time (LRT $\chi^2_1 = 7.95$, $p = 0.005$): slower-developing females had shorter mean lifespan, while the opposite effect was seen in males (Fig 3.2E). The predicted decrease in average lifespan between the fastest and slowest-developing females was 1.8 d (19.2 vs. 17.4 d, respectively), and the increase between the fastest and slowest-developing males was 3.5 d (17.1 vs. 20.6 d, respectively). While the effect of larval diet and its interaction with sex (LRT $\chi^2_3 = 7.72$, $p = 0.052$) approached significance, differences among treatments were small, and mortality did not change in a consistent direction in either sex as nutrient concentration decreased (Fig 3.2C). Finally, the scale parameter σ differed significantly between flies above and below the median wing length (LRT $\chi^2_1 = 7.16$, $p = 0.007$), indicating a slower rate of senescence in large relative to small flies (Fig 3.2D).

Discussion

In this chapter, I recorded longevity of female and male antler flies in the laboratory with two main goals. First, to characterize senescence in female antler flies for the first time, thereby providing insight into sex differences in aging. Second, to determine whether larval diet quality, which influences senescence in male antler flies (Angell et al. 2020a; Chapter 2), has the same effect on females or whether its effects are sex specific. In addition, the fact that I had to carry out this experiment in the laboratory means that I had the opportunity to compare aging and the effects of larval diet on males in captive and wild contexts.

In this study, female antler flies did display actuarial senescence, as do males in both the field and laboratory (Mautz et al. 2019). This is not surprising, as senescence has been reported in laboratory insects, including females, for decades (e.g., Rose and Charlesworth 1981), long before it was detected in wild insects (Bonduriansky and Brassil 2002). Depending on their body size, females reached daily mortality rates as high as 33.8% by their 38th day of adulthood.

Longevity and senescence rate did not differ significantly between females and males. This contrasts with laboratory studies in other insect species, which have often found sex differences in longevity and/or senescence. In captive insects, females often live longer and senesce slower than males (Kawasaki et al. 2008; Zajitschek et al. 2009a; Stojković and Savković 2011; Duxbury et al. 2017), as is also the case in mammals (Tidière et al. 2015). Although theory predicts that sex-specific selection, including sexual selection, should lead to decreased longevity and faster senescence in males (Promislow 2003; Bonduriansky et al. 2008; Maklakov and Lummaa 2013), I found no effect of sex on either trait when controlling for other variables such as body size.

The similarity of female and male longevity and senescence may indicate that selection on survival is concordant across the sexes. In other words, the selectively optimal mortality trajectory may be largely the same in females and males. However, I did not measure reproductive success in either sex, so I cannot be sure that the fitness effect of long vs. short life is the same in both sexes. It is possible that lifespan and/or senescence is under sexually antagonistic selection, but that intra-locus sexual conflict has constrained one or both sexes from achieving their optimal phenotype (Bonduriansky and Chenoweth 2009). Under intralocus sexual conflict, females and males may senesce more similarly than would be expected given sex specific selection (Bonduriansky et al. 2008; Maklakov and Lummaa 2013), and in extreme cases may not differ at all. Indian meal moths and bean beetles experience sexually antagonistic selection on longevity, but their intra-sex genetic correlation prevents them from reaching their optima (Lewis et al. 2011; Berg and Maklakov 2012).

Unlike in Chapter 2, I found no effect of larval diet quality on survival in this study. Unfortunately, I cannot infer this result as being a consequence of the difference in environment (i.e. captive vs. wild), because I also observed no effects of diet manipulation on body size or development time (as I did previously). Larval conditions were similar, but not identical, to those in Chapter 2. A major difference was the total amount of food, which I more than doubled in an attempt to decrease the impact of resource competition among larvae. It is possible that diet effects like those in Chapter 2, which were most pronounced in the poorest diet (identical composition to diet C here), only occur under resource competition, and thus, when food is abundant, larvae are able to compensate for decreased nutrient concentration by simply eating more. The other main difference between the two studies is that larvae and pupae in the previous study were relocated to the field partway through development, where they were exposed to natural fluctuations in temperature and humidity, whereas flies in this study were reared in stable laboratory conditions. The stress of maintaining physiological function under somewhat more natural conditions may have been disproportionately stressful for low-condition larvae and led to differences in performance, which were not observed in the lab.

Overall, the best predictor of longevity and actuarial senescence in the laboratory was neither sex nor larval diet, but body size (i.e., wing length). Regardless of sex, larger flies had lower overall mortality (Fig 3.2B) and senesced more slowly (Fig 3.2D) than small flies. Pooling across diet treatments, median lifespan was 2 d longer in females than males, which probably reflected the larger size of females on average (Fig 3.1B). Across animal species, large body size is positively correlated with longevity (Lindstedt and Calder 1976; Calder 1983). However, the opposite pattern is often observed within species (e.g., Hillesheim and Stearns 1992; Patronek et al. 1997), perhaps because a "high-condition" large body is more costly to maintain (Adler et al. 2016; Hooper et al. 2017). In male antler flies, Bonduriansky and Brassil (2005) found that large individuals lived longer in the wild but experienced faster reproductive senescence, and Angell et al. (2020a; Chapter 2) found that large body size and fast development (which is correlated with size) slightly decreased mortality in the wild, but fast development was associated with faster actuarial senescence. In contrast, in the laboratory, large individuals both lived longer and aged slower than small individuals. Perhaps this is because of the low population density in this study. In the wild, large males localize to the most highly contested territories, where male density is high and they are subjected to intense intrasexual combat (Bonduriansky and Brooks 1999). Accumulation of somatic damage while fighting probably contributes to actuarial senescence in this species. In contrast, population density was low in this study, which may have reduced the effect of combat on senescence.

Despite the lack of overall difference in longevity or senescence, there was a sex-specific relationship between wing length and longevity. Although large flies of both sexes lived longer, the effect was greater in females (Fig 3.2D). There was also a significant interaction of sex with development time, such that slow-developing males lived longer than fast developers, but fast-developing females lived longer than slow developers (Fig 3.2E). It is unclear why this this relationship differed in direction between the sexes. These sex differences were substantial in magnitude across the range of body sizes and development times observed in this study. The difference in predicted longevity of a large vs. small female was over 5 d greater than the difference observed in males, despite similar variance in body size within each sex.

Laying date significantly influenced body size and adult mortality. Offspring laid later grew larger (Fig. 3.1D) and had shorter lifespan (Table 3.2) than early-laid individuals. This pattern could result from a number of non-exclusive processes. Eggs were produced by the same cohort of parents from laying day one to 20, and differences between early- and late-laid eggs included the combination of at least three effects. First, reproductive senescence leads to reduced female fecundity in animals including insects (Moore and Moore 2001; Lemaître and Gaillard 2017), and this would have lowered larval density and potentially allowed late-laid individuals to exploit more resources and grow larger. Second, mortality occurred in the parental cages, which would have contributed to lower larval density as well. In addition, the genetic makeup of parents which survived to old age may have been different from that of the overall initial population (i.e., due to selective mortality), leading to genetic differences in early- and late-laid offspring. Finally, differences may represent senescence in non-genetic maternal and paternal effects, including egg provisioning and epigenetic factors (Moorad and Nussey 2016). I look more closely at how parental age influences offspring performance in Chapter 4.

In conclusion, despite relatively strong sexual selection in antler flies, females and males in the laboratory did not differ in their lifespan or actuarial senescence, when accounting for differences in body size. Increased body size, on the other hand, significantly improved longevity and decreased actuarial senescence rate, regardless of sex. This result is consistent with previous work suggesting that allometry is a major contributor to individual variation in survival and aging in this species (Bonduriansky and Brassil 2005).

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Table 3.1. Results of AICc model selection of survival distributions and scale parameter (σ) factors for the parametric survival regression. Values in parentheses represent ΔAICc relative to the best supported model (Gaussian) using the same scale parameter factor. The exponential distribution has only a single (location) parameter, so it only appears in the first "Intercept" column.

	Scale parameter factor							
Survival distribution	Intercept (one level)	Sex (two levels)	Larval diet treatment (four levels)	Binned dev. time (two levels)	Binned wing length (two levels)	Sex × larval diet (eight levels)	Sex × dev. time (four levels)	Sex × wing length (four levels)
Gaussian	1697.9 (0.0)	1699.0 (0.0)	1702.8 (0.0)	1700.2 (0.0)	1693.1 (0.0)	1706.4 (0.0)	1703.4 (0.0)	1695.1 (0.0)
Logistic	1705.8 (7.9)	1707.2 (8.2)	1710.9 (8.1)	1707.9 (7.7)	1701.3 (8.2)	1715.0 (8.6)	1711.6 (8.2)	1703.3 (8.2)
Weibull	1728.0 (30.1)	1731.2 (32.2)	1730.9 (28.1)	1730.3 (30.1)	1728.4 (35.3)	1737.3 (30.9)	1736.3 (29.9)	1732.0 (36.9)
Log-logistic	1753.6 (55.7)	1756.6 (57.6)	1756.5 (53.7)	1754.5 (54.3)	1754.5 (61.4)	1764.3 (57.9)	1759.8 (53.4)	1757.4 (62.3)
Log-normal	1760.8 (62.9)	1762.8 (63.8)	1763.4 (60.6)	1762.0 (61.8)	1762.1 (69.0)	1768.8 (64.2)	1766.2 (59.8)	1762.2 (67.1)
Exponential	1864.9 (167.0)	-	-	-	-	-	-	-

Table 3.2. Full model summary of the parametric Gaussian survival regression. All continuous variables were standardized to a mean of zero and standard deviation of 1. For the scale parameter, parameter estimates are presented on a log scale.

	Estimate ± standard error	*z*	*p*
Location parameter (μ)			
(Intercept)	18.4 ± 1.48	12.4	< 0.001
Sex (Male)	-0.234 ± 2.13	-0.11	0.913
Diet (B)	1.42 ± 2.25	0.63	0.528
Diet (C)	-1.07 ± 2.25	-0.47	0.641
Diet (D)	2.41 ± 2.30	1.05	0.294
Development time	-0.460 ± 0.944	-0.59	0.626
Wing length	3.35 ± 0.993	3.37	< 0.001
Lay date	-1.78 ± 0.666	-2.68	0.007
Sex (Male) × Diet (B)	1.97 ± 3.07	0.64	0.520
Sex (Male) × Diet (C)	2.91 ± 3.11	0.95	0.343
Sex (Male) × Diet (D)	2.05 ± 3.11	0.66	0.509
Sex (Male) × Development time	1.15 ± 1.21	0.95	0.342
Sex (Male) × Wing length	-1.32 ± 1.26	-1.04	0.301
Larval jar*	5×10^{-7}		
Scale parameter (ln(σ))			
Wing length (> median)	2.3 ± 0.74		
Wing length (≤ median)	2.04 ± 0.64		

*Variance explained by random effect. Despite low variance explained, LRT indicated that this random effect was significant (LRT $\chi^2_1 = 6.80$, $p = 0.009$).

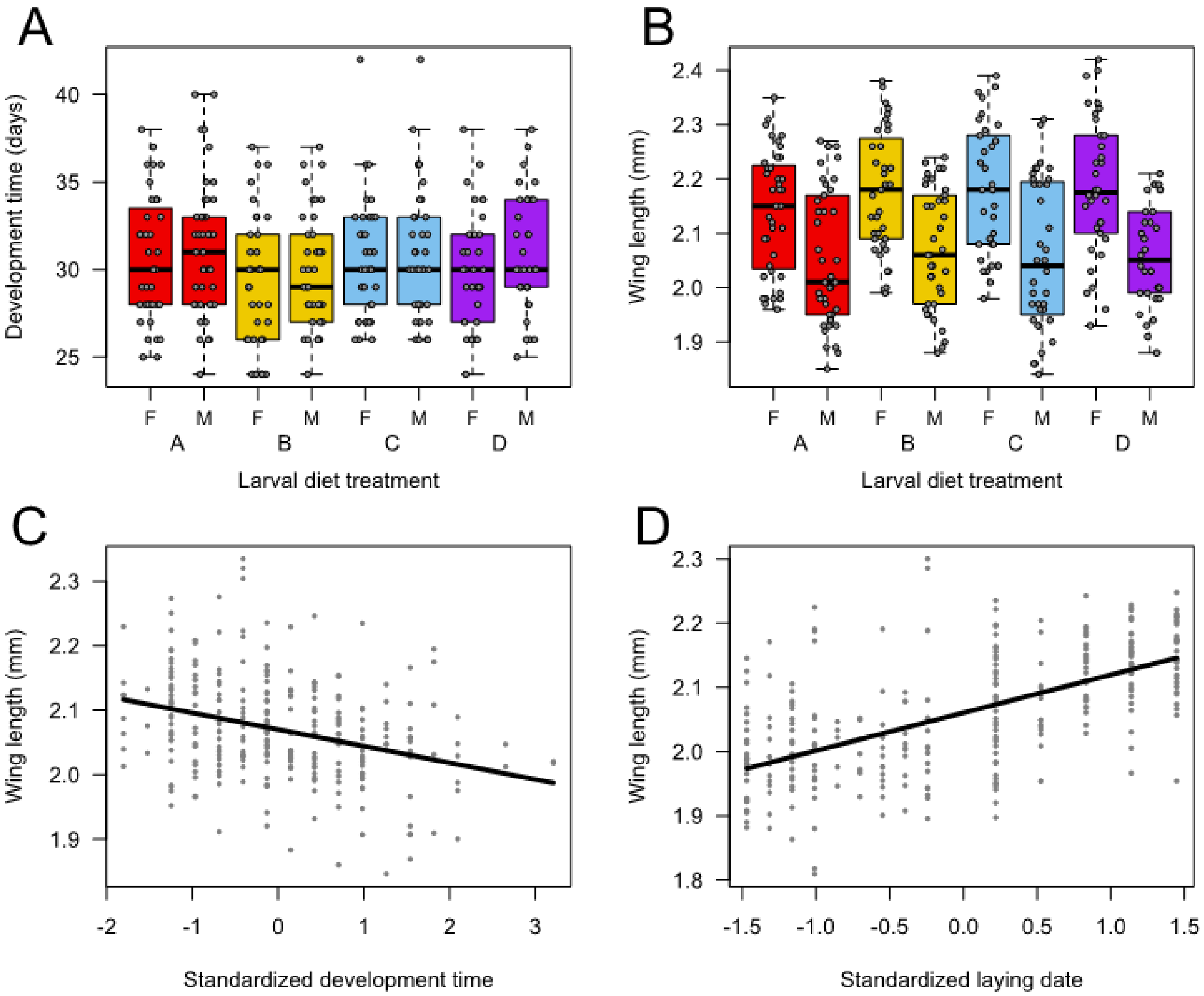

Fig. 3.1. Growth and development in female and male antler flies reared on four different larval diets. Each diet consisted of a different ratio of lean ground beef to inulin fibre (diet A: 100% beef, diet B: 89% beef, diet C: 87.5% beef, diet D: 86% beef). *A*, boxplots of egg-to-adult development time. Heavy horizontal line is the median and the box demarks the first and third quartile, and the whiskers extend to the most extreme point within 1.5 interquartile ranges. Points represent individual flies. *B*, boxplots of wing length. *C*, partial residual plot showing the effect of development time on wing length, controlling for other variables. *D*, partial residual plot showing the effect of laying date on wing length, controlling for other variables.

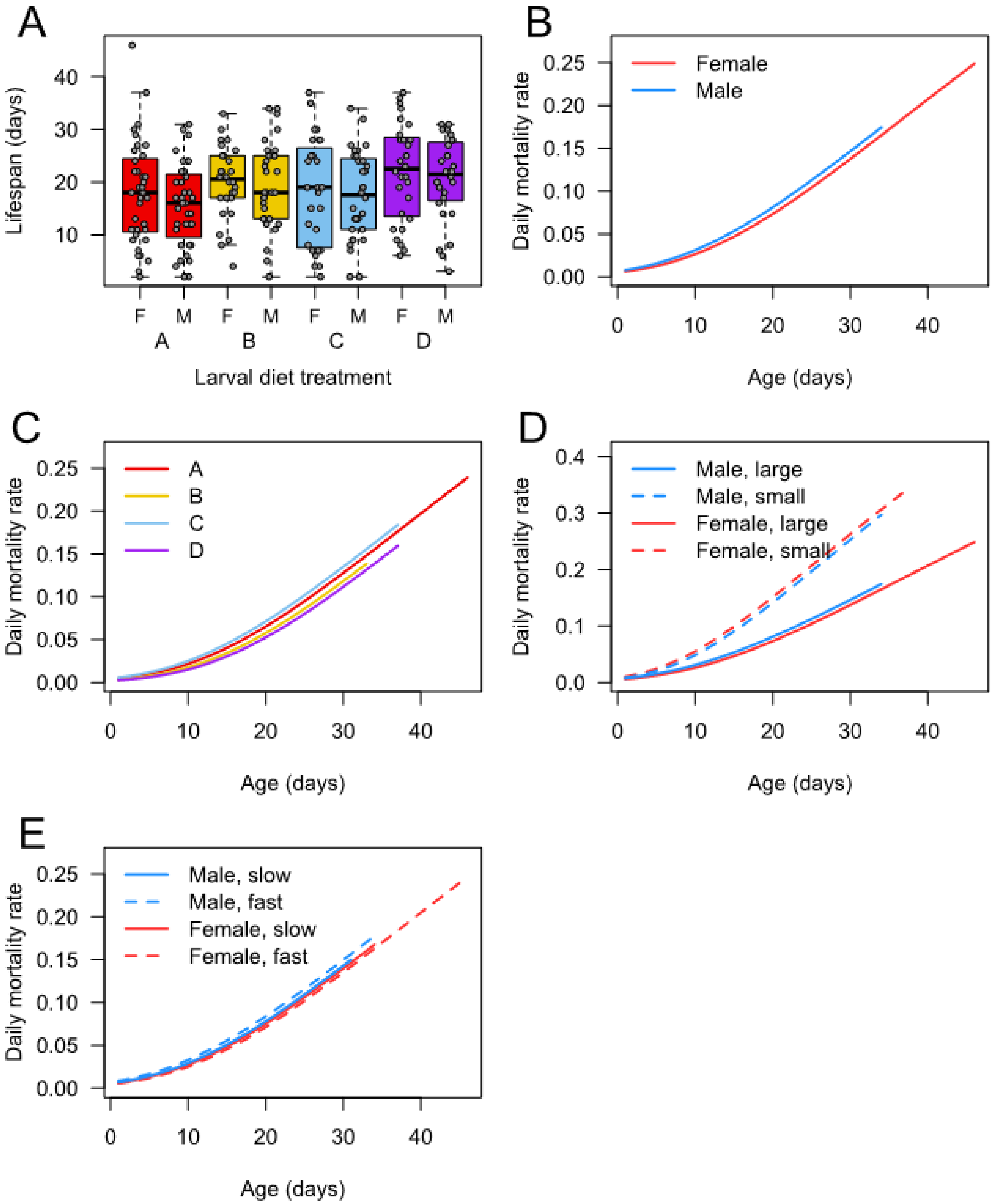
A
Lifespan (days)
10
20
30
40
F
M
F
M
F
M
F
M
A
B
C
D
Larval diet treatment
B
Daily mortality rate
0.00
0.05
0.10
0.15
0.20
0.25
Female
Male
0
10
20
30
40
Age (days)
C
Daily mortality rate
0.00
0.05
0.10
0.15
0.20
0.25
A
B
C
D
0
10
20
30
40
Age (days)
D
Daily mortality rate
0.0
0.1
0.2
0.3
0.4
Male, large
Male, small
Female, large
Female, small
0
10
20
30
40
Age (days)
E
Daily mortality rate
0.00
0.05
0.10
0.15
0.20
0.25
Male, slow
Male, fast
Female, slow
Female, fast
0
10
20
30
40
Age (days)

Fig 3.2. Longevity and actuarial senescence in female and male antler flies. *A*, boxplots of lifespan in each larval diet treatment. Each diet consisted of a different ratio of lean ground beef to inulin fibre (diet A: 100% beef, diet B: 89% beef, diet C: 87.5% beef, diet D: 86% beef). Heavy horizontal line is the median and the box demarks the first and third quartile, and the whiskers extend to the most extreme point within 1.5 interquartile ranges. Points represent individual flies. *B*, fitted mortality trajectories for females vs. males, based on the Gaussian survival regression. Curves are shown for "large" flies (75th percentile wing length, calculated globally) of diet A, for clarity. *C*, fitted mortality trajectories for the four larval diet treatments. Curves are only shown for "large" females. *D*, wing length. Curves are calculated for individuals of 25th and 75th percentile wing length (calculated globally), in diet A. *E*, development time. Curves were calculated for individuals of 25th and 75th percentile development time (calculated globally), "large" size (75th percentile wing length, calculated globally), and diet A.

Chapter 4: Maternal and paternal age effects on male antler flies in the wild

Introduction

Offspring produced by old parents often suffer survival or performance costs due to genetic deterioration in the parental germline (Monaghan and Metcalfe 2019), senescence in maternal and paternal effects (i.e., age-related declines in offspring provisioning, care, or epigenetic factors; Moorad and Nussey 2016), and/or genetic or phenotypic differences between old and young cohorts due to selective disappearance (Monaghan et al. 2020). Parental age effects have been reported in both laboratory studies (e.g., Lansing 1947; Priest et al. 2002; Fox et al. 2003, but see Ivimey-Cook and Moorad 2018) and natural populations (Bouwhuis et al. 2010; Schroeder et al. 2015; Fay et al. 2016), including humans (Gillespie et al. 2013; Arslan et al. 2017).

Parental age effects vary greatly in the traits affected and the nature of the effect (reviewed in Fay et al. 2016). Increasing parental age often decreases juvenile survival and performance (Fay et al. 2016), and effects can also carry over into adulthood, reducing offspring lifespan (Lansing 1947; Priest et al. 2002; Wylde et al. 2019) and reproductive success (Bouwhuis et al. 2010; Schroeder et al. 2015; Arslan et al. 2017). Negative parental age effects are most commonly reported, but, in some studies, offspring quality peaked at intermediate parental reproductive age and subsequently declined (Wang and vom Saal 2000; Rödel et al. 2009; Reichert et al. 2019), while in others offspring performed better with increasing parental age (Fox et al. 2003; Kroeger et al. 2020). Parental age effects can also depend on the sex of the parent (Fay et al. 2016; Wylde et al. 2019) and can interact with offspring sex (Fox et al. 2003; Schroeder et al. 2015).

Most laboratory studies of parental age effects have used short-lived model organisms, especially invertebrates, while studies of natural populations have largely, if not entirely, involved vertebrates. However, senescence differs strikingly across taxa (Jones et al. 2014) and between laboratory and natural environments (Kawasaki et al. 2008; Hämäläinen et al. 2014; Mautz et al. 2019). Maternal age effects appear to differ between wild and semi-captive contexts, but so far, laboratory and field studies remain highly confounded with phylogeny (Ivimey-Cook and Moorad 2020). Furthermore, manipulations of parental age in laboratory studies often vastly exceed the typical lifespan of the organism in the wild. Insect studies have often used parents ≥30 days of age (Priest et al. 2002; Bloch Qazi et al. 2017; Wylde et al. 2019), while estimates of average adult lifespan in wild insects range from 2.1–28.9 d (e.g., Fincke 1982; Bonduriansky and Brassil 2002; Kawasaki et al. 2008; Zajitschek et al. 2009).

Here I test whether parental breeding age influences male offspring performance in a wild insect, the antler fly, *Protopiophila litigata* (Diptera: Piophilidae), that lacks parental care. I investigate whether any parental age effects increase or decrease offspring performance, and whether they manifest differently depending on the sex of the aging parent. Male (but not female) antler flies have high site fidelity on the shed cervid antlers that host their mating aggregations, facilitating collection of longitudinal data under completely natural conditions (Bonduriansky and Brassil 2002). Taking advantage of this, I mated young (1–3 d) and old (10–13 d) lab-reared parents of both sexes in a two-way factorial design and recorded survival and mating success of male offspring released into the wild. This age manipulation is ecologically relevant, as my "old" age lies beyond the median adult lifespan for antler flies in nature (6–8 d) and approximates median lifespan in the laboratory (11–13 d; Mautz et al. 2019), but is well below the maximum recorded wild lifespan for this species (32 d; Bonduriansky and Brassil 2002).

Methods

Experimental procedure

My laboratory stock was derived from >200 individuals collected at the Algonquin Wildlife Research Station (AWRS), Algonquin Provincial Park, Ontario in 2017. They were maintained as a large, outbred population for 11 generations following Oudin et al. (2015).

Beginning 1 May 2018, I collected newly eclosed males and females, to serve as parents, from the stock over a period of 3–4 d. Adults were held in mixed-sex cages (35M:35F, 1.9–2.7 L) that contained sugar, water, and oviposition dishes containing ground beef and a sponge soaked with "beef solution" (see Oudin et al. 2015). Young parents were collected 9 d after old parents, so they were 1–3 d old when the old flies were 10–13 d old. Parents of each sex and age were mated to each other for 48 h in a 2×2 factorial design in 100M:100F cages (2.7 L, one per treatment combination) containing two oviposition dishes each which were collected and replaced after 24 h, yielding four dishes per cage. I repeated the entire procedure three times, 4–5 d apart, creating three staggered, genetically distinct blocks of offspring. Oviposition dishes were placed as pairs on 3–5 cm of coco peat in glass jars with a mesh cap and supplemented with up to 1.5 ml beef solution three times/week during larval development. All three temporal blocks were relocated to the AWRS on 22 May 2018 in portable Reptibator incubators (ZooMed Laboratories, Inc., USA) set to 23 °C (with ambient humidity and light).

Upon eclosion, offspring were immobilized without anaesthesia (Bonduriansky and Brooks 1997) and photographed under a microscope. Individuals that eclosed in the evening were held overnight in separate vials with moistened coco peat and *ad libitum* sugar prior to processing. Sugar supplementation has negligible effects on adult survival and mating success (Mautz et al. 2019). I used ImageJ v1.51 (Schneider et al. 2012) to measure wing length, a proxy for body size (Angell et al. 2020). Males—which, unlike females, have high site fidelity and can be tracked in the wild (Bonduriansky and

Brooks 2002)—were marked with individual codes on their thorax using enamel paint (The Testor Corporation, USA) following Bonduriansky and Brooks (1997). These males were subsequently released onto one of four shed moose (*Alces alces*) antlers that had been collected within the park and placed on 0.8 m-tall wooden stands in a natural forest environment. Antlers were not enclosed, so focal males were unrestricted in their movement and foraging, and they were exposed to natural weather conditions, predation, and variation in sex ratio and population density. Every 2 h, from 9:00–19:00, I observed the presence and mating status (single vs. copulating or mate guarding) of marked males on the antlers, and recorded sex ratio and the total number of antler flies present, including unmarked wild flies. Copulation and mate guarding last 2.3 h on average (Bonduriansky and Brooks 1998), so I observed nearly all matings. My survival and reproduction analyses include 147 males (young mother × young father: 41; young mother × old father: 32; old mother × young father: 43; old mother × old father: 31).

Statistical analyses

Statistical analyses were performed in R v3.6.3 (R Core Team 2020), and all continuous independent variables were standardized to mean of zero and standard deviation of one (Schielzeth 2010). I first investigated the effects of parental age treatment on offspring density (number of emerging adults per jar), development time, and wing length. I analyzed emergence with a two-way ANOVA of maternal and paternal age and determined significance via permutation, given observed heteroscedasticity, using the R package *lmPerm* (Wheeler and Torchiano 2016). Development time and wing length were each analyzed with a linear model that included fixed effects of parental ages, larval density, and larval block. I performed type-III *F*-tests with the R package *car* (Fox and Weisberg 2011).

I analyzed offspring survival with an interval-censored parametric survival regression in the R package *survival* (Therneau 2015). This package can fit survival regressions using one of six different survival distributions: the non-senescent, single-parameter exponential distribution, which has only a "location" parameter, and five two-parameter distributions that model senescence and that have "location" and "scale" parameters (i.e. Weibull, Gaussian, log-normal, logistic, and log-logistic).

Individuals were considered to have died in the inclusive interval between the day of their last sighting and the following day. Four males were alive at the end of the experiment and were right-censored. Males not observed at least one day following release were excluded to reduce effects of handling-related mortality. I performed the survival analysis in three steps: first I selected a survival distribution using AICc, second I selected a location parameter factor based on likelihood ratio tests (LRT), and finally I tested the significance of all variables in the selected model using LRT. These steps are explained in more detail below.

First, I used AICc model selection (Hurvitch and Tsai 1996), implemented in the R package *MuMIn* (Bartoń 2016), to choose which of the six survival distributions supported by *survival* provided the best fit to my data. To do so, I fit a series of survival regressions using each distribution. All models contained the following variables on the location parameter: maternal and paternal age, their interaction, development time, wing length, larval density (adult emergence per jar), lifetime average offspring adult density (flies/cm^2 on the antler), and block. Block was treated as a fixed effect rather than a random effect because there were only three levels, which is not sufficient to accurately estimate a random effect variance (Harrison et al. 2018).

The package *survival* can only accommodate a single categorical factor on the scale parameter, so the two-parameter distributions (i.e. all except the single-parameter exponential) were tested using the "strata" function to fit separate scale parameter values for each group of the following factors: maternal age, paternal age, maternal × paternal age, binned development time, and binned wing length. Development time and wing length, which have previously been shown to affect survival and senescence in this species (Bonduriansky and Brassil 2005; Angell et al. 2020), were converted to categorical variables for use on the scale parameter by binning them above and below the median value. Regardless of the scale factor, log-normal was always the top ranked survival distribution (see Results), so subsequent survival analyses used a log-normal distribution. The location parameter of the log-normal distribution ($\mu_{\log}$) represents mean lifespan on a log scale, and the scale parameter ($\sigma_{\log}$) represents the standard deviation of lifespan on a log scale.

Second, I selected among the above scale parameter factors by comparing a model where the scale parameter value was able to vary by the level of each factor to a model fitting a single global scale value, using LRT. Finally, I tested the significance of all variables using type-II LRT implemented in *car*. I calculated estimated marginal means using the R package *emmeans* (Lenth 2020).

Mating rate (the likelihood of mating at a given observation) was analyzed using a binomial generalized linear mixed model (GLMM) implemented in the R package *lme4* (Bates et al. 2015). When a male was mating in consecutive observations, the second record was disregarded to avoid double-counting. I included fixed effects of maternal and paternal age, their interaction, linear and quadratic effects of offspring age (to quantify reproductive senescence) and their two-way interactions with parental ages (to quantify treatment effects on reproductive senescence), as well as offspring adult density (flies/cm^2 at the time of observation), larval density, development time, wing length, antler, and block. I chose not to fit three-way interactions (e.g., maternal age × paternal age × offspring age) to reduce model complexity. I included random effects of individual identity and observation nested within day. I performed type-III Wald χ^2 tests on this model in *car*.

Finally, lifetime mating success (LMS; total number of matings observed) was analyzed using a negative-binomial generalized linear model (GLM) implemented in the R package *MASS* (Venables and Ripley 2002), including the same variables as the location parameter of the survival regression above as fixed effects. I performed type-III LRT on this model in *car*.

Results

Old mothers had fewer adult offspring emerging (permutation test: $p = 0.040$), indicating lower fecundity and/or reduced juvenile viability of their offspring when reared in the lab (Fig. 4.1A). There was no effect of paternal age ($p = 0.537$), nor did paternal age interact with maternal age ($p = 0.896$). Offspring from higher density jars took longer to develop (Fig. 4.1B) but eclosed adults did not differ in wing length, and parental age treatment did not affect development time or wing length when offspring density was accounted for (Table 4.1). Males were smaller than females on average (i.e., had shorter wings) but had similar development time (Table 4.1).

Survival of adult male offspring in the wild was best described by a log-normal distribution, regardless of the scale factor (ΔAICc = 0.1–1.8 for the next best supported distribution, Weibull; Table 4.2). Unlike the Weibull distribution, in which mortality rate continuously increases with age, the log-normal distribution describes an initial increase in mortality rate, followed by a decrease, which was very slight in this case (Fig. 4.2A). A non-senescent exponential model was a poor fit (ΔAICc = 115.4), providing strong evidence of actuarial senescence. No model in which the scale parameter ($\sigma_{\log}$) varied between groups was a significantly better fit than a model fitting a single global scale parameter (LRT: Maternal age: $\chi^2_1 = 0.0005$, $p = 0.982$; Paternal age: $\chi^2_1 = 0.963$, $p = 0.326$; Maternal × paternal age: $\chi^2_3 = 0.966$, $p = 0.809$; Binned development time: $\chi^2_1 = 0.010$, $p = 0.922$; Binned wing length: $\chi^2_1 = 0.150$, $p = 0.699$).

With respect to mean lifespan, there was a significant effect of paternal age (LRT: $\chi^2_1 = 4.39$, $p = 0.036$; Fig. 4.2A,C) but not maternal age (LRT: $\chi^2_1 = 1.51$, $p = 0.220$) on the location parameter ($\mu_{\log}$). Sons of old fathers lived slightly longer than sons of young fathers on average, and although this effect was driven by offspring of young mothers, the interaction was not significant (LRT: $\chi^2_1 = 2.07$, $p = 0.151$; Fig. 4.2B,D). Fast development (LRT: $\chi^2_1 = 10.2$, $p = 0.001$), low larval density (LRT: $\chi^2_1 = 4.439$, $p = 0.035$), and high average adult density (LRT: $\chi^2_1 = 46.4$, $p < 0.001$) were all significantly associated with increased lifespan, and there were significant differences among blocks (LRT: χ^2_3 = 11.4, p = 0.003). There was no effect of wing length on the location parameter (LRT: χ^2_1 = 1.96, p = 0.162).

There were significant linear (Wald χ^2_1 = 4.15, p = 0.042) and quadratic effects of age (Wald χ^2_1 = 11.93, p < 0.001) on male mating rate. Mating rate increased from release until age 9 d, followed by a subsequent decline (Fig. 4.3A). When excluding observations prior to the apparent peak at 9 d, the linear effect of age (actuarial senescence) was not significant (Wald χ^2_1 = 1.55, p = 0.213). Mating rate did not change with maternal (Wald χ^2_1 = 0.308, p = 0.579) or paternal age (Wald χ^2_1 = 0.047, p = 0.828), or their interactions with each other or offspring age (Maternal × paternal age: Wald χ^2_1 = 0.017, p = 0.897; Maternal age × offspring age: Wald χ^2_1 = 0.105, p = 0.747; Maternal age × offspring age^2: Wald χ^2_1 = 0.060, p = 0.807; Paternal age × offspring age: Wald χ^2_1 = 0.014, p = 0.970; Paternal age × offspring age^2: Wald χ^2_1 = 0.681, p = 0.409). Mating success increased with adult antler fly density (Wald χ^2_1 = 4.58, p = 0.032), but did not differ with larval density (Wald χ^2_1 = 0.594, p = 0.441), egg-to-adult development time (Wald χ^2_1 = 0.004, p = 0.952), wing length (Wald χ^2_1 = 0.232, p = 0.630), antler (Wald χ^2_3 = 3.68, p = 0.298), or block (Wald χ^2_2 = 2.14, p = 0.344).

Male LMS did not change significantly with maternal age (LRT: χ^2_1 = 0.030, p = 0.863), paternal age (LRT: χ^2_1 = 2.21, p = 0.138), or their interaction (LRT: χ^2_1 = 0.515, p = 0.473). Males that experienced a higher density of flies as adults had higher LMS (LRT: χ^2_1 = 2.57, p < 0.001). There was no effect of larval density (LRT: χ^2_1 = 0.515, p = 0.473), development time (LRT: χ^2_1 = 0.515, p = 0.473), wing length, or block (LRT: χ^2_1 = 0.515, p = 0.473) on LMS.

Discussion

Parental age effects on offspring longevity and performance are well known in humans and laboratory populations in other taxa (Bell 1918; Lansing 1947; Wang and vom Saal 2000), but research into such effects in wild animals has started only recently (reviewed in Fay et al. 2016). Here I use a manipulative field experiment to measure parental age effects on survival and reproduction of male antler flies under wild conditions. Under laboratory conditions, older females produced fewer adult offspring, indicating female reproductive senescence (i.e. a decline in fecundity with age; Moore and Moore 2001) and/or juvenile viability (Bloch Qazi et al. 2017), but I cannot separate these effects. There were no parental age effects on offspring size or development time. In the field, increased paternal age increased lifespan of adult offspring, although this effect was primarily driven by differences in offspring of young mothers. There were no significant maternal or parental age effects on offspring mating rate, reproductive senescence, or LMS.

Consistent with previous studies, I detected actuarial senescence in wild male antler flies (Bonduriansky and Brassil 2002). Overall mean lifespan, represented by the location parameter μ_{log} was most clearly affected by parental age (Fig. 4.2A). At 5 d, when the difference was greatest, I estimate 40% higher mortality in sons of young compared to old fathers, and I find a 20% difference in mean lifespan overall (Fig. 4.2C). However, there was no effect of maternal or paternal age on senescence rate, represented by the scale parameter σ_{log}. Differences in offspring survival were some of the earliest parental age effects reported (Bell 1918; Lansing 1947), suggesting these may be the strongest and easiest to detect. Frequently, offspring of old parents are short-lived (Bell 1918; Lansing 1947; Reichert et al. 2019), but some studies have found no effect (Schroeder et al. 2015; Arslan et al. 2017; Ivimey-

Cook and Moorad 2018) or a positive effect of parental age on offspring longevity (Priest et al. 2002; Fox et al. 2003), consistent with my findings in fathers.

Unlike for survival, there was no evidence for parental age effects on offspring mating rate. Previous studies have reported parental age effects on reproduction in the wild, but these typically measure offspring production (e.g., brood size or recruitment) rather than mating success (Bouwhuis et al. 2010; Schroeder et al. 2015; Kroeger et al. 2020).

Parental age effects can differ by parental sex (Fay et al. 2016) and can also interact with offspring sex (Fox et al. 2003; Schroeder et al. 2015). In wild house sparrows (*Passer domesticus*), increased parental age decreased lifetime reproduction of only same-sex offspring (Schroeder et al. 2015). Similarly, my results suggest stronger effects of paternal than maternal age on sons' survival and LMS, although I cannot say whether maternal age has a parallel effect on daughters. Female antler flies lack males' territorial behavior and can therefore only be studied in the laboratory.

Overall, these data demonstrate that large, costly parental age effects do not occur in wild male antler flies, at least over the span of ages used in my experimental manipulation. The only significant effect was a beneficial effect of a decrease in mortality in sons of old fathers (Fig. 4.2A). There was no evidence for any parental age effects on offspring mating rate or reproductive senescence. The positive paternal age effects I detected could represent a fundamentally positive relationship (Kroeger et al. 2020) or the beginning of an ultimately convex surface (Rödel et al. 2009; Ivimey-Cook and Moorad 2018). Additional parental ages would be required to verify that, but consider the natural history of antler flies: only 10 (6.8%) of males in this study lived beyond 13 d in the wild, and none lived >20 d. Laboratory parental age manipulations of months performed in laboratory studies of short lived species (Priest et al. 2002; Wylde et al. 2019) hardly represent late-life reproduction for wild insects.

Parental age effects can be caused by age-related changes in the parents (Moorad and Nussey 2016) or by selective disappearance of individuals differing in genetic makeup or reproductive strategy

(Ivimey-Cook and Moorad 2018; Monaghan et al. 2020). For example, alleles conferring longevity may have been overrepresented in those fathers that survived to old age, and thus in their offspring. Because my experiment was cross-sectional (i.e., using separate old and young cohorts) rather than longitudinal (i.e., tracking age-related changes within individuals), I cannot distinguish between these mechanisms.

In conclusion, my results begin to bridge the gap between laboratory-based understanding of parental age effects in short-lived invertebrates and vertebrate-based understanding of the consequences of parental age in the wild. My results suggest that apparent costs of increasing parental age that are often detected in the lab may be less prevalent in the wild. Studies of senescence in wild insects remain rare (Zajitschek et al. 2020), and further work in this area will hopefully reveal whether parental age effects in insects are as common under natural conditions as they appear to be in the laboratory.

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Table 4.1. Linear models testing for effects of parental age on development time and wing length of offspring.

	Development time (days)			Wing length (mm)		
Factor[1]	Estimate ± SE	*F* †	*P*	Estimate ± SE	*F* ‡	*P*
(Intercept)	29.0 ± 0.631	2110.1	< 0.001	2.27 ± 0.014	309.2	<0.001
Larval density	0.012 ± 0.004	8.51	0.004	-0.001 ± 0.000	1.49	0.223
Sex (Female)	-0.141 ± 0.244	0.382	0.537	0.049 ± 0.005	80.3	<0.001
Maternal age (Old)	0.184 ± 0.300	0.376	0.540	0.004 ± 0.007	0.342	0.559
Paternal age (Old)	0.085 ± 0..254	0.111	0.739	0.007 ± 0.006	1.57	0.211
Maternal × paternal age (Old:Old)	-0.457 ± 0.243	3.55	0.060	-0.003 ± 0.005	0.238	0.626
Block (2)	1.29 ± 0.400	7.39	< 0.001	-0.002 ± 0.009	4.32	0.014
Block (3)	-0.111 ± 0.467			-0.023 ± 0.010		

[1] Reference level is “young” for each parental age treatment, “male” for sex, and “1” for block.

† D.f. are 1, 440 for all variables except block, which has d.f. 2, 440.

‡ D.f. are 1, 425 for all variables except block, which has d.f. 2, 425.

Table 4.2. Model selection by AICc to choose a parametric survival distribution. ΔAICc values are calculated relative to the best supported model (log-normal) using the same scale variable.

	Scale parameter											
	Intercept (single level)		Development time (two levels)		Wing length (two levels)		Maternal age (two levels)		Paternal age (two levels)		Maternal × paternal age (four levels)	
	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc
Log-normal	779.9	0.0	782.2	0.0	782.2	0.0	782.3	0.0	781.7	0.0	786.5	0.0
Weibull	781.7	1.8	784.0	1.8	782.3	0.1	783.9	1.6	782.5	0.8	787.2	0.7
Log-logistic	783.6	3.7	786.0	3.8	785.6	3.4	786.0	3.7	785.0	3.3	789.9	3.4
Gaussian	802.7	22.8	803.9	21.7	805.0	22.8	805.1	22.8	805.0	23.3	807.3	20.8
Logistic	804.0	24.1	805.2	23.0	806.3	24.1	806.3	24.0	806.3	24.6	809.0	22.5
Exponential	885.5	105.6	-	-	-	-	-	-	-	-	-	-

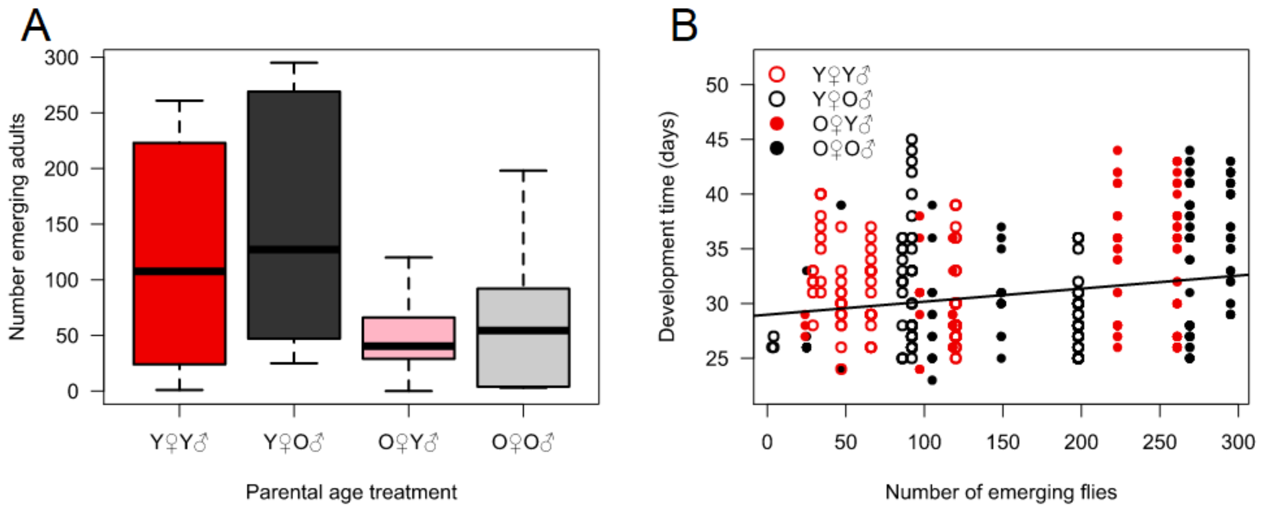

Figure 4.1. Offspring emergence and development time across parental age treatments. *A.* Boxplot of adult emergence per larval jar in each treatment. *B*, relationship between number of flies per jar and development time across treatments. Regression line based on the partial effect of larval density on development time in Table 1.

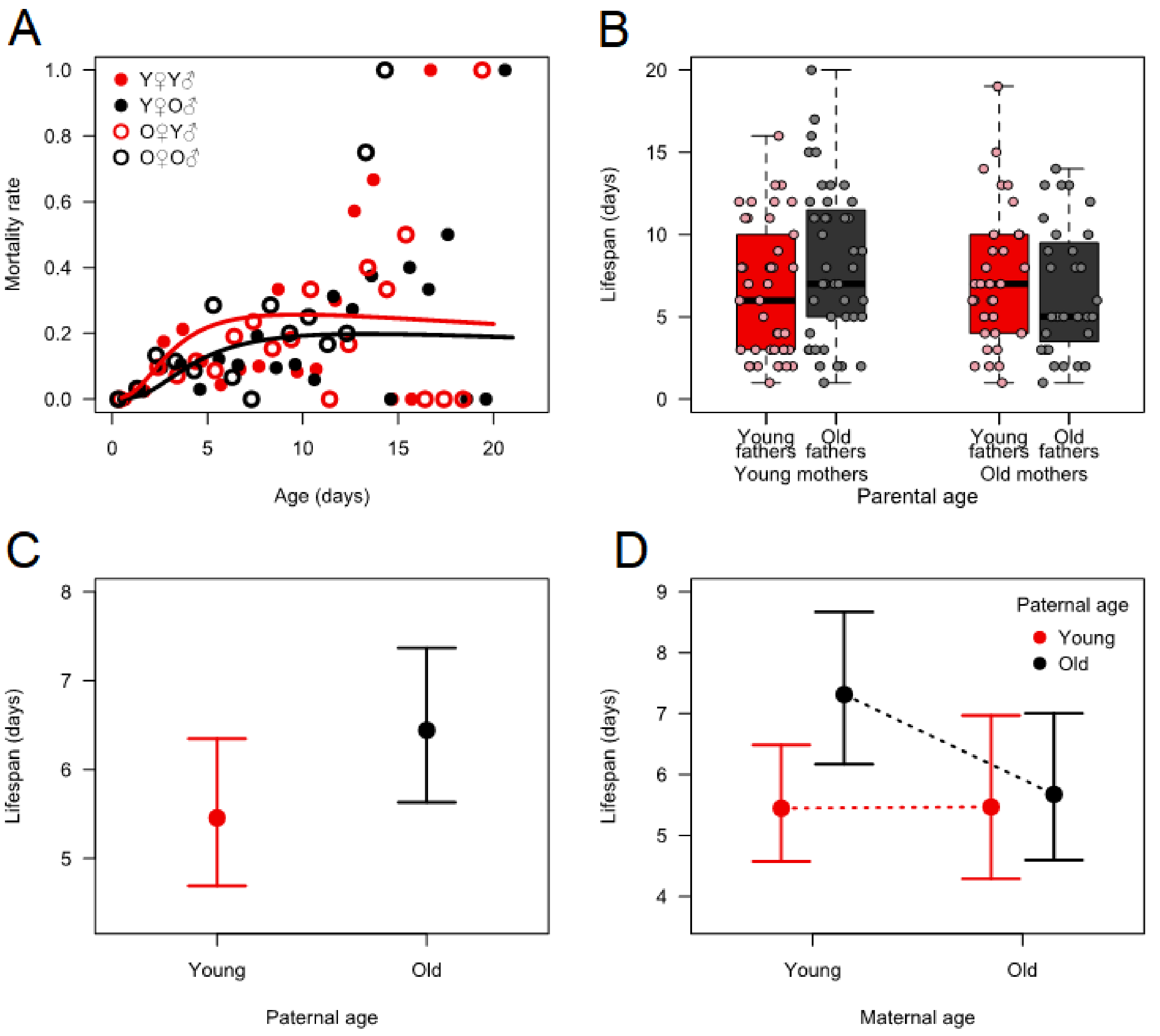

Figure 4.2. Survival in male antler flies with parents of different ages. *A*, age-specific mortality rate (actuarial senescence). Points represent observed daily mortality rates and curves represent predicted values based on the log-normal survival model. Red curve is sons of young fathers and black curve is sons of old fathers. *B*, observed lifespan. Thick lines represent the median and boxes demark the first and third quartiles. *C*, estimated marginal means for paternal age (i.e., accounting for the effects of other variables) on the location parameter ($\mu_{\log}$, representing mean lifespan on a log scale) from the log-

normal model, back-transformed into the original scale. *D*, estimated marginal means on the location parameter, back transformed into the original scale. Error bars represent 95% confidence intervals.

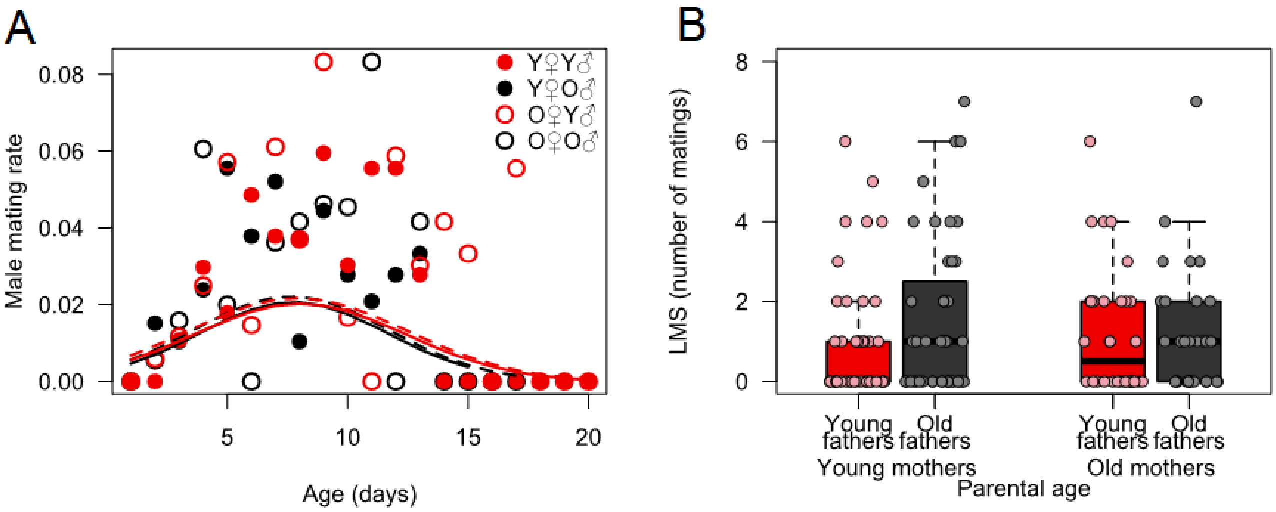

Figure 4.3. Mating success in male antler flies with parents of different ages. *A*, *c*hange in mating rate with age. Points represent observed mating rates (per observation period) in each treatment and the curve represents predicted values from the binomial GLMM in each treatment. Red vs. black lines represent young vs. old males respectively, and solid vs. dashed lines represent young vs. old females, respectively. *B,* lifetime mating success. Thick horizontal lines are the median and the boxes demark the first and third quartiles.

Chapter 5: How do natural differences in antler traits affect adult survival and performance of wild male antler flies?

Introduction

Variation in resource availability is a major contributor to individual variation in performance in wild populations (van Noordwijk and de Jong 1986). Life-history traits appear to be especially plastic, as evidenced by their low heritability (i.e. high proportion of phenotypic variance due to the environment) compared to other types of traits (Price and Schluter 1991). Resource availability (and environmental quality more generally) early in life can be especially important in shaping adult traits (e.g., Lindström 1999; Nussey et al. 2007; Hooper et al. 2017; Spagopoulou et al. 2020), a phenomenon known as the "silver spoon" effect (Grafen 1988).

Previous work in antler flies (*Protopiophila litigata*) has demonstrated that larval diet quality can influence adult morphology and performance (Oudin et al. 2015; Angell et al. 2020; Chapter 2). However, these studies used an artificial diet of ground beef mixed with varying amounts of fibre to alter the nutrient concentration. In nature, wild antler fly larvae feed within the bone matrix of shed moose and deer antlers (Bonduriansky 1995), where they presumably eat bone marrow and associated bacteria and fungi. A given antler will support multiple generations of developing antler flies over about four or five years. Antlers attract decreasing numbers of adults over that time, presumably because their food resources gradually become depleted (Bonduriansky 1996).

The nutritional composition of antler flies' natural larval diet is not characterized, and so it is unclear how well these artificial diets reflect biologically relevant variation in diet quality. The concentration and composition of macronutrients (carbon in the form of lipids and carbohydrates and nitrogen in the form of proteins) in ground beef may differ from that of antlers, and the nutrient composition of antlers may change through time as they become depleted, in addition to declines in nutrient concentration. Macronutrient composition and concentration interact to influence the expression of plastic life-history traits in many species (e.g., Maklakov et al. 2008; Gray et al. 2018). Finally, there may be other factors that contribute to larval environmental quality within the antler, such as antler size, water content, micronutrient content, population density, and toxins produced by microbes.

Angell and Cook (2019) found that body size, pupal development time, and sex ratio of antler flies differed among three larval antlers. Interestingly, the correlation of body size and development time differed in direction among antlers. The correlation was positive in the putatively freshest antler (the one that attracted the most adult males), suggesting a trade-off between fast development and large size. However, this correlation was negative in the two other, older antlers, consistent with the findings of lab studies of antler flies (Angell et al. 2020; Chapters 2 & 3). Unfortunately the study lacked sufficient replication (i.e., antlers) to differentiate among many correlated factors. Additionally, Angell and Cook (2019) did not compare differences in adult performance among flies that developed in each antler.

Here, in this final chapter, I build on previous work in antler flies by collecting larvae that developed in nine different antlers under natural conditions (i.e. in the wild). Antler fly larvae feed within the bone matrix, and then, from late May through July or August, they leave the antler to pupate in the soil during and after rain (Bonduriansky 2002; Angell and Cook 2019). I collected mature larvae as they left the antler to pupate and reared each individual through pupation to adulthood. Then, I tracked the survival and mating success of marked males in the wild to understand how natural variation in larval environmental quality contributes to adult performance and fitness in this species.

Methods

Experimental Procedure

Collection and Characterization of Antlers

Shed moose antlers were collected in the spring and summer of 2017-2019 in Algonquin Provincial Park, Ontario, Canada and relocated to the Algonquin Wildlife Research Station (AWRS) within the Park. In 2019, four of these were chosen as "observational" antlers onto which marked males would be released (described in detail below), and these were designated O1-O4. Twelve remaining ones were "source" antlers from which larval antler flies would be collected, designated S1-S12. After ten days of observation, it became apparent that male antler flies would not defend territories on antler O3, presumably because it was too old and resource depleted, so it was discarded and replaced with antler S5, which was subsequently designated O5.

Each antler was characterized in a number of ways (Table 5.1). The surface area of the antlers was measured by wrapping each antler in aluminum foil, and comparing the mass of the foil to a sheet of known surface area (100 cm^2). The percent of the surface area damaged by mammal chewing, which generally increases exponentially with antler age (Michael 1965; Bonduriansky 2002; Angell and Cook 2019), was measured similarly. The attractiveness of antlers to adult antler flies was quantified by the average daily density of adult males (flies/100 cm^2) on each antler during a check between 13:00-14:30, from 18 July to 25 July 2019, except for 19 July, which was skipped.

Finally, freshly dropped antlers (i.e., those which were shed the previous winter) can be identified through a combination of factors. Fresh antlers have a strong smell, high attractiveness to antler flies, and they yield no mature, pupating larvae until the middle of the summer. In contrast, older antlers are less attractive to adult flies, and they harbour overwintering larvae or eggs that begin leaving the antlers to pupate as early as May (Bonduriansky 1995). In this way I knew the precise age of six antlers collected over the years, and I estimated the approximate age of the remaining antlers by comparing them visually to the antlers of known age. My field work only lasted three years, so I did not have any antlers with known ages of four or more years that I could use as a basis to estimate the age of older antlers, so these are grouped into a single category (3+).

Rearing and Observation of Antler Flies

Carrion-feeding piophilids such as antler flies leave their larval environment when it rains to pupate in the soil (Bonduriansky 2002). I collected larvae between 29 May and 13 July 2019 by hand using soft forceps during or after rain, or after simulating rain by spraying the antlers with water and drumming on them with sticks and pens (Angell and Cook 2019). In addition to the source antlers, I occasionally collected larvae from the observational antlers O4 and O5 after rain, but I never sprayed them so as not to interfere with marked males' behaviour or performance. Larvae were placed in plastic vials containing 3-5 cm of moistened coco peat as a pupation substrate and plugged with a foam cap, in groups of up to five individuals from the same antler. I never collected more than 30 larvae from a given antler in one day to reduce over-representation of flies from the most larva-dense antlers, but I counted all additional larvae visible at the time of collection to estimate total larval density. Pupation vials were stored in a portable incubator ("Exo Terra Reptile Incubator," Rolf C. Hagen, Inc., Canada) at 22 °C to reduce environmentally-induced variation in development time.

Upon eclosion, adults were sexed based on their terminalia and males were placed in individual vials with moistened cocopeat until their cuticle was fully sclerotized (at least 6 hours or overnight). Then, males were photographed under a microscope and marked on the thorax with an individual symbol in enamel paint (The Testor Corporation, USA) using a trimmed paintbrush without anaesthesia, following Bonduriansky and Brooks (1997). Wing length, a proxy for body size (Angell et al. 2020), was measured in ImageJ v1.52a (Schneider et al. 2012). Males were released within 1 m of one of the four observational antlers. Observational antlers were placed on 0.8 m-high wooden stands (to facilitate monitoring both the upper and lower surface of the antler) in a natural forest environment at the AWRS.

Each antler was at least 40 m away from every other observational antler, and the source antlers were over 150 m away. Only a single fly was ever observed to migrate from the observational to the source antlers.

I monitored all observational antlers every two hours from 9:00-19:00 each day from 11 June to 31 July 2019. During observations, I recorded the presence and mating status (single vs. copulating or mate-guarding) of each marked male, as well as the total number of male and female antler flies on the antler at that time.

Statistical Analysis

I first validated the attractiveness of antlers (to adult males) as an indicator of antler quality by analyzing its correlation with chewing damage (a metric of antler age) and the number of larvae counted emerging from an antler. Second, I analyzed the effect of antler quality on adult male performance (mortality and mating) in the wild. Finally, because development time and body size are known to influence adult survival and mating success in antler flies (Bonduriansky and Brassil 2005; Angell et al. 2020; Chapters 2 & 3), I investigated whether these traits mediated any effects of antler quality on adult performance. To do so, I quantified the effects of antler quality on body size and pupal development time. Then, I tested whether either of these traits predicted adult performance and whether accounting for their effects altered that of antler quality. All statistical analyses were performed in R 3.6.3 (R Core Team 2020) and partial residual plots were produced in the R package *visreg* (Breheny and Burchett 2017).

Antler Attractiveness

Differences in antler attractiveness presumably reflect the adaptive ability of antler flies to distinguish higher quality, and hence desirable, antlers from low quality, less undesirable one, potentially by scent).

I expected quality, and thus attractiveness, to decrease with antler age. The extent of mammal chewing damage is a proxy for antler age (i.e. time since shedding); Bonduriansky (1996) reported a strong negative correlation between chewing damage and antler attractiveness on a $\log_{10}$–$\log_{10}$ scale ($R = -0.97$). I also expected the total number of larvae produced by an antler to be higher in more attractive antlers. Chewing damage, antler attractiveness, and the number of larvae were all highly skewed, so I used Spearman rank correlation tests to quantify their relationships. For the correlation between antler attractiveness and number of larvae, I excluded antler O1 and O2, as they produced no larvae because they were freshly shed, not because they were low quality.

Adult Mortality and Mating Success

I tested the effect of antler quality on two components of adult male performance in the wild: mortality and lifetime mating success (LMS). Compared to the previous chapters, this study had a much lower sample size because it was not feasible to collect as many wild larvae as can be reared in the lab, especially from low quality antlers. For this reason, I use a simpler set of statistical analyses of adult performance that do not quantify senescence. Males that did not survive at least one day post-release were removed to minimize the effect of handling-related mortality. This left 74 marked males that were resighted at least one day following their release. Continuous variables were standardized to a mean of zero and a standard deviation of one (Schielzeth 2010).

To test for effects on mortality and lifespan, I used a mixed-effects Cox proportional hazards regression, implemented in the R package *coxme* (Therneau 2020). A semi-parametric Cox regression quantifies the effects of variables on the overall hazard (mortality) rate, irrespective of the shape of the underlying hazard function. My model contained continuous fixed effects of $\log_{10}$-transformed larval antler attractiveness, mean adult density (both males and females) on the observational antler during the observations when the focal male was present, and mean adult sex ratio (proportion male) on the observational antler during the observations when the focal male was present. It also included a random effect of larval antler identity. Five males still alive at the end of the study were right censored. Significance testing was performed using type-II likelihood ratio tests (LRT) using the R package *car* (Fox and Weisberg 2011), as the design is not balanced and contrasts cannot be properly set for type-III tests in the *coxme* package.

To test for effects on LMS, I used a negative-binomial generalized linear mixed-effects model (GLMM) implemented in *lme4* (Bates et al. 2015). Copulation and mate guarding last about 2.3 h in antler flies (Bonduriansky and Brooks 1998), so I can be relatively confident that I observed nearly all matings by marked males. When a given male was observed mating or mate guarding at two consecutive observations, the second record was ignore to avoid double-counting. The GLMM included the same variables as the Cox regression above. Significance testing was performed using type-III LRT in *car*.

Effects Mediated by Pupal Development Time and Body Size

I quantified the contribution of antler quality to individual variation in pupal development time and wing length (a proxy for body size) using linear mixed-effects models (LMMs) implemented in the R package *lme4* (Bates et al. 2015). To retain antlers with zero attractiveness, I added an amount equal to the lowest non-zero male density divided by 10 to all values prior to transformation. The model for pupal development time included a fixed effect of $\log_{10}$ antler attractiveness and a random effect of larval antler. The model for wing length included fixed effects of pupal development time, $\log_{10}$ antler attractiveness, and their interaction, and a random effect of larval antler. I performed type-III *F*-tests in *car*.

Then I tested whether pupal development time and body size predicted adult performance and whether accounting for their effects altered that of antler quality. For mortality, I used a mixed-effects

Cox regression containing fixed effects of wing length, pupal development time, $\log_{10}$-transformed larval antler attractiveness, mean adult antler fly density on the observational antler during the observations where the male was present, and mean adult sex ratio (proportion male) on the observational antler during the observations where the male was present. For LMS, I used a negative-binomial GLMM containing the same variables. Significance testing was performed with LRT in *car*, as above.

Results

Antler Attractiveness

Antlers that were more extensively damaged by mammal chewing tended to attract fewer males ($\rho = -0.603$, $p = 0.015$; Fig. 5.1A) and more attractive antlers (i.e., those with higher adult male density) tended to produce more mature larvae ($\rho = 0.616$, $p = 0.025$; Fig 5.1B).

Adult Mortality and Mating Success

Adult mortality did not vary significantly with larval antler attractiveness (LRT: $\chi^2_1 = 0.223$, $p = 0.637$; Fig. 5.2A). Mortality was higher among males that experienced a more male-biased sex ratio on the observational antlers (LRT: $\chi^2_1 = 8.29$, $p = 0.004$), but it did not vary with adult density on the observational antlers (LRT: $\chi^2_1 = 0.411$, $p = 0.522$). LMS tended to be higher for males from more attractive antlers, but the trend was not significant (LRT: $\chi^2_1 = 1.80$, $p = 0.179$; Fig. 5.2B). LMS was significantly lower for males that experienced a more male-biased sex ratio on the observational antlers (LRT: $\chi^2_1 = 7.21$, $p = 0.007$), but it did not change with adult density on the observational antlers (LRT: $\chi^2_1 = 1.13$, $p = 0.287$).

Effects Mediated by Pupal Development Time and Body Size

Pupal development time was significantly shorter for flies from more attractive antlers ($F_{1,6.69} = 8.6$, $p = 0.023$; Fig. 5.3A). There was also a trend for wing length to increase with larval antler attractiveness (Fig. 5.3B), but it was marginally non-significant ($F_{1,201.6} = 3.03$, $p = 0.083$). Wing length did not vary with pupal development time ($F_{1,230.2} = 0.045$, $p = 0.833$) nor was there an interaction between attractiveness and pupal development time ($F_{1,230.0} = 2.23$, $p = 0.137$).

In the Cox model including pupal development time and wing length, mortality tended to decrease with increasing body size, although the effect was marginally non-significant (LRT: $\chi^2_1 = 3.71$, $p = 0.054$; Fig. 5.4A). However, there was no effect of pupal development time on morality (LRT: $\chi^2_1 = 1.31$, $p = 0.253$; Fig 5.4C). The effect of larval antler attractiveness remained non-significant (LRT: $\chi^2_1 = 0.216$, $p = 0.642$). As in the previous model, mortality was significantly higher among males that experienced a more male-biased sex ratio on the observational antlers as adults (LRT: $\chi^2_1 = 9.48$, $p = 0.002$), but it did not vary with average adult density on the observational antlers (LRT: $\chi^2_1 = 1.00$, $p = 0.316$).

In the GLMM including pupal development time and wing length, LMS increased significantly with wing length (LRT: $\chi^2_1 = 11.5$, $p < 0.001$; Fig 4.4B) but did not vary with pupal development time (LRT: $\chi^2_1 = 0.004$, $p =0.947$; Fig 4.4D). As in the previous analysis, LMS was non-significantly higher in males from more attractive antlers (LRT: $\chi^2_1 = 1.96$, $p = 0.161$). LMS decreased significantly for males whose average sex ratio on the observational antlers was more male biased (LRT: $\chi^2_1 = 6.16$, $p < 0.013$), but did not vary with average adult density on the observational antlers (LRT: $\chi^2_1 = 0.605$, $p = 0.437$).

Discussion

In this chapter, I monitored wild male antler flies that developed in nine different shed moose antlers in order to quantify how natural variation in larval environmental quality affects adult performance. This is the first such data in this species. I found that more attractive, and hence presumably higher quality, antlers yielded flies that developed significantly faster and that tended to be larger, although the latter was not significant ($p = 0.083$). Despite this, more attractive antlers were not associated with increased adult male longevity nor mating success, although larger males and those that experience less male-biased sex ratios tended to live longer and have greater mating success.

Unlike in Chapter 2, the early life environment (i.e. larval antler) did not appear to have a strong influence on adult life history. There are at least three possible, non-exclusive explanations for this result. First, it is possible that the range of environmental quality present in natural antlers was not great enough to affect antler fly life-history to the degree observed in Chapter 2 (but see Chapter 3). This could occur, for instance, if the nutrient concentration of undiluted ground beef used in the lab is far greater than that of natural antlers.

Second, it is likely that low-quality antlers attract fewer ovipositing females, and thus their reduced nutrient concentration may be somewhat balanced by less intense intraspecific resource competition among larvae. In Chapters 2 and 3, in contrast, females were not permitted to adjust their egg laying behaviour based on their perceptions of environmental quality. This explanation is consistent with the observations of Angell and Cook (2019). In that study, antler flies that grew in an unattractive antler containing few larvae were similar in size and development time compared to those from a more attractive antler, but flies from an unattractive antler containing many larvae were smaller and took longer to develop than either other antler.

Third, it is possible that male antler fly density is not a reliable proxy for antler quality. Although antler attractiveness was significantly correlated with both chewing damage and the number of larvae yielded by an antler, the relationships were not as strong as previously reported by Bonduriansky (1996). Some non-quality related variables, such as the precise location of the antlers, may have influenced male density, and some aspects of antler quality, such as larval density or presence of pathogens, may not be detectable to adult flies. Thus, if variation in survival and mating success does truly depend on antler quality, that relationship may have been obscured by noise in the attractiveness data.

Unfortunately, the larval environment of a moose antler is something of a black box. It is difficult to characterize its conditions while leaving it undisturbed for the larvae within. One way around this limitation would be to create naturalistic mesocosms which could be experimentally controlled and manipulated. To do so, one would have to characterize the range of nutritional content, larval population density, and other ecological conditions experienced by wild antler flies, and subsequently recreate those conditions as closely as is feasible. In this way, future researchers could test the individual and joint effects of different dimensions of environmental quality on individual performance in a systematic way. Because antler flies are easy to rear in captivity, such a study would be a great way to gain ecologically-relevant insight into life history plasticity in insects.

My results showed that larger body size significantly increased male LMS (Fig. 5.4B), and nearly significantly decreased mortality rate (Fig. 5.4A). These results are consistent with previous studies in antler flies demonstrating that large males live longer (Bonduriansky and Brassil 2005; Angell et al. 2020; Chapter 2) and have greater mating success (Bonduriansky and Brassil 2005; but see Angell et al. 2020; Chapter 2). Although the trend was non-significant, males that developed in more attractive antlers tended to be larger, and so antler quality may improve male fitness through its effect on body size. Interestingly, large male antler flies experience more rapid senescence in mating rate (Bonduriansky and Brassil 2005). Furthermore, rapid development, seen here in males from attractive antlers (Fig. 4.3A), is associated with faster actuarial senescence (Angell et al. 2020; Chapter 2). Thus, it seems likely that flies from high quality antlers senesce faster than flies from low quality antlers. Similar accelerated senescence in “high quality” individuals has been observed in other species as well (Adler et al. 2016; Hooper et al. 2017; Spagopoulou et al. 2020).

Similar to the findings of Angell et al. (2020; Chapter 2), larval antler quality influenced development time but not body size. Flies that grew in a high quality environment were able to undergo metamorphosis more rapidly (Fig 5.4A), suggesting that development rate may be energetically costly in antler flies (see Dmitriew 2011). Notably, unlike the previous chapters, I measured only pupal development time, rather than egg-to-adult development time. Pupation occurs outside the antler, with no further intake of food, so antler-related variation in the rate of metamorphosis must have been “set” by larval conditions, such as through differences in energy storage or metabolic rate.

There was a tendency for male flies from more attractive antlers to be larger (i.e., have greater wing length; Fig. 4.4B), although it was marginally non-significant. If real, this relationship is intuitive and would be consistent with studies of early life environmental quality in other species (e.g., Lindström 1999; Tigreros 2013; Hooper et al. 2017; McConnell and Judge 2018), although antler flies reared on artificial diets have not showed this effect (Oudin et al. 2015; Angell et al. 2020; Chapters 2 & 3). However, while fast egg-to-adult development was associated with large adult size in Chapters 2 and 3, I found no significant relationship between pupal development time and size. This could be because the strength and direction of this relationship differs among antlers (Angell and Cook 2019), although the interaction between pupal development time and antler attractiveness was also not significant.

In conclusion, antlers differed in their age, chewing damage, and attractiveness to adult flies, and there was some evidence that antler quality (as measured by attractiveness) influenced pupal development time and adult body size of wild antler flies. However, larval antler attractiveness did not significantly affect adult performance, although large-bodied males were more successful on average. It is possible that larval density is a key component of environmental quality that is not captured by the metric of antler attractiveness, which I also suggested in Chapter 3. Many factors can contribute to inter-individual life-history variation in wild populations, and much work remains to be done to characterize them in non-model invertebrates.

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Table 5.1. Characteristics of 16 shed moose antlers used in this study.

Antler	Antler age (years)*	Surface area (cm^2)	Chewing damage (%)	Mean adult male density (flies/100 cm^2)	Total larvae counted	Total larvae collected	Adults eclosed	Sex ratio at eclosion (proportion male)
O1	1	4255	0.011	0.856	-	-	-	-
O2	1	4924	0.219	1.62	-	-	-	-
O4	2	2361	1.47	2.03	218	45	19	0.526
S5 / O5	2	958	0.884	0.686	5	4	2	0.500
S9	2	4330	1.05	1.00	138	61	57	0.456
S10	(2)	2628	1.79	0.277	30	20	15	0.333
S12	(2)	4697	3.15	1.14	548	148	112	0.563
S8	3	3835	0.988	0.071	189	92	83	0.506
O3	(3+)	1766	0.253	-	-	-	-	-
S1	(3+)	2335	0.413	0.881	0	0	0	-
S2	(3+)	3599	6.42	0.174	96	70	54	0.453
S3	(3+)	3400	13.2	0.571	236	176	151	0.510
S4	(3+)	1135	18.9	0	0	0	0	-
S6	(3+)	2162	9.44	0.020	35	33	23	0.478
S7	(3+)	4758	1.84	0	0	0	0	-
S11	(3+)	2978	18.9	0	0	0	0	-

* Values in parentheses denote antlers that were not collected in their first year (i.e. when their age can be unambiguously assigned), so values are estimates based on comparisons to antlers of known age.

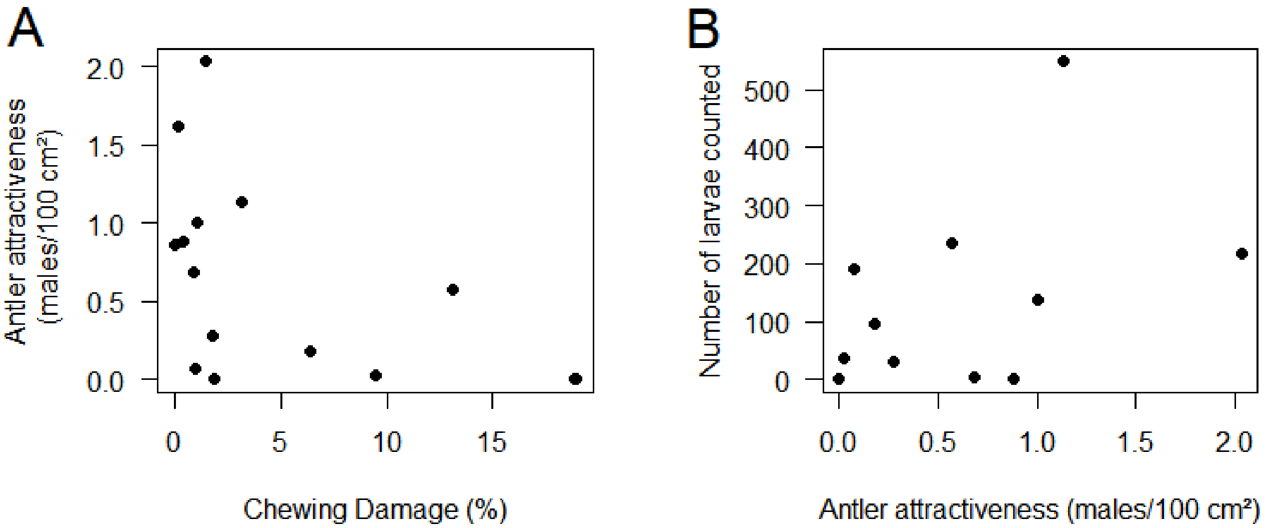

Fig. 5.1. Attractiveness to adult male antler flies as a metric of antler quality. *A*, antler attractiveness vs. extent of chewing damage (an index of antler age) (ρ = -0.603). *B*, number of larvae vs. antler attractiveness (ρ = 0.616).

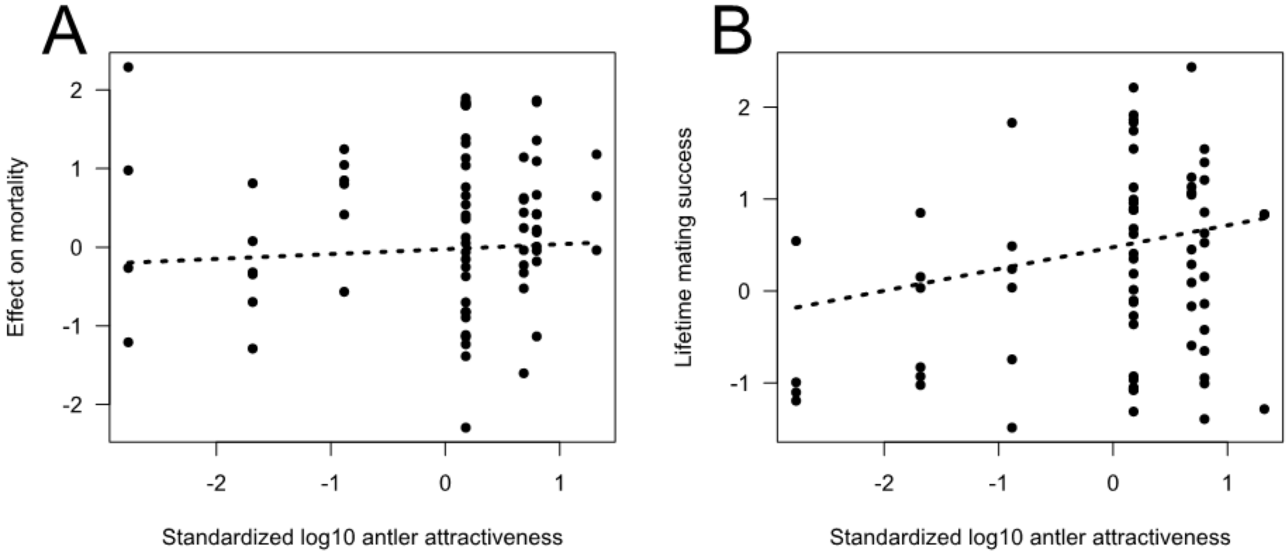

Fig. 5.2. Partial residual plots showing the effect of antler attractiveness on adult performance of wild male antler flies. *A*, partial effect of antler attractiveness on adult mortality based on the mixed-effects Cox proportional hazards regression not including wing length or development time (see Methods). Values represent log-transformed hazard ratio, such that positive values represent a proportionally higher mortality and negative values represent proportionally lower mortality relative to the intercept (mean antler attractiveness). *B*, partial effect of antler attractiveness on LMS in wild male antler flies, based on the negative binomial GLMM not including wing length or development time. Dotted lines represent non-significant estimates.

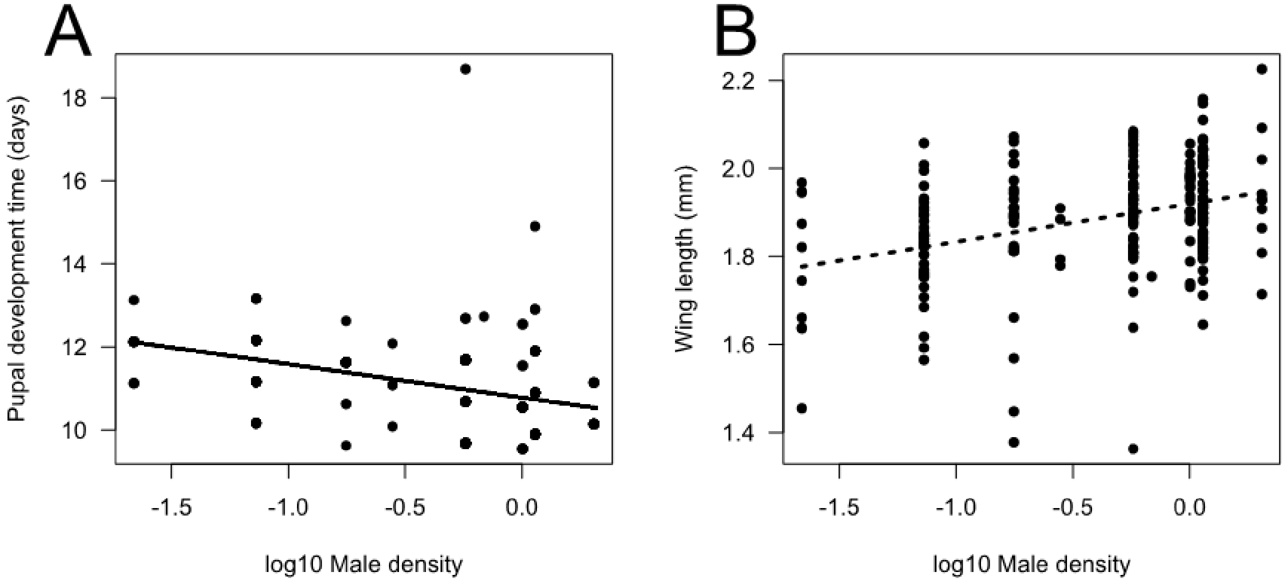

Fig. 5.3. Effect of antler attractiveness on growth and development of antler flies. Partial residual plots showing the partial effect of *A*, larval antler attractiveness (adult male density) on pupal development time, and *B* larval antler attractiveness on wing length of antler flies. Dotted lines represent non-significant estimates.

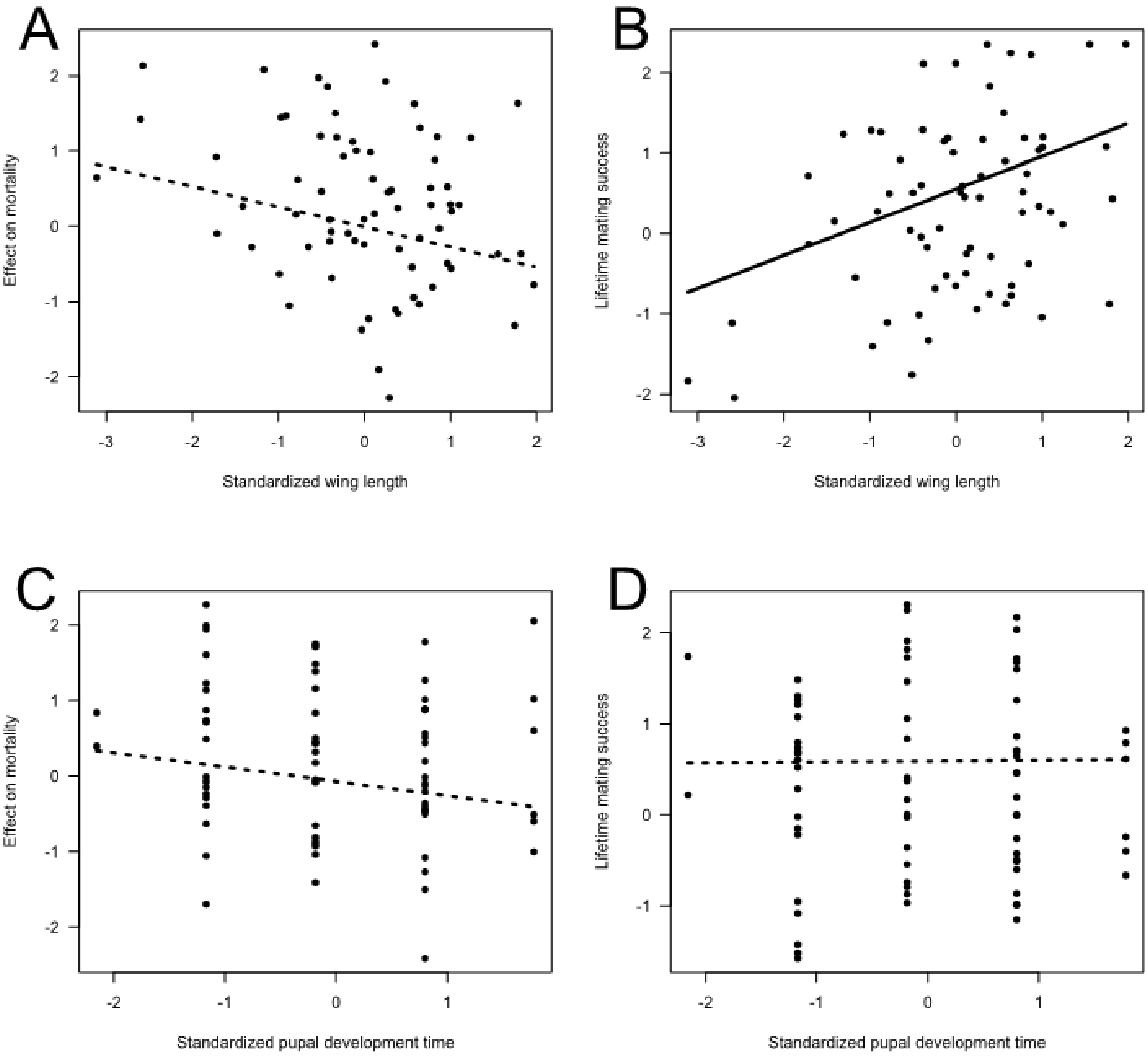

Fig. 5.4. Partial residual plots showing the effect of pupal development time and wing length on performance of adult male antler flies in the wild. Estimates and residuals are based on the Cox regression and GLMM including antler attractiveness, pupal development time, and wing length. *A*, partial effect of wing length on mortality. Values represent log-transformed hazard ratio, such that

positive values represent a proportionally higher mortality and negative values represent proportionally lower mortality relative to the intercept (mean wing length). *B*, partial effect of wing length on LMS. *C*, partial effect of pupal development time on mortality. Values represent log-transformed hazard ratios relative to the intercept (mean pupal development time). *D*, partial effect of pupal development time on LMS. Dotted lines represent non-significant estimates.

Chapter 6: Synthesis and Conclusion

Although senescence is a facet of the life history of most multicellular organisms (Jones et al. 2014), its causes and consequences remain incompletely understood. Furthermore, our knowledge of senescence in insects comes mostly from studies under controlled laboratory conditions, whereas studies of senesce in natural environments are uncommon (Zajitschek et al. 2020) despite knowing that such environments can cause important differences in the ageing process (Kawasaki et al. 2008; Hämäläinen et al. 2014; Mautz et al. 2019). In this thesis, I attempted to elucidate some of the proximate (plastic) causes of individual variation in senescence in the antler fly (*Protopiophila litigata*), and their fitness consequences, primarily in the field.

In Chapters 2, 3, and 5, I investigated variation in larval environmental quality, namely differences in larval diet. High-quality early life environments can improve many aspects of adult performance in animals (Lindström 1999; Tigreros 2013; Griffin et al. 2018; Macartney et al. 2018). However, while such "silver spoon" effects occasionally reduce senescence rate (Nussey et al. 2007; Fay et al. 2018), individuals from high quality environments sometimes senesce at the same rate or faster than those from low-quality environments (e.g., Adler et al. 2016; Hooper et al. 2017; Cooper and Kruuk 2018; Spagopoulou et al. 2020).

In my studies, a rich larval environment generally had positive effects on adult performance, but the results were complex. In Chapter 2, rich larval diet led to faster development, which in turn was associated with larger adult size and longer lifespan in the field. However, fast developers also senesced more rapidly and had a lower mating rate. In Chapter 3, larval diet quality did not affect development time, body size, longevity, or actuarial senescence rate in females or males in the laboratory, despite using a similar experimental manipulation to Chapter 2. This lack of an effect may reflect lower larval density (per gram of food) than the previous study such that reduced diet quality could be compensated through increased intake in the absence of intraspecific competition. In Chapter 5, males that developed in more attractive antlers did so faster and were (non-significantly) larger, while large flies lived (marginally non-significantly) longer and had significantly greater LMS. This study had lower power given the greater difficulty of collecting wild antler fly larvae compared to rearing them in the laboratory. For this reason, I was not able to make any inferences about senescence rate, and it is possible that the low sample size undermined my ability to detect significant effects of antler quality on adult performance. Overall, I found no evidence that high-quality early life environments lead to slower senescence in antler flies, and they can actually lead to faster actuarial senescence. Nevertheless, flies that developed in good conditions generally had better survival and mating success overall, consistent with the silver spoon hypothesis.

In Chapter 2, I also investigated sex differences in longevity, actuarial senescence, and plasticity. Sex differences in longevity and senescence are common, but not ubiquitous. It is not entirely clear what determines whether a species will be sexually dimorphic for these traits. The presence of strong sexual selection and a polygamous mating system have been proposed to lead to reduced lifespan and/or faster senescence in males (Bonduriansky et al. 2008; Maklakov and Lummaa 2013; Tidière et al. 2015), but these predictions are not always borne out (Lemaître et al. 2020). Although antler flies are not monogamous and males experience both inter- and intra-sexual selection in the wild (Bonduriansky and Brooks 1998, 1999; Angell et al. 2020), I found no differences in longevity or senescence between females and males in the laboratory. I also found no plasticity in these traits in either sex. Female and male antler flies may experience similar selection for survival, despite their different reproductive strategies, or they may be constrained from reaching their optima by intralocus sexual conflict. It is also possible that the lack of sexual dimorphism was an artifact of the controlled laboratory environment, which is known to plastically alter senescence in antler flies (Mautz et al. 2019).

In Chapter 4, I tested for effects of parental age at reproduction on their adult male offspring in the wild. Parental age effects are a manifestation of the aging process in parents, and can represent a form of transgenerational plasticity, although they can also be caused by genetic differences in the offspring of old and young parents (Monaghan et al. 2020). Bell (1918) and Lansing (1947) were some of the first researchers to report parental age effects, in the form of reduced longevity in the offspring of old parents. The expectation of negative consequence of parental breeding age remains common, but positive and hump-shaped relationships between parental age and offspring performance are reported as well (Wang and vom Saal 2000; Rödel et al. 2009; Kroeger et al. 2020; reviewed in Fay et al. 2016).

In my study, paternal, but not maternal, age significantly influenced the survival of male antler flies in the wild. Sons of old fathers tended to live longer, although this effect was primarily driven by differences in offspring of young mothers; sons of old mothers had similar lifespan regardless of paternal age. There was no effect of parental ages on offspring mating success or on actuarial or reproductive senescence rates. Old fathers in this study were 10-13 d old (post-eclosion), which is relatively old for an antler fly, but this is not their maximum wild lifespan (~32 d). Therefore, it is conceivable that offspring quality would decline at very old paternal age, but it is likely that the majority of parental age effects in wild antler flies operate at a shorter time scale. Unfortunately, the cohort-based design of my experiment makes it impossible to determine whether the effect of paternal age was a consequence of the aging process in old fathers or if it was caused by demographic changes due to selective disappearance of fathers in the "old" treatment.

In conclusion, antler fly life history, including senescence, is plastic and can be influenced by early life environmental quality and parental age. However, numerous other life history traits were affected simultaneously. Even though improved larval nutrition appears to accelerate senescence in antler flies, it had other beneficial consequences such that a positive "silver spoon" effect on longevity and/or LMS was observed overall. This highlights the fact that longevity and mating success are distinct from senescence (Péron et al. 2019), and that each is only a component of fitness.

Further ageing research on insects in nature, including antler flies, should take advantage of the fact that, unlike most vertebrates, it is possible to rear high numbers of individuals in captivity. Combining laboratory manipulation with field observation, as in Chapters 2 and 4, is a powerful approach for investigating life-history plasticity in response to different factors. For example, some of my results hint at a central role of larval density in determining environmental quality, and future work with antler flies could tease apart the effects of larval competition and nutrient concentration by systematically manipulating both. Such experiments should also take care to emulate the natural conditions under which species have evolved, as much as is feasible, to ensure the biological interpretability of their results. Future work in antler flies, and insects more generally, should continue to investigate causes of variation in life history traits, their correlations among one another, and their consequences for individual fitness.

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