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

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Abstract

High-quality developmental environments often improve individual performance into adulthood, but it is not clear what mediates these "silver spoon" effects. Furthermore, allocating toward early-life traits, such as growth, development rate, or reproduction, may lead to trade-offs with late life, so it is uncertain how a rich developmental environment will affect the ageing process (senescence). To investigate the effect of early-life environmental quality on life-history traits including senescence, and the traits mediating this, we reared larval antler flies (*Protopiophila litigata*) on four diets of varying nutrient concentration, then recorded survival and mating success of adult males released in the wild. Declining diet quality was associated with slower development, but had no effect on other life-history traits once development time was accounted for. Fast developing males were larger and lived longer, but experienced more rapid senescence in survival and lower mating rate than slow developers. Ultimately, larval diet, development time, and body size did not predict lifetime mating success. Thus, a rich environment led to a mixture of apparent benefits and costs, mediated by development time. Our results are largely inconsistent with the silver spoon hypothesis, and suggest that development time mediates the response of adult life-history traits to early-life environmental quality.

Key Words: Longevity, Mark-recapture, *Protopiophila litigata*, Senescence, Silver spoon, Trade-off.

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 [1,2]. A high-quality developmental environment is generally predicted to confer lasting benefits on individual performance [3]; this is known as the "silver-spoon" effect [4]. For instance, high quality environments in early life can lead to increased survival [5,6], fecundity [7], mating success [8–10], sperm quality and quantity [8,11,12], and immune function [13,14] 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 [15–19]. 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 [17,20–22]. 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 [23–25]. Faster growth also requires greater energy expenditure, leaving fewer resources available for subsequent somatic maintenance [2,26]. Some empirical studies have indeed found negative

phenotypic [21] or genetic correlations [27] between development rate and lifespan, although not all show this pattern [8,28]. Conversely, individuals with high resource acquisition may experience relaxed trade-offs [29] 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 [30,31]. Nevertheless, some studies have reported significant increases in lifespan and reduced senescence for individuals that experienced high quality developmental environments [6,9,32].

To determine the impact of early-life environmental quality on senescence in survival and mating success, we manipulated diet quality of antler fly larvae (*Protopiophila litigata*; Diptera: Piophilidae) 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 [33]. Males defend territories in large aggregations on the antler surface [34], and their high site fidelity and short adult lifespan make them well suited for studies of senescence in the wild because marked individuals 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 [35–37]. However, the effect of larval environment on this senescence pattern remain 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.

Materials and Methods

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 ref [38]. 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 [38] 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). The 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. Oviposition dishes were collected after each of nine consecutive 48 h laying periods beginning on May 2nd, 2013, creating nine temporal blocks of offspring.

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 cuticle to sclerotize. Each male was placed in a holding chamber [39] 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 [40]. 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 ($R = 0.99$; [38]). 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 [39]. 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. We released 179 males on antler A and 41 males on antler B (Table S1). Dispersal rate is low in this species, as only eleven individuals 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 [36]. Our analyses included 161 males tracked over 252 observation periods (7.04 ± 7.12 SD observations per male on average).

Statistical analyses

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 mixed-effects model (LMM), implemented in the R package *lme4* [41], that included oviposition dish as a random effect. To test for the effects of larval diet treatment and development time on wing length (our proxy for body size), we used an LMM that again included oviposition dish as a random effect. 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 actuarial and reproductive senescence. Our senescence analyses included additional confounding variables that could potentially affect male survival and mating success (see below for more details). Continuous variables were scaled to mean of zero and standard deviation of one prior to analysis [42]. All analyses were performed in R v3.5.1 [43]. Model selection was carried out using a backward and forward stepwise likelihood ratio test (LRT) procedure. 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.

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* [44] and *flexsurv* [45]. 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 [46] 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 the proportion of times a fly was observed on antler A, average population density, average sex ratio, and average mating rate (all as experienced over the lifetime of a given individual) as covariates, and block as a fixed factor. 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 selected the distribution that best fit the data using the corrected Akaike Information Criterion (AICc; [47]). We then performed stepwise model selection on each of the parameters of the model. The package *survival* was used to fit models with exponential, two-parameter Weibull, log-normal, log-logistic, and extreme value distributions, and *flexsurv* was used to fit the two-parameter Gompertz and three-parameter Weibull models. These packages allow fitting only a single factor to the shape parameter of the two- and three-parameter distributions (i.e. all except exponential), and any number of continuous 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 using the R package *MuMIn* [48]. 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 always provided the best fit to our data (ΔAICc > 9; Table S2) and was therefore used in subsequent model selection. The scale parameter (λ) of the Weibull model is the intercept of the relationship between age and mortality rate on a log-log scale and represents the time at which ~63% of the

individuals are dead, while the shape (α) is the slope on a log-log scale and 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 [49].

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 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.

Mating rate and reproductive senescence

To test whether early diet affected male mating rate or reproductive senescence, we used generalized linear mixed-effects models (GLMM) using *lme4.* 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 approximately 137 ± 52 min [50], and a given male was never observed mating at 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. We also included potential confounding variables in all our models. Antler fly density estimated at the time of observation and lifespan were included as covariates, while antler, hour of day, and larval block were included as fixed factors. We included observation (nested within day) and male identity as random effects in all models. Sex ratio was not included, as it was undefined during observation periods with zero flies present on an antler, but results

were qualitatively similar when only considering observations with defined sex ratios (results not shown). 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), but no fixed effects, converged on the same model as backward selection.

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* [51].

The initial model for backward selection contained the following terms: diet treatment, development time, wing length, antler, lifetime average density, lifetime average sex ratio, and larval block. Forward selection from an initial model containing only an intercept term converged on the same model.

Results

Effect of diet on development time and wing length

Egg-to-adult development time increased with decreasing diet quality (LMM: $F_{3,19.9} = 7.26$, $p = 0.002$, Fig. 1A), with a 28% increase in mean time between highest- and lowest-quality diets, but there was also substantial variation within each diet. Males that developed more slowly tended to be smaller (i.e.

had shorter wings; $F_{1,114.3} = 5.65$, $p = 0.019$; Fig. 1B). After accounting for the effect of development time, diet quality did not affect wing length (LMM: $F_{3,140.2} = 0.91$, $p = 0.436$) and there was no interaction between diet and development time on wing length ($F_{3,143.97} = 1.19$, $p = 0.315$).

Actuarial senescence

For Weilbull shape, the model including an effect of binned development time provided the best fit to the data ($\chi^2_1 = 6.01$, $p = 0.014$; diet: $\chi^2_3 = 0.733$, $p = 0.865$; wing length: $\chi^2_1 = 2.92$, $p = 0.087$ compared to a model with only an intercept). 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 a significant effect of only development time ($\chi^2_1 = 20.96$, $p < 0.001$), yielding a best fit model that included effects of development time on both the scale and shape of actuarial ageing (Table 1). These 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; shape parameter $\alpha = 2.33$ vs. 1.66 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: 4–20.25]) than slow developers (8 days [95% CI: 2–20.78]). There was no significant effect of larval diet, wing length, antler, sex ratio, density, or average mating rate on the scale of actuarial senescence (Table S3).

Mating rate and reproductive senescence

Males that developed more slowly had significantly higher mating rates (Fig. 3, Table 2), but larval diet did not significantly affect average mating rates ($\chi^2_3 = 4.047$, $p = 0.256$) when accounting for the effect of development time (Table S3). In addition, mating rate was higher at high density and on antler B. There was no significant relationship between mating rate and wing length, longevity, hour of day, or block (Table S3). Mating rate was not affected by age ($\chi^2_1 = 0.738$, $p = 0.390$), nor did age interact with either larval diet, 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] = -0.071 ± 0.083 SE).

Lifetime mating success

Larval diet did not affect LMS, nor did development time or wing length (all $p > 0.05$; Table S3). LMS was significantly affected by the average fly density ($\chi^2_1 = 5.29$, $p = 0.021$) and the average sex ratio experienced over a male's life ($\chi^2_1 = 14.9$, $p < 0.001$), such that males that experienced higher density and less male-biased sex ratios tended to have higher LMS. In addition, LMS did not differ among blocks or between antlers (Table S3).

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 [3,4], 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 did develop faster, and fast-developing males tended to reach greater adult sizes and live 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.

Our results demonstrate that early-life diet does not have a consistent "silver spoon" effect on adult traits in male antler flies: fast development, partially caused by high diet quality, was associated with extended adult lifespan and larger size, but also faster senescence and lower average mating rate. Other studies have reported similarly complex phenotypic effects of early life environmental quality: rich larval diets can lead to increased reproductive effort and a shortened lifespan [17,20,21], although we observed the opposite effect here. 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 [30,31].

We did not detect strong evidence of trade-offs between early and late life in 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 or genetic quality among individuals [29]. 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 [36]. 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 [52]. 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 [18].

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 from an 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. Using this more conservative approach, development time remained a significant predictor of the shape and scale of actuarial senescence, and of average mating rate, alongside larval diet which was now, unsurprisingly, also significant (Tables S4, S5). 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 [35–37].

Development time had a complex effect on actuarial senescence. Rapid larval development was associated with a low Weibull scale parameter, reflecting a low initial mortality rate (Table 1, Fig. 2). 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 (Table 1, Fig. 2). The co-occurrence of rapid development and rapid aging is consistent with physiological trade-offs between early- and late-life performance [23,24,27]. 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 17% of males survived beyond 15 days, the point at which age-specific mortality for fast developers exceeded that of slow developers (Fig. 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.

Slow-developing male antler flies had a higher average mating rate than fast developers (Fig. 3). This result is surprising, especially since slow developers were smaller on average and large male antler flies are more successful in territorial combat [34] and are preferred by females [53]. Furthermore, a previous study of male mating success in antler flies found that larger males had a high daily mating rate [36]. 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, and apparently lower-quality, 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 [54]. Small male antler flies may similarly localize to areas of the antler where males do not defend territories, such as oviposition sites or the underside [34]. They may also be more willing to accept matings from less fecund females that high-quality males would reject [53].

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 [55]. These males might be more susceptible to copulatory take-overs by rivals [50], be willing to accept less fecund females [53], lose paternity due to sperm expulsion by females [50], or produce semen with a reduced stimulatory effect on egg production (see ref [56]). 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 [35–37]. Previous studies have also reported reproductive senescence in this species [35–37], 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. [37] 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 no effect on male actuarial ageing (Weibull shape or scale) or average mating rate in our results. Bonduriansky and Brassil [36] found that increasing male size was associated with greater longevity and mating success, but faster reproductive senescence in antler flies. Interestingly, Mautz et al. [37] 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. 1B; [57]). Thus, the significant effects of body size on lifespan, mating success, and senescence

reported by Bonduriansky and Brassil [36] 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 [58] and one of the first to investigate early–late life trade-offs in wild insects (but see ref. [18]). 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 fitness consequences of variation in development time were mixed and were not consistent with a silver spoon effect on all adult traits. 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. 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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Author Contributions

MO and HR conceived the study design with input from RB. MO and BM performed the experiment and collected data. CA and NR performed data analysis. CA and MO drafted the manuscript. All authors contributed to interpretation and critically revised the manuscript.

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Table 1. Variables affecting actuarial senescence in male antler flies. Scale and shape effects from the reduced Weibull survival model are summarized below. The significance of individual terms was determined by LRT when each was dropped from the reduced model. Estimates for the scale effects are on a log scale, and continuous variables were standardized to mean of zero and unit standard deviation.

	Estimate	SE	χ^2_1	p
Scale effect (λ)				
Intercept	2.33	0.044		
Development time	-0.223	0.046	21.0	< 0.001
Shape effect (α)				
Development time			7.44	0.006
< Median	2.33	0.060		
≥ Median	1.66	0.024		

Table 2. Variables affecting mating rate in male antler flies. Fixed and random effects from the reduced binomial GLMM are summarized below. The significance of individual fixed effect terms was determined by LRT when each was dropped from the reduced model. Estimates are on a logit scale, and continuous variables were standardized to mean of zero and unit standard deviation.

Fixed effect	Estimate	SE	χ^2_1	p
Intercept	-4.48	0.158		
Development time	0.337	0.100	10.52	0.001
Antler			38.66	< 0.001
Antler B	1.44	0.241		
Fly density	0.410	0.103	15.38	< 0.001
Random effect	Variance	SD		
Male identity	0.281	0.531		
Observation (within day)	0.413	0.643		

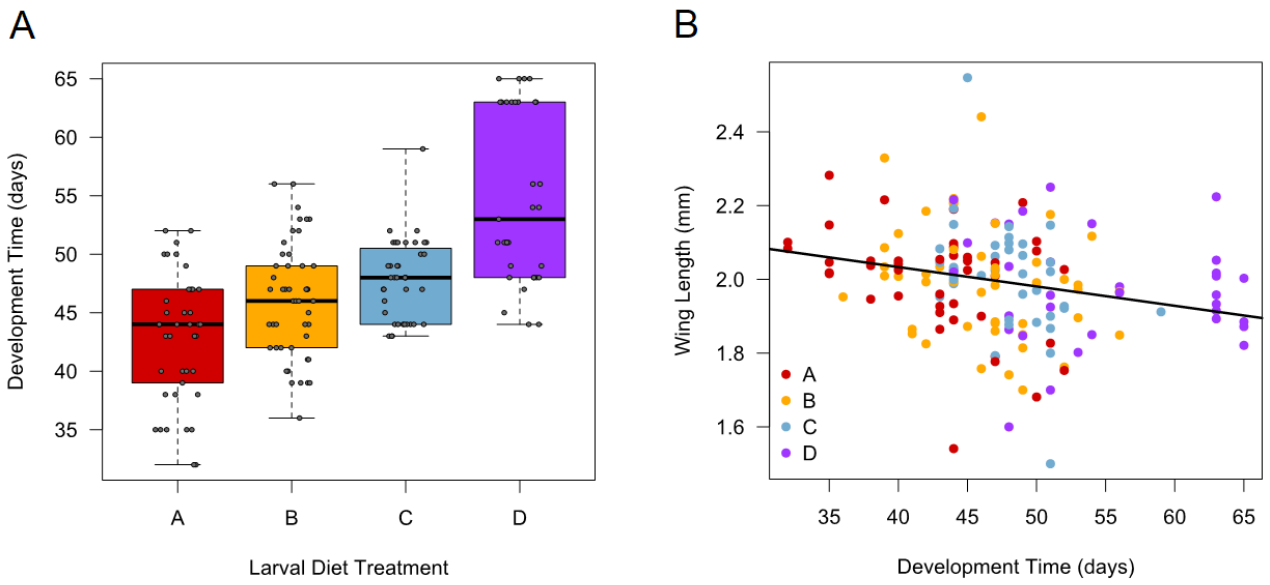

Fig. 1. Variation in egg-to-adult development time and wing length within and among larval diet treatments. *A*, boxplot of development time in each diet. Thick horizontal lines denote the median, boxes demarcate the first and third quartiles and whiskers indicate the minimum and maximum values. *B*, wing size as a function of developmental time across all larval diet treatments. The regression line is fit on the pooled data set ($F_{1,159} = 9.39$, $p = 0.003$), 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).

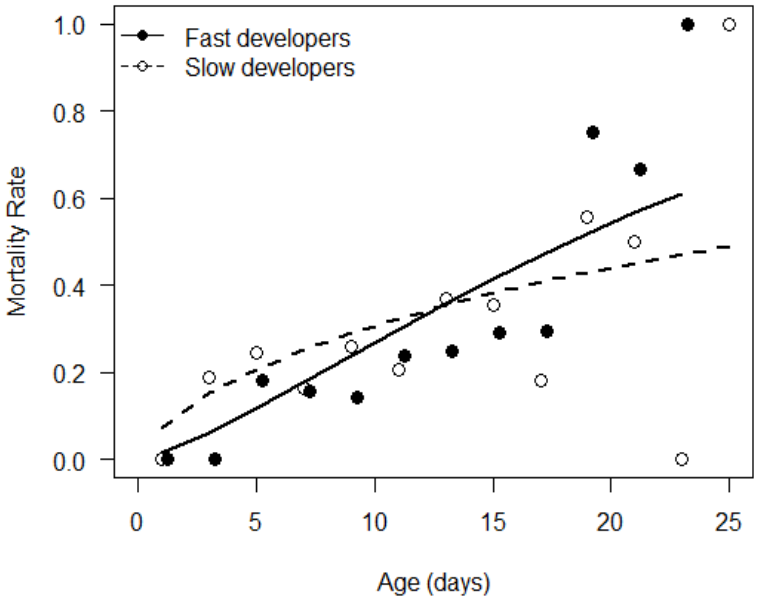

Fig. 2. The effect of egg-to-adult development time on actuarial senescence in male *Protopiophila litigata*. The effect of development time on the scale parameter was analyzed as a continuous variable, but is plotted as mortality curves for males above or below the median trait value.

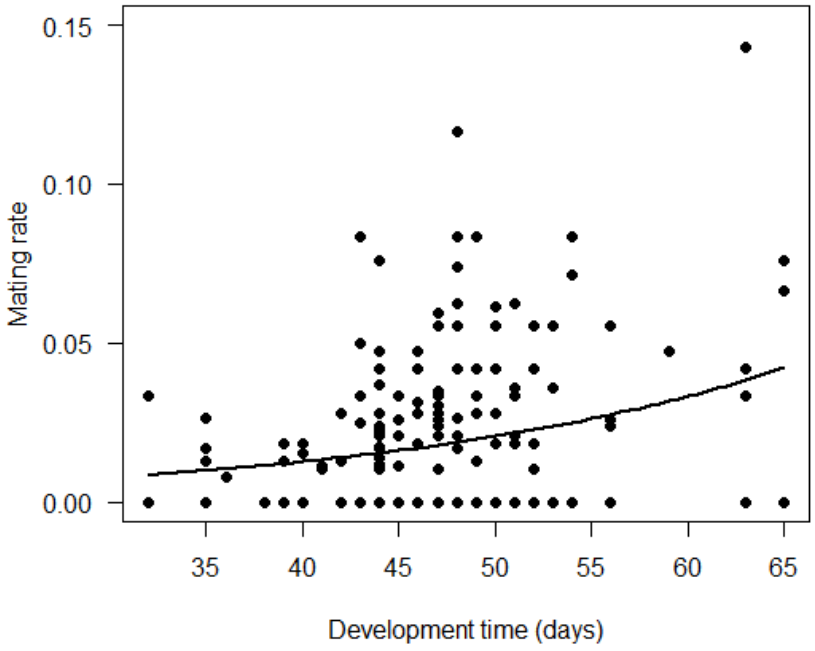

Fig. 3. Relationship between egg-to-adult development time and average mating rate in male antler flies.

Supplementary Information

Table S1. Number of males from each treatment released on antlers A and B.

Antler	Treatment	Flies released	Total
A	A	49	179
	B	48	
	C	42	
	D	40	
B	A	11	41
	B	10	
	C	10	
	D	10	

Table S2. ΔAICc values from survival distribution selection, calculated relative to the best fit model with the same factor on the shape parameter (either a single global shape parameter or separate shape values for one of three different factors). NA: the Exponential distribution is characterized by a single parameter so that the shape is constrained to be 1.

	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	982.8	9.9	989.8	9.7	981.2	11.7	983.9	11.3
Gompertz	1012.9	40.0	998.5	18.4	996.2	26.7	996.6	24.0
Extreme value	1026.7	53.1	1029.4	49.3	1028.9	59.4	1029.1	56.5
Log-logistic	993.0	20.1	999.5	19.4	988.8	19.3	993.1	20.5
Log-normal	986.6	13.7	992.5	12.4	980.6	11.1	986.1	13.5
Gaussian	1003.6	30.7	1006.2	20.5	1006.0	36.5	1006.2	33.6
Exponential	1067.2	94.3	NA	NA	NA	NA	NA	NA

Table S3. Significance of additional factors and covariates dropped during model selection, based on LRT between the final model and a model with the variable added.

Variable	χ^2	df	*p*
Actuarial senecence (Weibull model)			
Larval diet treatment	5.26	3	0.153
Wing length	2.25	1	0.133
Time spent on antler A	0.527	1	0.468
Average fly density	0.060	1	0.806
Average sex ratio	0.247	1	0.619
Average mating rate	0.895	1	0.344
Block	13.63	8	0.092
Mating rate (binomial GLMM)			
Age	0.738	1	0.390
Larval diet treatment	4.047	3	0.256
Wing length	0.043	1	0.836
Longevity	0.080	1	0.390
Hour	8.12	5	0.150
Block	12.63	8	0.125
Lifetime mating success (negative binomial GLM)			
Larval diet treatment	1.68	3	0.640
Development time	0.427	1	0.513
Wing length	0.291	1	0.589
Time spent on antler A	0.006	1	0.936
Block	8.51	8	0.385

Table S4. Reduced Weibull survival model using residual development time and residual wing length. The significance of individual terms was determined by LRT when each was dropped from the reduced model. Estimates for the scale effects are on a log scale.

	Estimate	SE	χ^2	df	*p*
Scale effect (λ)					
Intercept	2.34	0.08			
Diet treatment			8.94	3	0.03
Diet B	0.052	0.109			
Diet C	0.083	0.115			
Diet D	-0.262	0.120			
Residual development time	-0.037	0.008	18.5	1	< 0.001
Shape effect (α)					
Residual development time			4.09	1	0.05
< Median	2.20	0.048			
≥ Median	1.71	0.028			

Table S5. Reduced binomial GLMM for mating rate, using residual development time and residual wing length. The significance of individual fixed effect terms was determined by LRT when each was dropped from the reduced model. Estimates are on a logit scale.

Fixed effect	Estimate	SE	χ^2	df	p
Intercept	-4.50	0.210			
Larval diet treatment			9.60	3	0.022
Diet B	-0.230	0.238			
Diet C	-0.109	0.244			
Diet D	0.490	0.254			
Residual development time	0.045	0.018	7.50	1	0.006
Antler			39.6	1	< 0.001
Antler B	1.46	0.243			
Fly density	0.420	0.104	15.8	1	< 0.001
Random effect	Variance	SD			
Male identity	0.264	0.514			
Observation (within day)	0.420	0.648			

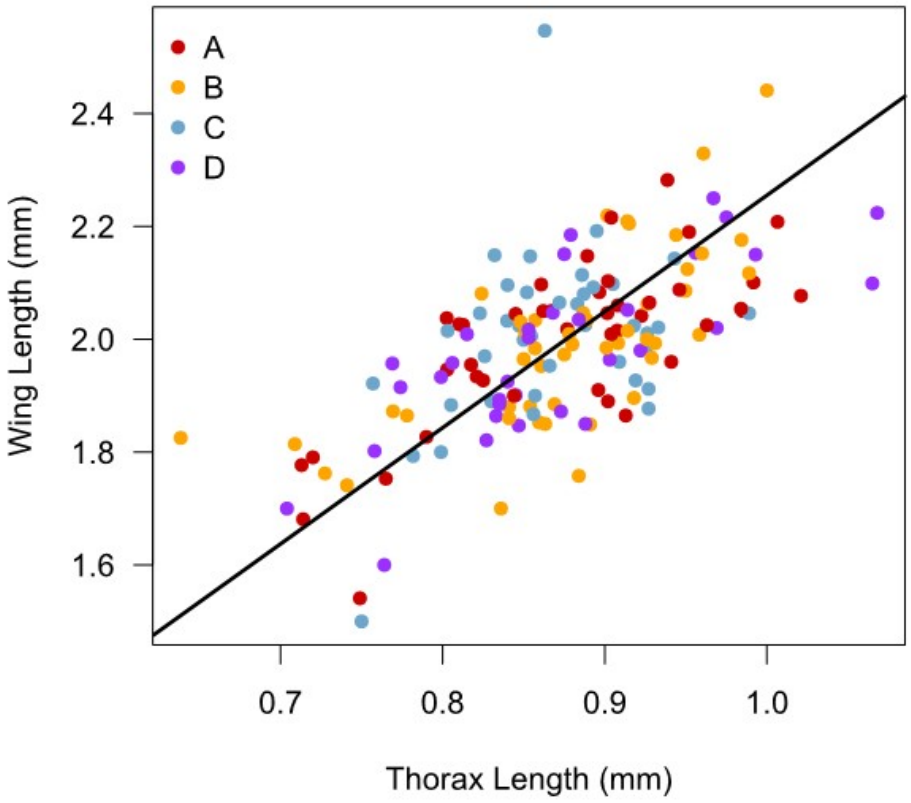

Fig. S1. Relationship between wing length (mm) and thorax length (mm) in male antler flies across four larval diet treatments (colors). Treatments did not differ significantly in slope or intercept, so the overall reduced major axis fit is represented by the black line ($r = 0.645$, $p < 0.001$).