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 allocating toward early-life traits, such as growth, development rate, and reproduction, may lead to trade-offs with late life performance. It is therefore uncertain how a rich developmental environment will affect the ageing process (senescence), particularly in wild insects. To investigate the effects of early-life environmental quality on insect life-history traits, including senescence, 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 average mating rate compared to 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 indicate that "silver spoon" effects can be complex and 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.

1. 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 [17,20–23]. 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 [24–26]. Faster growth also requires greater energy expenditure, leaving fewer resources available for subsequent somatic maintenance [2,27]. Some empirical studies have indeed found negative

phenotypic [21] or genetic correlations [28] between development rate and lifespan, although not all show this pattern [8,29]. Conversely, individuals with high resource acquisition may experience relaxed trade-offs [30] 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 [31,32]. Nevertheless, some studies have reported significant increases in lifespan and reduced senescence for individuals that experienced high quality developmental environments [6,9,33].

While studies of insect life histories and senescence in captivity are common (e.g. [34–36]), studies of senescence in wild populations have focused mainly on vertebrates [37,38]. Patterns of survival and performance can differ markedly between wild and captive animals, including insects [39–41], 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 [42]. A few field studies have detected trade-offs linking body size and reproductive effort to senescence rates in insects [18,43], 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 [44]. Males defend territories in large aggregations on the antler surface [45], 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 [39,43,46]. 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.

2. Material and Methods

(a) Experimental procedure

(i) 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 [47]. 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).

(ii) 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.

(iii) 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 [48] 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 [49]. 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 ($R = 0.99$; [47]). 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 [48]. 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 S1). Dispersal among antlers is generally low in this species [50], 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 [43]. Our analyses included 161 males tracked over 251 observation periods (7.04 ± 7.12 SD observations per male on average).

(b) Statistical analyses

All analyses were performed in R v 3.6.3 [51].

(i) 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

166 treatment and larval block, as well as a second LM containing diet treatment, development time (a
167 continuous variable), their interaction, and larval block. We performed type III *F*-tests using the R
168 package *car* [52].
169
170 *(ii) Adult performance and senescence*
171 Development time (number of days between egg laying and adult emergence) varied among diet
172 treatments (see Results), but there was also substantial independent variation within treatment levels
173 such that we were able to discriminate the respective effects of diet and development time on male
174 performance and actuarial and reproductive senescence. These analyses included additional
175 confounding variables that could potentially affect male survival and mating success (see below for
176 details). Continuous variables were scaled to mean of zero and standard deviation of one prior to
177 analysis [53]. Model selection was carried out using a backward and forward stepwise likelihood ratio
178 test (LRT) procedure. If the two selected models differed, a LRT was used to compare them, and the
179 significance of all terms was assessed using LRTs relative to the final model.
180
181 *(iii) Actuarial senescence*
182 The effects of diet treatment, development time, and body size on male actuarial senescence were
183 analyzed using parametric survival models, implemented in the R packages *survival* [54] and *flexsurv*
184 [55]. We chose this approach over semi-parametric Cox proportional hazards regression because Cox
185 models only test for differences in overall mortality rate, but cannot detect differences in aging rates
186 among groups. We used an interval-censored survival model [56] in which we assumed death occurred
187 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* [57] to select the survival distribution that best fit the data based on the corrected Akaike Information Criterion (AICc; [58]). 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* packageto 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). 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 [59].

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 final model.

(iv) 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* [60]. 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 [61], 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 (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) and a fixed effect of block, converged on the same model.

(v) 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, 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 model.

(vi) 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

254 independent effects. We calculated residual development time from a one-way ANOVA among diets—
255 thereby representing only within-diet treatment variation in development time—and residual wing
256 length from a regression against development time—representing the effect of body size independent of
257 development time. We then performed model selection for survival, mating rate, and LMS as above,
258 using residual development time and residual wing length instead of the 'raw' variables. An effect of
259 residual development time and/or residual wing length would infer the importance of that variable even
260 when diet or development time respectively is allowed to account for all shared variation.
261
262 **3. Results**
263 ***(a) Effect of diet on development time and wing length***
264 Egg-to-adult development time increased with decreasing diet quality ($F_{3,149} = 23.0$, $p < 0.001$, Fig.
265 1A), with a 28% increase in mean time between highest- and lowest-quality diets, but there was also
266 substantial variation within each diet. Larval diet treatment did not significantly influence male wing
267 length when considered alone ($F_{3,149} = 0.431$, $p = 0.731$). When considering development time and diet
268 treatment together, wing length was negatively related to development time ($F_{1,145} = 13.4$, $p < 0.001$;
269 Fig. 1B), diet quality still did not affect wing length ($F_{3,145} = 1.26$, $p = 0.289$), and there was no
270 interaction between diet and development time on wing length ($F_{3,145} = 1.52$, $p = 0.212$).
271
272 ***(b) Actuarial senescence***
273 A Weibull survival distribution was a consistently best fit to the data (Table S2) and an effect of binned
274 development time on the Weibull shape parameter significantly improved the fit compared to an
275 intercept-only model ($\chi^2_1 = 6.01$, $p = 0.014$). Effects on the shape parameter of diet ($\chi^2_3 = 0.733$, $p =$

0.865) and wing length ($\chi^2_1 = 2.92$, $p = 0.087$) did not improve fit (see also AICc values in Table S2). 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 ($\chi^2_1 = 11.5$, $p < 0.001$) and wing length ($\chi^2_1 = 3.85$, $p = 0.0498$), but did not include diet treatment ($\chi^2_3 = 3.71$, $p = 0.294$). There was also no significant effect of antler, sex ratio, density, or average mating rate on the scale of actuarial senescence (Table S3a). 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. 2a; Table S4; 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. 2b).

(c) Mating rate and reproductive senescence

Males that developed more slowly had significantly higher mating rates ($\chi^2_1 = 11.5$, $p < 0.001$; Fig. 3; Table S5), but diet treatment did not significantly affect average mating rates ($\chi^2_3 = 2.65$, $p = 0.449$) 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 S3b). Mating rate was not affected by age ($\chi^2_1 = 1.74$, $p = 0.187$), nor did age interact with either diet treatment, development time, or wing length (all $p > 0.05$).

298 Therefore, we do not detect reproductive senescence in our data. If an age term is added to the final
299 GLMM, the estimate of its effect on mating success is negative, as would be expected for reproductive
300 senescence, but it is non-significant (reduced model + age: β [logit scale] = -0.112 ± 0.086 SE).
301
302 ***(d) Lifetime mating success***
303 Diet treatment did not affect LMS, nor did development time or wing length (all $p > 0.05$; Table S3c).
304 LMS was significantly affected by the average fly density ($\chi^2_1 = 7.11$, $p = 0.008$) and the average sex
305 ratio experienced over a male's life ($\chi^2_1 = 19.6$, $p < 0.001$), such that males that experienced higher
306 density and less male-biased sex ratios tended to have higher LMS (Table S6). LMS did not differ
307 among blocks or between antlers (Table S3c).
308
309 ***(e) Analyses of residual development time and residual wing length***
310 Our supplementary analysis using resiudal development time and residual wing length allowed diet
311 treatment to account for all shared variation with development time. Consequently, residual
312 development time represented only development time variation within diet treatment levels, and
313 residual wing length reflected only size variation that was independent of development time. As
314 expected, the previously non-significant effect of larval diet became significant when it was allowed to
315 explain all shared variation with development time, with decreasing nutrient concentration being
316 associated with both higher mortality (Table S7a; Table S8) and greater average mating rate (Table S7b;
317 Table S9). However, the previously significant effects of development time persisted such that males
318 with shorter residual development time had reduced mortality (Table S8) and had lower average mating
319 rates (Table S9), consistent with the main analyses. Also consistent with the main analyses, residual

wing length had a small effect on survival (Table S7a; Table S8), but not mating success (Table S7b; Table S9). 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 S7a). Again, none of the variables of interest influenced LMS (Table S7c).

5. 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 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

342 developers, although they may ultimately have had somewhat higher fitness due to potential differences
343 in postcopulatory performance (see below). Other studies have similarly reported complex phenotypic
344 effects of early life environmental quality: rich larval diets can lead to increased reproductive effort and
345 a shortened lifespan and/or accelerated senescence [17,20,21,23], although we observed the opposite
346 effect on lifespan and reproduction as previous studies. Given the complex influence of early-life
347 conditions reported in this and other studies, it is not surprising that two recent meta-analyses failed to
348 detect consistent silver spoon effects on lifespan or actuarial senescence in laboratory or wild
349 populations [31,32].

350 We did not detect strong evidence of trade-offs between early and late life performance in our
351 antler flies. Fast development was associated with longer lifespan, not shorter, and there was no
352 significant relationship between longevity and average mating rate. Furthermore, body size, which
353 depends on allocation toward growth in the larval stage, was not significantly associated with survival,
354 mating success, or senescence rate. This positive correlation of life-history traits suggests high
355 variation in resource acquisition and/or genetic quality among individuals [30]. Nevertheless,
356 development time had opposing effects on average mating rate and survival, which could arise from an
357 underlying survival–reproduction trade-off. This would be consistent with a previous study of this
358 species that reported a significantly higher average mating rate in short-lived males [43]. Although it
359 can be difficult to detect trade-offs in nature, studies of wild vertebrates have often identified trade-offs
360 between early and late life [38]. However, wild field crickets (*Gryllus campestris*) experience no
361 apparent trade-offs between early reproduction and survival, and only a modest effect of early
362 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 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 S7a-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; Table S4). 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; Table S4). The co-occurrence of rapid development and rapid aging is consistent with physiological trade-offs between early- and late-life performance [24,25,28]. 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). 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. 3). This result is surprising, especially since slow developers were smaller on average and large male antler flies are more successful in territorial combat [45] and are preferred by females [62]. Furthermore, a previous study of male mating success in antler flies found that larger males had a high daily mating rate [43]. 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 [63]. 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) [45]. They may also be more willing to accept matings from less fecund females that high-quality males would reject [62].

406 Despite their high average mating rate, slow-developing males may not have achieved equal
407 fitness as their peers. We only recorded mating success, which does not take into account variation in
408 female fecundity or postcopulatory effects including sperm viability, sperm competition, and female
409 choice [64]. These males might be more susceptible to copulatory take-overs by rivals [61], be willing
410 to accept less fecund females [62], lose paternity due to sperm expulsion by females [61], or produce
411 semen with a reduced stimulatory effect on egg production (see ref. [65]). If these mechanisms of
412 postcopulatory selection act against slow-developing males, their siring success could be lower than
413 other males, despite similar LMS.
414 Our detection of actuarial senescence in male antler flies in the wild is consistent with multiple
415 previous studies and further reinforces the existence of senescence in a short-lived insect in nature
416 [39,43,46]. Previous studies have also reported reproductive senescence in this species [39,43,46], but
417 we did not find a significant decline in male mating rate with age, although the trend was negative.
418 Reproductive declines may simply be difficult to detect at smaller sample sizes, as Mautz et al. [39]
419 detected clear reproductive senescence in male antler flies in one year (n = 432 males), but found only
420 low support in the other (n = 219) in which sample size was similar to the current study.
421 Wing length had a small effect on male actuarial senescence (Weibull scale) and no effect on
422 average mating rate in our results. In our study, large males tended to live longer. Similarly,
423 Bonduriansky and Brassil [43] found that larger male size was associated with greater longevity and
424 mating success, but faster reproductive senescence in antler flies. Interestingly, Mautz et al. [39]
425 reported differing effects of body size between years: large males experienced substantially higher
426 mortality in one year, but slightly lower mortality in the other, and slightly higher mating rate in both
427 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; [66]). Thus, the significant effects of body size on lifespan, mating success, and senescence reported by Bonduriansky and Brassil [43] 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 [42] 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 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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451

452 **Author Contributions**

453 MJO and HDR conceived the study design with input from RB. MJO and BSM performed the
454 experiment and collected data. CSA and NOR performed data analysis. CSA and MJO drafted the
455 manuscript. All authors contributed to interpretation and manuscript revisions.

456

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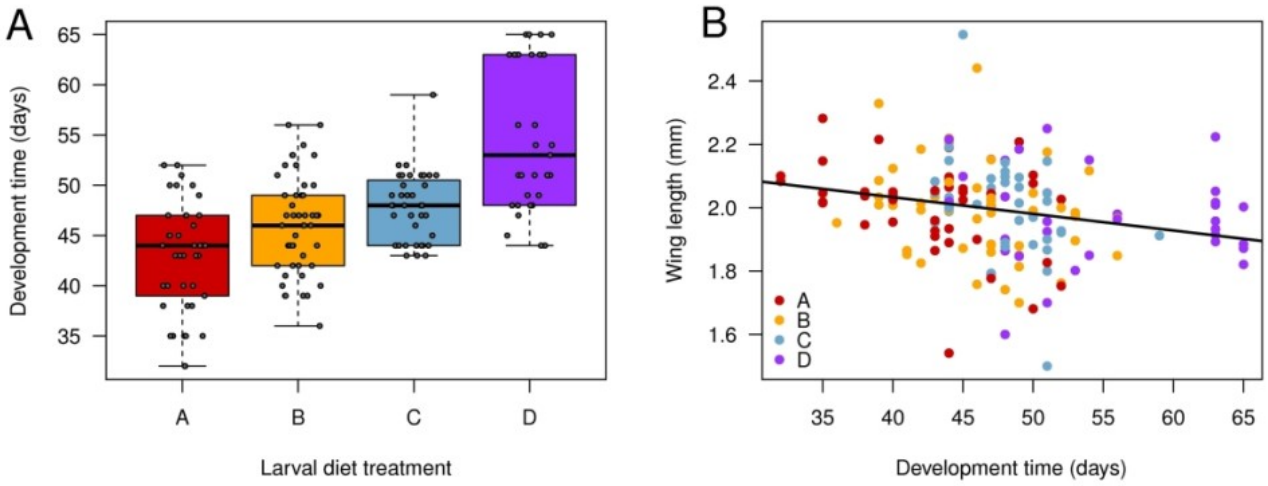
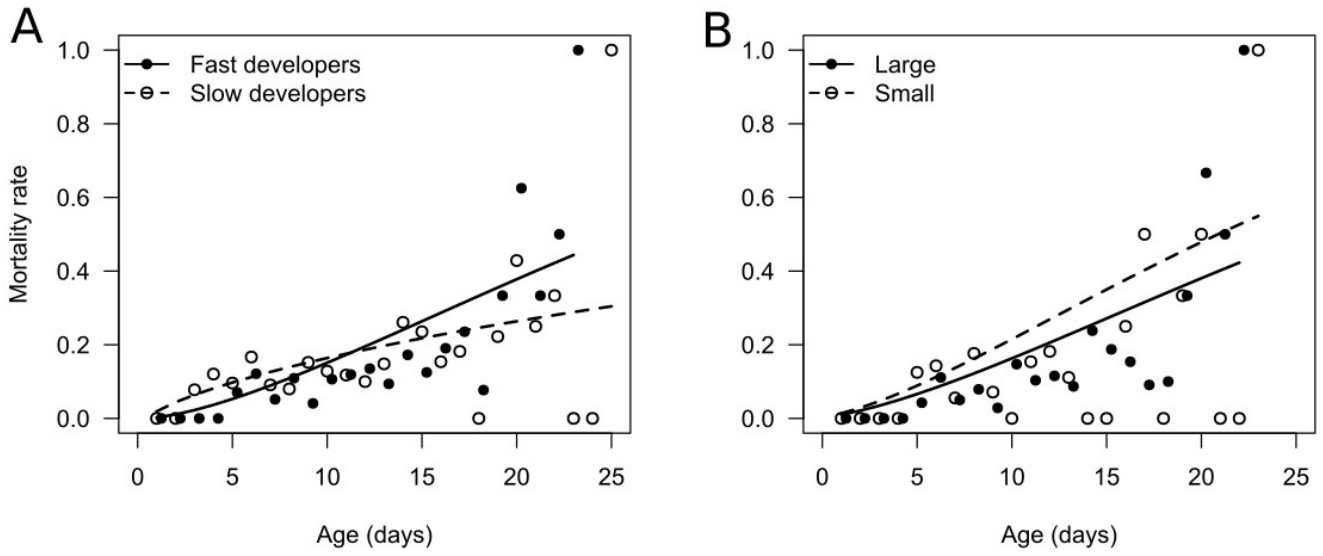


461 Fig. 1. Variation in egg-to-adult development time and wing length within and among larval diet
462 treatments. *A*, boxplot of development time in each diet. Thick horizontal lines denote the median,
463 boxes demarcate the first and third quartiles and whiskers indicate the minimum and maximum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

468



470 Fig. 2. The effect of *A*, egg-to-adult development time and *B*, wing length (body size) on actuarial
471 senescence (daily mortality rate) in male *P. litigata*. The effect of development time and wing length on
472 the scale parameter were analyzed as continuous variables, but are plotted as mortality curves for males
473 above or below the median trait value. Symbols are observed daily mortality rates for the two groups,
474 while the lines represent fitted mortality curves based on the best supported Weibull survival model
475 (weighted means across blocks). Due to the shape effect of development time, panel *B* shows mortality
476 rates for fast developers only.

477

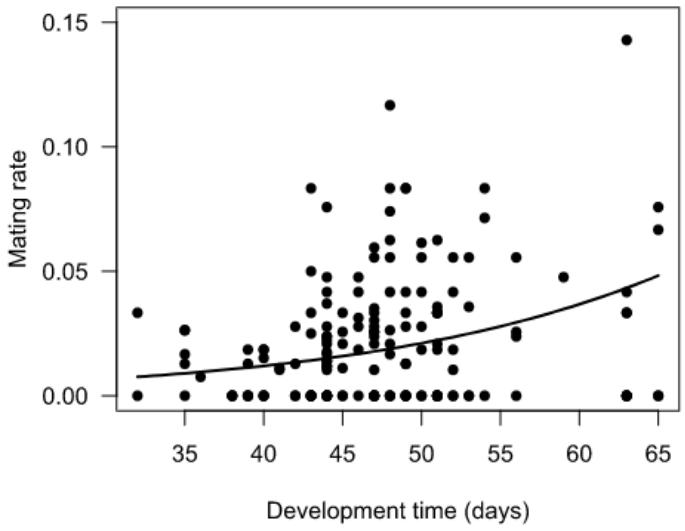


Fig. 3. Relationship between egg-to-adult development time and average mating rate in male antler flies. Points represent lifetime average mating rate for each male and the line represents predicted values from the best fit GLMM (weighted mean across antlers and blocks).

Supplementary Information for

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

Christopher S. Angell, Mathieu J. Oudin, Nicolas O. Rode, Brian S. Mautz, Russell Bonduriansky, and Howard D. Rundle

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* [1] and *flexsurv* [2] 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 [3] using the R package *MuMIn* [4], considering models with $\Delta AICc < 2$ to be equally well supported [5]. 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) and was therefore used in subsequent model selection.

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Table S1. 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. 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 S3. 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 final model, relative to a model lacking this term. Terms in bold were present in the final 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

523 Table S4. Parameter estimates from the final parametric Weibull survival model. Estimates for the scale
524 parameter are on a log scale, and covariates were standardized to a mean of zero and a standard
525 deviation of one. The reference level for “Block” was block 1.
526

	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 S5. 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 S6. 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 S7. Significance of all fixed effects in the residual model selection, based on LRT relative to the best fit model (or to a model lacking the given term). Terms in bold are included in the final model.

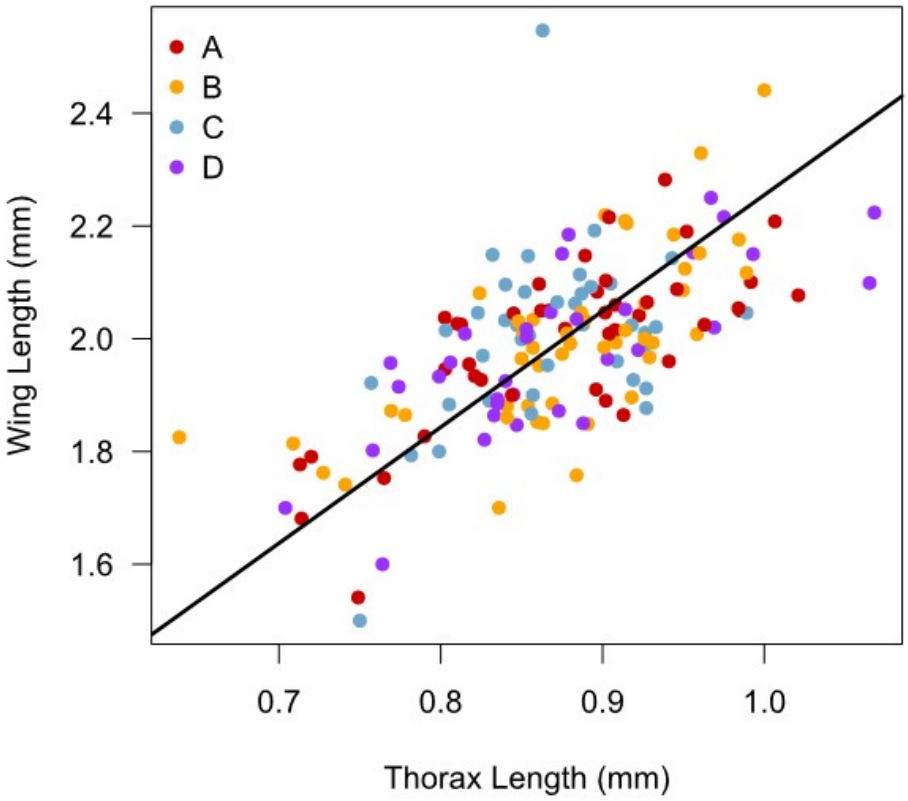
Variable	χ^2	df	*p*
A. Actuarial senescence (Weibull regression)			
Larval diet treatment	**12.0**	**3**	**0.007**
Residual development time	**13.6**	**1**	**< 0.001**
Residual wing length	**3.88**	**1**	**0.049**
Block	**16.2**	**8**	**0.040**
Lifetime average sex ratio	2.36	1	0.125
Lifetime average 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 S8. 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 S9. 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		



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