Title

Causes and consequences of variation in development time in a field cricket

Short Running title

Development time and crickets

Susan N. Gershman[1], Owen G. Miller[,2] and Ian M. Hamilton[2,3]

[1]*Department of Evolution, Ecology and Organismal Biology, 1465 Mount Vernon Ave, The Ohio State University at Marion, Marion, OH, 43302 USA*

[2]*Department of Evolution, Ecology and Organismal Biology, The Ohio State University*

[3]*Department of Mathematics, The Ohio State University*

Abstract

Variation in development time can affect life history traits that contribute to fitness. In *Gryllus vocalis*, a non-diapausing cricket with variable development time, we used a path analysis approach to determine the causative relationships between parental age, offspring development time and offspring life history traits. Our best-supported path model included both the effects of parental age and offspring development time on offspring morphological traits. This result suggests that offspring traits are influenced by both variation in acquisition of resources and trade-offs between traits. We found that crickets with longer development times became larger adults with better phenoloxidase-based immunity. This is consistent with the hypothesis that crickets must make a trade-off between developing quickly to avoid predation before reproduction and attaining better immunity and a larger adult body size that provides advantages in male-male competition, mate choice, and female fecundity. Slower-developing crickets were also more likely to be short-winged (unable to disperse by flight). Parental age has opposing direct and indirect effects on the body size of daughters, but when both the direct and indirect effects of parental age are taken into account, younger parents had smaller sons and daughters. This pattern may be attributable to a parental trade-off between the number and size of eggs produced with younger parents producing more eggs with fewer resources per egg. The relationships between variables in the life history traits of sons and daughters were similar, suggesting that parental age and development time had similar causative effects on male and female life history traits.

Keywords

Development time, parental effects, parental age, body size, immunity, wing morph, field cricket, trade-off

Introduction

Seasonal variation in temperature and rainfall represents a physiological challenge for many organisms. Invertebrates have been able to colonize and persist in areas with seasonally unsurvivable conditions by delaying growth during these times of year. Some insects diapause (a temporary pause in the growth and development of an organism due to adverse environmental conditions) as either an obligate or facultative strategy (Mosseau and Roff 1989) while other insects vary in development time without diapause (Danks 2007). Optimal development time can depend on both density-independent abiotic factors (e.g. temperature and availability of food) and density-dependent biotic factors (e.g. competition, predation risk and parasitism risk) (Danks 1997, Roff 1992). The longer the development time, the more time an individual has to acquire biomass, but it also has increased opportunity to die before reproducing. Organisms may vary in development time for two non-mutually-exclusive reasons: variation in their ability to acquire resources (acquisition) or variation in how resources are allocated to different traits (trade-offs) (van Noordwijk and de Jong 1986). If variation in development time is maintained by trade-offs between traits, the benefits of rapid development are offset by other fitness costs. If differences in acquisition are maintaining variation in development time, then higher-condition individuals (Clancey and Byers 2014) that have the ability to acquire or integrate more resources would both have faster development and also have other trait values associated with higher fitness.

Variation in resources (acquisition) can be driven by parental contributions. Well-provisioned offspring may be able to devote resources to multiple traits while poorly-provisioned offspring may need to make trade-offs between traits (Fox and Dingle 1994). Although many factors affect the quality of parental provisions (Mousseau and Fox 1998), in this paper, we will focus on the effects of parental age on offspring traits. In different insect taxa, as adults age, their stores of nutritional resources may either become depleted (Rivero et al. 2001) or increase (Stachschmidt et al. 2013). Parental nutritional resources can affect offspring morphological traits like development time and body size (Bonduriansky and Head 2007). Further, in seasonal species, older parents may have a shorter window for reproduction than younger parents. In species with diapause, older parents are more likely to produce diapausing eggs (Danks 1997). In non-diapausing seasonal species, older parents may produce either faster-growing offspring (Phelan and Frumhoff 1991, Zehnder et al. 2007) or slower-growing large offspring (Benton et al. 2008). Parental age can also have a direct effect on offspring body size (Opit and Throne 2007, Qazi et al 2017), fecundity (Opit and Throne 2007, Hercus and Hoffmann 2000, Nystrad and Dowling 2014) and immunity (Rossiter et al. 1990). Here, we investigate the effect of field cricket parental age and offspring development time on four offspring life history traits: adult body size, fecundity, immunity and wing morphology.

In crickets, adult males and females with a larger body size are consistently at a fitness advantage. Larger males are more likely to win fights (Brown et al. 2006, Briffa 2008).

Females prefer larger males (Gray 1997, Bertram and Rook 2011, Deb et al. 2012). And larger males produce more offspring (Zeng et al. 2018). As in most species (Roff 1992, Honek 1993), larger female crickets lay more eggs (Simmons and García-González 2007). Larger female crickets are also more likely to mate than smaller females (Brown 2008, del Castillo 2015). Previous studies on many taxa have found that larger adults have slower development times (Roff 1992, Roff 2000). Although, in rare instances, individuals have the ability to both develop rapidly and become large adults (Reznick et al. 2000). If development time trades off with adult body size, individuals should either develop quickly into small adults with lower reproductive success or develop slowly into large adults with higher reproductive success. If variation in development time is maintained by individual differences in access to resources, high-quality individuals would be expected to develop quickly into large adults while low-quality individuals develop slowly into smaller adults.

Unlike the relatively straightforward relationship between development time and body size, the relationship between development time and immunity is contradictory and complex. Some studies have found a trade-off between development time and immunity, with individuals either developing rapidly at a cost to immunity or maintaining immunity through slower growth (Rantala and Roff 2005, van der Most et al. 2011). In other studies, some individuals have both rapid development time and superior immunity, while other individuals suffer with slow development time and poor immunity (Rantala and Roff 2005); this outcome could be explained by variation in acquisition among individuals (Lee et al. 2008). If development time trades off with immunity, individuals could either develop quickly into adults with poor immunity or develop slowly into adults with superior immunity. If variation in development time is maintained by individual differences in acquisition of resources, high-quality individuals would be expected to develop quickly and also have superior immunity while low-quality individuals would develop slowly and have poor standing immunity. However, because both development time and immunity are correlated with other life history traits, immunity may be indirectly affected by development time via another trait. For example, reproductive effort is associated with both immunity (Schwenke et al. 2016) and development time (Roff 2000). Trade-offs between effort spent on reproduction and immunity are common (Lochmiller and Deerenberg 2000, Schwenke et al. 2016). These potentially complex interactions also need to be taken into consideration.

In many field crickets, there is variation in the length of male and female hindwings (wing morph). Individuals with short hindwings cannot fly. However, young long-winged adults may fly to a new location then re-allocate effort from wing muscle to reproductive organ mass (Johnson 1969). The long-term persistence of this wing polymorphism suggests that each wing morph confers equal fitness (Roff and Fairbairn 1991, Roff 1994). Wing morph has a heritable basis (Roff and Fairbairn 2001) but is also strongly affected by developmental environment (Harrison 1980). The benefits of dispersal depend on biotic and abiotic factors that vary seasonally, and only young adult crickets can disperse by flying. Thus, development time, which determines when in the

season individuals eclose, may affect how advantageous it would be to be long-winged versus short-winged. Because little has been published on the relationship between development time and wing morph (Begin and Roff 2002), in this study, we make no *a priori* predictions about the relationship between development time and wing morphology.

Males and females may experience different selection pressures, leading to differences in life history and life history traits (Roff 1992). In insects, sex-specific differences in development time and body size are common (Teder 2013). Males and females are also predicted to differ in their investment in reproduction versus other life history traits like somatic maintenance (Glass and Stahlschmidt 2019). This can cause males and females to show differences in immunity (Zuk and McKean 1996). In crickets, there are known sex-specific differences in immunity, as well as sex-specific trade-offs between immunity and reproductive effort (Adamo et al. 2001, Gershman 2008, Kerr et al. 2010). In this study, we will investigate whether there are sex-specific differences in the relationship between parental age and offspring traits.

Although crickets are a model system for facultative diapause (Mosseau and Roff 1989, Bradford and Roff 1993), not all species of cricket diapause (Danks 1997, Masaki 1997). Non-diapausing cricket species may also respond to environmental stressors by slowing their development and growth (Lyn et al. 2012, Gutierrez et al. 2020). The consequences of variation in development time in non-diapausing crickets has received little attention. In this paper, we examine the causes and consequences of variation in development time on a non-diapausing cricket: *Gryllus vocalis*, the vocal field cricket (Weissman and Gray 2019). In the field, although adults are most commonly found in May-July, some adults can be found year-round. Under laboratory conditions of 12 light : 12 dark and constant temperature, this species does not diapause (SNG, pers. obs.), but there is substantial individual variation in the time between hatching and adulthood (2-6 months; SNG, pers. obs.). This combination of lab and field observations suggests that in the field, individual *G. vocalis* may vary in development time which can affect when they eclose into adults. Thus, variation in development time can affect the ability of individuals to survive and reproduce in the field. In this paper, we will explore the complex causative relationships between parental age, offspring development time and other offspring life history traits using path analysis. Path analysis is a statistical approach that evaluates causal models of variables and evaluates which causal pathways can explain observed patterns of variation in dependent variables (Shipley 2016). Here, we will address three related questions:

Question 1: Which path model best describes the causative relationships among parental age, development time, adult body size, immunity, fecundity, and wing morph?

Question 2: How do development time and parental age affect offspring body size, immunity, fecundity, and wing morph? Does the relationship between development

time, parental age, and other life history traits suggest trade-offs, or variation in overall offspring resources?

Question 3: Are male and female offspring life history traits affected similarly or differently by parental age and development time?

Methods

Colony maintenance

The lab colony of *G. vocalis* crickets are descendants of 50 adults collected from the University of California Riverside Botanic Gardens in May 2014, supplemented by 50 adults collected from the same location in May 2015. Experiments described in this paper were conducted in 2016. In the laboratory, crickets are maintained on a long day 15:9 light cycle at a constant 25°C. Size cohorts of crickets are maintained in 8 50-L plastic boxes. Each box contains egg carton flats, *ad libitum* ground (for nymphs) or whole (for adults) alfalfa pellets, and plastic vials capped with cotton for water. Weekly, adults are grouped into a box with cotton oviposition substrate. Oviposition cotton is collected weekly and incubated for 10 days at 25°C until hatching. Monthly, individuals are size-sorted and sometimes culled for density to ensure that each box contains a healthy density of nymphs that are of homogeneous size. Size-sorting prevents slow-developing crickets from being outcompeted for food or cannibalized by faster-developing crickets and preserves the natural variation in development time.

Experimental design

To create the parental generation, 20 males and 20 females were collected from the colony (Fig. 1). All individuals had eclosed into adults within 24 hours of collection and all adults were collected on the same day. Thus, all adults were the same number of days post-adult eclosion. The length of time that it took each parent to develop from an egg into an adult is unknown, but reflects the variation in development time within the colony. These 40 adults were housed together continuously for the duration of their participation in the experiment on a long day 15:9 light cycle at a constant 25°C with *ad libitum* food (alfalfa pellets) and water. At five days after adult eclosion, *G. vocalis* are capable of mating. Seven days after adult eclosion, a petri dish filled with moist cotton was provided as oviposition substrate. Females were given access to this oviposition substrate for exactly 24 hours, then the oviposition pad was removed and incubated. A second oviposition pad was provided 14 days after adult eclosion for 24 hours. A third oviposition pad was provided 21 days after adult eclosion for 24 hours. In the intervals between oviposition pads, no oviposition substrate was provided and females did not lay eggs. In crickets, females fertilize eggs as they are oviposited, using stored sperm. When oviposition substrate is temporarily unavailable, females continue to produce and provision unfertilized eggs, but lay few eggs (SNG, personal obs.). In summary, three batches of eggs were collected. Parents of the eggs were 7, 14 or 21 days old. Offspring shared the same pool of 40 parents. Offspring from each oviposition pad were within 24 hours of the same age. Two replicate blocks of this experiment were performed.

To measure development time in offspring, oviposition pads were incubated (constant 25°C) until hatching. Hatchlings from each oviposition pad were reared in separate 50-L boxes on a long day 15:9 light cycle at a constant 25°C with *ad libitum* ground alfalfa pellets and water. Periodically, crickets were randomly culled to ensure approximately equal density among boxes. Density and crowding has been found to affect development, adult body size, wing morph, and overall health in the crickets *Gryllus bimaculatus*, *Allonemobius socius and Gryllus integer* (Iba et al. 1995, Olvido et al. 2003, Niemelä et al. 2012). Boxes were inspected every 24 hours for eclosing adults. Each box yielded approximately 200 adult crickets. Exact sample sizes are listed in Table S1. As each cricket eclosed into an adult, it was collected and housed individually. To perform phenoloxodase (PO) immunity assays, 7 days after adult eclosion, hemolymph was extracted from a subset of crickets. To ensure that all hemolymph extraction procedures could be performed at approximately the same time of day, hemolymph was extracted from 14 or fewer crickets on any given day. So, on days when more than 14 crickets eclosed, 14 crickets were randomly chosen for hemolymph extraction. Sample sizes are included in Table S1. We also measured female fecundity on the subset of females that donated hemolymph. After hemolymph extractions on day 7, two colony adult males were introduced into each female's home container. The three individuals were housed together for 7 days (until day 14 after adult eclosion). Also on day 7, moist rolled cheesecloth for oviposition substrate was added to each female home container. This oviposition substrate was available continuously for 14 days (day 21 after adult eclosion). Every 3-4 days, the cheesecloth roll was removed and replaced with a fresh one. For each female, four cheesecloth oviposition pads were provided and collected. Methods for counting eggs are described below. All crickets were killed by freezing and their bodies were stored at -20°C until morphological measurements (pronotum width and wing length) could be collected.

Phenoloxidase assay

To determine the ability of individual offspring to coat pathogens in melanin, one component of the insect immune system, we performed an assay of phenoloxidase (PO) activity. Insect hemolymph contains a precursor of PO, the enzyme that catalyzes the rate-limiting step in the production of melanin. (Söderhäll and Cerenius 1998) In this assay, we added the hemolymph of each cricket to dopamine (L-DOPA) to determine how well each individual can activate the PO in their hemolymph and use it to convert the L-DOPA to melanin.

We extracted 3uL of hemolymph from under the pronotum of each live cricket using a 10-ul micropipetter, immediately mixed the hemolymph with 40 uL of 1x PBS in a 1.5 mL microcentrifuge tube, then froze it at -20°C for at least 2 weeks to lyse cells. We pipetted 5-uL of the hemolymph-PBS solution into each of 84 wells of a 96-well spectrophotometer plate, blocked by treatment, block, and sex. One row of 12 wells in each plate contained 5-uL of PBS alone as a control for variation in spectrophotometer runs. We added and mixed 90-ul L-DOPA to each well of the 96-well plate, then

immediately inserted the plate into the spectrophotometer. Every 10 minutes for 200 minutes, the spectrophotometer agitated the plate then took an optical density (OD) reading at a wavelength of 490. As L-DOPA in the hemolymph solutions became melanized, the hemolymph in the wells darkened. For each well of the plate, we calculated the slope of the regression line of OD readings over time. This slope represents PO activity over time. We subtracted each insect's PO slope from the average control slope for that plate. PO activity was not normally distributed. For log transformation, all values must be positive. We subtracted the minimum PO value (-0.00011) from all slopes to make them positive, then took the log of these values.

Female fecundity

To determine the fecundity (number of eggs produced) of female offspring, each female was housed with two colony males for 24 hours. Each female was then returned to her home container. The water vial in her home container was swapped for a 18 cm x 30 cm piece of cheesecloth, rolled into a cylinder which provided moisture and oviposition substrate. Every 2-3 days for 2 weeks, cheesecloth was removed and replaced with a new piece of cheesecloth. Four cheesecloths per female were collected. Used cheesecloth was incubated for 7 days and then frozen at -20C. Over the next few months, these frozen cheesecloths were unrolled over a piece of plexiglass marked with a graph-paper grid and all eggs were counted.

Morphological measurements

To determine offspring skeletal body size, we measured cricket pronotum width. Pronotum width is a better proxy for body size than weight because daily and weekly patterns of hydration and oviposition cause individual weight to have low repeatability (SNG pers obs.) After crickets were killed by freezing, crickets were thawed, pinned to a dissection board and photographed through a dissection scope. The magnification was fixed at 10x and the field of vision fixed at 23 mm. A graticule was photographed to calibrate the sizes of images in mm. We used ImageJ to measure the length of each pronotum in pixels, then converted this measurement to mm. For both egg counting and morphological measurements, all researchers were blind to information about experimental treatment. All researchers were trained until they achieved statistically significant repeatability with measurements from previously trained researchers.

315 Path analysis methods
316
317 To determine which path model best describes the causative relationships among
318 parental age, offspring development time, and offspring morphological traits (Question
319 1), we used path analysis to test three *a priori* models that differed in which life history
320 variables were directly affected by parental age (Fig. 2). We constructed separate
321 models for males and females because fecundity was only included in the female
322 models. All models include direct effects of block on all offspring traits (not shown on
323 Fig. 2). Model 1 ("Acquisition") tests the hypothesis that offspring life history traits are
324 causally independent of one another but are causally dependent on parental age. In this
325 model, parental age directly affects all offspring life history traits except the fecundity of
326 female offspring. In female models, body size directly affects fecundity (Honek 1993). If
327 there is a trade-off among life history traits, then this model should be rejected. Model
328 2 ("Trade-off") tests the hypothesis that offspring morphological traits are causally
329 dependent on development time, and that parental age indirectly affects these traits
330 through development time. If there is a trade-off between development time and other
331 traits, then we expect that faster development times of offspring result in smaller adult
332 body size and lower PO activity. If parental age mediates an acquisition effect, for
333 example, offspring of younger (or older) parents are able to both grow quickly and
334 attain large size, then this model should be rejected. Model 3 ("Acquisition and trade-
335 off") tests the hypothesis that offspring morphological traits are causally dependent on
336 development time and parental age. In this model, parental age directly affects
337 development time and the morphological traits of offspring body size or wing
338 morphology. If there is a trade-off between development time and other traits, then we
339 expect that faster development times of offspring result in smaller adult body size and
340 lower PO activity, controlling for the effects of other variables like parental age. If
341 variation in parental age mediates an acquisition effect, then we expect that this model
342 will not be rejected. The direct effects of age on life history traits should be such that
343 younger (or older) parents are able to both grow quickly and attain large size.
344
345 Our models necessarily make several assumptions. First, because parental age and block
346 precede offspring development, offspring adult body size, offspring wing morphology,
347 offspring immunity and the fecundity of female offspring, we assume that parental age
348 and block cause the offspring variables, and not the reverse. Similarly, because offspring
349 development from egg to adult precedes adulthood, we only test models in which
350 development time causes variation in adult body size, wing morph, immunity, and
351 female fecundity, and not the reverse. Finally, because adult body size and wing morph
352 do not change after adult eclosion, whereas immunity and fecundity can be influenced
353 by events after adult eclosion, we only test models in which morphological variables
354 cause variation in immunity and female fecundity, and not the reverse.
355
356 Each model was fit with the *sem* procedure in the R-package *lavaan*, using the "MLR"
357 estimator. We used the MLR test statistic to test whether the model could be rejected
358 at an alpha value of 0.05. We also assessed two measures of approximate fit for each

359 model: Bentler's comparative fit index (CFI) and root mean square error of
360 approximation (RMSEA).
361
362 To further explore the direct and indirect effects of parental age and development time
363 on offspring life history variables (Question 2), we constructed a 'full' model based on
364 the general structure of Model 3 "Acquisition and trade-off" (Fig. 3a). In this model,
365 parental age and block are causal to development time, parental age, block and
366 development time are causal to the morphological variables of body size and wing
367 morph, and all of these are causal to immunity and, in females, fecundity. This model
368 includes all possible direct causal pathways. Female and male models were fit
369 separately, as fecundity was only measured for females. Models were fit with the *sem*
370 procedure in the R-package *lavaan*, using the "MLR" estimator. Direct effects of
371 parental age on other model variables were the standardized regression coefficients of
372 age on the target variables. Indirect effects were the product of the standardized
373 regression coefficients along a pathway (e.g. for the path A→B→C, the indirect path
374 estimate would be path estimate A→B x path estimate B→C). Total effects of age or
375 development time on a target variable were the sums of all direct and indirect effects
376 (e.g. the total path estimate for paths between A and C would be path estimate
377 A→B→C + path estimate A→C). Nonparametric bootstrap confidence intervals for
378 standardized parameter estimates of direct and indirect effects were obtained using the
379 *boot.ci* procedure in the *boot* package in R. Significance of model parameters was
380 assessed by inspecting whether the 95% confidence interval for each parameter
381 overlapped 0.
382
383 To test whether the direct and indirect effects of parental age and block on other model
384 variables differed between male and female offspring (Question 3), we constructed the
385 acyclic directed graph in Fig. 3a, excluding fecundity as it was not measured in males.
386 The model was fit using data from male and female offspring, with sex as a grouping
387 variable. We initially constrained all model parameters to be equal for males and
388 females ("fully constrained model"). We then allowed one path coefficient to vary freely
389 between males and females ("free path model"). To test whether allowing paths to vary
390 between males and females improved the fit of the model, we compared the goodness
391 of fit of each free path model with that of the fully constrained model using a likelihood
392 ratio tests (LRT). We performed eleven separate LRTs, one for each regression
393 parameter in the model. We adjusted for multiple comparisons by using a Bonferroni
394 adjusted α-value of 0.00455. More detailed path analysis methods are included in
395 supplementary materials.
396
397 Results
398
399 Question 1: Which path model best describes the causative relationship between
400 parental age, development time, adult body size, immunity, fecundity, and wing morph?
401

For both female and male offspring, the best-supported model (Fig. 2, Model 3: "Acquisition and trade-off") included a direct causative effect of parental age on development time, wing morph and adult body size of offspring, the effect of development time on morphological traits, and the effect of development time and morphological traits on the other life history traits (immunity and fecundity). We failed to reject Model 3 for females and for males ($p > 0.05$, Table S2). In addition, CFI for Model 3 was greater than 0.95 and RMSEA less than 0.05 for females and for males, indicating good approximate fit between data and models. Models 1 and 2 were rejected for females and for males ($p < 0$ .05; Table S2). For Models 1 and 2, CFI was less than 0.95 and RMSEA greater than 0.05, indicating poor approximate fit between data and models. Path coefficients are reported in Table S3.

Question 2: How do development time and parental age affect offspring body size, immunity, fecundity, and wing morph?

For both daughters and sons, slower-growing offspring eclose into larger adults (Path estimate for daughters = 0.11, $p = 0.021$; Path estimate for sons = 0.12, $p = 0.002$).

Slower-growing daughters and sons had better PO-based immunity. Development time had a direct effect on PO-based immunity (Path estimate for daughters = 0.25, $p = 0.001$; Path estimate for sons = 0.27, $p < 0.001$). Indirect paths from development time to PO-based immunity (development time $\rightarrow$ body size $\rightarrow$ PO; development time $\rightarrow$ wing morph $\rightarrow$ PO) were not statistically significant (Table S5). However, the total path coefficients indicate that overall, slower-growing offspring had higher PO-based immunity (Total path estimate for daughters = 0.23, $p = 0.001$; Total path estimate for sons = 0.28, $p < 0.001$).

Slower-developing daughters and sons were more likely to be short-winged (Path estimate for daughters = -0.347, $p<0.001$; Path estimate for sons = -0.221, $p<0.001$). Development time did not have an effect on the fecundity of daughters, but larger daughters laid more eggs (Path estimate for daughters = 0.133, $p=0.037$; Table S5).

For both female and male offspring, parental age has opposing direct and indirect effects on adult body size. Following the direct path between parental age and offspring body size, younger parents have smaller offspring (Path estimate for daughters = 0.150, $p = 0.0011$; Path estimate for sons = 0.165, $p < 0.001$). Following the indirect path from parental age via offspring development time to offspring body size, (parental age $\rightarrow$ development time $\rightarrow$ offspring body size) younger parents had slower-developing offspring and slower-developing offspring eclosed into larger adults; but this indirect path was not statistically significant for either daughters or sons (Table S4). The total path coefficient from parental age to offspring body size is positive, indicating that if both direct and indirect effects are taken into account, younger parents have smaller offspring (Total path estimate for daughters = 0.134, $p = 0.003$; Total path estimate for sons = 0.159, $p < 0.001$).

For female offspring, there were indirect effects of parental age via offspring development time on offspring PO-based immunity (parental age $\rightarrow$ development time $\rightarrow$ PO): younger parents had offspring with superior PO-based immunity (Indirect path estimate for daughters = -0.035, p= 0.020), however, the total path effects were not statistically significant for either daughters or sons (Table S5).

There were also indirect effects of parental age on the wing morph of daughters (parental age $\rightarrow$ development time $\rightarrow$ wing morph), with younger parents having more short-winged daughters (Indirect path estimate for daughters = 0.047, p = 0.003); the total path coefficient was also positive (Total path estimate for daughters = 0.105, p = 0.016). Parental age did have a statistically significant effect on the wing morph of sons (Table S5).

Question 3: Are male and female offspring life history traits affected similarly or differently by parental age and development time?

We reject the null hypothesis of no difference between male and female models (Table S5). However, allowing path coefficients to vary freely between males and females significantly improved model fit for only one path: from experimental block to offspring development time (Table S5). When we allowed free variation in those paths that linked pairs of life history traits, model fit was not significantly improved (Table S5).

There was no difference in development time between male and female offspring (ANOVA $F_{1,1268} = 3.82$, $P = 0.051$). Male offspring were larger than females (ANOVA $F_{1,1246} = 144.5$, $P < 0.0001$). Female offspring had better PO-based immunity than males (ANOVA $F_{1,364} = 8.86$, $P = 0.0031$), and females had longer wings than males (ANOVA $F_{1,1244} = 22.6$, $P < 0.0001$). As previously indicated, the effect of development time on body size, immunity and wing morph was similar in male and female offspring. Parental age had similar direct effects on female and male offspring body size. However, parental age only affected the development time of daughters, not sons (Table S4).

Discussion

Question 1: Which path model best describes the causative relationship between parental age, development time, adult body size, immunity, fecundity, and wing morph?

The best-supported model (Model 3: Acquisition and trade-off) included a direct causative effect of parental age on both development time and offspring morphological traits. The model (Model 1: Acquisition) that described parental age as directly affecting all offspring life history traits (except the fecundity of female offspring) did a poor job of characterizing the causative relationship between variables. The model (Model 2: Trade-off) that included a direct effect of parental age on development time but not on offspring morphological traits also was rejected, although fit indices were better than for Model 1. However, including the effect of both acquisition (parental effects) and trade-offs between offspring traits best characterized the relationships between variables. Although many studies examine parental effects, and many studies examine interactions between development time and morphological traits, few studies of life history evolution include both parental effects and the effects of variance in development time on other life history traits. These results highlight the need for more studies that include both variables to understand the relative impact of each on downstream life history traits that affect fitness. For example, in studies on flies, rice weevils, and gypsy moths that found an effect of parental age on offspring body size (Opit and Throne 2007, Qazi et al 2017), fecundity (Opit and Throne 2007, Hercus and Hoffmann 2000, Nystrad and Dowling 2014) and immunity (Rossiter et al. 1990), effects of parental age may be mediated by offspring development time. Further, parental age alone may explain only a small portion of the variance in offspring traits. Conversely, most studies that examine the effects of development time on offspring traits (Roff 1992, Roff 2000, van der Most et al 2011) either limit or ignore variation in parental age. Ignoring the effect of adult age (e.g. blocking for adult age among treatments) may bias data. Limiting adult age may reduce variation in offspring development time and downstream offspring traits and represent only a subset of possible offspring phenotypes.

Question 2: How do development time and parental age affect offspring body size, immunity, fecundity, and wing morph?

In this study, we found that crickets with longer development times became larger adults. Many previous studies have found a similar relationship between development time and adult body size (Roff 1992, Roff 2000). Our result is consistent with the hypothesis that crickets must make a trade-off between developing quickly to avoid predation before reproduction and attaining a larger adult body size to gain advantages in male-male competition, female choice, male choice, and female fecundity. We found that both male and female crickets that develop more slowly become larger adults. This result is striking because males and females gain fundamentally different advantages from large adult body size: the advantages of large body size for males are primarily due

to inter- and intra-specific sexual selection, while the advantages of large female body size are primarily due to natural selection for increased fecundity. It seems likely that covariances between male and female trait values may constrain the ability of either sex to evolve independently. However, in this instance, rather than creating antagonistic coevolution between males and females, these covariances creates synergistic positive effects on the fitness of each sex.

Although we found a positive relationship between the development time and body size of female offspring, and a positive relationship between female body size and fecundity, we did not find a statistically significant relationship between development time and fecundity. In a meta-analysis of quantitative genetic studies, Roff (2010) found that the relationship between development time and female fecundity is not predictable: although development time frequently trades off with adult body size, and body size is often positively correlated with fecundity, the correlation between development time and fecundity could be either positive or negative (Roff 2000). Roff also found that in many studies (similar to our study), the lack of statistically significant correlation between development time and fecundity could be attributed to the high variances associated with these two variables (Roff 2000).

For both male and female offspring, crickets that took longer to develop had superior PO-based immunity than crickets that developed more quickly. This result confirms what has been found in some previous results (Rantala and Roff 2005, van der Most et al. 2011), although not others (Rantala and Roff 2005). Our result suggests that crickets may be making a trade-off between developing rapidly and investing effort in PO-based immunity. On a proximate level, crickets that develop slowly have more opportunities for exposure to pathogens than crickets that develop rapidly. Thus, slower-growing crickets may also benefit more from investment in immunity than faster-growing crickets. As components of immunity can interact differently with other morphological and life history traits (Adamo 2004, Rantala and Roff 2005, Gershman et al. 2010), it would be valuable to explore the relationship between development time and other facets of immunocompetence.

We found that younger parents had smaller sons and daughters. The effect of parental age on sons was relatively straightforward: parental age had a direct effect on the body size of sons, but not an indirect effect on body size via development time. In contrast, parental age had opposing direct (positive) and indirect (negative) effects on the body size of daughters. However, path analysis indicated that the total effect of parental age on the body size of daughters was positive: younger parents have smaller daughters. Proximately, trade-offs and differences in acquisition can explain why younger parents have smaller offspring. In field crickets, female daily fecundity peaks within the first few weeks of sexual maturity, and then gradually declines (Lorenz 2007). It is possible that younger parents have smaller offspring due to a trade-off that parents are making between the size and number of eggs that they produce (Fox and Czesak 2000); if younger parents lay more eggs and allocate fewer resources per egg than older parents,

this could potentially affect the size that adult offspring are able to achieve. In *Gryllus pennsylvanicus* field crickets, previous studies have documented maternal effects on cricket egg size and nymphal development: larger eggs develop more quickly into heavier nymphs (Roff 1992, Roff and Sokolovska 2004). Alternatively, Stachschmidt et al. (2013) found that adult *Gryllus texensis* crickets increased in body condition as they aged, which suggests that older parents may have the nutritional capacity to lay better provisioned eggs than younger parents. These better-provisioned eggs may have the capacity to develop into larger adults. Ultimately, it may be beneficial for adults to produce offspring that eclose into adults within the short window of time at the beginning of the reproductive season: in the field, offspring that eclose in May will have ample access to resources and mates, and a relatively long period of time in which to lay their own eggs. Our finding that younger parents produce slower-developing daughters and older parents produce faster-developing daughters reduces variation in when daughters are likely to eclose into adults, concentrating adult eclosion times within a relatively short window of time. It is surprising that parental age does not have a similar effect on the development time of sons. However, previous studies in other taxa have also found sex-specific parental effects (Lind et al. 2015).

Although there was not a direct effect of parental age on the wing morph of daughters, parental age via development time affected wing morph, with younger parents having more short-winged daughters. This indirect effect was substantial enough that overall (total path) parental age had a positive effect on the wing morph of daughters. Parental age did not have an effect on the wing morph of sons, either directly or indirectly. Short-winged females have a higher lifetime fecundity than long-winged females, primarily because short-winged females are able to start reproducing immediately, rather than waiting to disperse by flight and then reallocate their reserves from flight to reproduction (Roff and Fairbairn 1991). However, long-winged females have the ability to disperse to new locations if resources are depleted due to overcrowding. It may be beneficial for young parents to produce short-winged daughters who can immediately take advantage of the available food and oviposition substrate to maximize egg production. As older parents produce offspring later in the season, it may be beneficial to produce daughters who can disperse to areas with more available resources. It is surprising that parental age does not similarly affect the wing morphs of sons, as sons make the same trade-offs between devoting their reserves to reproduction versus flight (Crnokrak and Roff 1995).

We found block effects on offspring development time, body size, wing morph and immunity. Although we cannot definitively know why block had an effect, the most likely biological cause is time of year. The crickets in this study had been reared for 4-8 generations in a temperature- and light- controlled windowless room. However, it is possible that either parents or offspring had not fully lost their sensitivity to time of year. As experimental blocks were performed sequentially rather than simultaneously, seasonality may have influenced our results.

Question 3: Are male and female offspring life history traits affected similarly or differently by parental age and development time?

We found that female offspring had better PO-based immunity than male offspring. This result is consistent with previous studies on crickets (Gershman 2008, Gershman et al. 2010) and also most other taxa (Zuk and McKean 1996). Female invertebrates are posited to have superior immunity to males because females gain more benefit more from investing in immunity than males: males only have to live long enough to mate, whiles females need to live long enough to mate, locate appropriate oviposition substrate, and oviposit. We found that female offspring were more likely to be long-winged than male offspring. This result has been found in previous studies (Roff 1990). We also found that although male offspring were larger than female offspring, male and female offspring did not differ in development time. This result is also logical, as a mismatch between male and female development times would result in lower reproductive success for all individuals. There were not differences in the relationships among biological variables between the male and female path models. This suggests that life history traits of male and female offspring are similarly affected by variation in parental age and development time.

Overall, this path analysis approach has allowed us to identify a sequence of linked biological causes that contribute to variation in life history traits. Variation in development time can influence a suite of life history trait values that may be more beneficial when they occur together. We found that slower-growing offspring are larger, with better immunity and shorter wings. This combination of traits could be advantageous to non-dispersing offspring that remain in densely-populated areas where large body size confers higher sexually-selected fitness and disease transmission is more likely. Conversely, we found that faster-growing offspring are more likely to have long wings, with smaller body size and poorer immunity. These dispersing individuals would likely experience lower population density, thus body size and immunity may be less important to their fitness. Future field-based studies could provide valuable information about the fitness consequences of these suites of traits.

In this study, we measured the effect of parental age and offspring development time on a limited suite of offspring physical traits. In future studies, it would be valuable to measure how parental age and development time affect behavioral traits important to sexual and natural selection, as well as other unmeasured physical traits important to fitness. A path analysis approach is instrumental in allowing researchers to understand the causative effects of morphological and behavioral traits over the lifetime of an individual.

649 Literature cited
650
651 Adamo SA 2004. How should behavioural ecologists interpret measurements of
652 immunity? *Anim. Behav*. 68, 1443–1449. doi:10.1016/j.anbehav.2004.05.005
653
654 Adamo SA, Jensen M, Younger M. 2001. Changes in lifetime immunocompetence in
655 male and female *Gryllus texensis* (formerly *G. integer*): trade-offs between
656 immunity and reproduction. *Anim Behav*. 62, 417–425. doi 10.1006/anbe.2001.1786
657
658 Andersson M. 1994. *Sexual Selection*, Princeton University Press.
659
660 Begin M, Roff DA, Debat V. 2004. The effect of temperature and wing morphology on
661 quantitative genetic variation in the cricket *Gryllus firmus*, with an appendix examining
662 the statistical properties of the Jackknife-manova method of matrix comparison. *J. Evol.*
663 *Biol.* 17, 1255–1267. doi 10.1111/j.1420-9101.2004.00772.x
664
665 Bégin M, Roff DA. 2002 The common quantitative genetic basis of wing
666 morphology and diapause occurrence in the cricket *Gryllus veletis*. *Heredity* 89, 473–
667 479.
668
669 Benton TG, St Clair JJH, Plaistow SJ. 2008. Maternal effects mediated by maternal age:
670 from life histories to population dynamics. *J. Anim. Ecol.* 77, 1038–1046. doi
671 10.1111/j.1365-2656.2008.01434.x
672
673 Bertram SM, Rook V. 2011. Jamaican field cricket mate attraction signals provide age
674 cues. *Ethology* 117, 1050–1055. doi 10.1111/j.1439-0310.2011.01958.x
675
676 Bonduriansky R, Head M. 2007. Maternal and paternal condition effects on offspring
677 phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). *J. Evol. Biol*. 20, 2379–2388.
678 doi 10.1111/j.1420-9101.2007.01419.x
679
680 Bradford MJ and Roff DA. 1993. Bet hedging and the diapause strategies of the cricket
681 *Allonemobius fasciatus*. *Ecology* 74, 1129–1135.
682
683 Brown WD, Smith AT, Moskalik B., Gabriel J. 2006.Aggressive contests in house crickets:
684 size, motivation and theinformation content of aggressive songs. *Anim. Behav*. 72, 225–
685 233.
686
687 Brown WD. 2008. Size-biased mating in both sexes of the black-horned tree cricket,
688 *Oecanthus nigricornis* Walker (Orthoptera: Gryllidae: Oecanthinae). *J. Insect Behav*. 21,
689 130–142.
690

691 Briffa M. 2008. Decisions during fights in the house cricket, *Acheta domesticus*: mutual
692 or self assessment of energy, weapons and size? *Anim. Behav.* 75, 1053–1062. doi
693 10.1016/j.anbehav.2007.08.016
694
695 Clancey E, Byers JA. 2014. The definition and measurement of individual condition in
696 evolutionary studies. *Ethology* 120, 845–854. doi 10.1111/eth.12272
697
698 Crnokrak P, Roff DA. 1995. Fitness differences associated with calling behaviour in the
699 two wing morphs of male sand crickets, *Gryllus firmus*. *Anim. Behav*. 50, 1475–1481.
700
701 Danks HV 1997. *Insect Dormancy: An Ecological Perspective*. Biological Survey of Canada
702 (Terrestrial Arthropods), Ottawa. ISBN: 0969272707.
703
704 Danks HV 2007. The elements of seasonal adaptations in insects. *Canad. Entomol*. 139,
705 1–44.
706
707 Deb R, Bhattacharya M, Balakrishnan R. 2012. Females of a tree cricket prefer larger
708 males but not the lower frequency male calls that indicate large body size. *Anim. Behav*.
709 84, 137–149. doi.org/10.1016/j.anbehav.2012.04.020
710
711 del Castillo RC. 2015. Body size, fecundity, and sexual size dimorphism in the neotropical
712 cricket *Macroanaxipha macilenta* (Saussure) (Orthoptera: Gryllidae). *Neotrop. Entomol.*
713 44, 116–22. doi 10.1007/s13744-014-0266-1
714
715 Fox CW, Dingle H. 1994. Dietary mediation of maternal age effects on offspring
716 performance in a seed beetle (Coleoptera: Bruchidae). *Funct. Ecol.* 8, 600–606.
717
718 Fox CW, Czesak ME. 2000. Evolutionary ecology of progeny size in arthropods. *Annu.*
719 *Rev. Entomol.* 45, 341–369.
720
721 Gershman SN. 2008. Sex-specific differences in immunological costs
722 of multiple mating in *Gryllus vocalis* field crickets. *Behav. Ecol*. 19, 810–815. doi
723 10.1093/beheco/arn040.
724
725 Gershman SN, Barnett CA, Pettinger AM, Weddle CB, Hunt J, Sakaluk SK. 2010. Give 'til it
726 hurts: trade-offs between immunity and male reproductive effort in the decorated
727 cricket, *Gryllodes sigillatus*. *J. Evol. Biol*. 23, 829–839. https://doi.org/10.1111/j.1420-
728 9101.2010.01951.x
729
730 Glass JR, Stahlschmidt ZR. 2019. Should I stay or should I go? Complex environments
731 influence the developmental plasticity of flight capacity and flight-related trade-offs.
732 *Biol. J. Linnean Soc*. 128, 59–69. https://doi.org/10.1093/biolinnean/blz073.
733

734 Gray DA. 1997. Female house crickets, *Acheta domesticus*, prefer the chirps of large
735 males. *Anim. Behav*., 54, 1553–1562.
736
737 Gutierrez Y, Fresch M, Ott D, Brockmeyer J, Scherber C. 2020. Diet composition and
738 social environment determine food consumption, phenotype and fecundity in an
739 omnivorous insect. *R. Soc. Open Sci*. 7, 200100. http://dx.doi.org/10.1098/rsos.200100
740
741 Harrison RG. 1980. Dispersal polymorphisms in insects. *Ann. Rev. Syst.* 11, 95–118.
742
743 Hercus MJ, Hoffmann AA. 2000. Maternal and grandmaternal age influence offspring
744 fitness in *Drosophila*. *Proc. R. Soc. Lond.* B 267, 2105–2110. doi 10.1098/rspb.2000.1256
745
746 Honek A. 1993. Intraspecific variation in body size and fecundity in insects: a general
747 relationship. Oikos 66, 483–492.
748
749 Iba M, Nagao T, Urano A. 1995. Effects of population density on growth, behavior and
750 levels of biogenic amines in the cricket, Gryllus bimaculatus. Zool. Sci. 12, 695–702. doi
751 10.2108/zsj.12.695
752
753 Johnson CG. 1969. *Migration and Dispersal of Insects by Flight*. London: Methuen.
754
755 Kerr AM, Gershman SN, Sakaluk SK. 2010. Experimentally induced spermatophore
756 production and immune responses reveal a trade-off in crickets. *Behav. Ecol.* 21, 647–
757 654. doi 10.1093/beheco/arq035
758
759 Lee KP, Simpson SJ, Wilson K. 2008. Dietary protein-quality influences melanization and
760 immune function in an insect. *Funct. Ecol.* 22, 1052–1061.
761
762 Lind MI, Berg EC, Alavioon G, Maklakov AA. 2015. Evolution of differential maternal age
763 effects on male and female offspring development and longevity. *Funct. Ecol.* 29, 104–
764 111. doi 10.1111/1365-2435.12308
765
766 Lochmiller RL and Deerenberg C. 2000 Trade-offs in evolutionary immunology: just what
767 is the cost of immunity? *Oikos* 88, 87–98.
768
769 Lorenz MW. 2007. Oogenesis-flight syndrome in crickets: Age-dependent egg
770 production, flight performance, and biochemical composition of the flight muscles in
771 adult female *Gryllus bimaculatus*. *J. Insect Physiol.* 53: 819-832.
772
773 Lyn J, Aksenov V, LeBlanc Z, Rollo CD. 2012. Life History Features and Aging Rates:
774 Insights from intra-specific patterns in the cricket *Acheta domesticus*. *Evol. Biol*. 39, 371–
775 387. doi 10.1007/s11692-012-9160-0
776

777 Masaki, S. 1997. Geographical variation of life cycles in crickets (Ensifera: Grylloidea).
778 *Eur. J. Entomol.* 93, 281–302.
779
780 Mousseau TA, Fox CW. 1998. The adaptive significance of maternal effects. *TREE* 13,
781 403–407.
782
783 Mosseau TA, Roff DA. 1989. Adaptation to seasonality in a cricket: patterns of
784 phenotypic and genotypic variation in body size and diapause expression along a cline in
785 season length. *Evolution* 43, 1483–1496. doi 10.1111/j.1558-5646.1989.tb02598.x
786
787 Niemelä PT, Vainikka A, Lahdenperä S, Kortet R. 2012. Nymphal density, behavioral
788 development, and life history in a field cricket. *Behav. Ecol. Sociobiol*. 66, 645–652. doi
789 10.1007/s00265-011-1312-1
790
791 Nystrad M, Dowling DK. 2014. Transgenerational interactions involving parental age and
792 immune status affect female reproductive success in *Drosophila melanogaster*. *Proc. R.*
793 *Soc*. B 281: 20141242.
794
795 Olvido AE, Elvington ES, Mousseau TA. 2003. Relative effects of climate and crowding on
796 wing polymorphism in the southern ground cricket, *Allonemobius socius* (Orthoptera:
797 Gryllidae). *Florida Entomol*. 86, 158–164.
798
799 Opit GP, Throne JE. 2007. Influence of maternal age on the fitness of progeny in the Rice
800 Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). *Environ. Entomol*. 36, 83–89.
801
802 Phelan JP, Frumhoff PC. 1991. Differences in the effects of parental age on offspring
803 life history between tropical and temperate populations of milkweed bugs (*Oncopeltus*
804 spp.). *Evol. Ecol*. 5, 160–172.
805
806 Qazi MCB, Miller PB, Poeschel PM, Phan MH, Thayer JL, Medrano, CL. 2017.
807 Transgenerational effects of maternal and grandmaternal age on offspring viability and
808 performance in *Drosophila melanogaster*. *J. Insect. Physiol*. 100, 43–52.
809
810 Rantala MJ, Roff DA. 2005. An analysis of trade-offs in immune function, body size and
811 development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. *Funct. Ecol.*
812 19, 323–330.
813
814 Reznick D, Nunney L, Tessier A. 2000. Big houses, big cars, superfleas and the
815 costs of reproduction. *TREE* 15, 421-425.
816
817 Rivero A, Giron D, Casas J. 2001. Lifetime allocation of juvenile and adult nutritional
818 resources to egg production in a holometabolous insect. *Proc. R. Soc. Lond* B 268,1231-
819 1237. doi 10.1098/rspb.2001.1645.
820

Roff DA. 1990. Selection for changes in the incidence of wing dimorphism in *Gryllus firmus*. *Heredity* 65, 163–168.

Roff DA. 1992. *The evolution of life histories: theory and analysis*. Routledge, Chapman and Hall, New York.

Roff DA. 1994. Habitat persistence and the evolution of wing dimorphism in insects. *Am. Nat.* 144, 772–798.

Roff DA. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *J. Evol. Biol*. 13, 434–445.

Roff DA, Fairbairn DJ. 1991. Wing dimorphisms and the evolution of migratory polymorphisms among the Insecta. *Amer. Zool.* 31, 243–251.

Roff DA, Fairbairn DJ. 2001. The genetic basis of dispersal and migration, and its consequences for the evolution of correlated traits. In: Clobert J, Danchin E, Dhondt AA, Nichols JD (eds). *Dispersal*, Oxford University Press: Oxford, 191–202.

Roff DA, Sokolovska N. 2004. Extra-nuclear effects on growth and development in the sand cricket *Gryllus firmus*. *J. Evol. Biol.* 17, 663–671. doi:10.1046/j.1420-9101.2003.00673.x

Rossiter MC, Yendol WG, Dubois, NR. 1990.Resistance to *Bacillus thuringiensis* in gypsy moth (Lepidoptera: Lymantriidae): genetic and environmental causes. *J Econ. Entomol.* 83, 2211–2218. doi-org.proxy.lib.ohio-state.edu/10.1093/jee/83.6.2211

Schwenke RA, Lazzaro BP, Wolfner MF. 2016. Reproduction-immunity trade-offs in insects. *Annu. Rev. Entomol.* 61, 239–256. doi 10.1146/annurev-ento-010715-023924

Shipley, B. 2016. *Causes and Correlation in Biology: A Users Guide to Path Analysis, Structural Equations and Causal Inference in R.*, 2nd edn. Cambridge: Cambridge University Press.

Simmons LW, García-González F. 2007. Female crickets trade offspring viability for fecundity. *J. Evol. Biol.* 20, 1617–1623. doi 10.1111/j.1420-9101.2007.01320.x

Söderhäll K, Cerenius L. 1998. Role of the prophenoloxidase activating system in invertebrate immunity. *Curr. Opin. Immunol*. 10, 23–28. doi: 10.1016/s0952-7915(98)80026-5

Stahlschmidt ZR, Rollinson N, Acker M, Shelley A. 2013. Are all eggs created equal? Food availability and the fitness trade-off between reproduction and immunity. *Funct. Ecol*. 27, 800–806. doi: 10.1111/1365-2435.12071

Teder T. 2013. Sexual size dimorphism requires a corresponding sex difference in development time: a meta-analysis in insects. *Funct. Ecol*. 28, 479–486. doi: 10.1111/1365-2435.12172

van Noordwijk AJ, de Jong G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* 128, 137–142.

van der Most PJ, de Jong B, Henk K. Parmentier HK, Verhulst S. 2011. Trade-off between growth and immune function: a meta-analysis of selection experiments. *Funct. Ecol.* 25, 74–80. doi 10.1111/j.l365-2435.2010.01800.x

Weissman DB, Gray DA. 2019. Crickets of the genus *Gryllus* in the United States (Orthoptera: Gryllidae: Gryllinae). *Zootaxa* 4705, 1–277.

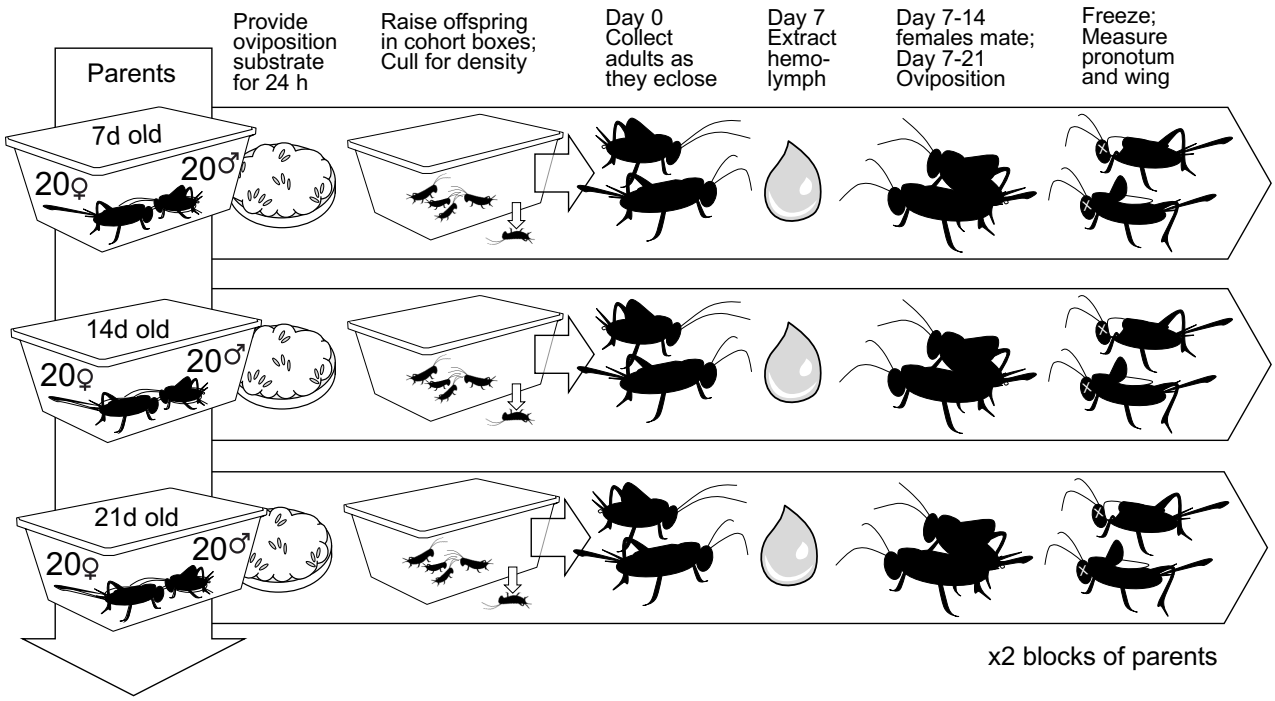
Zehnder CB, Parris, MA, Hunter MD. 2007. Effects of maternal age and environment on offspring vital rates in the Oleander aphid (Hemiptera: Aphididae). *Environ. Entomol.* 36, 910–917.

Zeng Y, Zhou F-H, Zhu D-H. 2018. Fight outcome briefly affects the reproductive fitness of male crickets. *Sci. Reports* 8, 9695. doi 10.1038/s41598-018-27866-4

Zera AJ, Denno RF. 1997. Physiology and ecology of dispersal polymorphism in insects. *Ann. Rev. Entomol.* 42, 207–230.

Zuk M, McKean KA. 1996. Sex differences in parasite infections: Patterns and processes. *Internat. J. Parasit.* 26, 1009-1023. doi 10.1016/S0020-7519(96)80001-4

892



893

894 Figure 1. Summary of experimental design.

895

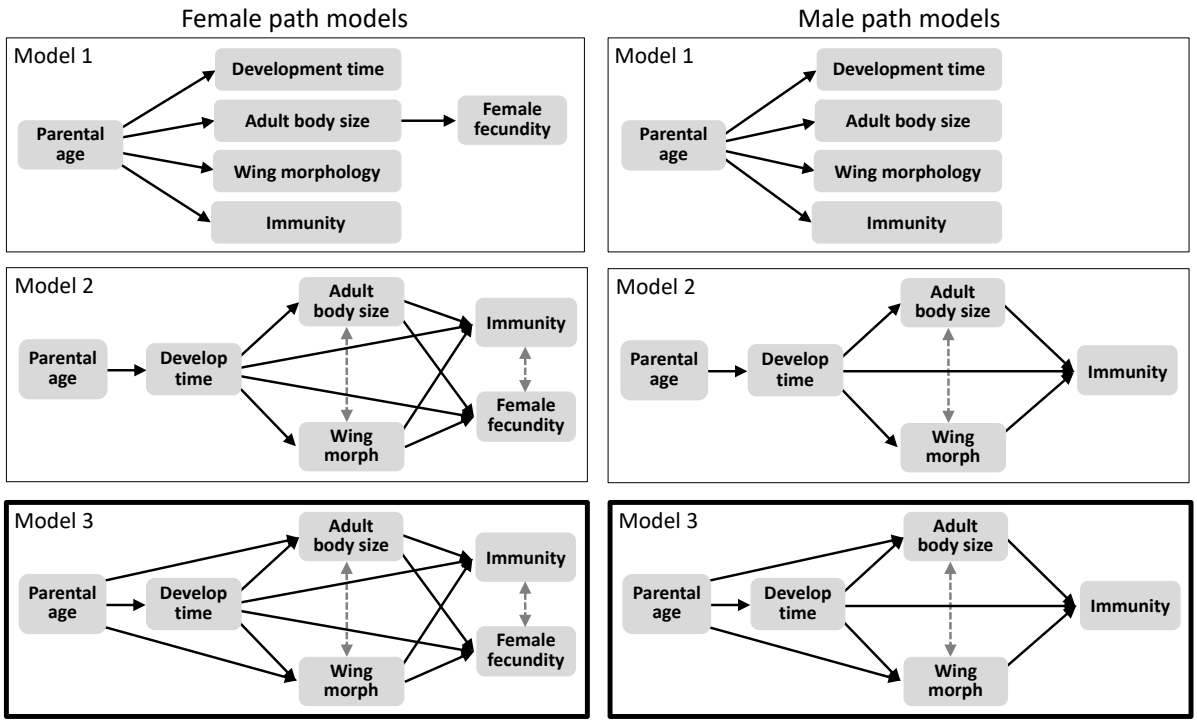
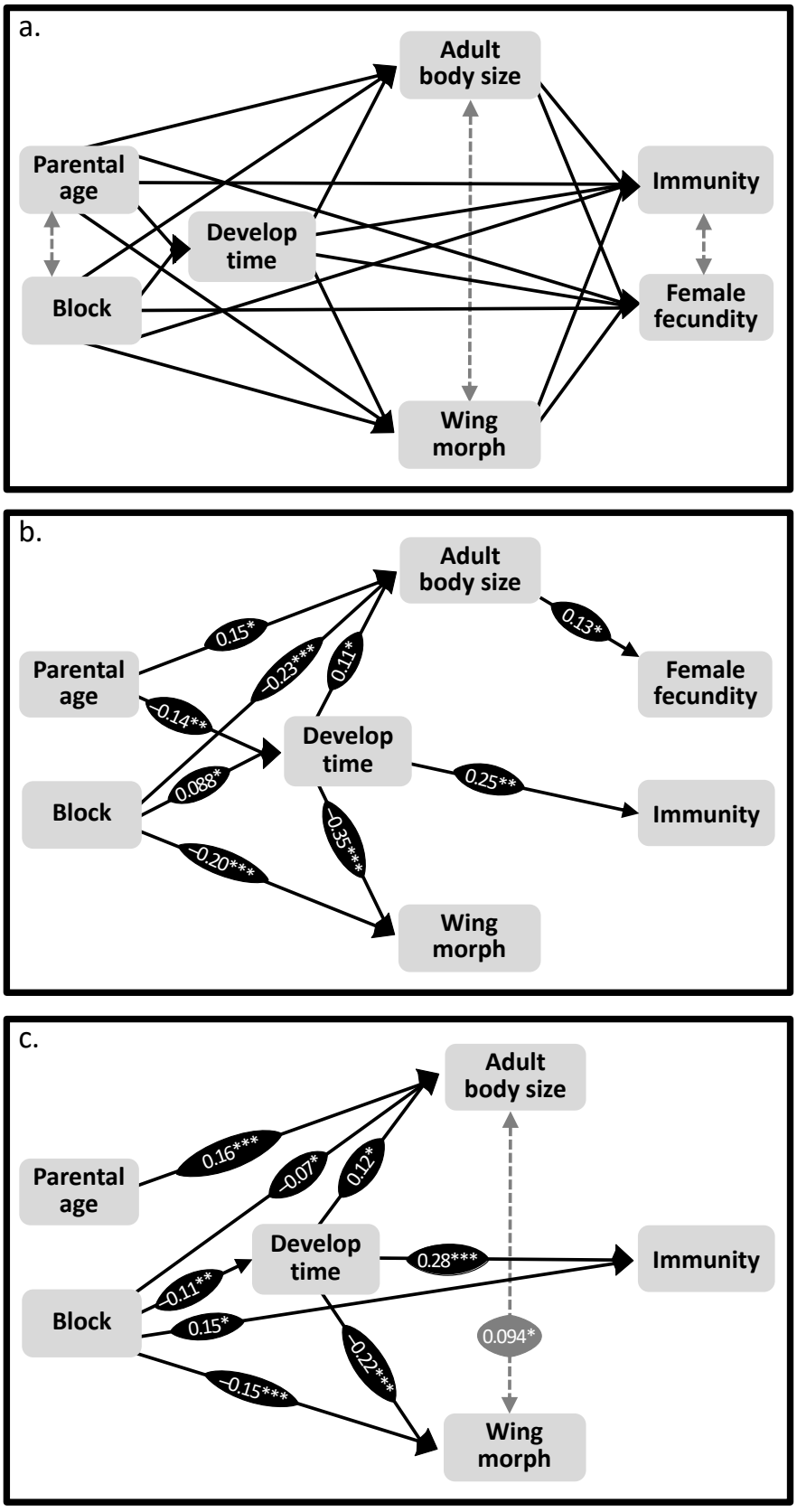


Figure 2. Path models for female and male offspring. Arrows indicate causal relationships between variables (cause –> effect). For both males and females, Model 3: ("Acquisition and trade-offs"), in bold outline, is the best-supported model.


a.
Adult body size
Parental age
Immunity
Develop time
Block
Female fecundity
Wing morph
b.
Adult body size
0.15*
0.13*
Parental age
−0.23***
0.11*
Female fecundity
−0.14**
Develop time
0.088*
0.25**
Block
Immunity
−0.35***
−0.20***
Wing morph
c.
Adult body size
0.16***
Parental age
−0.07*
0.12*
Develop time
0.28***
Immunity
−0.11**
Block
0.15*
0.094*
−0.22***
−0.15***
Wing morph

902
903

Figure 3. Analysis of full path model. (a) Path diagram for the full path model. Regressions are indicated by solid lines, covariances by dotted lines. The model for male offspring does not include female fecundity. Path analysis results for (b) female offspring and (c) male offspring. Arrows shown represent causal links (solid lines) and covariances (dotted lines) with a $p < 0.05$. All path coefficients are listed on Table S4 in the supplementary material. Values represent standardized coefficients. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$.

Author Contribution

SNG and OGM designed the study with feedback from IMH. OGM carried out the experiments. SNG, with the assistance of many undergraduate students, measured the morphological traits and assayed immunity. SNG and IMH planned the statistical approach. IMH researched and performed all of the path analyses. SNG wrote the manuscript.

Acknowledgements

Thanks to the OSU students who assisted in the laboratory work for this project: Mitchell Cheung, Ellen Dunkle, Heather Fair, Alejandro Figueroa-Ripoll, Lauren Filippidis, Kelsi Parson, Abigail Ratcliff. This work was funded by a Theodore J. Cohn Research Fund grant from the Orthopterists' Society, an OSU Marion Research Development Grant, and a Regional Campus Faculty Research/Creative Activity grant from The OSU College of Arts & Sciences.