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Phenotypes optimized for early-life reproduction exhibit faster somatic deterioration with age, revealing a latent cost of high condition

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Abstract

High condition enables individuals to express a phenotype with greater reproductive potential. However, life-history theory predicts that reproduction will trade off with somatic maintenance and viability, and several studies have reported faster age-related decline in performance in high-condition individuals, suggesting that high condition in early life is associated with accelerated somatic deterioration. This trade-off may be especially pronounced in males, which often express condition-dependent secondary sexual traits that can impose viability costs during development and through damage-inflicting adult sexual behaviours. To test this prediction, we reared larvae of the neriid fly *Telostylinus angusticollis* on diets of varying nutrient content and quantified somatic deterioration in solitary males, males housed in all-male or mixed-sex groups and immobilized males subjected to mechanical stress. We found that males reared on a nutrient-rich larval diet (high-condition males) suffered a higher rate of somatic deterioration with age, particularly when housed in groups. Perhaps as a result of accelerated somatic deterioration, high-condition males did not outlive low-condition males. In addition, high-condition males housed in all-male groups experienced a greater reduction in escape response with age than males housed in mixed-sex groups, suggesting that male–male combat promotes somatic deterioration. However, even when immobilized, high-condition males were still found to be more susceptible to somatic damage than low-condition males. Our findings suggest that a high-condition male phenotype is more prone to somatic damage, both as a result of associated behaviours such as combat, and because of the inherent fragility of the high-condition body.

Introduction

Resource availability during development is thought to be a key determinant of lifetime fitness, often establishing adult phenotypes and reproductive strategies. For some organisms, the availability of resources in development determines the fixed adult morphology. Holometabolous insects fall into this category: they must acquire all the nutrients used for somatic growth and structural development in the larval stage, such that access to plentiful nutritional resources in development (hereafter, 'high condition') tends to result in adult phenotypes with greater reproductive potential. Indeed, high-condition males typically express larger secondary sexual traits (Emlen, 1994, 1997; Griffith *et al.*, 1999; Cotton *et al.*, 2004; Bonduriansky & Rowe, 2005; Bonduriansky, 2007), and high-condition females typically exhibit greater fecundity (Blanckenhorn, 2000). Individuals in low condition tend to adopt strategies that maximize their fitness given their limited access to resources (Gross, 1996; Roff, 1996; West-Eberhard, 2003), although which traits will suffer is likely to depend largely on which nutrients are scarce (Boggs, 2009). Little is known, however, about the effects of early-life condition on ageing, and it is possible that

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variation in condition may account for some of the unexplained variation in ageing rates observed within populations (Nussey *et al.*, 2008).

The term 'condition' is conventionally defined as the quantity of metabolic resources available to an organism for investment in all fitness-enhancing functions (Andersson, 1982; Nur & Hasson, 1984; Rowe & Houle, 1996). Although metabolic resource availability, and an individual's ability to convert resources into fitness, may change over an individual's lifetime, here we use the term 'condition' to refer to the phenotypic state of the newly emerged adult, which, in holometabolous insects, is determined to a large extent by the nutrient content of the larval diet. Contrasting predictions can be made about the relation between early-life condition and the rate of ageing (Bonduriansky & Brassil, 2005; Bonduriansky *et al.*, 2008; Adler and Bonduriansky 2014). Because high condition has been shown to confer enhanced performance in many contexts (Hill, 2011), individuals that start life in high condition may be expected to maintain their soma better with advancing age than individuals that start out in low condition. This could be the case if the additional resources available to high-condition individuals are allocated to both reproduction and somatic repair (van Noordwijk & de Jong, 1986; Reznick *et al.*, 2000), and has been demonstrated in some species in which individuals that invest more in sexually selected traits also live longer (Jennions *et al.*, 2001; Houslay *et al.*, 2015). However, this may not always be possible. Life-history theory suggests that the optimization of reproductive traits in high-condition individuals may come at the cost of reduced ability to maintain the soma with advancing age (Hughes & Reynolds, 2005; Ljubuncic & Reznick, 2008). This is because reproduction-related traits and activities are thought to trade off with somatic maintenance and survival (Williams, 1966; Stearns, 1989). These trade-offs can take the form of *metabolic opportunity costs*, which occur when resources used for one function are unavailable for alternate functions, or can result from *contrasting selection*, which occurs when a trait favoured in one context entails costs in another (Agrawal *et al.*, 2010; Houslay & Bussiere, 2012). These two types of trade-offs can occur separately or at the same time, as discussed below.

Even if high-condition individuals invest more in somatic maintenance, the greater reproductive effort associated with high condition could impose a higher rate of damage (an example of a trade-off resulting from contrasting selection), and it is likely that some of this damage cannot be repaired. For example, insects are unable to repair or replace their adult exoskeletons (Downer & Laufer, 1983; Bonduriansky *et al.*, 2008), and most mammals cannot repair or replace their adult teeth (Ungar, 2010). As a result, strategies that allow high-condition individuals to convert resources into reproductive success, such as male–male combat and intersexual interactions, may be associated with a higher susceptibility to somatic damage, resulting in faster somatic deterioration with age ('somatic ageing'). Additionally, high-condition individuals could experience metabolic opportunity costs, if they preferentially invest resources into reproduction-related traits and those resources are then unavailable for investment in the soma. This could also lead to faster somatic ageing, either because somatic structures are initially built less sturdily, or because resources are unavailable later in life for somatic maintenance and repair.

Indeed, there is evidence that high condition entails trade-offs in terms of reduced ability to maintain performance with age. Hunt *et al.* (2004) found that male black field crickets in high condition invested more in sexual advertisement throughout their lives, but died sooner (but see Zajitschek *et al.*, 2009). Bonduriansky & Brassil (2005) found that large male body size conferred high mating rate in early life but was also associated with a heightened rate of reproductive ageing in a wild population of antler flies (also see Robinson *et al.*, 2006). It is not known, however, what proximate factors mediate this apparent association between high condition and rapid ageing. If high-condition individuals engage in more damage-inflicting behaviour, then faster somatic deterioration might be seen in high-condition individuals only when exposed to other individuals and reproductive opportunities. Alternatively, if high-condition bodies are inherently more susceptible to somatic damage, then faster somatic deterioration will be observed in individuals that start life in high condition even when deprived of opportunities for interaction and reproduction.

In order to investigate the relation between initial condition and somatic ageing, we exposed some larvae of *Telostylinus angusticollis* (Diptera, Neriidae; Fig. 1) to a nutrient-restricted diet in development ('poor' larval diet) while providing others with nutrients *ad libitum* ('rich' larval diet). When reared on a rich larval diet, *T. angusticollis* individuals of both sexes are larger as adults than their siblings reared on a poor larval diet, but larval diet quality has a particularly pronounced effect on males in terms of adult body size and the expression of secondary sexual traits, including elongated heads, antennae and legs (Bonduriansky, 2007). *T. angusticollis* males fight for access to females, and laboratory assays show that sexual selection favours large male body size and enhanced secondary sexual trait expression (Fricke *et al.*, 2015). Females reared on a rich larval diet produce larger clutches (Bonduriansky & Head, 2007; Adler & Bonduriansky, 2013).

In order to quantify somatic deterioration, we measured two traits that are likely to be important to survival and reproduction – wing damage, and escape response following a simulated predator attack. Wing damage represents an irreparable injury for insects (Downer & Laufer, 1983; Bonduriansky *et al.*, 2008),

Fig. 1 Male neriid flies, *Telostylinus angusticollis*, size each other up before a fight by locking antennae. Male–male combat can be intense, particularly among large, high-condition males (with access to plentiful nutrients in development). Body size, antennae length and leg length are all elongated in high-condition males and appear to function in both intra- and intersexual interactions. Photograph by Russell Bonduriansky.

and probably results from mechanical wear and tear in part from activities related to reproduction such as fighting and mating (Mühlhäuser & Blanckenhorn, 2002). Wing damage has been shown to accumulate with age in insects, and has been commonly employed as a reliable technique to determine the age of individuals (reviewed in Burkhard *et al.*, 2002). Wing damage has also been shown experimentally to be a causative factor of mortality in bumble bees (Carter, 1992), and has been used to determine parity in female mosquitoes (Corbet, 1960). A reduction in escape response is likely to be associated with somatic deterioration, such as wing damage, but may also result from flight muscle histolysis resulting in a reduction or loss of flight ability (Marden, 2000; Engqvist *et al.*, 2011), from neurosensory deterioration or from a lack of energy available for movement. Escape response is clearly important in the wild, where predation is a major source of mortality.

To determine whether effects of condition on somatic maintenance are mediated by behaviour and social interactions or by the properties of the body itself, we manipulated the social environment – housing some males on their own, some in mixed-sex groups and some in all-male groups. This allowed us to assess the effects of reproduction, male–male fighting and social interaction in general on somatic damage. In order to gauge the relevance of these measures to wild populations, we also measured somatic damage and escape response in wild-caught *T. angusticollis* individuals. In addition, in a separate experiment, we subjected immobilized males to mechanical stress and measured wing vein thickness, in order to determine whether condition directly affects somatic fragility and structural robustness independently of any condition-dependent variation in behaviour. We focused our study on males because this sex exhibits an especially pronounced developmental response to nutrient availability at the larval stage, in terms of body size as well as the expression of morphological secondary sexual traits and reproductive behaviours such as combat (Bonduriansky, 2007).

Materials and Methods

Source and rearing of flies

The laboratory stock used in this experiment was derived from > 100 individuals of *Telostylinus angusticollis* collected from aggregations on the trunks of *Acacia longifolia* trees in Fred Hollows Reserve in Sydney, Australia, and maintained in the laboratory as a large, outbred population for about 25 generations, supplemented annually with new wild-collected individuals. Larval/oviposition medium for our stock flies consisted of 30 mL blackstrap sugarcane molasses, 30 mL liquid barley malt and 32 g soya protein powder per litre of dry coco peat hydrated with 800 mL of water (see Bonduriansky, 2007 for product details). The food mixture was homogenized thoroughly using a handheld blender and frozen at −20 °C until the day of use. However, larvae in our stock cages experience a range of conditions depending on the age of the medium at the time of oviposition and the density of larvae, and this results in phenotypic variation comparable to that seen in the wild source population. Thus, our captive population is unlikely to have adapted to any particular nutrient concentration in the artificial larval diet.

Our larval diet (condition) manipulation appears to be ecologically relevant. *T. angusticollis* males and females both show great variability in body size in the wild (Bonduriansky, 2006). Such phenotypic variation can be greatly reduced under common-garden conditions in the laboratory, but re-created by varying larval diet quality (Bonduriansky, 2007). It appears that natural larval environments are highly variable in nutrient quality and availability, which could result from variation in spatial and temporal availability of nutrients, as well as varying degrees of larval crowding (M. Adler & R. Bonduriansky, personal observations). Thus, our larval diet (condition) manipulation is likely to reveal evolved plasticity in the flies' response to the

nutritional environment in development, making this species a good candidate in which to study how condition affects life-history traits.

Somatic deterioration experiment

The experiment was conducted in two consecutive blocks. To obtain flies for each block of the experiment, eggs were collected from stock cages and transferred alternately into 250-mL containers of fresh 'rich' or 'poor' larval medium provided *ad libitum*, with 50 eggs transferred into each container. Rich larval medium was prepared in the same way as the oviposition medium provided to the adult stock flies, described above, but all eggs were transferred to this medium when it was fresh. Poor medium contained the same ingredients, but with the nutrients reduced to one-third of the rich medium concentrations. Flies emerging from the rich larval medium are referred to below as 'high condition', and flies from the poor medium as 'low condition'. Adult flies, which attain sexual maturity within a few days and can live for several months in the laboratory (Adler *et al.*, 2013), were assigned randomly, within each condition treatment, to adult experimental treatments immediately after emergence. Flies assigned to group treatments were transferred into 1-L cages in groups of 10 ($N = 480$ flies in 48 cages), and male flies assigned to individual treatments were transferred into 250-mL cages ($N = 109$ males in 109 cages). Group treatments consisted of virgin males in groups of 10 ('all-male groups'; $N = 240$ flies in 24 cages) and mixed-sex groups of five males and five females ('mixed-sex groups'; $N = 240$ flies in 24 cages). See Table 1 for sample sizes per block and condition treatment.

Experimental cages were covered in mesh stockings to allow for ventilation. White polyester fabric wool was used to cover the bottom of each cage and moistened regularly to maintain high humidity and provide a source of water. The rich larval medium described above was provided to adult flies in all treatments as a source of food as well as an oviposition medium, and changed every 10 days. Group cages were provided with 70 mL of larval medium, whereas individual cages received a 12-mL petri dish full of medium. As an additional source of food for adults, all cages also contained separate dishes filled with brown sugar and instant dried yeast. Sugar and yeast dishes were changed if they became mouldy. All cages were watered and checked every 3 days for dead flies, and the experiment was terminated when > 90% of the flies had died (surviving flies were censored in the lifespan analysis). Dead flies were removed from group cages. Flies were kept on a 12-h:12-h light–dark cycle using a combination of broad-spectrum fluorescent and incandescent lighting, at approximately 26 °C and 50% humidity.

Table 1 Number of flies in each social environment and condition treatment, in blocks 1 and 2 of the experiment.

Social environment treatments	Block 1		Block 2	
	High condition	Low condition	High condition	Low condition
Individual males	30 flies in 30 cages	28 flies in 28 cages	25 flies in 25 cages	26 flies in 26 cages
All-male groups	70 flies in 7 cages	50 flies in 5 cages	60 flies in 6 cages	60 flies in 6 cages
Mixed-sex groups	70 flies in 7 cages	50 flies in 5 cages	60 flies in 6 cages	60 flies in 6 cages

Wing damage and escape response assays

At day 0 and day 28 following adult emergence, all male flies were examined for wing damage and their escape response was assessed. On day 0, each fly was transferred (without CO_2) into a scintillation vial, inspected for somatic damage and subjected to the escape response assay before being transferred into its experimental cage. Flies that showed signs of damage or developmental abnormalities (frayed wings or missing legs, $N = 5$) were excluded from the experiment. On day 28, a point at which all flies would have been mature for at least 10 days (M. Adler and R. Bonduriansky, personal observations) but approximately 65% of the flies in both diet treatments were still alive, each fly was removed from its experimental cage, examined for wing damage and subjected to the escape response assay, and then returned to the same cage. Wing damage was determined by visual examination and each fly given a score of 0, 1 or 2. A score of 0 indicates the wings are fully intact, 1 indicates damage to ~ 10% or less of the wing area, and a score of 2 indicates damage to > 10% of the wing area. This technique for scoring wing damage is based on the standard technique used for ageing tsetse flies, which has been shown to be as effective as a more complicated technique using a map wheel and camera lucida (Hayes & Wall, 1999). The technique was also chosen as it allowed for assessment of damage with minimal handling of the flies, whereas more precise measurements of damage tend to necessitate knocking out or killing the animal (as in Burkhard *et al.*, 2002). Escape response was measured by inverting the scintillation vial so that the fly landed upright inside the lid, then tapping rapidly 10 times on the side of the lid with a metal instrument, causing the fly to startle and attempt to fly to the other end of the vial. Flies that flew away from the lid end of the vial were given a score of 2, flies that could climb only partway out of the lid end of the vial were given a score of 1 and flies that were unable to climb out of the lid end of the vial at all were given a score of 0. Performance in this assay therefore reflects locomotory ability and vigour.

Somatic damage susceptibility in immobilized males

To determine whether our condition treatment resulted in differential somatic fragility (independently of any differences between treatment groups in behaviour), we conducted a separate experiment in which high- and low-condition males were killed and then exposed to varying types and degrees of somatic stress. Flies in this experiment were reared on rich and poor larval diets, as described above. Twenty-four hours after eclosion, flies from each larval diet treatment were killed through exposure to CO_2, then checked under a microscope for any evidence of pre-existing damage, photographed using a Leica DFC420 (Leica Microsystems, Wetzlar, Germany) camera mounted on a Leica MS5 stereoscope, and their thorax length was measured as a proxy for body size using ImageJ (Rasband, 1997–2009). Flies from each diet treatment were then exposed to one of three durations of either a shaking or a blowing assay. The blowing assay was conducted by placing a single fly at the bottom of a chamber connected to the end of a hairdryer. The hairdryer was then turned on, without heat, at maximum strength for 30, 60 or 90 s, causing the fly to rotate rapidly inside the chamber. For the shaking assay, a single fly was placed in a glass scintillation vial, inserted into a vortex mixer and then shaken at maximum speed for 2, 3 or 4 min (see Table S1 for sample sizes). Afterwards, the flies were examined under a microscope and wing damage quantified based on a 6-point scale, where 0 indicates no damage and 5 indicates more than 50% of the wing was damaged. This damage estimate was more precise than that used in the main experiment because the flies were dead and thus able to be viewed under a microscope.

Measurements of wing vein thickness

To determine whether differences in wing fragility between high- and low-condition males reflect structural differences in their wings, we removed wings from 20 low-condition and 25 high-condition individuals, mounted the wings in glycerol on microscope slides and measured the thickness of wing veins near the wing tip (where wing damage typically occurs) at six landmarks (Fig. S1). The tip of each wing was imaged using a Leica DFC420 camera mounted on a Zeiss Axioskop 40 (Carl Zeiss, Oberkochen, Germany) compound microscope. Wing vein thickness was then measured from the images using ImageJ software (Rasband, 1997–2009). Two measurements were obtained for each vein in each individual in order to estimate measurement repeatability (Table S2).

Measurements of somatic deterioration in wild flies

To gauge the occurrence of wing damage and reduced escape performance in wild flies, 54 male *T. angusticollis* were collected from our field site at Fred Hollows Reserve in Sydney, Australia, and assessed *in situ*. Each fly was caught by covering it with a scintillation vial (taking care to avoid injury), and somatic deterioration and escape response assays were performed immediately, in the manner described above for flies in the laboratory experiment.

Statistical analysis

Lifespan data were analysed using the R package MCMCglmm (Hadfield, 2010). A poisson error structure was used, and censored data (flies that escaped during the experiment, or that were still alive at the end of the experiment) were incorporated using the 'cenpoisson' option. Parameter estimates were based on a Markov chain Monte Carlo process with 13 000 iterations, including a 3000-iteration burn-in phase, with effective samples taken every 10th iteration. Autocorrelation between samples did not exceed the level recommended by Hadfield (2014). We initially fit a model with larval diet treatment (rich vs. poor), social environment treatment (grouped vs. individually housed) and their interaction as fixed effects, block as a fixed effect and replicate cage as a random effect. We also fitted a version of this model with wing damage added as a fixed covariate to test for effects of wing damage at 28 days of age on subsequent survival. Next, we fitted a model to the data for grouped flies to investigate effects of group sex ratio (all-male groups vs. mixed-sex groups), diet and their interaction on lifespan, with diet treatment, sex ratio and their interaction as fixed effects, block as a fixed effect and cage as a random effect. Block was modelled as a fixed effect because, with only two blocks, it was not possible to obtain a reliable estimate of the variance component for this factor.

Wing damage and escape response at age 0 were not analysed, because all flies used in the experiment had a perfect score at emergence for these measures. Wing damage and escape response at 28 days of age were analysed using cumulative link models for ordinal data, with a logit link function, using the R package 'ordinal' (Christensen, 2015). For each response variable, we first fit a model with larval diet, social environment (grouped vs. individually housed) and their interaction and block as fixed effects and replicate cage as a random effect. Next, to test the effects of sex ratio on somatic deterioration, we fit a model to the data on group-housed flies only, with larval diet, social environment (all-male groups vs. mixed-sex groups) and their interaction and block as fixed effects, and replicate cage as a random effect. We also tested the effect of diet for individually housed males only. Ordinal models were also used to analyse wing fragility of immobilized flies. We fitted separate models for each stress type (shaking and blowing), with larval diet (rich vs. poor),

stress duration and their interaction as fixed effects, and thorax length (as an index of body size) included as a fixed covariate. Thorax length was standardized (i.e. converted to z-scores: mean = 0, variance = 1) within larval diet treatments prior to the analysis in order to eliminate redundancy between the categorical effect of larval diet treatment and the body size covariate. This covariate therefore tested the effect of body size on somatic fragility within larval diet treatment groups. All models were simplified by removing non-significant interactions.

Effects of larval diet on wing vein thickness, and repeatabilities for the wing vein measurements, were estimated using linear mixed models fitted by restricted maximum likelihood (REML), with larval diet (rich vs. poor) as a fixed effect and individual identity as a random effect. Covariation among measures of deterioration (wing damage, escape response), and between measures of deterioration and longevity, was tested using Spearman's rank correlations. These analyses were carried out separately on data for individually housed flies, and on cage means for group-housed flies. All analyses were conducted using R version 3.0.1 (The R Foundation for Statistical Computing, Vienna, Austria).

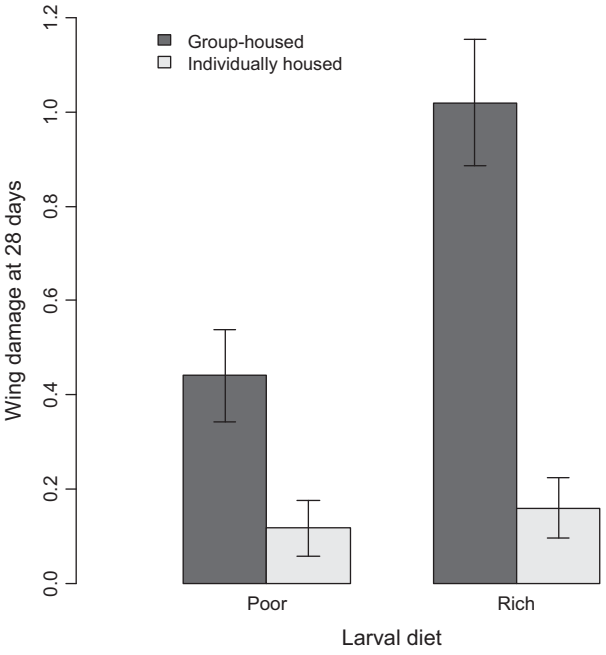

Fig. 2 Males reared on a rich larval diet accumulated more wing damage with age when housed in groups. Bars show mean wing damage for males reared on poor or rich larval diet and housed individually (grey) or in groups (black), with standard errors of replicate means.

Results

Effects of condition and social environment on the rate of somatic ageing

Wing damage

For wing damage at 28 days of age, we detected an interaction of larval diet × social environment, whereby wing damage was especially high in males reared on a rich larval diet and also housed in groups (estimate = 1.62, SE = 0.80, $P = 0.044$; Fig. 2). The effect of larval diet on wing damage was nonsignificant in individually housed males (estimate = 0.42, SE = 0.68, $P = 0.54$) but, among group-housed males, the degree of wing damage was greater in males reared on a rich larval diet (estimate = 1.95, SE = 0.58, $P < 0.001$). However, there was no effect of sex ratio (all-male groups vs. mixed-sex groups) on wing damage (estimate = −0.31, SE = 0.56, $P = 0.58$), nor a larval diet × sex ratio interaction (estimate = 0.46, SE = 0.71, $P = 0.51$). In analyses of grouped males and all males, we also detected significant effects of experimental block (estimates > 1.9, SE < 0.40, $P < 0.001$).

Escape response

Escape response at 28 days of age was lower in males reared on a rich larval diet (estimate = −2.19, SE = 0.94, $P = 0.020$), and lower in males maintained in groups (estimate = −2.47, SE = 1.15, $P = 0.032$; Fig. 3). For individually housed males, there was almost no variation in escape response (a reduced response was observed for a single individual reared on a rich larval diet), so the larval diet effect was not analysed. For males housed in groups, the larval diet × sex ratio interaction was marginally nonsignificant (estimate = −3.29, SE = 1.95, $P = 0.092$), and we detected no main effects of larval diet (estimate = 0.22, SE = 1.49, $P = 0.88$) or sex ratio (estimate = 1.33, SE = 1.64, $P = 0.42$). The experimental block effect was significant in these models (estimates > 3.46, SE < 1.17, $P < 0.003$).

Lifespan

Flies housed individually lived longer than flies housed in groups (posterior mean = −0.32, 95% confidence limits: −0.56, −0.11, $P = 0.008$; Fig. 4). However, there was no effect of larval diet on lifespan (posterior mean = 0.15, 95% confidence limits: −0.12, 0.37, $P = 0.25$), nor a larval diet × social environment interaction (posterior mean = −0.07, 95% confidence limits: −0.39, 0.21, $P = 0.64$). We tested the effect of wing damage at 28 days on subsequent survival by adding wing damage to the above model, fitted to a reduced data set consisting of individuals that survived to age 28 days ($N = 315$ males). This analysis showed a negative association between wing damage and survival (posterior mean = −0.10, 95% confidence limits: −0.20, −0.01, $P = 0.024$).

For males housed in groups, lifespan was not affected by sex ratio (posterior mean = −0.07, 95% confidence

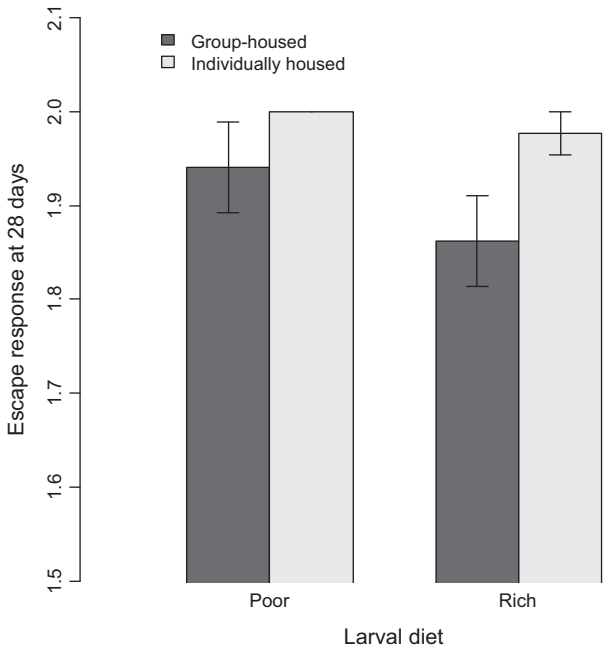

Fig. 3 Escape response at 28 days was lower in males reared on a rich larval diet and in males housed in groups. Bars show mean wing damage for males reared on poor or rich larval diet and housed individually (grey) or in groups (black), with standard errors of replicate means. Note there is no standard error bar for the individually housed poor males because there was no variance in this group – all flies had perfect scores for escape response at 28 days.

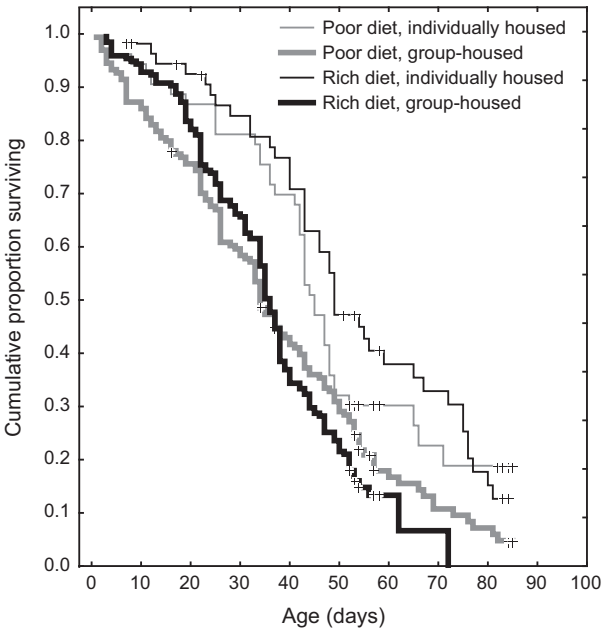

Fig. 4 Social interaction reduced lifespan, but larval diet had no effect. The proportion of male flies, housed individually (thin lines) or in groups (thick lines), surviving as a function of age, with separate lines for males reared on a rich (black lines) or poor (grey lines) larval diet. Crosses indicate censored individuals.

limits: −0.41, 0.25, $P = 0.63$), larval diet (posterior mean < 0.01, 95% confidence limits: −0.31, 0.37, $P = 0.98$) or their interaction (posterior mean = 0.12, 95% confidence limits: −0.32, 0.56, $P = 0.54$). There was no significant effect of block in any analysis of lifespan.

Somatic fragility in immobilized males

When immobilized and subjected to mechanical stress, males reared on a rich larval diet accumulated more wing damage than did males reared on a poor larval diet (Fig. 5), both when stress was applied by shaking (estimate = 1.41, SE = 0.28, $P < 0.0001$) and blowing (estimate = 1.90, SE = 0.30, $P < 0.0001$). The degree of wing damage increased with stress duration (shaking: estimate = 0.57, SE = 0.17, $P = 0.0006$; blowing: estimate = 0.79, SE = 0.18, $P < 0.0001$). The interaction of larval diet treatment and stress duration was not significant for either stress type and was removed from the model. Within larval diet treatments, the degree of wing damage was not related to body size when stress was applied by shaking (estimate = 0.20, SE = 0.14, $P = 0.16$), but damage increased with body size when stress was applied by blowing (estimate = 0.31, SE = 0.14, $P = 0.0267$).

The greater wing fragility of immobilized males reared on a rich larval diet did not result from thinner wings. At each of the six measurement landmarks, males reared on a rich larval diet had thicker wing veins than males reared on a poor larval diet (Table S2).

Somatic deterioration in wild flies

Of 54 male flies collected from a natural population, four individuals (7.5%) had damaged wings. All of the wild-captured individuals received the maximum score in the escape response assay (i.e. no wild-captured flies demonstrated compromised escape ability).

Discussion

Our study provides several lines of evidence that a high-condition phenotype is associated with heightened susceptibility to somatic damage. We found that males that started life in high condition sustained more wing damage by 28 days of age than males that started life in low condition when permitted to interact with other individuals. High-condition males also tended to suffer a greater reduction in escape response by 28 days of age. We did not detect a difference in wing damage between high- and low-condition males that were individually housed, suggesting that social interaction

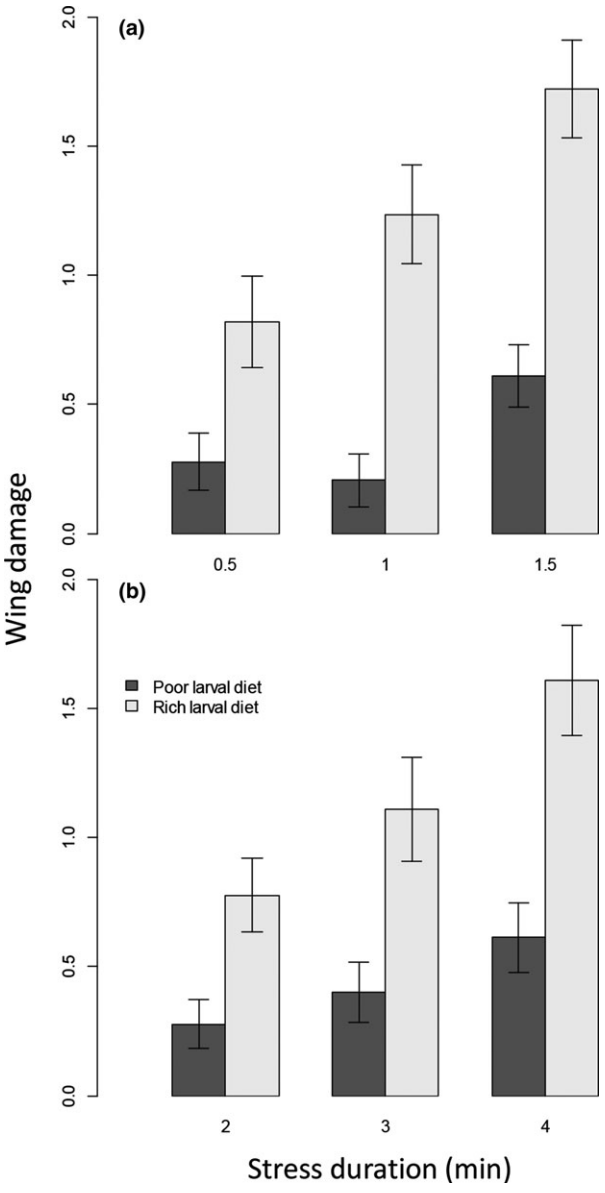

Fig. 5 Males reared on a rich larval diet were more susceptible to mechanical stress than males reared on a poor larval diet. Bars show mean wing damage sustained by males reared on poor (black) or rich (grey) larval diet when subjected to mechanical stress from blowing air (a) or shaking (b), with standard errors of individual data.

contributes substantially to somatic deterioration in high-condition males. Nonetheless, even when immobilized, high-condition males were more prone to somatic damage from mechanical stress. Our results therefore suggest that high-condition males deteriorate more rapidly with age both because their bodies are inherently more damage-prone, and because they are more likely to engage in damaging interactions with other individuals. Hence, in *Telostylinus angusticollis*, accelerated somatic ageing appears to be a cost of expressing a high-condition phenotype optimized for reproduction.

Somatic ageing was strongly influenced by social interaction: flies housed in groups had more wing damage at 28 days of age than flies housed individually. Flies housed in groups also suffered a greater reduction in escape response with age, compared with individually housed flies. Because flies housed individually showed little evidence of deterioration by 28 days of age, we found no overall effect of larval diet quality (which determines early-life condition) for wing damage. However, our results showed that rich larval diet promotes wing damage with age in group-housed flies. In addition, we found that escape response at 28 days of age was lower for flies reared on a rich larval diet. Interestingly, there was no effect of sex ratio (mixed-sex vs. all-male) on wing damage, suggesting that male combat may be equally intense irrespective of female presence or that mating and fighting result in a similar degree of wing damage for males. These results present something of a contrast to effects found in *Teleogryllus commodus* crickets, in which the jump and bite performance of males shows little evidence of age-related decline, and surprisingly, only males kept alone as virgins showed any evidence of performance-related senescence (Lailvaux *et al.*, 2011). That study manipulated adult diet, which was found to have no effect on performance or its age-related decline.

We found no overall difference in lifespan between low- and high-condition flies. Because the degree of wing damage at 28 days was a significant predictor of subsequent survival, the more rapid somatic deterioration of high-condition males may have negated any survival advantages associated with high early-life condition [e.g. *T. angusticollis* flies in high condition have previously been shown to survive longer than low condition flies when exposed as adults to starvation (Adler *et al.*, 2013)]. These results thus suggest that in terms of adult viability, the advantages of greater resource availability may be at least partially negated by the costs of expressing a high-condition phenotype. However, social interaction strongly affected lifespan. Individually housed flies lived longer than group-housed flies, a finding consistent with results of a previous study (Adler & Bonduriansky, 2011). Reduced survival in group-housed males may result from the fact that social interaction is known to entail costs such as competition for food and space (Boyd, 1982; Krause & Ruxton, 2002), increased stress and increased exposure to parasites (Alexander, 1974; Krause & Ruxton, 2002).

As lifespan of *T. angusticollis* in the laboratory is much longer than in the wild (with median lifespan in the wild 3.5 days and median lifespan in the laboratory 35 days, Kawasaki *et al.*, 2008), flies in the wild may rarely live long enough to accumulate somatic damage. However, we found that wing damage is also

observable in wild male flies, with 7.5% of males in our sample exhibiting readily visible wing damage. Interestingly, we found no flies with a reduced escape response. This suggests that there is strong selection against reduced escape response in the wild, or alternatively, that this measure of deterioration may be associated with advanced age, a relatively rare phenomenon in wild insects (see Bonduriansky & Brassil, 2002).

Our results show that condition and social environment interact to affect somatic ageing: high-condition flies housed in groups have more wing damage at 28 days of age than any other treatment combination. This is likely due in part to the fact that in *T. angusticollis*, high-condition males engage in male–male combat whereas low-condition males rarely do so (Bath *et al.*, 2012) and that combat interactions involving high-condition males are much more likely to escalate into drawn-out, potentially damaging contests (R. Bonduriansky & A. Hooper, unpublished data). Such behavioural differences between high- and low-condition males are likely due to contrasting selection, where high-condition males tend to gain a fitness advantage by engaging in more agonistic behaviour, but this entails a somatic cost later in life. However, the trade-off in high condition cannot be due entirely to a difference in behaviour, as demonstrated by our results for immobilized males, in which high-condition flies accumulated more damage than low-condition flies when subjected to mechanical stress. This suggests that high-condition bodies are inherently more damage-prone, pointing to a role for trade-offs resulting from metabolic opportunity costs as well as those resulting from contrasting selection.

Interestingly, the greater fragility of high-condition wings does not appear to be driven by a simple structural constraint, insofar as we found that high-condition flies have thicker wing veins than low-condition flies. This result, however, does not completely rule out the idea that high-condition flies are structurally weaker, particularly in relative terms. Although their wing veins are thicker in absolute terms, high-condition flies (which are larger and heavier) are likely to experience greater forces on their wings [which exhibit a tight, slightly negative allometry with body size (Bonduriansky, 2006, 2007)] during flight or physical contact with surrounding objects, and it is not clear whether the greater thickness of their wings compensates fully for these greater forces. The simple fact of having a larger, heavier body and greater wingspan may increase vulnerability to damage and perhaps make it more difficult to avoid obstacles. This would be especially noticeable in a crowded social environment, where there are more individuals to come into contact with. Enlarged morphological traits can impose locomotion-related costs. For example, in stalk-eyed flies, male flight performance is reduced in species with particularly elongated eye stalks (Swallow *et al.*, 2000), and in fiddler crabs, males with enlarged 'major' claws have reduced running endurance compared with males lacking major claws (Allen & Levinton, 2007). It is not clear whether absolute enlargement of the wings of neriid flies can impede movement or promote somatic damage, but biomechanical principles suggest that this is indeed possible (Koehl, 1996).

It is also possible that a scarcity of nutrients during development for low-condition males might trigger greater investment into dispersal mechanisms, which could be associated with greater strength and durability of wings and flight muscles (despite wing veins that are thinner in absolute terms), in order to produce a phenotype that is optimized to seek out an adult environment with more resources or fewer conspecific competitors (Dingle, 2001; Zera & Harshman, 2001; Dmitriew & Rowe, 2011). Alternatively, the wings of high-condition males could be optimized for sexual signalling. Shevtsova *et al.* (2011) reported striking stability in colour patterns, formed by variable membrane thickness, in the wings of Diptera and Hymenoptera, which the authors suggested was likely a result of sexually selected visual signals. It is possible that the wings of high-condition *T. angusticollis* males are built, in part, for sexual signalling functions, whereas the wings of low-condition males are optimized for viability-related functions such as dispersal, and therefore more resistant to mechanical damage.

Overall, our findings provide strong evidence that nutrient availability in development, which determines early-life condition, also affects somatic ageing rate in adults. As opposed to the traditional view that high condition translates to increased performance across all fitness-related traits (reviewed in Rowe & Houle, 1996; Hill, 2011), we found that high condition enhances investment in traits that tend to increase reproductive success in early life, such as large body size and enlarged secondary sexual traits, but also imposes latent costs manifested as somatic ageing. This trade-off may weaken overall selection for traits associated with high condition, such as enlarged secondary sexual characters and reproductive behaviours (Bonduriansky & Brassil, 2005).

Our results may help to explain the findings of several previous studies showing that high condition was associated with a relatively rapid reduction in performance with advancing age (Bonduriansky & Brassil, 2005; Hunt *et al.*, 2004; also see Robinson *et al.*, 2006). Our results suggest that high early-life condition may promote senescence via accelerated accumulation of structural damage, taking the form, in insects, of irreparable exoskeletal deterioration. Interestingly, we found that this deterioration resulted not only from the tendency of high-condition males to engage in damaging behaviours, but also from the greater fragility of the high-condition male body itself. Variation in condition may therefore contribute to intrapopulation variation

in the rate of senescence (reviewed in Nussey *et al.*, 2008). Notably, in contrast with the above-mentioned studies on the condition dependence of ageing rate, several studies have reported positive genetic correlations between early- and late-life performance (reviewed in Charmantier *et al.*, 2006; Maklakov *et al.*, 2015). More research is needed to understand the contributions of genes and environment to intrapopulation variation in ageing rate.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Sample sizes, thorax length means (mm) ± standard errors and mean wing damage scores for *Telostylinus angusticollis* males reared on rich and poor larval diets, killed by CO_2 and subjected to mechanical stress by blowing air from a hand-held hair dryer without heat, or shaking in a test-tube shaker.

Table S2 Wing-vein measurements on male flies reared on rich (n = 25) and poor (n = 20) larval diets.

Figure S1 Landmark locations of wing-vein thickness measurements (see Table S2).

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