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Noriyoshi Kawasaki
Evolution and Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney 2052, New South Wales, Australia

Chad Brassil
University of Nebraska - Lincoln, cbrassil2@unl.edu

Robert Brooks
Evolution and Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney 2052, New South Wales, Australia

Russell Bonduriansky
Evolution and Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney 2052, New South Wales, Australia

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Environmental Effects on the Expression of Life Span and Aging: An Extreme Contrast between Wild and Captive Cohorts of *Telostylinus angusticollis* (Diptera: Neriidae)

Noriyoshi Kawasaki,[1] Chad E. Brassil,[2] Robert C. Brooks,[1] and Russell Bonduriansky[1,*]

1. Evolution and Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney 2052, New South Wales, Australia;
2. School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588

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abstract: Most research on life span and aging has been based on captive populations of short-lived animals; however, we know very little about the expression of these traits in wild populations of such organisms. Because life span and aging are major components of fitness, the extent to which the results of many evolutionary studies in the laboratory can be generalized to natural settings depends on the degree to which the expression of life span and aging differ in natural environments versus laboratory environments and whether such environmental effects interact with phenotypic variation. We investigated life span and aging in *Telostylinus angusticollis* in the wild while simultaneously estimating these parameters under a range of conditions in a laboratory stock that was recently established from the same wild population. We found that males live less than one-fifth as long and age at least twice as rapidly in the wild as do their captive counterparts. In contrast, we found no evidence of aging in wild females. These striking sex-specific differences between captive and wild flies support the emerging view that environment exerts a profound influence on the expression of life span and aging. These findings have important implications for evolutionary gerontology and, more generally, for the interpretation of fitness estimates in captive populations.

Keywords: aging, senescence, environment, natural population, field study, phenotypic plasticity, *Telostylinus angusticollis*.

* Corresponding author; e-mail: r.bonduriansky@unsw.edu.au.

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> From the standpoint of evolutionary biology an animal's expectation of life in its natural surroundings is much more significant than the degree of decrepitude to which it may be nursed in laboratory or zoo. (P. B. Medawar 1946)

Empirical research on life span and aging has largely relied on captive populations of short-lived animals like *Drosophila melanogaster*, *Caenorhabditis elegans*, and mice (see Rose and Charlesworth 1981; Partridge and Barton 1993*a*, 1993*b*; Charlesworth and Hughes 1996; Jucker and Ingram 1997; Partridge and Mangel 1999; Kirkwood and Austad 2000; Hughes et al. 2002). In contrast, we know remarkably little about life span and aging of short-lived organisms in their natural environments (see Medawar 1946; Partridge 1988; Williams et al. 2006). This gap in knowledge is especially problematic because the interpretation of a wide range of evolutionary studies often rests on the assumption that laboratory results are at least qualitatively informative of patterns that occur in wild populations, where the traits of interest evolved (Harshman and Hoffmann 2000). Because life span and aging (i.e., the pattern of age-related changes in the probability of survival and reproduction) are major components of fitness, the assumption that laboratory results can be generalized to natural environments may be violated if life span and aging differ substantially between natural settings and laboratory settings. In particular, if environmental effects on the expression of life span and aging interact with phenotypic variation (e.g., sex, age cohort, genetic or conditional morph, etc.), then exposure to laboratory environments may yield patterns that are qualitatively different from those occurring in natural environments. Here we provide evidence of large, sex-dependent differences in life span and aging between cohorts observed in fully natural environments versus genetically similar cohorts observed in laboratory environments. We argue that such effects may complicate the interpretation of many laboratory studies and highlight the need for a better understanding of environmental effects on the expression of life span and aging.

Currently available evidence yields contradictory clues about how wild and captive populations differ in aging rates and life spans. For example, in wild rodents it remains unclear whether aging occurs at all (Slade 1995), whereas Bonduriansky and Brassil (2002) observed rapid aging in wild antler flies. Curiously, recent studies based on common-garden assays have suggested that wild *D. melanogaster* and mice have the genetic potential to age more slowly and to live longer than their laboratory-adapted counterparts (Sgrò and Partridge 2000; Linnen et al. 2001; Miller et al. 2002). However, in relation to the question of how wild organisms and captive organisms differ in life span and rate of aging, the relevance of these common-garden studies is unclear because aging and life span are highly plastic traits. The radically different environments experienced by wild organisms and captive organisms could have large effects on the expression of these traits, and such effects could either negate or amplify any genetic differences between wild and laboratory-adapted populations. Genotype × environment interactions for life span have been observed in *D. melanogaster* (Vieira et al. 2000; Marden et al. 2003) and *C. elegans* (Shook and Johnson 1999; Van Voorhies et al. 2005). Life span and aging rate are affected by diet in many animals (Medawar 1946; Nishiyama et al. 1997; Masoro 2005); even the scent of food affects life span in *Drosophila* (Libert et al. 2007). In rats, an enriched environment reduces the rate of brain aging (Saito et al. 1994; Nakamura et al. 1999). In ungulates, aging rate in wild males appears to be affected by population density (Mysterud et al. 2001). Consequently, comparisons of wild and captive populations must take into account environmental effects on the expression of aging and life span (Williams et al. 2006). The few studies that have tested for such effects—in plants, birds, and baboons—have observed considerable differences in life span between laboratory and wild populations (Ricklefs 2000; Roach 2001; Bronikowski et al. 2002). However, no previous study of any animal species has compared both life span and aging rate of genetically similar populations living in fully natural environments versus laboratory environments.

Our lack of knowledge about aging and life span in short-lived organisms in the wild largely reflects technical impediments. Small animals are difficult to mark, are often difficult to recapture, and, given their high extrinsic mortality rates, large samples are generally required to detect aging. Currently available phenotypic indices of aging, such as age-related changes in pteridine concentrations, are imprecise and are strongly affected by environmental factors (Hayes and Wall 1999; Robson et al. 2006). These impediments can be partly overcome, however, through the use of an effective individual marking technique (Bonduriansky and Brooks 1997; Hagler and Jackson 2001) and through the choice of model species with a sufficient site fidelity to yield reasonably high individual resighting rates, such as insects that form stable mating aggregations (see Bonduriansky and Brassil 2002, 2005).

We employed a mark-release-resight method to investigate life span and actuarial aging (i.e., a change in mortality rate with age, henceforth "aging") in the wild in *Telostylinus angusticollis* (Neriidae), a large fly that forms mating aggregations on damaged tree trunks (Bonduriansky 2006, 2007; fig. 1). At the same time, we estimated life span and aging under a variety of conditions (e.g., individual housing vs. grouped housing, same-sex groups vs. mixed-sex groups) in a laboratory stock that was recently established from the same wild source population. We also experimentally tested for effects on mortality of our marking technique as a result of mechanical injury or paint toxicity. Modeling of mortality rate as a function of age using maximum likelihood techniques revealed a striking contrast between wild and captive flies in life span, rate of aging, and effects of sex.

Methods

Study Animals

We studied a wild population of *Telostylinus angusticollis* (Enderlein) in the Fred Hollows Reserve, Sydney, Australia. The flies formed mixed-sex aggregations on two beetle-damaged *Acacia longifolia* trees and could be found sporadically on several other trees and a wooden fence nearby. *Telostylinus angusticollis* adults fed, mated, and oviposited

Figure 1: Wild female *Telostylinus angusticollis* marked with an individual code on the thoracic notum, perched on *Acacia longifolia* bark.

on the *A. longifolia* trunks, and larvae developed in the rotting bark. We established a laboratory population from ~100 individuals collected from this wild population. The laboratory stock was maintained in four 10-L population cages with a layer of moist "cocopeat" (Galuku, Sydney) for two generations prior to this study. Brown sugar and soy protein were provided as food for adult flies. Cages were maintained at 26°C, 50% humidity, and a 14L : 10D cycle and were illuminated using a combination of broad-spectrum fluorescent and incandescent lights.

Larval medium for oviposition was provided in a 250-mL container at the bottom of each cage. Larval medium consisted of 30 mL of blackstrap sugar cane molasses (Conga Foods, Preston, Victoria, Australia), 30 mL of liquid barley malt (Select Foods, Smithfield, New South Wales, Australia), and 32 g of soy protein powder (PharmaCare, Warriewood, New South Wales, Australia) per liter of dry cocopeat hydrated with 800 mL of purified water. The mixture was thoroughly homogenized using a handheld blender and was frozen at −20°C until the day of use. Every 20 days, each container of oviposition medium was supplemented with approximately 250 mL of fresh larval medium and transferred to a mesh-covered 2-L jar. Adults emerging from the 2-L jars were transferred to population cages.

Laboratory Study

Aging rate in the laboratory was estimated under several different conditions. Assay A contained 208 flies (102 females and 106 males) that were maintained individually in 75-mL cages. Assay B contained 200 flies (98 females and 102 males) that were maintained in 40 same-sex groups (20 groups of 4 or 5 females each and 20 groups of 5 males each) in 250-mL cages. Assay C contained 800 flies that were maintained in 40 same-sex groups of 10 individuals each (20 groups of females and 20 groups of males), and 40 mixed-sex groups of 10 individuals each (5 females and 5 females) in 2-L cages (life span could not be determined for a few individuals; see "Results" for actual sample sizes). The flies used in these assays were obtained as newly emerged individuals from the laboratory stocks. Cages contained a layer of hydrated cocopeat and small dishes of brown sugar and dry yeast (soy protein in assay C) for adult food. In assay C, mixed-sex cages also contained 75-mL jars of oviposition medium that was replaced every 2 weeks. All cages were watered regularly to keep the cocopeat moist. Cages were checked daily for dead flies.

Field Study

To investigate aging in the wild, we collected 80 females from the wild population, marked their adult F_1 progeny with individual codes, and released them near the *T. angusticollis* aggregations at the field site (fig. 1). Adults in the F_1 generation were marked using a modified version of the technique of Bonduriansky and Brooks (1997). The marking device consisted of a cylinder and piston constructed from a 75-mL specimen container (Sarstedt, Technology Park, South Australia, Australia). The cylinder was covered at one end with a 4-mm-grid wire mesh. One fly at a time was released into the cylinder, and the piston was inserted and adjusted so as to immobilize the fly with its thoracic notum protruding through an opening in the mesh. The device was then placed on the stage of a Leica MS5 stereoscope and an individual code was applied to the thoracic notum in white enamel paint (Model Master, Testor, Rockford, IL) using a brush consisting of a pointed strip of index card paper with a single short hair glued on its tip. Individual codes consisted of two-figure combinations of arabic numerals, Latin letters, and Japanese letters. Marked flies were then released near an aggregation site on damaged *A. longifolia* trees in the Fred Hollows Reserve (50% of flies released at each site). In total, 914 flies (424 males and 490 females) were released between December 27, 2005, and March 27, 2006 (92% of marked flies were released within 3 days of adult emergence). There is no evidence that laboratory-reared flies differed from wild flies in any phenotypic trait, including condition. Laboratory-reared flies were morphologically indistinguishable from wild-caught flies, and a pilot mark-recapture study using wild-caught flies of unknown age produced similar results (not shown) to the findings reported here for laboratory-reared flies.

The trunks and major branches of the two *A. longifolia* trees where major aggregations occurred were inspected daily for the presence of marked individuals between December 27, 2005, and April 25, 2006. A ladder was used to access the canopy. Other trees and fence posts within 5 m of the aggregation trees were also inspected. The identity and location of each marked individual were recorded.

Treatment Effects within Laboratory Assays

To determine whether marked flies experience an elevated mortality rate as a result of mechanical injury or paint toxicity, we marked half of the individuals used in laboratory assays A and B. Of the 40 groups in assay B, 20 groups contained 2 marked and 3 unmarked individuals, and the other 20 groups contained 3 marked and 2 unmarked individuals. We recorded mortality in marked and unmarked individuals. Within the laboratory assays, we

tested for effects of marking on life span using survival analysis. We also tested for effects on life span of assay, sex, and marking by factorial ANOVA. Life span data from the laboratory assays were approximately normally distributed. We used survival analysis to test for an effect of reproduction on male and female life spans in assay C (same-sex groups vs. mixed-sex groups). Because our design was balanced, the number of groups (blocks) was large, and the number of flies per block was small, we omitted block effects from the analyses reported here. Inclusion of block effects has no qualitative effect on the results (not shown).

Aging Analysis

Daily mortality for laboratory and wild flies was modeled as a linear term within an inverse logit function. The model was fit to binomially distributed survival data using maximum likelihood techniques (Hilborn and Mangel 1997). Because aging in the wild was studied using mark-recapture techniques with animals of known age, we used classical mark-recapture models in which the probability c of resighting of a live fly was estimated (Lebreton et al. 1992) in addition to parameters describing mortality. We tested for "actuarial" aging (senescence), defined as a continuous increase with age in the probability of death (Carey 2001). For non-sex-specific analysis, the Gompertz model and a linear inverse logit model fit the data equally well (change in Akaike Information Criterion [ΔAIC] = .05). A family of inverse logit models was used for subsequent analysis because of the ease with which terms can be included and the computational ease with which algorithms can fit maximum likelihood estimates. Given the inherent limitations of demographic data from a wild population, the detection of subtle differences between patterns of senescence at late ages is beyond the scope of this study, making distinctions between various aging models largely irrelevant. In fact, traditional senescence models do not have robust mechanistic derivations (Abrams and Ludwig 1995). Our choice of model was therefore utilitarian: our objectives were to determine whether senescence occurs and to detect differences in rates of senescence between flies inhabiting different environments and between sexes. Models of mortality for the wild flies included δ, a parameter related to the additional loss of flies through death or dispersal on the day of release that is above and beyond the normal mortality rate. Models were constructed using a forward/backward stepwise approach with a focus on examining sex-specific aging parameters within each lab assay and in the wild. Subsequently, comparisons of parameter differences were made among lab assays and between lab assays and the wild. We used AIC to compare models (Burnham and Anderson 2002), and likelihood ratio tests yielded P values for nested comparisons. Median and maximum life spans were estimated from the best-fit model, with median life span defined as the age by which 50% of the cohort is dead and maximum life span defined as the age by which 99% of the cohort is dead (ignoring δ, which is assumed to reflect an artifact of the mark-release procedure).

Results

Life Span and Aging in the Wild

Estimates of aging and life span parameters derived from the best-fit models for wild and captive flies are summarized in table 1. For the wild flies, the best-fit model included aging in males but no change in mortality rate with age in females,

$$\mu_{\mathrm{f}}(d) = \begin{cases} \dfrac{1}{1+\exp(-a_{\mathrm{f}})}, & d > 1, \\ \dfrac{1}{1+\exp(-[\delta + a_{\mathrm{f}}])}, & d = 1, \end{cases}$$

$$\mu_{\mathrm{m}}(d) = \begin{cases} \dfrac{1}{1+\exp(-[a_{\mathrm{m}} + b_{\mathrm{m}}d])}, & d > 1, \\ \dfrac{1}{1+\exp(-[\delta + a_{\mathrm{m}} + b_{\mathrm{m}}d])}, & d = 1, \end{cases} \tag{1}$$

where $\mu_i(d)$ is the probability of daily mortality for sex i on day d, a_i is the background mortality parameter for sex i, b_i is the aging rate (slope) for sex i, and δ is the additional death or dispersal parameter for the day of release. These parameters are estimated in conjunction with an estimation of the daily probability of resighting a live fly ($c = 0.22$). On the day of release, the probability of a fly's total disappearance from the population was $1/\{1 + \exp[-(a + \delta)]\}$, which gives values of 0.60 for females and 0.40 for males. Similarly, the daily probability of mortality at age 0, which is equivalent to the background or extrinsic mortality rate after factoring out δ, is $1/[1 + \exp(-a)]$, which gives values of 0.23 for females and 0.12 for males. Thus, the additional probability of death or dispersal immediately following release is the difference between these probabilities, which gives values of 0.37 for females and 0.28 for males. Male-specific aging (b_{m}) in the wild was strongly supported in the best-fit model (AIC = 2,043.4) compared with a model that did not include male aging (AIC = 2,058.2). However, the inclusion of a female aging term (b_{f}) in the model had little support (AIC = 2,045.4) relative to the best-fit model with no female senescence (AIC = 2,043.4). Thus, aging was not detected in wild females.

Post hoc power analysis supported the conclusion that aging rate is negligible in wild females. We used two ap-

proaches. First, we simulated data sets with an increased value of b_f but with all other parameters maintained at their best-fit estimates. We found that, when b_f becomes large enough to be detected, overall mortality rate becomes so high that all statistical power is lost. This suggests that, given the high baseline mortality rate of wild females, we have little information about their rate of aging. Second, we investigated the possibility that the rate of background mortality for females is inflated as a result of failure to detect aging. We found that, if average life span is held constant such that an increase in b_f results in a concomitant decrease in a_f, there is a >95% probability of detecting $b_f > 0.0268$. This detection threshold is paired with $a_f = -1.31$, representing a small decrease in daily background mortality from 23% in the best fit model to 21% at the power analysis threshold. The detection threshold for b_f is 84% less than the best-fit estimate of b_m. Both analyses suggest that, in the wild, aging contributes much less to overall mortality rate in females than in males.

Life Span and Aging in Captivity

We found no evidence of a consistent effect of marking on life span in laboratory flies (fig. 2). In assay A, survival analysis showed a positive effect of marking on life span in females ($N_{unmarked} = 51$, $N_{marked} = 51$; Cox-Mantel test: test statistic $= -2.61$, $P = .009$) but a negative effect in males ($N_{unmarked} = 55$, $N_{marked} = 51$; Cox-Mantel test: test statistic $= 2.69$, $P = .007$). In assay B, we found no significant effect of marking on life span in either females ($N_{unmarked} = 49$, $N_{marked} = 49$; Cox-Mantel test: test statistic $= 1.95$, $P = .052$) or in males

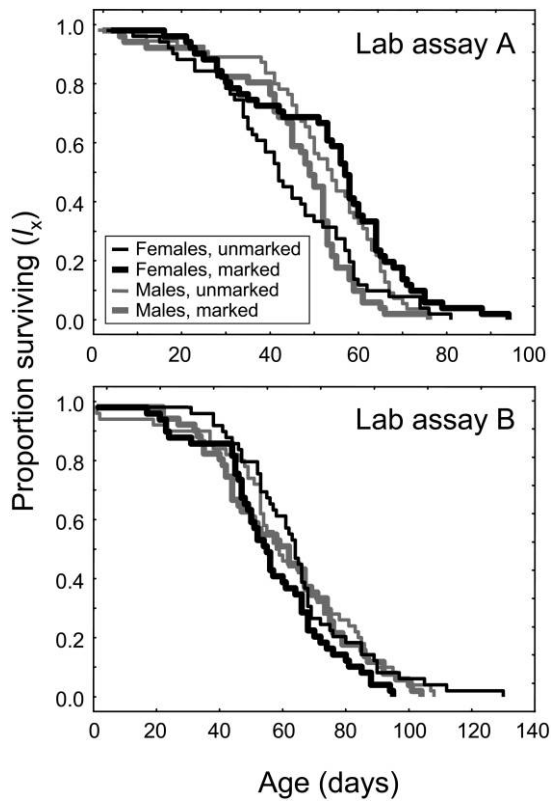

Figure 2: Proportion of the cohort alive as a function of age (days) for marked and unmarked female and male flies in laboratory assays A and B.

Table 1: Parameter estimates from best-fit models for wild and laboratory flies

Assay, sex	a	b	δ	c	L_{med}	L_{max}
Wild:						
Female	−1.20 (.23)	0	**1.6** (.37)	**.22**	3	18
Male	−2.00 (.12)	.170	**1.6** (.28)	**.22**	4	10
Laboratory assay A:						
Female	−5.77 (.003)	.051	…	…	36	63
Male	−6.88 (.001)	.079	…	…	37	56
Laboratory assay B:						
Female	**−5.97 (.003)**	**.041**	…	…	**45**	**78**
Male	**−5.97 (.003)**	**.041**	…	…	**45**	**78**
Laboratory assay C:						
Female	−4.83 (.008)	.047	…	…	26	53
Male	−4.07 (.017)	.036	…	…	21	49

Note: a = Background mortality parameter, with corresponding daily background mortality in parentheses; b = rate of increase in mortality with age; δ = additional death or dispersal parameter for the wild population on the day of release, with corresponding death or dispersal rate in parentheses; c = daily probability of resighting a live fly in the wild; L_{med} = median life span (days); L_{max} = maximum life span (days), for females and males in the wild population and each laboratory assay. Parameter values in bold represent common estimates for both sexes within an assay.

($N_{\text{unmarked}} = 50$, $N_{\text{marked}} = 51$; Cox-Mantel test: test statistic = 0.56, $P > .5$). Likewise, ANOVA failed to detect a significant effect of marking (table 2). We found no effect of reproduction on life span in the laboratory (assay C), either in females ($N_{\text{nonreproducing}} = 196$, $N_{\text{reproducing}} = 193$; Cox-Mantel test: test statistic = −0.01, $P > .5$) or in males ($N_{\text{nonreproducing}} = 198$, $N_{\text{reproducing}} = 202$; Cox-Mantel test: test statistic = −0.35, $P > .5$; fig. 3). Because neither marking nor reproduction had consistent effects on life span, we omitted these factors from the aging model for laboratory flies to maximize power to detect effects of environment on aging.

The best-fit model for the laboratory flies was similar to that for the wild flies, with the exclusion of δ and c,

$$\mu_{i,j}(d) = \frac{1}{1 + \exp(-[a_{i,j} + b_{i,j}d])}, \tag{2}$$

where subscript i denotes the sex and subscript j denotes the laboratory assay. Significant differences between the sexes were found for senescence rates in laboratory assay A (individual cages; ΔAIC = 8.88, $P < .0001$) and laboratory assay C (same-sex and mixed-sex groups; ΔAIC = 4.94, $P = .002$). In laboratory assay B (same-sex groups), however, the non-sex-specific model (AIC = 10,006.0) was a better fit than a model with sex-specific senescence rates (AIC = 10,009.9). The aging rate for males in assay A was greater than the male aging rate in assay B (ΔAIC = 23.2) and assay C (ΔAIC = 35.74). Female aging rate did not differ among laboratory assays. Estimating female senescence rates across all assays as a single parameter was a better model (AIC = 10,008.4) than using assay-specific senescence rates (AIC = 10,009.0).

Comparison of Wild and Captive Flies

For males, aging rate in the wild was greater than aging rate in any of the laboratory assays (ΔAIC ≥ 2.4, $P \leq .03$; fig. 4). The estimated aging rate for wild males was more than twice the estimated aging rate for laboratory assay A and over fourfold higher than the estimates for laboratory assays B and C. For females, there was no significant difference between a model with a common senescence rate for the wild study and assay A as compared with a model with separate senescence rates for the wild study and assay A ($P = .5$). The same is true for the wild study and assay C ($P = .3$). However, in each case, although not significantly different, the best-fit model was the one in which the senescence rate of wild females was set to zero and the senescence rate of laboratory females was set to a positive value (ΔAIC = 1.7 and ΔAIC = 1.4, respectively). These results indicate a lack of power to detect senescence in wild females.

Survival (l_x) curves for the wild flies and for each laboratory assay are shown in figure 5. Median and maximum life spans for flies of both sexes in the wild were far lower than any of the estimates for the captive flies (table 1).

Table 2: ANOVA for effects of assay, sex, and marking on life span of *Telostylinus angusticollis* in the laboratory

Variable	Sum of squares	df	Mean squares	F	P
Assay	15,247	1	15,247	38.512	<.001
Sex	4	1	4	.010	.919
Marking	341	1	341	.861	.354
Assay × sex	10	1	10	.025	.873
Assay × marking	1,266	1	1,266	3.199	.074
Sex × marking	330	1	330	.834	.362
Error	157,965	399	396	…	…

Note: In assay A, flies were maintained individually; in assay B, flies were maintained in same-sex groups.

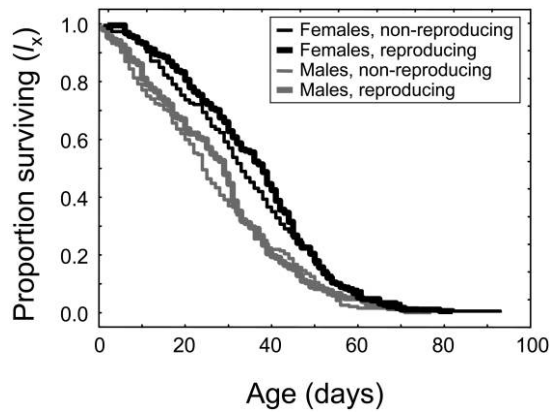

Figure 3: Proportion of the cohort alive as a function of age (days) for female and male flies in same-sex (nonreproducing) and mixed-sex (reproducing) groups in laboratory assay C.

Discussion

We observed a striking contrast in life span and aging rate between wild flies and a captive stock recently established from the same wild population. Despite significant differences among laboratory assays, all demographic estimates for the laboratory flies were clustered together, in contrast with those for the wild flies. In the wild, males aged far more rapidly than their captive counterparts, but wild females showed no significant change in mortality rate with age. Wild flies of both sexes exhibited far higher extrinsic (age-independent) mortality rates and much shorter median and maximum life spans than did captive flies. These results demonstrate the profound influence of

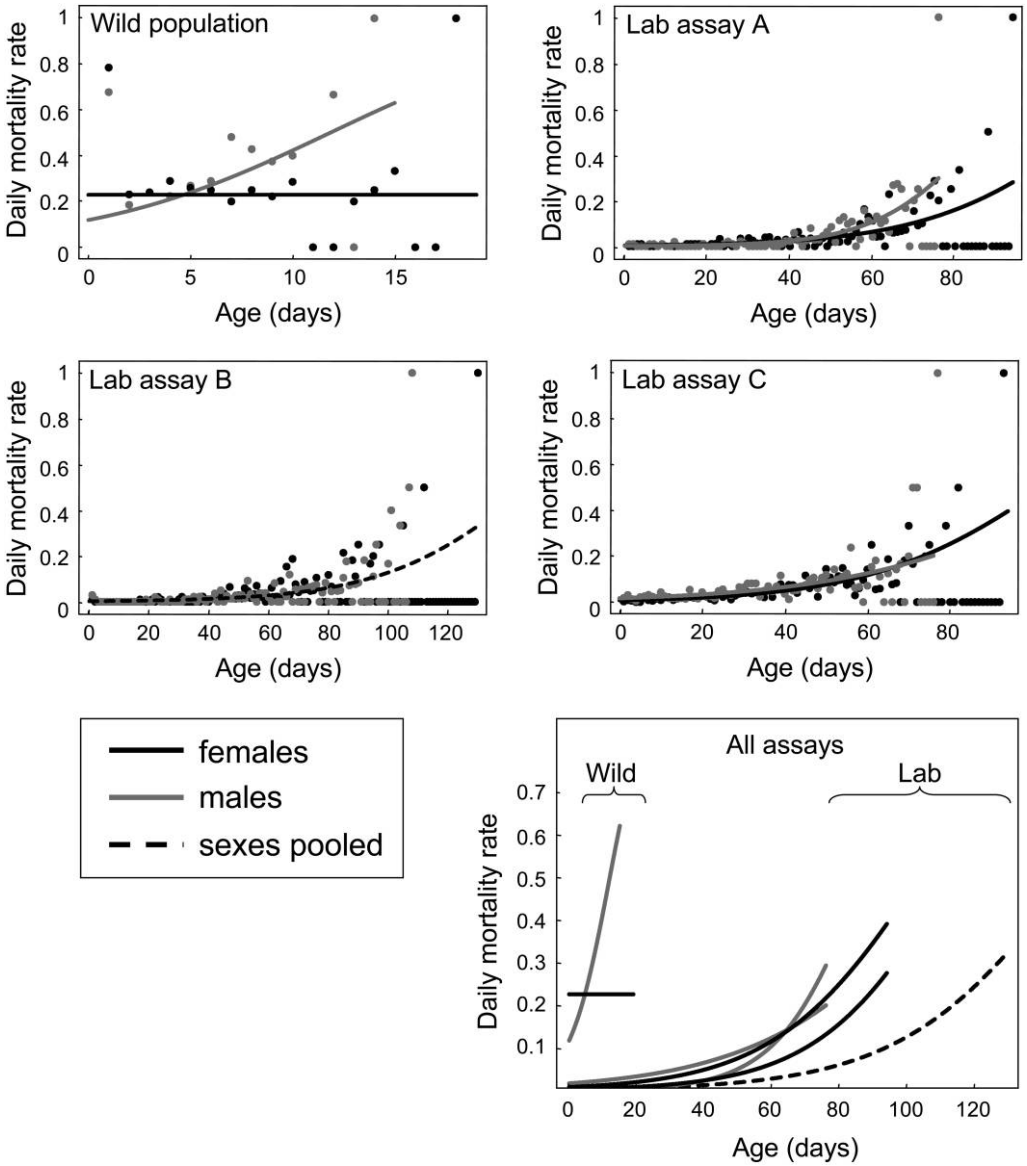

Figure 4: Aging patterns in laboratory assays and the wild population. In the wild, males exhibited very rapid aging, whereas no aging was detected in females. In laboratory assay A, males aged more rapidly than females did. In laboratory assay B, we detected no sex difference in aging rate. In laboratory assay C, females and males likewise exhibited very similar (albeit significantly different) rates of aging. Hazard rate functions from all laboratory assays and the wild population are plotted on common axes in the panel on the bottom right, illustrating the contrast between wild flies and captive flies.

environment on aging rate and life span and suggest that the findings of laboratory studies on short-lived organisms like *Drosophila melanogaster*, *Caenorhabditis elegans*, and mice may be strongly influenced by benign laboratory conditions.

Our design relied on the use of an individual marking technique and we found no consistent evidence for effects of marking on mortality via mechanical damage or paint toxicity. Our experiment does not exclude the possibility that marking increases predation risk in the wild because marked flies may be more conspicuous to visually oriented predators such as birds. Marking could thus inflate estimates of age-independent (background) mortality rate and perhaps affect estimates of aging rate, although it could not create an artifactual aging pattern because the added visibility is age independent. However, in our population,

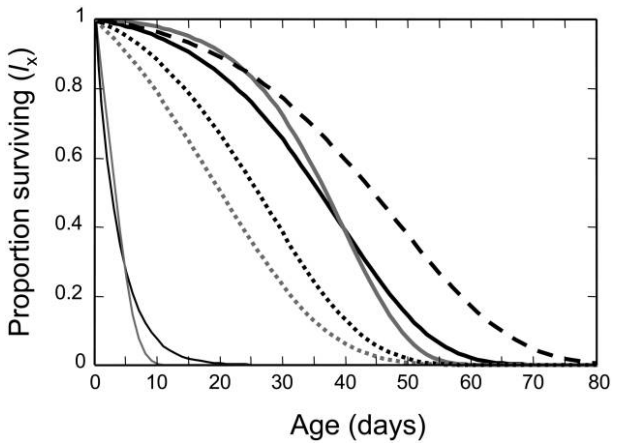

Figure 5: Proportion of the cohort alive as a function of age (days) for female and male flies in the wild population and in the laboratory assays. Curves are based on mortality rates estimated from the best-fit models (not including δ, which is assumed to represent an artifact of the mark-release procedure). Thin solid lines represent wild flies (*black line*, females; *gray line*, males). Thick solid lines represent lab assay A (*black line*, females; *gray line*, males). Thick dashed line represents lab assay B (both sexes). Dotted lines represent lab assay C (*black line*, females; *gray line*, males).

the effect of marking on mortality is likely to be minor because the dominant predators of these flies are small skinks (*Eulamprus tenuis*) that approach their prey with their bellies pressed to the bark (N. Kawasaki and R. Bonduriansky, unpublished data) and thus typically see *Telostylinus angusticollis* individuals (which have extremely long legs) from below or from the side. We observed numerous successful and unsuccessful predation attempts by these skinks but never observed predation attempts by birds (N. Kawasaki and R. Bonduriansky, unpublished data). In addition to added mortality, the initial disorientation and stress associated with marking and release could render flies temporarily more vulnerable to predation and other hazards. Such an effect is one possible cause of the substantial rate of disappearance of marked individuals from the population on the day of release, accounted for in our models by a separate parameter (δ).

As in any mark-release-resight study, individuals disappearing from the population are assumed to have died, but it is usually impossible to exclude the possibility of migration from the study site. However, migration from the study site is unlikely in our population for several reasons. First, we studied an isolated population within a small (~1 ha) urban park surrounded by built-up areas. The nearest *T. angusticollis* population outside this park is ~1.5 km away (R. Bonduriansky, unpublished data). Second, the rate of migration between locations within the study site was very low: ~1% of marked individuals (5 females and 4 males) migrated between the two release sites (~70 m apart), and ~2% of individuals (13 females and 3 males) migrated between a major aggregation tree and other trees or to a fence ~10 m away. Thus, immigration probably contributed very little to the disappearance of marked individuals from our study population. Moreover, migration rates between the two release sites were similar for males and females, so it is unlikely that differential migration rates contributed to observed sex differences in life span and aging rate of wild flies.

The far more risky and stressful environment encountered by wild flies clearly accounts for the much higher background mortality rate observed in the wild. But how can we account for the difference in aging rate (i.e., rate of increase in mortality rate with age) between males in the wild and those in the laboratory? Two nonexclusive processes could contribute to this difference. First, somatic deterioration may proceed at the same rate in wild flies and captive flies, but the harsh environment experienced by wild males may impose intense selection on somatic condition and, thus, expose the somatic effects of aging to a greater extent in wild flies. Wild males must avoid predators and deal with temperature changes and inclement weather while competing for mates. A slight decline in somatic condition may therefore result in a far greater increase in the risk of death in the wild than in the sheltered, benign laboratory environment. Second, somatic deterioration may actually proceed more rapidly in the harsh environment experienced by wild flies. For example, stress associated with changing temperature, unstable food supply, parasites, and the vigorous physical activity involved in predator avoidance and sexual competition could result in rapid accumulation of somatic damage and cause accelerated aging in individuals in the wild compared with individuals in the laboratory.

Curiously, we were unable to detect a significant increase in mortality rate with age in wild females. This apparent difference between the sexes is likely to reflect contrasting reproductive strategies (see Vinogradov 1998; Bonduriansky et al. 2008) and, in particular, stronger condition dependence of mortality risk in males than in females. Wild males spend much of their time walking through aggregation sites on tree trunks, fighting rivals, and attempting to copulate with females (see Bonduriansky 2006, 2007). Several predatory skinks (*E. tenuis*) were typically seen hunting for *T. angusticollis* on each tree where flies aggregated (N. Kawasaki and R. Bonduriansky, unpublished data). These visual predators may target males disproportionately because their greater activity makes them more visible. Similarly, male-male combat may contribute to the accumulation of somatic damage (see Bonduriansky and Brassil 2005). Both factors may strengthen the condition dependence and age dependence of mortality risk

(as well as our power to detect aging) in males relative to females, who spend much of their time sitting nearly motionless while lapping up resin near damaged areas of tree trunks (N. Kawasaki and R. Bonduriansky, unpublished data). Although we suspect that we would have detected aging in wild females given a sufficiently large sample size, our results and power analysis suggest that aging is of relatively little importance for wild females at the population level because age-independent mortality overwhelms the signal of age dependence in mortality rate. This null result thus suggests an interesting biological difference between the sexes that calls for further investigation. Females also exhibited slower aging and longer median life spans than males in two of the three laboratory assays.

Our findings suggest that a consideration of environmental effects can reconcile apparently contradictory predictions and observations from the literature regarding aging in wild populations. Life-history theory predicts that animals who are subject to high extrinsic mortality rates will evolve rapid aging rates (Williams 1957; Hamilton 1966; Kirkwood and Rose 1991). However, many gerontologists have argued that aging will be difficult or impossible to detect in short-lived animals in the wild because very few individuals survive to old age and advanced senescence (Comfort 1979; Hayflick 2000; Kirkwood and Austad 2000). This reasoning is based on the implicit assumption that mortality rate increases at the same rate in wild populations as in captive ones. For example, if the maximum observed life span in a wild insect population is 20 days, it is assumed (on the basis of observations of 20-day-old captive animals) that the increase in mortality rate with age in the oldest age class observed in the wild will be negligible. Our findings suggest, however, that whether aging can be detected in a wild cohort of reasonable size may depend on the balance between condition-independent extrinsic mortality, which tends to obscure aging by eliminating older individuals, and the tendency for environmental factors to expose or accelerate somatic deterioration, which will strengthen the signal of aging. The expectation that aging will be undetectable as a result of high extrinsic, condition-independent mortality agrees with our results for wild *T. angusticollis* females, whereas the prediction of rapid aging is supported by our findings for wild males.

A consideration of the role of environment may also reconcile direct evidence of rapid aging in wild insects (this study; Bonduriansky and Brassil 2002) with evidence from common-garden experiments with *D. melanogaster* and mice indicating that lines recently derived from wild populations possess the genetic capacity for relatively slow aging and long potential life spans compared with laboratory-adapted lines (Sgrò and Partridge 2000; Linnen et al. 2001; Miller et al. 2002). Strong selection on condition in wild populations may favor genes with pleiotropic effects that result in relatively slow aging rates and long life spans under benign laboratory conditions (Abrams 1993; Linnen et al. 2001; Williams and Day 2003; Reznick et al. 2004; Bronikowski and Promisiow 2005). However, our results suggest that the harsh environment experienced by wild populations may strengthen the age dependence of mortality rate and greatly reduce life expectancy, and that such effects may be large enough to negate the genetic potential for slow aging and long life in wild-adapted organisms. A combination of approaches may therefore be required. Inferences about age-dependent traits or fitness components rely on assumptions about life expectancy and aging rate in natural environments, necessitating direct estimates of these parameters in wild populations. Empirical evidence suggests that wild animals suffer higher mortality rates and, sometimes, more rapid aging than their captive counterparts do (this study; Ricklefs 2000; Bonduriansky and Brassil 2002; Bronikowski et al. 2002), that mortality rates and aging rates can covary with phenotypic traits such as body size (Bonduriansky and Brassil 2005) and differ between the sexes (this study), and that different components of performance may decline at different rates (Reznick et al. 2004). However, common-garden experiments (Sgrò and Partridge 2000; Linnen et al. 2001; Miller et al. 2002; Reznick et al. 2004) can expose genetic differences in potential aging rate and life span and can shed light on the evolution of life-history strategies under contrasting selection.

Although they are smaller than the contrasts between the wild population and the laboratory populations, differences between laboratory assays were substantial; the causes of this variation, however, are unclear. Assays A and B were performed simultaneously, albeit using different-sized cages (75 mL in assay A vs. 250 mL in assay B), whereas assay C was performed later. Thus, both housing conditions and random temporal variation in ambient temperature, humidity, and diet may have contributed to variation in life expectancy among assays. Like the difference between wild and captive flies, the variation among laboratory assays reflects the sensitivities of aging and life span to environmental conditions. Interestingly, we failed to detect any survival cost of reproduction in assay C, a result that appears to run counter to the expectation that reproductive activity accelerates aging and reduces life span through both latent and immediate costs (Ernsting and Isaaks 1991; Kirkwood and Rose 1991; Tatar et al. 1993; Kotiaho 2001). However, the costs of reproduction may be low and difficult to detect in the benign and resource-rich laboratory environment (Harshman and Hoffmann 2000; Lindstrom 2001; Marden et al. 2003; Messina and Fry 2003; Barbraud and Weimerskirch 2005) and were

perhaps countered to a substantial degree by as yet unknown costs of virginity (Carey et al. 2002). The presence of the oviposition medium in the mixed-sex treatment only may also have enhanced the longevity of reproducing flies in assay C.

Three previous studies have reported direct comparisons of aging rate and life span in the wild and in laboratory environments. A study on the perennial plant *Plantago lanceolata* reported a more rapid increase in mortality rate with age in the wild than in a greenhouse (Roach 2001). In contrast, an analysis of data on 28 species of birds showed much higher background mortality rates in the wild but little evidence of a difference in aging rate between wild and captive populations (Ricklefs 2000). Similarly, in baboons, a demographic comparison of two wild populations with a captive population reported a substantially higher background mortality rate in one wild population but similar mortality rates in the other wild and captive populations, and little difference between the three populations in aging rates (Bronikowski et al. 2002). Because the studies on birds and baboons could not control for genetic factors, it is unclear to what extent the observed interpopulation variation reflects environmental factors versus genetic factors. Moreover, the study on baboons compared demographic parameters for females only. More studies of this kind (ideally, comparing demographic parameters of genetically similar cohorts in contrasting environments) are needed to illuminate variation between sexes and taxa in the effects of environment on life span and aging.

Several previous studies have reported mean or median life spans for wild insects (Fincke 1982, 1986; McCauley 1983; Banks and Thompson 1985; Hafernik and Garrison 1986; Elgar and Pierce 1988; Bonduriansky and Brassil 2002). These estimates—for several damselfly species, a milkweed beetle, and a piophilid fly—range from 2 to 16 days. To our knowledge, only one previous study formally tested for aging in a wild insect population: Bonduriansky and Brassil (2002) observed a rapid increase in mortality rate and a decrease in reproductive rate with age, similar to our study's results for male *T. angusticollis*. The study of aging and life span in wild populations (particularly in small animals) presents considerable technical challenges. The strong plasticity of aging rate and life span presents the additional challenge of accounting for temporal and spatial variation in environmental effects on the expression of these traits.

This study provides direct evidence that environmental effects on the expression of life span and aging result in large differences between wild and captive animals and that these environmental effects are sex specific (i.e., wild males age much more rapidly than do captive males, but no such pattern is evident in females). These findings point to the possibility that many laboratory comparisons of net fitness or components of fitness may be substantially off the mark. For example, many laboratory studies have reported sex differences in life span or aging, but our findings suggest that a comparison of the sexes in the wild (or even under altered conditions in the laboratory) could yield qualitatively different results. We suggest that the same problem could potentially confound within-sex comparisons of net fitness or fitness components for different genotypes, phenotypes, age classes, species, and so forth; if these subjects are also affected differently by environmental factors, then laboratory populations and wild populations could yield qualitatively different patterns. Such effects may thus complicate the interpretation of a wide range of laboratory studies, including those that compare fitness components such as early-life and late-life performance (e.g., Carey et al. 2002; Hunt et al. 2004) or direct and indirect selection on mating preferences (e.g., Hosken and Tregenza 2005), those that relate fitness to genetic or phenotypic variation (e.g., Arnqvist and Nilsson 2000), and those that compare fitness components between sexes (e.g., Chippindale et al. 2001; Ballard et al. 2007). Our findings underscore the importance of investigating life span and aging in the wild as well as under a range of laboratory conditions.

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