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Reproductive and post-reproductive life history of wild-caught *Drosophila melanogaster* under laboratory conditions

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Abstract

The life history of the fruit fly (*Drosophila melanogaster*) is well understood, but fitness components are rarely measured by following single individuals over their lifetime, thereby limiting insights into lifetime reproductive success, reproductive senescence and post-reproductive lifespan. Moreover, most studies have examined long-established laboratory strains rather than freshly caught individuals and may thus be confounded by adaptation to laboratory culture, inbreeding or mutation accumulation. Here, we have followed the life histories of individual females from three recently caught, non-laboratory-adapted wild populations of *D. melanogaster*. Populations varied in a number of life-history traits, including ovariole number, fecundity, hatchability and lifespan. To describe individual patterns of age-specific fecundity, we developed a new model that allowed us to distinguish four phases during a female's life: a phase of reproductive maturation, followed by a period of linear and then exponential decline in fecundity and, finally, a post-ovipository period. Individual females exhibited clear-cut fecundity peaks, which contrasts with previous analyses, and post-peak levels of fecundity declined independently of how long females lived. Notably, females had a pronounced post-reproductive lifespan, which on average made up 40% of total lifespan. Post-reproductive lifespan did not differ among populations and was not correlated with reproductive fitness components, supporting the hypothesis that this period is a highly variable, random 'add-on' at the end of reproductive life rather than a correlate of selection on reproductive fitness. Most life-history traits were positively correlated, a pattern that might be due to genotype by environment interactions when wild flies are brought into a novel laboratory environment but that is unlikely explained by inbreeding or positive mutational covariance caused by mutation accumulation.

Introduction

Understanding variation in fitness-related traits, the direct targets of natural selection, is the major aim of studies in life-history evolution (Stearns, 1992; Flatt & Heyland, 2011). Traditionally, many life-history studies have been performed in the laboratory because it is practically very difficult, or even impossible, to measure life-history traits by following many individuals in the wild over their lifetime while at the same time controlling for confounding factors (Pekkala *et al.*, 2011).

The probably most frequently used organism in laboratory studies of life history and ageing is the fruit fly, *Drosophila melanogaster*, due to its short life cycle, ease of culture and powerful genetic tools (Prasad & Joshi, 2003; Flatt & Schmidt, 2009). Life-history studies using

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this model include a vast number of experiments on *de novo* mutations and standing genetic variation (Houle *et al.*, 1994; Pletcher *et al.*, 1998); phenotypic plasticity and genotype by environment (G×E) interactions (Chippindale *et al.*, 1993); pleiotropy and trade-offs (Stearns & Partridge, 2001; Flatt, 2011); experimental evolution and artificial selection (Luckinbill *et al.*, 1984; Zwaan *et al.*, 1995; Partridge *et al.*, 1999; Stearns *et al.*, 2000; Rose *et al.*, 2004); and the molecular mechanisms of ageing (Tatar *et al.*, 2003; Partridge *et al.*, 2005; Paaby & Schmidt, 2009).

An important consideration in experiments of *Drosophila* life history is whether to study populations collected directly from the wild in the laboratory or whether to perform assays on long-established laboratory-adapted stocks (Harshman & Hoffmann, 2000). Fly strains freshly brought into the laboratory from the wild experience a novel environment, which can lead to selection for laboratory adaptation (Matos *et al.*, 2000; Sgrò & Partridge, 2000), confounding G×E interactions and/or spurious correlations among life-history traits (Service & Rose, 1985; Clark, 1987). For example, whereas many quantitative genetic studies and selection experiments based on laboratory stocks found robust evidence for negative correlations consistent with trade-offs (for a review see Stearns & Partridge, 2001), several studies using wild-caught flies found either positive or no correlations (Giesel *et al.*, 1982; Giesel, 1986). However, some of these studies used wild stocks that were inbred after they were brought into the laboratory, which might give rise to spurious positive correlations among fitness components (Rose, 1984). Moreover, exposing wild flies to a novel laboratory environment can cause confounding G×E interactions that might change the sign of correlations. These issues prompted Service & Rose (1985) and Clark (1987) to argue that wild-caught flies may not be suitable for life-history studies; instead, they suggested experiments should be performed using laboratory-adapted stocks, which are near or at evolutionary equilibrium, especially when estimating life-history correlations or performing artificial selection.

There is, however, a major flip side to the use of long-term laboratory stocks. An important problem is that such stocks are typically maintained on a discrete 2-week culture interval, which imposes inadvertent selection for rapid development and increased fecundity (Sgrò & Partridge, 2000; Houle & Rowe, 2003), but which completely relaxes selection on the later part of adult life, thereby allowing the accumulation of late-acting deleterious mutations (Promislow & Tatar, 1998). Although it is likely that, in natural populations, reproductive success remains high beyond 4 days of adult age, alleles that affect life history after 4 days of adulthood are not directly exposed to selection under such a regime (Promislow & Tatar, 1998). This might in turn predispose fly stocks to exhibit negative correlations between early- and late-age fitness traits. Several observations are consistent with this notion. For example, laboratory adaptation of wild-caught flies can lead to an increase in early fecundity and a decline in longevity (Sgrò & Partridge, 2000). Similarly, Linnen *et al.* (2001) found that the lifespan of a wild-caught strain was almost identical to that of a line that had undergone nearly 20 years of laboratory selection for increased lifespan, suggesting that selection restored wild-type levels of lifespan by removing deleterious mutations that had accumulated under long-term 2-week culture. Thus, by using laboratory-adapted strains maintained in standard culture, one might risk to observe negative correlations that would not be found in strains freshly derived from the wild, calling for a re-evaluation of the notion that one must preferentially use laboratory-adapted stocks in studies of *Drosophila* life history (Linnen *et al.*, 2001). Indeed, the majority of life-history studies in *Drosophila* have been performed with laboratory stocks, so that relatively little is known about the life history of wild-caught, non-laboratory-adapted populations (but see, e.g. Draye *et al.*, 1994; Schmidt & Paaby, 2008).

Here, we provide a detailed analysis of basic but still poorly understood patterns of reproductive and post-reproductive life history of wild-caught, non-laboratory-adapted *D. melanogaster*. In brief, we followed the life histories of a large number of individual females, derived from three recently caught, geographically distinct wild populations, over their lifetime. We measured ovariole number, fecundity, hatchability and lifespan of these flies under optimal laboratory conditions and, from these data, estimated individual lifetime fecundity, total lifetime production of viable eggs and the length of the reproductive and post-reproductive period. Although our experiments did not permit us to exclude G×E interactions caused by the novel laboratory environment, all life-history assays were performed on outbred wild flies shortly after they were brought into the laboratory, thus allowing us to avoid confounding effects of long-term laboratory culture and adaptation, inbreeding and mutation accumulation. We were primarily interested in using our data to investigate three problems.

First, previous studies of wild-caught flies have suggested that wild flies often exhibit superior performance as compared to long-term laboratory stocks (Dobzhansky *et al.*, 1964; Giesel, 1986; Stearns, 1992), but whether this is a result of inbreeding, mutation accumulation or long-term laboratory adaptation of the laboratory stocks remains largely unclear. Because our experimental design allowed us to exclude confounding effects of inbreeding, mutation accumulation and long-term laboratory adaptation, we asked whether exposure of wild flies to a novel laboratory environment might be sufficient to lead to predominantly positive life-history correlations (Service & Rose, 1985). A novel laboratory environment might generate positive correlations in two, not mutually exclusive ways. The first possibility is that genotypes that are either fortuitously pre-adapted or maladapted

to the novel environment have generally improved or decreased fitness, respectively, which would produce positive correlations (Service & Rose, 1985). The second possibility is that negative correlations are absent or masked under presumably optimal and protected laboratory conditions, for example because resource acquisition is not limiting (van Noordwijk & de Jong, 1986). Even though unmanipulated laboratory stocks can show the same phenomenon and the absence of negative phenotypic correlations does not necessarily imply the absence of genetic trade-offs, finding predominantly positive phenotypic correlations would support the notion that novel environments bias life-history correlations towards positive values (Service & Rose, 1985).

Second, fecundity data are typically collected by counting the total number of eggs produced by cohort of females in each age class, but by averaging across many individuals, this method obscures variation in age-specific fecundity among individuals (Novoseltsev *et al.*, 2004; Khazaeli & Curtsinger, 2010a). This approach therefore limits insights into individual patterns of age-specific fecundity, reproductive senescence and post-reproductive lifespan. Consequently, several studies have analysed individual variation in fecundity by fitting a simple model with three stages (Novoseltsev *et al.*, 2002, 2003, 2004, 2005): (1) a period during which egg production is zero, (2) a phase during which egg production plateaus at a steady-state level and (3) a phase during which the rate of egg production decreases exponentially. Because this model has so far only been applied to laboratory stocks, we were interested in testing whether it also provides a good description of individual fecundity among wild-caught females. Visual inspection of our data revealed, however, that this model does not fit our data well, and we therefore examined a series of simple, heuristic fecundity models to determine which model describes our data best.

Third, a small number of studies have reported that females of long-term laboratory stocks terminate egg production a few days prior to death (Rogina *et al.*, 2007; Mueller *et al.*, 2009; Khazaeli & Curtsinger, 2010a), yet the evolutionary significance of this pattern remains unclear. Moreover, it remains unknown whether wild-caught flies kept under protected laboratory conditions can also outlive their reproductive potential, or whether post-reproductive lifespan is an artefact of laboratory adaptation and long-term culture. In organisms such as fruit flies, where there can be no maternal post-reproductive contribution to offspring fitness (i.e. no maternal care for last-born offspring, no fitness benefits through caring for offspring of relatives via kin selection), post-reproductive lifespan is thought to represent a nonadaptive, variable and random 'add-on' at the end of the life history (Reznick *et al.*, 2006). If so, we might expect that post-reproductive lifespan represents an indirect correlate of selection for fitness components that are adaptive earlier in life, or a by-product of different rates of ageing of the soma and the reproductive system (Reznick *et al.*, 2006). To examine these predictions, we asked whether populations that differ in overall life history exhibit significant variation in the length of post-reproductive lifespan and whether post-reproductive lifespan is correlated with early life-history components.

Materials and methods

Fly populations and maintenance

We used three recently collected outbred wild populations of *D. melanogaster* from: (i) Austria (Kahlenberg, Vienna: 48.28°N, 16.33°E; collected by P. Klepsatel in October 2010), (ii) South Africa (Phalaborwa: 23.93°S, 31.12°E; collected by J. Pool in July 2010) and (iii) Zambia (Siavonga: 16.53°S, 28.72°E; July 2010; collected by J. Pool in July 2010). Prior to life-history assays, we maintained flies as outbred populations in population cage culture with overlapping generations, with a generation time of approximately 3 weeks, to avoid inadvertent selection by, and laboratory adaptation to, standard 2-week culture (Promislow & Tatar, 1998). For the Austrian population, 200 freshly collected females and males were introduced into a population cage and maintained for two overlapping generations prior to the assays; for the Zambian and South African populations, cages were initiated with ten females and ten males from each of 30 (Zambia) or seven (South Africa) isofemale lines and maintained for four overlapping generations prior to the assays. Adaptation to laboratory conditions ('domestication') can occur quite rapidly in *D. melanogaster*, that is, on the order of 8–10 generations or perhaps less (Frankham & Loebel, 1992), but given the small number of nonoverlapping generations (2–4) prior to our assays, it is unlikely that our populations experienced strong laboratory adaptation. Flies were maintained on a standard cornmeal–agar–yeast (2%) diet supplemented with active yeast, at 25 °C and 60% relative humidity, with a 12 h:12 h light/dark cycle.

Life-history assays

We followed individual females throughout their lifetime and recorded the following life-history traits: daily fecundity, age at death, ovariole number and the hatchability of eggs laid by experimental females. From these data, we estimated lifetime fecundity, total lifetime production of viable eggs, the length of the reproductive and post-reproductive period and total lifespan. Due to the large number of flies involved, we could not measure all populations simultaneously. South African and Zambian flies were assayed simultaneously; however, Austrian flies were assayed 2 months earlier.

To obtain experimental individuals for life-history assays, we collected for each population 500 eggs laid within 2 h and placed them into vials, each vial with

50 eggs, thereby avoiding larval crowding. Upon eclosion, we collected adult flies over a 24-h period and set up vials with one female and two males per vial ($N = 200$ vials for both Austria and Zambia; $N = 150$ for South Africa) on a standard medium with active yeast sprinkled on top of the medium (roughly 10 mg per vial), at 25 °C and 60% relative humidity, with a 12 h:12 h light/dark cycle. Flies were transferred to fresh vials daily after 24 h, at which time we also counted eggs laid since the last transfer, scored vials for dead females and recorded female age at death. Females that escaped or got stuck in the medium were removed for statistical analysis. Dead or escaped males were replaced immediately. Every 3 weeks, we replaced *all* males with younger ones (adult age 1–14 days) to ensure that female fecundity and offspring viability were not affected by a failure of old males to successfully fertilize females; males were also replaced in case that the eggs laid by a given female were not viable.

After egg counting, all eggs laid by a given female were kept to determine the number of hatched vs. unhatched eggs 48 h later. For the Austrian population, we measured hatchability (i.e. the proportion of eggs that hatched and survived to larval instar 1) at days 5, 15 and 25 of adulthood and then every day thereafter; however, because hatchability usually decreased quite rapidly to zero as a function of age (typically within < 25 days), we could not precisely determine the end of the reproductive period for all flies from this population. We therefore excluded the Austrian population from analyses of reproductive and post-reproductive period and total lifetime production of viable eggs. For the Zambian and South African populations, we determined hatchability in 5-day intervals until day 20 of adulthood and then daily thereafter. For these populations, we also inspected vials daily by eye: whenever we observed an obvious decrease in hatchability for a given vial (presence of > 5 nonviable eggs), we measured hatchability daily. For the Zambian and South African populations, we interpolated missing data in between measurements until day 20 of adulthood by using the values from the next measurement.

Upon death, we dissected all females in water under a dissecting stereo microscope and counted the number of ovarioles in each ovary. Ovariole number was defined as the sum of ovarioles from both ovaries. We also visually inspected the physiological state of the ovaries (e.g. the stage of egg chamber development; egg retention); egg chambers were staged following King (1970).

Models of individual fecundity and hatchability

We fitted eight-three-stage individual fecundity models to our data on age-specific egg production, where stage 1 was characterized by a linear increase in fecundity or by zero egg production; stage 2, by a reproductive plateau or a linear decrease in fecundity; and stage 3, by an exponential decrease in fecundity or by zero egg production (for details see Supporting Materials and Methods).

The best-fitting model was model 8 ('linear-linear-exponential'), with a linear increase in fecundity at stage 1, followed by a linear decrease in fecundity at stage 2 and an exponential decrease in fecundity at stage 3. The model is given by the following equation:

$$f_8(t) = I_{[t<\mathrm{On}_1]}\frac{t}{\mathrm{On}_1}S_0 + I_{[\mathrm{On}_1 \le t<\mathrm{On}_2]}(S_0 + c(t-\mathrm{On}_1)) + I_{[\mathrm{On}_2 \le t]}(S_0 + c(\mathrm{On}_2-\mathrm{On}_1))\exp(-\alpha(t-\mathrm{On}_2))$$

where f_8 is fecundity at time (age) t; I is the indicator function; On_1 represents the length of the period of 'reproductive maturation', which is characterized by a steep linear increase in fecundity up to a maximum level called 'peak fecundity', S_0; c denotes the rate of linear decrease in fecundity; On_2 represents the onset of the phase of exponential decrease in fecundity; and α represents the rate of exponential decrease in fecundity. Together, c, On_2 and α characterize the period of 'reproductive senescence'. Note that this phase of senescent decline in fecundity ultimately leads to a period of zero egg production at the end of life, the 'post-oviposition period', which is followed by death.

We used the daily fecundity data to estimate the parameters of all eight models, with all parameters being estimated for each fly independently. Parameters were chosen so that the squared error of the prediction for a given model m was minimized:

$$\mathrm{Err}_m = \sum_{t=1}^{D}(f_m(t) - O(t))^2$$

where D is the day of death (age at death), and $O(t)$ is the observed fecundity at day (age) t. The error for a given model was calculated as the sum of errors for all flies from a given population. Error minimization was performed using the function 'optim' in R v.2.12.2 with the default algorithm from Nelder & Mead (1965), which is suitable for nondifferentiable functions. For each fly and model, we used at least 10 starting values to avoid potential local minima. Furthermore, to improve convergence towards biologically meaningful values, we set the following bounds on parameter values: $\mathrm{On}_1 \ge 1.5$, $\mathrm{On}_2 > \mathrm{On}_1$, $\alpha > 0.01$, $c \le 0$ and $f > 0$. Parameter estimates were analysed statistically using best-fit parameter estimates from single flies within a given population. In rare cases, we could not properly estimate parameter values for particular individuals and treated them as missing values.

To investigate patterns of senescent decline in egg hatchability as function of maternal age, we fitted two simple heuristic models to our hatchability data from

the South African and Zambian populations (see Supporting Materials and Methods for details).

Statistical analysis

To test for among-population variation in life-history traits (except survival) and in model parameter estimates, we used univariate one-way ANOVA, with 'population' as a fixed factor, followed by Tukey's HSD *post hoc* tests; note that treating population as a random factor did not qualitatively change the results. For mortality data, we first determined the best-fitting mortality distribution and then used the Kaplan–Meier method to estimate survivorship; pairwise differences in survival among populations were tested using nonparametric log-rank and Wilcoxon rank-sum tests, followed by Bonferroni correction for multiple testing.

For traits measured repeatedly on the same individuals (age-specific fecundity over time; hatchability as a function of maternal age), we used repeated-measures MANOVA (von Ende, 2001), using 'population' as the among-treatment factor, 'time' (age) as the within-treatment factor, including the 'population × time' interaction. Hatchability data were arcsine-transformed prior to repeated-measures MANOVA.

To examine linear relationships between life-history traits, we used ANCOVA, with 'population' as a fixed factor, the covariate (i.e. the independent or 'predictor' trait), and including the 'population × covariate' interaction (homogeneity of slopes test). In addition, we also calculated Spearman rank correlations between pairs of traits for each population separately, as well as between life-history traits and model parameter estimates. Because we estimated a large number of correlations, we corrected for multiple testing by applying the conservative Bonferroni correction to each correlation table. Note that applying this correction to *all* tests across *all* tables did not qualitatively change the results. All analyses were performed with JMP v.8.0.2 (SAS, Raleigh, NC, USA).

Results

Phenotypic variation and covariation in life-history traits

We first characterized variation and covariation for a suite of major life-history traits among the three populations; here, we just give a brief summary of these results (see Supporting Results and Discussion for details). Populations varied in several components of reproductive life history, including ovariole number, different measures of age-specific fecundity, total fecundity, as well as egg production per ovariole (Supporting Results and Discussion, Figs S1 and S2; for an analysis of *individual* fecundity profiles see below). Populations did not differ in egg hatchability, which declined with increasing maternal age (Supporting Results and Discussion, Table S1, Fig. S3). Variation for total lifespan among populations was negligible, with a weak trend towards differences in early adult survival (Supporting Results and Discussion, Fig. S4; for an analysis of reproductive vs. post-reproductive lifespan see below). From our data on fecundity, hatchability and adult survival, we were also able to estimate lifetime reproductive success (LRS, i.e. lifetime number of viable offspring) and observed that populations varied significantly in this proxy measure of individual fitness (Supporting Results and Discussion).

Most life-history traits were positively correlated with each other in all populations (Supporting Results and Discussion, Tables S2 and S3). For example, as expected, ovariole number was positively correlated with early daily fecundity (Supporting Results and Discussion, Fig. S2), and lifespan was positively correlated with total lifetime fecundity (Supporting Results and Discussion, Fig. S5). However, trait pairs typically expected to exhibit negative correlations (phenotypic trade-offs), such as early fecundity and lifespan, *also* showed positive correlations (see Supporting Results and Discussion). Thus, we failed to find evidence for trade-offs at the phenotypic level.

Individual patterns of age-specific fecundity

In addition to our analysis of variation and covariation in life-history traits based on *average* estimates across many individuals, we also analysed *individual* life-history patterns by fitting simple models to the age-dependent trajectories of fecundity and hatchability (see Supporting Results and Discussion) of individual females.

Of the eight models we examined, age-specific fecundity was best described by a model with a quite steep linear increase in egg production early in life, followed by a slow linear decrease in fecundity and ending with a phase of exponential decrease in fecundity late in life (model 8, 'lin-lin-exp'; see Table S4; Fig. 1). On the basis of our best-fit model, we can therefore biologically distinguish four phases during a female's life: (i) a period of reproductive maturation, characterized by a steep linear increase in egg production levels up to a maximum value ('peak fecundity'), which is typically reached within the first 3–4 days of adulthood; (ii) a period of slow linear decrease in fecundity; (iii) a period of exponential decrease in fecundity; and, finally, (iv) a post-ovipository period, during which no eggs are laid anymore and which is followed by death.

Notably, the model proposed by Novoseltsev *et al.* (2002, 2003, 2004, 2005) (model 1, '0-plateau-exp'; see Supporting Materials and Methods) did not fit our data well. In particular, our empirical data and best-fit model failed to confirm the existence of a fecundity plateau postulated by these authors; instead, we found clear evidence for a pronounced fecundity peak (Fig. 1). The lack of a plateau is also apparent from Fig. 2,

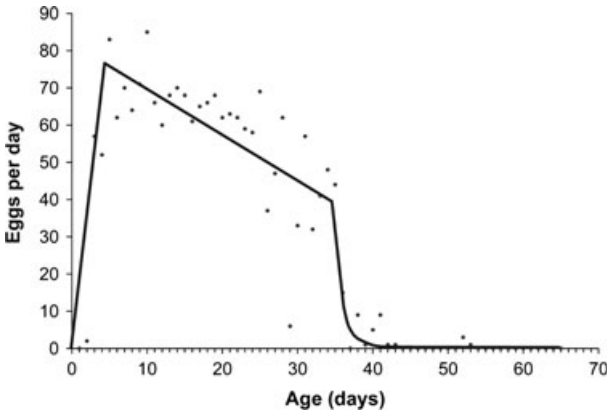

Fig. 1 Best-fit model of individual fecundity (model 8). Daily fecundity scores are shown as black dots. Our model biologically distinguishes four distinct periods in a female's life: (i) a steep linear increase in egg production levels ('reproductive maturation') up to a maximum ('peak fecundity'); (ii) a slow linear decrease in fecundity; (iii) an exponential decrease in fecundity; and (iv) a period during which no eggs are laid anymore ('post-ovipository' period), followed by death. The example shown here is from a single, representative female.

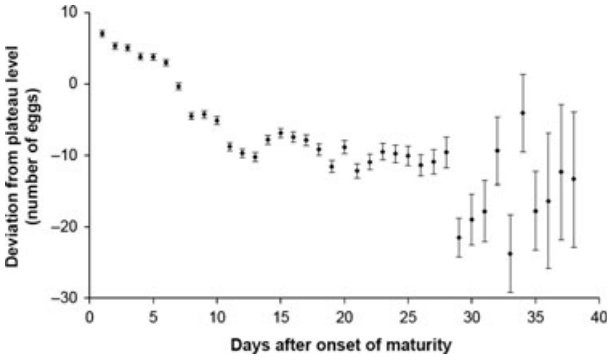

Fig. 2 Average deviation of age-specific fecundity scores from a fecundity plateau. There is a clear lack of fit to a plateau; our data and model thus fail to confirm the existence of a fecundity plateau. Error bars represent standard deviations.

which shows the deviation of age-specific fecundity values from a plateau. Thus, even at the individual level, female flies exhibit clear-cut fecundity peaks.

We next estimated the parameters from our best-fit model and compared these estimates among populations. Populations differed significantly in the length of their reproductive maturation period; Austrian flies had the shortest (2.91 ± 0.05 days); South African, intermediate (3.6 ± 0.06); and Zambian flies, the longest period of maturation (4.1 ± 0.06) ($F_{2,471} = 119.69$, $P < 0.0001$; Tukey's HSD: $P < 0.05$, A ≠ SA ≠ Z); thus, Austrian flies reached peak fecundity earliest as compared to South African and Zambian flies. In line with our results on early daily fecundity (see Supporting Results and Discussion), populations also differed consistently in peak fecundity (A: 103.46 ± 1.63 eggs, SA: 94.9 ± 1.89, Z: 88.5 ± 1.68; $F_{2,484} = 20.61$, $P < 0.0001$; Tukey's HSD: $P < 0.05$, A ≠ SA ≠ Z). Whereas populations did not vary in the rate of linear decrease in fecundity ($F_{2,471} = 1.15$, $P = 0.32$), they differed in the onset of the period of exponential decrease in fecundity (A: 25.0 ± 0.62 days, SA: 22.65 ± 0.76, Z: 16.94 ± 0.64; $F_{2,441} = 42.3$, $P < 0.0001$; Tukey's HSD: $P < 0.05$, A ≠ SA ≠ Z) and the rate of exponential decrease in fecundity (A: 4.48 ± 0.56; SA: 6.07 ± 0.69; Z: 2.82 ± 0.59; $F_{2,441} = 6.51$, $P = 0.0016$; Tukey's HSD: $P < 0.05$, SA ≠ A = Z), suggesting that South African flies had the strongest senescent decline in fecundity.

We also investigated correlations between these parameter estimates and life-history traits (Table S5). Peak fecundity was positively correlated with both early daily fecundity and early daily fecundity per ovariole, but not significantly correlated with the length of the ovipository period, the duration of the reproductive period or total lifespan. Peak fecundity was negatively correlated with the slope of the linear decrease in fecundity, suggesting that flies with higher peak fecundity exhibit a faster rate of senescent decline in egg production. As expected, flies with a later onset or a lower rate of exponential decline in fecundity had significantly longer ovipository and reproductive periods as well as longer total lifespan. When we used our data to estimate correlations between life-history traits and parameter estimates obtained from the model of Novoseltsev *et al.* (2002, 2003, 2004, 2005), we found the correlations typically to be much weaker or absent as compared to our best-fit model (details not shown).

Reproductive and post-reproductive lifespan

Next, we examined the length of the reproductive and post-reproductive period. In a first approach, we operationally defined reproductive lifespan as the 'ovipository' period (the period from eclosion until the day the last egg is laid) and post-reproductive lifespan as the 'post-ovipository' period (the period from the day the last egg is laid until death). Populations differed significantly in the length of both the ovipository (A: 31.85 ± 0.83 days, SA: 28.86 ± 0.95, Z: 25.39 ± 0.83; $F_{2,503} = 15.09$, $P < 0.0001$; Tukey's HSD: $P < 0.05$, A ≠ SA ≠ Z) and post-ovipository period (A: 6.12 ± 0.52 days, SA: 8.44 ± 0.59, Z: 9.36 ± 0.52; $F_{2,503} = 10.27$, $P < 0.0001$; Tukey's HSD: $P < 0.05$, A ≠ SA = Z). Thus, females died approximately 6–10 days after having laid their last egg, suggesting that the onset of the post-ovipository period is a relatively good predictor of the time of death. In support of this, visual inspection of a life-history graph (Carey *et al.*, 1998), which depicts the relationship between the end of the ovipository period and female age at death for individual females, suggests that the length of the post-ovipository period, and thus the timing of death, is on average remarkably independent of how long individual females lived (Fig. 3).

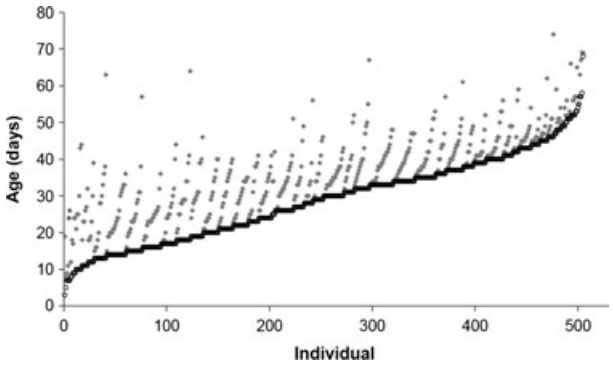

Fig. 3 A life-history graph (see Carey *et al.*, 1998) depicting the relationship between the end of the ovipository period and female age at death for individual females. Individual females are displayed along the *x*-axis; individuals on the far left of the *x*-axis had the shortest ovipository period and those on the far right the longest. Black open circles depict the end of the ovipository period for a given fly; grey diamonds depict the death of a given fly. For any given female, the vertical distance between the black open circle and the corresponding grey diamond represents the length of the post-ovipository period. Notably, the length of the post-ovipository period is on average remarkably independent of how long individual females lived.

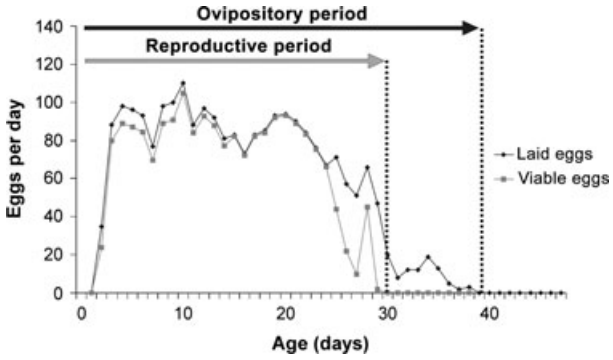

Fig. 4 Difference between the length of the ovipository and the (true) reproductive period. The reproductive period, which specifically accounts for fertility (production of viable eggs only), is typically shorter than the ovipository period, which does not distinguish whether eggs are viable or not. The example shown here is from a single female, which is representative of the overall pattern observed in our data.

We also used an alternative approach to examine reproductive and post-reproductive lifespan. Because the proportion of egg hatch typically declined to zero several days before egg production stopped, the majority of eggs laid towards the end of the ovipository period were not viable, suggesting that quantifying egg production without accounting for hatchability might overestimate realized reproductive success and thus indirectly also the length of the reproductive period (Fig. 4; also see Supporting Results and Discussion). The fact that many eggs laid late in life were not viable was likely caused by senescence of the female reproductive system rather than by a senescent decline of male reproductive capacity or infertility because in our experiment, males were regularly replaced with younger males. This prompted us to redefine the length of the reproductive and post-reproductive period as follows: the period from eclosion until the day the last viable egg is laid represents the (true) reproductive period, whereas the period from the day the last viable egg is produced until death represents the (true) post-reproductive period (this period may thus include nonviable eggs). Using this definition, South African flies had a longer reproductive lifespan than Zambian flies (SA: 22.62 ± 0.75, Z: 20.41 ± 0.67; $F_{1,302} = 4.81$, $P = 0.029$), but did not differ in post-reproductive lifespan (SA: 14.93 ± 0.82, Z: 14.76 ± 0.72; $F_{1,302} = 0.02$, $P = 0.88$), confirming the prediction that post-reproductive lifespan is unlikely to contribute to differences in fitness among populations (Austrian flies were excluded from this analysis, see Material and Methods.). Hence, when reproductive lifespan is defined appropriately, by considering fertility rather than fecundity, the length of the reproductive period was 20–22 days (approximately 60% of the total lifespan), which is about 5–6 days shorter than the ovipository period. Similarly, post-reproductive lifespan was 14–15 days, which represents a substantial proportion (approximately 40%) of total lifespan and which is about 5–6 days longer than the post-ovipository period (cf. Fig. 4). Thus, under optimal, protected laboratory conditions, wild-caught fruit fly females can apparently exhibit a very long post-reproductive phase.

The ovipository, the 'true' reproductive and the 'true' post-reproductive periods were all positively correlated with lifespan (Table S1). Most importantly, post-reproductive lifespan was not significantly correlated with any other life-history traits, suggesting that post-reproductive lifespan does not represent a fitness correlate (Table S1).

Ovarian phenotype at female death

Finally, to further explore patterns of female reproductive senescence, we inspected the ovaries of experimental females upon death. Most females (65%; 316 of 487 flies) retained at least two mature eggs per ovariole in the majority (> 50%) of ovarioles (Fig. S5). In some cases, ovarioles contained up to five retained eggs; only 5% of females had ovaries without a single mature retained egg (24 of 487 flies). None of the ovarioles examined contained vitellogenic (stages 8–12) egg chambers.

We also inspected ovaries of old but still alive females that were accidentally stuck to the medium and which were excluded from statistical analysis (see Materials and Methods). Similar to females that died 'naturally', 'accidental' females displayed egg retention; by contrast, however, their ovaries also contained vitellogenic stage 8/9 chambers (but none of the higher stages). These observations might suggest that vitellogenesis was still

active in these old flies, but that egg chamber development was arrested at stage 8/9, possibly by programmed cell death (PCD). The rapid senescent decline in oviposition prior to death might thus be due to both retention of mature eggs and PCD at mid-oogenesis. In contrast, young females during the time of peak fecundity contained egg chambers of all development stages, with no more than one mature egg per ovariole; degeneration of egg chambers by PCD was absent.

Discussion

Because most studies of *Drosophila* life history have used long-term laboratory stocks, the life history of wild flies is still poorly understood. Here, we have provided a detailed description of the reproductive and post-reproductive life history among wild-caught, non-laboratory-adapted *D. melanogaster*. We were primarily interested in addressing three problems: (i) What are the signs of correlations between life-history traits in wild-caught flies measured in a novel laboratory environment? (ii) What are the patterns of individual fecundity and reproductive senescence among wild-caught females? (iii) What are the patterns and evolutionary significance of post-reproductive lifespan among wild-caught females? To make our findings general, we followed the life histories of individual females derived from three populations of different geographical origin – a tropical population from Zambia, a temperate population from Austria and an intermediate population from South Africa (from the temperate/tropical boundary) – under presumably optimal, protected laboratory conditions.

Under laboratory conditions, wild-caught flies do not exhibit phenotypic trade-offs

The majority of phenotypic correlations between different traits in our data were positive. Despite the prediction of trade-offs among at least some of these traits, this is a commonly observed pattern for life-history traits, even at the level of genetic covariance (Charlesworth, 1990; Houle, 1991, 2001; Stearns, 1992). Thus, our results confirm that this overall pattern holds for wild-caught, non-laboratory-adapted flies measured under laboratory conditions.

For example, one of the best documented life-history trade-offs is the negative correlation between early fecundity and lifespan, which can be observed at the genetic, physiological or phenotypic level (Stearns, 1992; Stearns & Partridge, 2001; Flatt *et al.*, 2008; Flatt, 2011). A negative genetic correlation between these traits in *Drosophila* has been documented in numerous studies of correlated responses to artificial selection for increased lifespan or when genetic correlations were estimated through breeding experiments (Stearns & Partridge, 2001; Rose *et al.*, 2004; Flatt, 2011). However, studies of phenotypic (rather than genetic) correlations between early fecundity and lifespan have often failed to find evidence for the existence of a trade-off (Stearns, 1992), and this has usually been attributed to confounding effects of inbreeding, mutation accumulation or G×E interactions (Service & Rose, 1985; Clark, 1987). Under mutation accumulation, for instance, mutational effects of *de novo* mutations often exhibit positive pleiotropic effects on fitness components (Houle *et al.*, 1994). Indeed, we failed to find a negative phenotypic relationship between early fecundity and lifespan in our experiment (also see Aigaki & Ohba, 1984; Khazaeli & Curtsinger, 2010b). Our finding adds to a growing number of examples suggesting that the trade-off between reproduction and survival can be uncoupled under certain conditions (Flatt, 2009, 2011; Grandison *et al.*, 2009; Khazaeli & Curtsinger, 2012).

Importantly, because we took care to avoid confounding effects of inbreeding, mutation accumulation and laboratory adaptation, it is unlikely that these factors would have masked phenotypic trade-offs in our experiment. The most parsimonious explanation for our results is that the predominance of positive correlations is a result of the exposure of wild-caught flies to a novel (laboratory) environment (Service & Rose, 1985). One reason for this could be that under optimal laboratory conditions, wild-caught flies might exhibit unconstrained physiological performance (cf. van Noordwijk & de Jong, 1986; Reznick *et al.*, 2000), an interpretation that is consistent with previous observations (Dobzhansky *et al.*, 1964; Giesel, 1986; Stearns, 1992; Draye *et al.*, 1994).

The question of what is the appropriate environment in which to measure life-history traits is a general methodological problem. Inevitably, a novel laboratory environment might represent an unnatural, unrealistic environment, a fact that might lead one either to miss relevant phenotypes or to misinterpret the relevance of laboratory-based findings for natural environments. For example, it has been found that flies selected for increased lifespan in one laboratory may not show the longevity phenotype when measured in a different laboratory because of subtle differences in assay conditions from laboratory to laboratory (Ackermann *et al.*, 2001). On the other hand, as discussed in the introduction (also see Harshman & Hoffmann, 2000), the use of stocks adapted to the laboratory environment has its own problems. Because it is practically impossible to measure the relevant life-history traits of wild-caught flies in the wild, we must perform the assays in the laboratory. Although this is a fundamental limitation, the upside is that it allows us to ask biologically important questions about GxE and the effects of novel (laboratory) environments. For example, although unmanipulated laboratory stocks can show the same phenomenon and although we cannot rule out the existence of underlying negative genetic correlations, our results clearly support

the notion that the effect of a novel laboratory environment is to bias life-history correlations towards positive values (Service & Rose, 1985). If this turns out to be a general phenomenon, it might tell us something important about the physiology underlying life-history trade-offs (cf. van Noordwijk & de Jong, 1986; Reznick *et al.*, 2000).

Individual females exhibit clear-cut fecundity peaks

To examine individual fecundity, we fitted a series of heuristic models to our data on age-specific egg production. These models represent modifications of the model proposed by Novoseltsev *et al.* (2002, 2003, 2004, 2005). The main feature of the model by Novoseltsev and colleagues is that it assumes a period during which individual fecundity first increases but then plateaus at a steady-state level before decreasing during a phase of 'reproductive senescence'. According to Novoseltsev *et al.* (2003, 2004), the rationale underlying the assumption of a plateau is that typically individual age-specific fecundity trajectories do not exhibit a clear fecundity peak; the authors argue that fecundity peaks, which are commonly observed at the population or cohort level, are an artefact of averaging across many individual trajectories. However, when we compared our models with that of Novoseltsev and colleagues, we found that their model, or any other models that assume a reproductive plateau (models 1–4; see Supporting Materials and Methods, Table S4), fitted our data much worse than models that assume a fecundity peak (models 5–8; Supporting Information, Table S4). Based on our best-fitting fecundity model (model 8), we can biologically distinguish four phases during a female's life: (i) a period of reproductive maturation, characterized by a sharp linear increase in fecundity up to a maximal value (peak fecundity); (ii) a period of slow linear decrease in fecundity; (iii) a period of exponential decrease in fecundity; and (iv) a post-ovipository period during which no eggs are laid anymore, followed by death. Thus, our results suggest the existence of a pronounced fecundity peak in *D. melanogaster*, even at the level of individual females (cf. Kindlmann *et al.*, 2001).

Several factors might explain the discrepancy between our data and those of Novoseltsev *et al.* (2002, 2003, 2004, 2005). First, we did not impose any constraints on the number of model parameters; our models thus tend to be more complex than the one by Novoseltsev and colleagues. Second, we used wild-caught flies rather than laboratory stocks, whereas Novoseltsev and colleagues fitted data from laboratory stocks or selection lines to their model. Third, whether a clear peak is observed might depend upon the diet used in a given experiment. Clearly, further studies are required to determine how general fecundity peaks are among individual *Drosophila* females and to identify the factors that might influence them.

Reproductive decline is not simply a function of old age

When we analysed our data on individual egg production, we found that the phase of reproductive maturation, which leads up to the fecundity peak, usually extended over the first three to 4 days of adulthood, whereas the period of linear decline in fecundity lasted much longer, that is, on average approximately 18 days. Indeed, the majority of eggs were laid during this latter phase; the length of this period was therefore positively correlated with both the length of the ovipository period and lifespan (Table S5). This phase of linear decline was followed by an accelerated, exponential decline in fecundity, a pattern that has already previously been observed (Rauser *et al.*, 2005; Rogina *et al.*, 2007) and that has been called the 'death spiral' by Mueller *et al.* (2007, 2009). Remarkably, females showed an exponential decline in fecundity independently of how long they lived, thus implying that onset of this phase is not simply an absolute function of old age.

That female fecundity declines sharply as a function of age is well known (David *et al.*, 1974; Zhao *et al.*, 2008), and the linear and exponential decrease in fecundity might thus be thought of as reflecting functional senescence of the female reproductive system, for example caused by the age-dependent loss of ovarian germ line and somatic stem cells (Margolis & Spradling, 1995; Pan *et al.*, 2007; Zhao *et al.*, 2008). Margolis & Spradling (1995), for instance, found that half of all ovarian stem cells were lost 22 days after egg-laying had begun, and many ovarioles of aged females lacked developing egg chambers. However, in our experiment, the dramatic decline in oviposition and egg production among females prior to death was not exclusively caused by a loss of germ line stem cells with age: when we dissected the ovaries of dead females, ovarioles very often exhibited egg retention, and vitellogenic development was arrested, whereas old but still alive females contained egg chambers at vitellogenic stage 8/9.

Consistent with the findings of Zhao *et al.* (2008), our observations might indicate that egg chambers of ageing females are subject to developmental arrest at mid-oogenesis, presumably due to PCD. The decline in the rate of germ line stem cell proliferation and the increase in both ovarian cell death and egg retention therefore all seem to contribute to the decline in female oviposition and egg production with age. Because egg retention and PCD are often triggered by diverse stresses (Gruntenko *et al.*, 2003; McCall, 2004), we hypothesize that reproductive decline is at least partly due to a build-up of ovarian stress levels over time.

In addition to the senescent decline in oviposition and egg production, we also found that fertility, measured as the number of viable eggs, decreased strongly with maternal age, as has been found previously (David *et al.*, 1975). Such an age-dependent decline in fertility is well

known in mammals (Velde & Pearson, 2002), but has also been described for invertebrates such as the nematode *Caenorhabditis elegans* (Hughes *et al.*, 2007; Luo *et al.*, 2009, 2010; Luo & Murphy, 2011). In mammals, the senescent decrease in fertility is due to a decline in oocyte quality, which is thought to result from an increased rate of aneuploidy due to nondisjunction during meiosis (Broekmans *et al.*, 2007). Because nondisjunction rates are known to increase with age in *Drosophila* (Tokunaga, 1970), a higher rate of aneuploidy among the offspring of aged mothers might explain the senescent decline in fertility among fruit fly females.

It is in principle possible that the reproductive decline we have observed is due to males ceasing to court and mate old females because female attractiveness decreases with age (Kuo *et al.*, 2012). However, we think it is more likely that females might reject to mate and stop to lay eggs because of their deteriorated physiological state prior to death.

Post-reproductive lifespan is a nonadaptive 'add-on' at the end of life

Female death in our experiment was preceded by a pronounced post-ovipository period (cf. Rogina *et al.*, 2007; Mueller *et al.*, 2009; Khazaeli & Curtsinger, 2010a), with females typically dying within 6–10 days after having laid their last egg. Remarkably, females exhibited a terminal, post-ovipository period irrespective of their age at death (Fig. 3), suggesting that the length of the post-ovipository period appears to be an accurate predictor of death (Rogina *et al.*, 2007). The death of such females may thus not be a sudden age-related accident but the end point of a time-progressive physiological process (Rogina *et al.*, 2007). In contrast to our data, Rogina *et al.* (2007) reported that females stopped laying eggs only about 1–2 days before death; such differences in the length of the post-ovipository period among studies might reflect differences among genotypes/stocks or diet.

Most females in our experiment laid nonviable eggs for about 5–6 days before entering the post-ovipository period. When accounting for this loss of fertility by defining the post-reproductive lifespan as the period from the day the last *viable* egg was laid until death, we observed that this period represents a substantial fraction (approximately 40%, or 14–15 days) of total lifespan. Because fruit flies are thought to be very short-lived in the wild, with a mean adult life expectancy of about 1.3–6.2 days (Rosewell & Shorrocks, 1987), presumably due to high levels of extrinsic mortality, it seems obvious that the extended post-reproductive lifespan we have observed might be a consequence of maintaining flies under optimal, protected laboratory conditions.

A pronounced post-reproductive lifespan is expected to evolve when post-reproductive females make a significant contribution to the fitness of their offspring or to that of relatives (Hawkes *et al.*, 1998; Shanley & Kirkwood, 2001; Shanley *et al.*, 2007; Kirkwood & Shanley, 2010). Conversely, in organisms without maternal post-reproductive contribution to offspring fitness, post-reproductive lifespan likely represents a nonadaptive, variable and random 'add-on' at the end of the life history (Reznick *et al.*, 2006). If so, post-reproductive lifespan might be either an indirect correlate of selection for fitness components that are adaptive earlier in life, or a by-product of different rates of ageing of the soma and the reproductive system (Reznick *et al.*, 2006; also see Cohen, 2004; Luo *et al.*, 2009; Luo & Murphy, 2011). A related idea is that, if the length of both somatic and reproductive lifespan is variable and unpredictable, it might pay off to evolve a somatic lifespan that is longer than the reproductive lifespan because a longer life expectancy reduces the risk of accidental death before reproduction has ceased (Tully & Lambert, 2011). Under this model, one might expect that the reproductive system ages at a faster rate than the soma (Cohen, 2004; Luo *et al.*, 2009) and that there is a positive correlation between the variance in the duration of total lifespan and the length of the post-reproductive period (Tully & Lambert, 2011).

Consistent with the notion that post-reproductive lifespan does not directly contribute to individual fitness, we failed to find differences in post-reproductive lifespan among populations, similar to Reznick *et al.*'s observations (2006) in guppies. Moreover, post-reproductive lifespan was positively correlated with total lifespan in our data. The long post-reproductive lifespan of *Drosophila* maintained under laboratory conditions might thus be a correlate of extended total lifespan under optimal, protected conditions (Reznick *et al.*, 2006; Kirkwood & Shanley, 2010). However, in contrast to Reznick *et al.* (2006), we failed to find a correlation between post-reproductive lifespan and other reproductive fitness components, such as fecundity or reproductive lifespan. Our results therefore indicate that in fruit flies, post-reproductive lifespan is a highly variable, random 'add-on' at the end of reproductive life, but they do not support the hypothesis that it is a correlate of selection on reproductive fitness components (Reznick *et al.*, 2006) (Table S1).

As discussed by Kirkwood & Shanley (2010), artefactual post-reproductive lifespan might be observed when disease (e.g. due to deleterious mutations) has accelerated ovarian depletion, or if intensive breeding or strong laboratory adaptation has selected for increased early fecundity, which might in turn lead to more rapid ovarian exhaustion. Because we have attempted to avoid confounding effects of laboratory adaptation, inbreeding and mutation accumulation, we think that it is unlikely that these factors explain the substantial post-reproductive lifespan we have observed.

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References

Ackermann, M., Bijlsma, R., James, A.C., Partridge, L., Zwaan, B.J. & Stearns, S.C. 2001. Effects of assay conditions in life history experiments with *Drosophila melanogaster*. *J. Evol. Biol.* **14**: 199–209.

Aigaki, T. & Ohba, S. 1984. Individual analysis of age-associated changes in reproductive activity and lifespan of *Drosophila virilis*. *Exp. Gerontol.* **19**: 13–23.

Broekmans, F.J., Knauff, E.A.H., Velde, E.R.T., Macklon, N.S. & Fauser, B.C. 2007. Female reproductive ageing: current knowledge and future trends. *Trends Endocrinol. Metab.* **18**: 58–65.

Carey, J.R., Liedo, P., Müller, H.G., Wang, J.L. & Vaupel, J.W. 1998. A simple graphical technique for displaying individual fertility data and cohort survival: case study of 1000 Mediterranean Fruit Fly females. *Func. Ecol.* **12**: 359–363.

Charlesworth, B. 1990. Optimization models, quantitative genetics, and mutation. *Evolution* **44**: 520–538.

Chippindale, A.K., Leroi, A.M., Kim, S.B. & Rose, M.R. 1993. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. *J. Evol. Biol.* **6**: 171–193.

Clark, A.G. 1987. Senescence and the genetic-correlation hang-up. *Am. Nat.* **129**: 932–940.

Cohen, A.A. 2004. Female post-reproductive lifespan: a general mammalian trait. *Biol. Rev. Camb. Philos. Soc.* **79**: 733–750.

David, J.R., Biémont, C. & Fouillet, P. 1974. Sur la forme des courbes de ponte de *Drosophila melanogaster* et leur adjustment á des modèles mathématiques. *Arch. Zool. Exp. Gen.* **115**: 263–277.

David, J.R., Cohet, Y. & Fouillet, P. 1975. The variability between individuals as a measure of senescence: a study of the number of eggs laid and the percentage of hatched eggs in the case of *Drosophila melanogaster*. *Exp. Gerontol.* **10**: 17–25.

Dobzhansky, T., Lewontin, R.C. & Pavlovsky, O. 1964. The capacity for increase in chromosomally polymorphic and monomorphic populations of *Drosophila pseudoobscura*. *Heredity* **19**: 597–614.

Draye, X., Bullens, P. & Lints, F.A. 1994. Geographic variations of life history strategies in *Drosophila melanogaster*. I. Analysis of wild-caught populations. *Exp. Gerontol.* **29**: 205–222.

von Ende, C.N. 2001. Repeated-measures analysis: growth and other time-dependent measures. In: *Design and Analysis of Ecological Experiments* (S.M. Scheiner & J. Gurevitch, eds), pp. 134–157. Oxford University Press, Oxford.

Flatt, T. 2009. Ageing: diet and longevity in the balance. *Nature* **462**: 989–990.

Flatt, T. 2011. Survival costs of reproduction in *Drosophila*. *Exp. Gerontol.* **46**: 369–375.

Flatt, T. & Heyland, A. (eds) 2011. *Mechanisms of Life History Evolution – The Genetics and Physiology of Life History Traits and Trade-Offs*. Oxford University Press, Oxford.

Flatt, T. & Schmidt, P.S. 2009. Integrating evolutionary and molecular genetics of aging. *Biochim. Biophys. Acta* **1790**: 951–962.

Flatt, T., Min, K.J., D'Alterio, C., Villa-Cuesta, E., Cumbers, J., Lehmann, R. *et al.* 2008. *Drosophila* germ-line modulation of insulin signaling and lifespan. *Proc. Natl. Acad. Sci. USA* **105**: 6368–6373.

Frankham, R. & Loebel, D.A. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: rapid genetic adaptation to captivity. *Zoo Biol.* **11**: 333–342.

Giesel, J.T. 1986. Genetic correlation structure of life history variables in outbred, wild *Drosophila melanogaster*: effects of photoperiod regimen. *Am. Nat.* **128**: 593–603.

Giesel, J.T., Murphy, P.A. & Manlove, M.N. 1982. The influence of temperature on genetic interrelationships of life history traits in a population of *Drosophila melanogaster*: what tangled data sets we weave. *Am. Nat.* **119**: 464.

Grandison, R.C., Piper, M.D.W. & Partridge, L. 2009. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* **462**: 1061–1065.

Gruntenko, N.E., Bownes, M., Terashima, J., Sukhanova, M. & Raushenbach, I.Y. 2003. Heat stress affects oogenesis differently in wild-type *Drosophila virilis* and a mutant with altered juvenile hormone and 20-hydroxyecdysone levels. *Insect Mol. Biol.* **12**: 393–404.

Harshman, L.G. & Hoffmann, A.A. 2000. Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends Ecol. Evol.* **15**: 32–36.

Hawkes, K., O'Connell, J.F., Jones, N.G.B., Alvarez, H. & Charnov, E.L. 1998. Grandmothering, menopause, and the evolution of human life histories. *Proc. Natl. Acad. Sci. USA* **95**: 1336–1339.

Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* **45**: 630–648.

Houle, D. 2001. Characters as the units of evolutionary change. In: *The Character Concept in Evolutionary Biology* (G.P. Wagner, eds), pp. 109–140. Academic Press, San Diego.

Houle, D. & Rowe, L. 2003. Natural selection in a bottle. *Am. Nat.* **161**: 50–67.

Houle, D., Hughes, K.A., Hoffmaster, D.K., Ihara, J., Assimacopoulos, S., Canada, D. *et al.* 1994. The effects of spontaneous mutation on quantitative traits. I. Variances and covariances of life history traits. *Genetics* **138**: 773–785.

Hughes, S.E., Evason, K., Xiong, C.J. & Kornfeld, K. 2007. Genetic and pharmacological factors that influence reproductive aging in nematodes. *PLoS Genet.* **3**: 254–265.

Khazaeli, A.A. & Curtsinger, J.W. 2010a. Post-reproductive survival and the Faulkner Effect in *Drosophila melanogaster*. *Dros. Inf. Serv.* **93**: 154–155.

Khazaeli, A.A. & Curtsinger, J.W. 2010b. Life history variation in an artificially selected population of *Drosophila melanogaster*: pleiotropy, superflies, and age-specific adaptation. *Evolution* **64**: 3409–3416.

Khazaeli, A.A. & Curtsinger, J.W. 2013. Pleiotropy and Life History Evolution in *Drosophila melanogaster*: Uncoupling Life Span and Early Fecundity. *J. Gerontol. A Biol. Sci. Med. Sci.* **68**: 546–553.

Kindlmann, P., Dixon, A.F.G. & Dostálková, I. 2001. Role of ageing and temperature in shaping reaction norms and fecundity functions in insects. *J. Evol. Biol.* **14**: 835–840.

King, R.C. 1970. *Ovarian Development in Drosophila melanogaster*. Academic Press, New York, NY.

Kirkwood, T.B.L. & Shanley, D.P. 2010. The connections between general and reproductive senescence and the evolutionary basis of menopause. *Ann. N. Y. Acad. Sci.* **1204**: 21–29.

Kuo, T.-H., Yew, J.Y., Fedina, T.Y., Dreisewerd, K., Dierick, H.A. & Pletcher, S.D. 2012. Aging modulates cuticular hydrocarbons and sexual attractiveness in *Drosophila melanogaster*. *J. Exp. Biol.* **215**: 814–821.

Linnen, C., Tatar, M. & Promislow, D. 2001. Cultural artifacts: a comparison of senescence in natural, laboratory-adapted and artificially selected lines of *Drosophila melanogaster*. *Evol. Ecol. Res.* **3**: 877–888.

Luckinbill, L.S., Arking, R., Clare, M.J., Cirocco, W.C. & Buck, S.A. 1984. Selection for Delayed Senescence in *Drosophila melanogaster*. *Evolution* **38**: 996–1003.

Luo, S. & Murphy, C.T. 2011. *Caenorhabditis elegans* reproductive aging: regulation and underlying mechanisms. *Genesis* **49**: 53–65.

Luo, S., Shaw, W.M., Ashraf, J. & Murphy, C.T. 2009. TGF-beta Sma/Mab signaling mutations uncouple reproductive aging from somatic aging. *PLoS Genet.* **5**: e1000789.

Luo, S., Kleemann, G.A., Ashraf, J.M., Shaw, W.M. & Murphy, C.T. 2010. TGF-beta and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. *Cell* **143**: 299–312.

Margolis, J. & Spradling, A. 1995. Identification and behavior of epithelial stem-cells in the *Drosophila* ovary. *Development* **121**: 3797–3807.

Matos, M., Rose, M.R., Rocha Pité, M.T., Rego, C. & Avelar, T. 2000. Adaptation to the laboratory environment in *Drosophila subobscura*. *J. Evol. Biol.* **13**: 9–19.

McCall, K. 2004. Eggs over easy: cell death in the *Drosophila* ovary. *Dev. Biol.* **274**: 3–14.

Mueller, L.D., Rauser, C.L. & Rose, M.R. 2007. An evolutionary heterogeneity model of late-life fecundity in *Drosophila*. *Biogerontology* **8**: 147–161.

Mueller, L.D., Shahrestani, P. & Rauser, C.L. 2009. Predicting death in female *Drosophila*. *Exp. Gerontol.* **44**: 766–772.

Nelder, J.A. & Mead, R. 1965. A simplex method for function minimization. *Computer J.* **7**: 308–313.

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

Novoseltsev, V.N., Arking, R., Novoseltseva, J.A. & Yashin, A.I. 2002. Evolutionary optimality applied to *Drosophila* experiments: hypothesis of constrained reproductive efficiency. *Evolution* **56**: 1136–1149.

Novoseltsev, V.N., Novoseltseva, J.A. & Yashin, A.I. 2003. What does a fly's individual fecundity pattern look like? The dynamics of resource allocation in reproduction and ageing. *Mech. Ageing Dev.* **124**: 605–617.

Novoseltsev, V.N., Arking, R., Carey, J.R., Novoseltseva, J.A. & Yashin, A.I. 2004. How an individual fecundity pattern looks in *Drosophila* and medflies. *Ann. N. Y. Acad. Sci.* **1019**: 577–580.

Novoseltsev, V.N., Arking, R., Carey, J.R., Novoseltseva, J.A. & Yashin, A.I. 2005. Individual fecundity and senescence in *Drosophila* and medfly. *J. Gerontol. A Biol. Sci. Med. Sci.* **60**: 953–962.

Paaby, A.B. & Schmidt, P.S. 2009. Dissecting the genetics of longevity in *Drosophila melanogaster*. *Fly* **3**: 29–38.

Pan, L., Chen, S.Y., Weng, C.J., Call, G., Zhu, D.X., Tang, H. *et al.* 2007. Stem cell aging is controlled both intrinsically and extrinsically in the *Drosophila* ovary. *Cell Stem Cell* **1**: 458–469.

Partridge, L., Prowse, N. & Pignatelli, P. 1999. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B Biol. Sci.* **266**: 255–261.

Partridge, L., Gems, D. & Withers, D.J. 2005. Sex and death: what is the connection? *Cell* **120**: 461–472.

Pekkala, N., Kotiaho, J.S. & Puurtinen, M. 2011. Laboratory relationships between adult lifetime reproductive success and fitness surrogates in a *Drosophila littoralis* population. *PLoS ONE* **6**: e24560.

Pletcher, S.D., Houle, D. & Curtsinger, J.W. 1998. Age-specific properties of spontaneous mutations affecting mortality in *Drosophila melanogaster*. *Genetics* **148**: 287–304.

Prasad, N.G. & Joshi, A. 2003. What have two decades of laboratory life-history evolution studies on *Drosophila melanogaster* taught us? *J. Genet.* **82**: 45–76.

Promislow, D.E. & Tatar, M. 1998. Mutation and senescence: where genetics and demography meet. *Genetica* **102–103**: 299–314.

Rauser, C.L., Abdel-Aal, Y., Shieh, J.A., Suen, C.W., Mueller, L.D. & Rose, M.R. 2005. Lifelong heterogeneity in fecundity is insufficient to explain late-life fecundity plateaus in *Drosophila melanogaster*. *Exp. Gerontol.* **40**: 660–670.

Reznick, D., Nunney, L. & Tessier, A. 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol. Evol.* **15**: 421–425.

Reznick, D., Bryant, M. & Holmes, D. 2006. The evolution of senescence and post-reproductive lifespan in guppies (*Poecilia reticulata*). *PLoS Biol.* **4**: e7.

Rogina, B., Wolverton, T., Bross, T.G., Chen, K., Muller, H.G. & Carey, J.R. 2007. Distinct biological epochs in the reproductive life of female *Drosophila melanogaster*. *Mech. Ageing Dev.* **128**: 477–485.

Rose, M.R. 1984. Genetic covariation in *Drosophila* life history: untangling the data. *Am. Nat.* **123**: 565–569.

Rose, M.R., Passananti, H.B. & Matos, M. 2004. *Methuselah Flies – A Case Study in the Evolution of Aging*. World Scientific, Singapore.

Rosewell, J. & Shorrocks, B. 1987. The implication of survival rates in natural populations of *Drosophila* – capture recapture experiments on domestic Species. *Biol. J. Linn. Soc.* **32**: 373–384.

Schmidt, P.S. & Paaby, A.B. 2008. Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. *Evolution* **62**: 1204–1215.

Service, P.M. & Rose, M.R. 1985. Genetic covariation among life-history components: the effects of novel environments. *Evolution* **39**: 943–945.

Sgrò, C.M. & Partridge, L. 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *Am. Nat.* **156**: 341–353.

Shanley, D.P. & Kirkwood, T.B.L. 2001. Evolution of the human menopause. *BioEssays* **23**: 282–287.

Shanley, D.P., Sear, R., Mace, R. & Kirkwood, T.B. 2007. Testing evolutionary theories of menopause. *Proc. R. Soc. Lond. B Biol. Sci.* **274**: 2943–2949.

Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.
Stearns, S.C. & Partridge, L. 2001. The genetics of aging in *Drosophila*. In: *Handbook of the Biology of Aging* (E. Masoro & S. Austad, eds), pp. 353–368. Academic Press, San Diego.
Stearns, S.C., Ackermann, M., Doebeli, M. & Kaiser, M. 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. *Proc. Natl. Acad. Sci. USA* **97**: 3309–3313.
Tatar, M., Bartke, A. & Antebi, A. 2003. The endocrine regulation of aging by insulin-like signals. *Science* **299**: 1346–1351.
Tokunaga, C. 1970. The effects of low temperature and aging on nondisjunction in *Drosophila*. *Genetics* **65**: 75–94.
Tully, T. & Lambert, A. 2011. The evolution of postreproductive life span as an insurance against indeterminacy. *Evolution* **65**: 3013–3020.
Velde, E.R.T. & Pearson, P.L. 2002. The variability of female reproductive ageing. *Hum. Reprod. Update* **8**: 141–154.
Zhao, R., Xuan, Y., Li, X. & Xi, R. 2008. Age-related changes of germline stem cell activity, niche signaling activity and egg production in *Drosophila*. *Aging Cell* **7**: 344–354.
Zwaan, B., Bijlsma, R. & Hoekstra, R.F. 1995. Direct selection on life span in *Drosophila melanogaster*. *Evolution* **49**: 649–659.

Supporting information

Additional Supporting Information may be found in the online version of this article:
Figure S1 Egg production.
Figure S2 Linear relationship between ovariole number and mean early daily fecundity between days 1 and 10.
Figure S3 Average deviation of maternal-age-specific hatchability scores from a plateau.
Figure S4 Adult female survivorship (fraction of flies alive at age x) as a function of age x.
Figure S5 Linear relationship between total lifetime fecundity and lifespan.
Figure S6 Ovaries of a dead female exhibiting egg retention.
Table S1 Spearman rank correlation coefficients (ρ) between life history traits.
Table S2 Average proportion egg hatchability over time with standard errors.
Table S3 Spearman rank correlation coefficients (ρ) between viable egg production and life history traits for the South African and Zambian populations.
Table S4 Sum of errors for the different models of individual fecundity.
Table S5 Spearman rank correlation coefficients (ρ) between parameters of our fecundity model (model 8) and different life history traits.

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