Reproductive potential predicts longevity of female Mediterranean fruitflies

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Reproduction exacts a price in terms of decreased survival. Our analysis of the interplay between age patterns of fecundity and mortality for individual female medflies (*Ceratitis capitata*) revealed that individual mortality is associated with the time-dynamics of the egg-laying trajectory. In a sample of 531 medflies, we found that each individual has a characteristic rate of decline in egg laying with age. This defines an individual's rate of reproductive exhaustion. This rate was shown to predict subsequent mortality. The larger the remaining reproductive potential, the lower the subsequent mortality. An increased mortality risk was seen in flies for which egg production declined rapidly early on, irrespective of the level of egg production. Thus, reproductive potential and lifetime are coupled in such a way that those flies which are able to profit most from an extended life span in terms of increased egg output are indeed likely to live longer.

Keywords: reproductive clock; mortality; depletion of eggs; egg-laying trajectory; modelling; bootstrap

1. INTRODUCTION

The purpose of this study was twofold: to establish patterns of reproductive activity as measured in terms of egg laying for a cohort of female Mediterranean fruitflies (medflies) and then to correlate those patterns with longevity. As it turns out, egg-laying trajectories at the individual level follow simple exhaustion or decay dynamics. We demonstrate here that the best predictor for subsequent mortality is the rate of decline of egg laying, i.e. the rate at which the egg supply is exhausted, rather than intensity of reproduction.

The cost of reproduction concept has been established by many researchers (Williams 1957; Partridge & Farquhar 1981; Partridge 1987; Kirkwood & Rose 1991; Partridge & Barton 1993; Abrams & Ludwig 1995; Chapman *et al.* 1998; Westendorp & Kirkwood 1999). In this paper, we argue that individual egg-laying data for medflies point to the critical role played by the remaining reproductive potential which quantifies the degree of egg depletion for an individual fly. As the reproductive potential declines, subsequent mortality increases. This adds an important dimension to the concept of cost of reproduction for medflies. The classical cost of reproduction concept envisions damage incurred by reproduction which leads to a shortened life span. In the light of our findings, reproduction itself leads to a decline in reproductive potential which is then associated with increased subsequent mortality. In particular, we found that the rate of decline of reproduction and not intensity of reproduction proved to be the best predictor of subsequent mortality. Thus, an unqualified concept of cost of reproduction incurred by competition for limited available resources between reproduction and maintenance proves too simplistic.

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2. MATERIAL AND METHODS

Individual egg-laying counts were recorded daily for 1000 mated female medflies at the mass-rearing facility in Metapa, Mexico. In addition, time of death was recorded for each fly. Five hundred and thirty-one egg-laying subjects who lived beyond day 26 were selected from these flies. The flies were held in individual cups and were fed a full diet of protein and glucose *ad libitum*. Details of how the flies were reared and a description of other features of the experiment can be found in Carey *et al.* (1998*a*).

The Cox proportional hazards model, nonlinear least squares, bootstrap tests and smoothed hazard function estimates were used for statistical analysis of the data. Details of the two bootstrap tests, which were developed for testing for an association between the reproductive clock and life span, are given in Appendix A.

3. RESULTS

(a) *Constant rate of decline of egg laying*

Individual egg-laying trajectories rose sharply after egg laying began 5–17 days after emergence, reached a peak and then slowly declined. The rate of decline varied between individuals but one of our findings was that this rate was approximately constant for each individual.
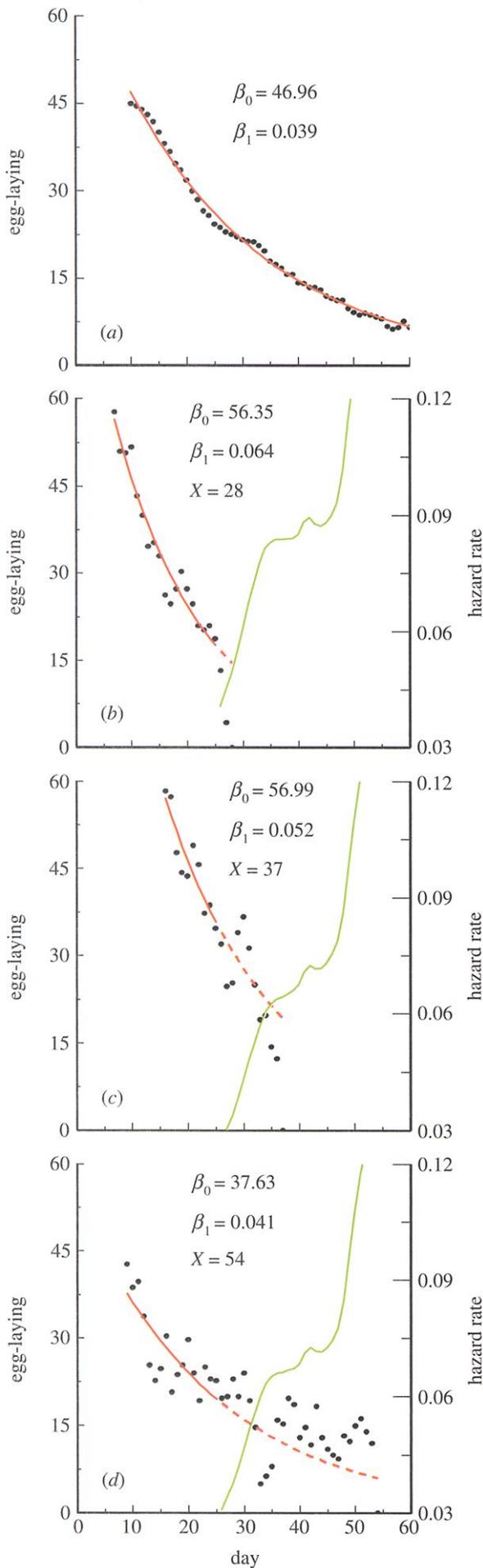
The age trajectory of reproductive decline for each fly was accordingly modelled by the exponential function

$$f(x) = \beta_0 \exp(-\beta_1(x - \theta)), \tag{1}$$

where $f(x)$ is the fecundity (measured by daily egg count) of the fly at age x days and θ is the age at peak egg laying (sample mean 11.09 ± 3.55(s.d.)).

The two parameters β_0 (mean 57.25 ± 16.70), the peak height of the trajectory and β_1, the rate of decline (mean 0.090 ± 0.093), varied considerably from fly to fly (figure 1). A modest but significant negative correlation between β_0 and β_1 ($r = -0.15$ and $p < 0.05$) indicated that

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(a) $\beta_0 = 46.96$ $\beta_1 = 0.039$

(b) $\beta_0 = 56.35$ $\beta_1 = 0.064$ $X = 28$

(c) $\beta_0 = 56.99$ $\beta_1 = 0.052$ $X = 37$

(d) $\beta_0 = 37.63$ $\beta_1 = 0.041$ $X = 54$

fecundity tends to decline more slowly for flies with higher peak fecundity. We found that the protracted decline in egg laying after the initial sharp rise was reasonably well predicted by the exponential model (figure 1).

A consequence of these simple egg-laying dynamics was that, for any age x, we could predict the fraction of remaining eggs relative to the total number of eggs by

$$\pi(x) = \int_x^\infty f(s)\mathrm{d}s \Big/ \int_\theta^\infty f(s)\mathrm{d}s = \exp(-\beta_1(x - \theta)).$$

(2)

This function, with values declining from 1 to 0 as the fly ages, provides a simple measure of reproductive exhaustion at age x in terms of remaining (relative) reproductive potential. It could be loosely described as an individual's reproductive clock, which advances at a speed determined by the rate of decline β_1.

(b) Association between mortality and exhaustion of reproductive potential

In our experiment the likelihood that a fly died increased as the fly's reproductive potential was exhausted and the reproductive clock advanced. The finding of an association between mortality and exhaustion of reproductive potential was our main result. It leads to a new perspective on the relationship between reproduction and longevity.

The association between remaining reproductive potential and longevity was established in three different ways. The first was a bootstrap test using randomly resampled lifetimes and quantifying the number of eggs that would have been produced under a random exchange of lifetimes. The idea was that, if there was no association between reproductive potential and life span, a random exchange of lifetimes would not alter the total number of eggs produced by all flies in the sample.

The second analysis provided a similar quantification of the total number of eggs laid if flies in randomly formed pairs exchanged lifetimes. Again, if there was no association the number of eggs would remain unaffected by such an exchange. The third analysis provided for a direct prediction of subsequent mortality of an individual fly, based on a hazard regression model and using reproductive potential as a predictor variable.

Obviously, fecundity and mortality are strongly correlated with age. As fecundity decreases with older age, mortality increases. Hence, in order to prevent confounding effects from this association, we fitted the trajectories by only using data prior to day 25, whereas longevity was measured as remaining lifetime after day 25. Thus, the fitted trajectories of fecundity were predicted

Figure 1. Trajectories of fecundity and mortality. The trajectories of fecundity are fitted to data from the peak to day 25 (red solid line) by nonlinear least squares and predicted thereafter (red dashed line). Age at death is indicated by X. (*a*) Trajectory fitted to average counts of daily eggs for the sample of 531 medflies. Daily egg counts, reproductive trajectory and predicted smoothed (Müller *et al.* 1997*a*) individual hazard function (green) for a fly with life span at (*b*) the 10% quantile, (*c*) the 50% quantile, and (*d*) the 90% quantile.
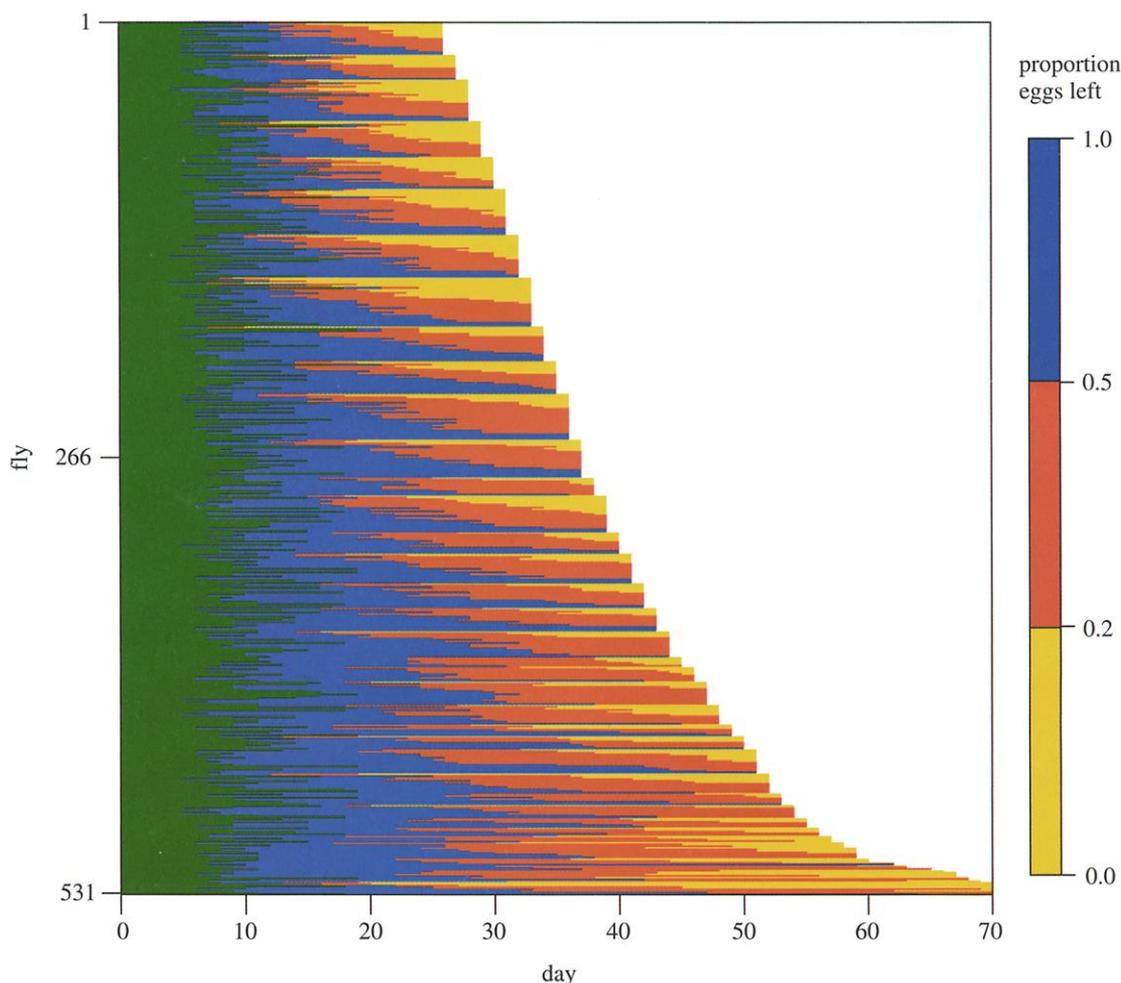
Figure 2. Lifetime and proportion of eggs left. All 531 flies are ordered according to life span, which defines the length of the coloured horizontal bar for each fly. Taken together, the bars provide the empirical survival function. The colours within each bar indicate the remaining relative reproductive potential $\pi(x)$. The initial phase (green) marks the time elapsed between eclosion and peak reproduction. Flies with the same lifetime are ordered according to remaining reproductive potential.

after age 25 days based on the above model. This guaranteed that the fitted trajectories were not influenced by a fly's life span and allowed bona fide predictions of subsequent mortality.

(c) *Confirming the association between reproductive potential and longevity via the bootstrap*

Suppose that, instead of dying at the actually observed age at death X, each fly was assigned a new life span of X^* chosen at random from the sample of 531 observed life spans. If longevity and reproductive clock or, equivalently, remaining reproductive potential at death are linked, then this random reassignment should tend to increase the remaining reproductive potential at death on average (measured by the average value of $\pi(X^*)$).

The null hypothesis that no such change occurs corresponds to no link between reproductive potential and lifetime. Using the bootstrap method (Efron & Tibshirani 1993; Manly 1997), we devised a bootstrap test for this null hypothesis (see Appendix A for details). This bootstrap test provided strong evidence against the null hypothesis of no link ($p = 0.0004$) and in favour of the alternative that the occurrence of death becomes ever

more likely as the reproductive potential of a fly is exhausted.

(d) *Graphical confirmation of the association via the event history diagram*

The result for the association between reproductive clock and lifetime is illustrated by an event-history diagram (figure 2). The event history diagram (Carey *et al.* 1998*b*) is based on fitting exponential trajectories to egg laying over the entire life span.

This diagram demonstrates graphically that there will be a loss in actual eggs when lifetimes are randomly rearranged. The event-history diagram demonstrates the close relationship between reproductive potential and life span for all 531 flies. For example, almost half of the flies (262 out of 531) died with fewer than 20% of their eggs left (yellow zones indicating an advanced state of the reproductive clock) and 91% (482 out of 531) died with fewer than 50% of their eggs left (blue zones). Thus, early death is less likely in the presence of a large remaining reproductive potential and random reassignment of lifetimes will tend to increase remaining reproductive potential at death.

As flies in the upper half of the graph with relatively short life spans are likely to be assigned increased life spans in a random reshuffling of life spans, not much will be achieved for these flies in terms of additional egg laying due to the near exhaustion of their egg-laying potential. The same reshuffling probably includes the random assignment of shorter life spans to the relatively long-lived flies in the lower half of the graph. Their death will then occur while they are still in the blue or red zones with sizeable remaining egg-laying potential. Their lifetime output in eggs will therefore decline sharply. These losses in terms of eggs not laid relative to egg-laying potential will dominate the, at best, meagre gains the flies in the upper half might achieve. The net result is therefore a decrease in the conversion of egg-laying potential into actual eggs on average.

(e) *Exchanging lifetimes between flies in randomly selected pairs: a thought experiment*

In another thought experiment, we assume that flies are randomly grouped into pairs and that, for each pair, life spans are exchanged but reproductive clocks and fecundity trajectories are not. Testing the null hypothesis of no change in the average value of π at death against the alternative of an increase in this value, we found that an exchange of life spans is detrimental to the flies' total egg-laying output ($p = 0.000\,02$). The details of this bootstrap test are given in Appendix A.

For example, for the pair of flies whose egg-laying data are shown in figure 1*b,c*, their average remaining egg-laying potential at death (corresponding to eggs not laid) would increase by 4.8% under life span exchange. Their predicted combined output in terms of eggs would decline by *ca.* 130 eggs, a loss of 9.6%, as the longer-lived fly of figure 1*b* would lose more in egg output than the shorter-lived fly of figure 1*c* would gain as a consequence of the hypothetical exchange of lifetimes.

(f) *Predicting subsequent mortality from early patterns of egg laying*

We fitted a Cox proportional hazards model for mortality after day 25 with the function π as a time-varying predictor and obtained a highly significant ($p < 0.001$) relative risk function of $\exp(-1.37\pi(x))$ (Cox 1972; Andersen *et al.* 1993). Flies with only 5% of their egg-laying potential left are 3.42 times as likely to die as flies with 95% of their potential remaining and 1.85 times as likely to die as flies with 50% left. Using the predicted mortality for days 26–30 to form low-, medium- and high-risk groups of 177 flies each, we found observed death rates of 14 out of 177, 26 out of 177 and 61 out of 177, respectively ($p < 0.005$). This demonstrated a highly significant prediction of subsequent mortality from early reproductive patterns based solely on the rate of exhaustion of reproductive potential.

In accordance with other medfly experiments (Müller *et al.* 1997*b*), hazard rates rose rapidly, reached a shoulder and then rose further (figure 1*b–d*). It is noteworthy that the hazard regression model with π as a predictor of mortality was better than alternative models according to the Akaike (1973) information criterion (see Appendix A) and also in terms of prediction error. Therefore, it appears that the remaining egg-laying potential function

π was indeed providing the link with longevity. It is thus the dynamic features of egg laying and not the absolute number of eggs laid that matters.

4. DISCUSSION

A substantial body of theoretical and experimental research on the costs of reproduction (Partridge & Farquhar 1981) and on reproductive determinism (Maynard Smith 1958; Minchella & Loverde 1981; Bell 1984; Bell & Koufopanou 1986; Carey *et al.* 1986) has shown that reproduction can decrease survival and that exhaustion of reproductive capacity plays a role in ageing. Our findings point to a fundamental link between reproductive dynamics and survival. Recently, a 'delayed wave of death' following reproduction was reported (Sgro & Partridge 1999) and, while this finding reinforces the concept of a reproductive clock, our findings do not support the idea that there is a direct cost of reproduction. The link between mortality and reproduction is carried by the dynamics of reproduction and not by the absolute magnitude of reproduction, as measured in the number of eggs produced. For example, a high reproduction rate with slowly declining reproductive potential is associated with a longer life span according to our findings. In contrast, the classical cost-of-reproduction hypothesis would associate high reproduction rates with shortened life spans.

In particular, our analysis provides a detailed description of the nature of the linkage between the dynamics of the reproductive trajectory and subsequent mortality. We established the primacy of the rate of reproductive decline over absolute levels of reproduction regarding this link. It is quite amazing that, based solely on knowledge of early reproductive patterns, our approach allows a reasonable prediction of the increase in subsequent death rates at the level of the individual. A possible interpretation of this finding is that the rate of reproductive decline is a good indicator of the speed of ageing of an organism. In this sense, the reproductive clock is synchronized with an individual's biological age as contrasted to chronological age.

Reproductive decline thus serves as an indicator of not only reproductive exhaustion and gonadal ageing, but also of senescence. Individual medflies experience age-specific fecundity and mortality trajectories that are linked in such a way that flies are generally enabled to lay most of their potential eggs before death. Flies that rapidly exhaust their egg-laying potential tend to die early, while flies that experience slowly declining egg-laying trajectories live longer. These flies have a more slowly advancing reproductive clock and their increased longevity coupled with higher levels of daily egg laying leads to an abundance of eggs as compared with flies with a faster advancing reproductive clock who tend to live shorter lives with rapidly declining daily egg-laying yields. It is tempting to classify individuals into groups displaying various degrees of 'vitality' or 'frailty', which expresses itself in both longevity and level of reproductive activity.

The pace of an individual's reproductive exhaustion and a fly's survival chances may be jointly determined by pleiotropic genetic factors. It is also possible that both egg-laying and mortality patterns are affected by

micro-environmental conditions early in life, conveying physiological strength or frailty (Giesel 1976; Wagner & Altenberg 1996).

Such phenotypic adaptability is plausible as this link enables a fly to produce more offspring on average and because experiments have shown that, depending on whether conditions are favourable for reproduction, medflies can switch between radically different egg-laying and mortality trajectories (Vaupel *et al.* 1998).

APPENDIX A: STATISTICAL METHODS

(a) *Bootstrap test via resampling from lifetimes*

We constructed a bootstrap sample of size 531 by sampling randomly with replacement from the observed 531 lifetimes. The bootstrap sample of lifetimes was denoted by X_i^*, $i = 1, \ldots, 531$. Then we recorded $Q = (1/531) \sum_{i=1}^{531} \pi_i(X_i^*)$. In order to obtain π_i, the fitted parameters β_0 and β_1 for the ith fly were used and the ith bootstrap sample lifetime X_i^* was inserted as the argument. Thus, for each of the 531 flies, the remaining reproductive potential was calculated for an assumed lifetime X_i^*, which was determined according to the bootstrap sample. The bootstrap sampling process was repeated 50 000 times and the empirical distribution of the 50 000 values of Q thus obtained defined the quantile of the observed average remaining potential $\pi_{obs} = (1/531) \sum_{i=1}^{531} \pi_i(X_i)$, within this distribution, X_i being the actually observed 531 lifetimes. This quantile and, thus, the p-value was 0.0008.

(b) *Bootstrap test via exchanging lifetimes in random pairs*

We constructed bootstrap samples consisting of 265 randomly selected pairs of flies by sampling with replacement from a randomly constructed partition of the flies into 265 distinct pairs. For each bootstrap sample we computed the average difference in remaining reproductive potentials when the flies in each of the pairs exchanged their lifetimes, i.e. $X_{i,1}$, $X_{i,2}$, while retaining their reproductive dynamics $\pi_{i,1}(x)$, $\pi_{i,2}(x)$:

$$D^* = \frac{1}{265} \sum_{i=1}^{265} ([\pi_{i,1}(X_{i,2}) + \pi_{i,2}(X_{i,1})]$$
$$- [\pi_{i,1}(X_{i,1}) + \pi_{i,2}(X_{i,2})]). \tag{A1}$$

The relevant quantile and, thus, the p-value is the quantile of zero within the empirical distribution of D^* from 100 000 bootstrap samples and it was found to be 0.000 02.

(c) *Akaike (1973) information criterion*

This criterion (Akaike 1973; Klein & Moeschberger 1997) can be used for model selection in a Cox proportional hazards model. The selected model minimizes $-2 \log L + 2p$, where L is the partial likelihood and p is the number of parameters in the model.

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