

**REPRODUCTIVE POTENTIAL PREDICTS LONGEVITY OF
FEMALE MEDITERRANEAN FRUIT FLIES**

**(reproductive clock/mortality/depletion of eggs/egg-laying
trajectory/modeling/bootstrap)**

Hans-Georg Müller^{1}, James R. Carey², Deqing Wu³, Pablo Liedo⁴ and James W.
Vaupel³*

Version May 9, 2000

¹Division of Statistics, University of California, Davis, One Shields Ave., Davis, CA 95616, USA

²Department of Entomology, University of California, Davis, One Shields Ave., Davis, CA 95616, USA.

³Max Planck Institute for Demographic Research, Doberaner Str. 114, D-18057 Rostock, Germany.

⁴El Colegio de la Frontera Sur, Carreterra Antiguo Aeropuerto, Tapachula, Chiapas, Mexico.

*To whom correspondence should be addressed (e-mail: mueller@wald.ucdavis.edu).

ABSTRACT

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*) reveals that individual mortality is associated with the time dynamics of the egg-laying trajectory. We find, in a sample of 531 medflies, 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 is shown to predict subsequent mortality. The larger the remaining reproductive potential, the lower is the subsequent mortality. Increased mortality risk is seen in flies for which egg production declines 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 lifespan in terms of increased egg output indeed are likely to live longer.

INTRODUCTION

The purpose of this study is twofold: To establish the patterns of reproductive activity as measured in terms of egg-laying for a cohort of female Mediterranean fruit flies (medflies) and then to correlate those patterns with longevity. As it turns out, the egg-laying trajectories on 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 a damage incurred by reproduction which leads to shortened lifespan. In the light of our findings, reproduction itself leads to a decline in reproductive potential which then is associated with increased subsequent mortality. In particular, we find that the rate of decline of reproduction and not intensity of reproduction proves 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.

MATERIALS AND METHODS

For 1000 mated female medflies individual egg-laying counts were recorded daily at the mass-rearing facility in Metapa, Mexico. In addition, time of death was recorded for each fly. From these flies, 531 egg-laying subjects were selected who lived beyond day 26. 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. (1998a).

For the statistical analysis of the data, the Cox proportional hazards model, non-linear least squares, bootstrap tests and smoothed hazard function estimates were used. Details for the two bootstrap tests which were developed to test for an association between reproductive clock and lifespan are given in a separate Appendix.

RESULTS

Constant Rate of Decline of Egg-Laying Individual egg-laying trajectories sharply rise after egg-laying begins 5-17 days after emergence, reach a peak, and then slowly decline. The rate of decline varies among individuals but one of our findings is that this rate is approximately constant for each individual.

The age-trajectory of reproductive decline for each fly is accordingly modeled by the exponential function

$$f(x) = \beta_0 \exp(-\beta_1(x - \theta)),$$

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 (standard deviation)).

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), vary considerably from fly to fly (Fig. 1). A modest but significant negative correlation between β_0 and β_1 ($r = -.15, p < .05$) indicates that fecundity tends to decline more slowly for flies with higher peak fecundity. We find that the protracted decline in egg-laying after the initial sharp rise is reasonably well predicted by the exponential model (Fig. 1).

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

$$\pi(x) = \int_x^\infty f(s)ds / \int_\theta^\infty f(s)ds = \exp(-\beta_1(x - \theta)).$$

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 .

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 is our main result. It leads to a new perspective on the relation between reproduction and longevity.

The association between remaining reproductive potential and longevity was established in three different ways. The first is 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 is that if there is no association between reproductive potential and lifespan, a random exchange of lifetimes would not alter the total number of eggs produced by all flies in the sample.

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

Obviously, fecundity and mortality are strongly correlated with age: As fecundity decreases with older age, mortality increases. Hence, to prevent confounding effects from this association, we fit the trajectories by only using data prior to day 25, whereas longevity is measured as remaining lifetime after day 25. Thus, the fitted trajectories of fecundity are predicted after age 25, based on the above model. This guarantees that the fitted trajectories are not influenced by a fly's lifespan, and allows bona fide predictions of subsequent mortality.

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 is assigned a new lifespan of X^* , chosen at random from the sample of 531 observed lifespans. 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. Utilizing the bootstrap method (Efron & Tibshirani (1993); Manly (1997)), we devise a bootstrap test for this null hypothesis (see Appendix for details). This bootstrap test provides strong evidence against the null hypothesis of no link ($p = 0.0004$) and in favor of the alternative that the occurrence of death becomes ever more likely as the reproductive potential of a fly is exhausted.

Graphical Confirmation of the Association via the Event History Diagram. The result on the association between reproductive clock and lifetime is illustrated by an event history diagram (Fig. 2). The event history diagram (Carey et al. 1998b)) 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 (262/531) died with fewer than 20% of their eggs left (yellow zones indicating an advanced state of the reproductive clock) and 91% (482/531) 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 re-assignment 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 re-shuffling of life spans, not much in terms of additional egg-laying will be achieved for these flies due to the near exhaustion of their egg-laying potential. The same re-shuffling likely 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 meager 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 the average.

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 find that an exchange of life spans is detrimental to the flies' total egg-laying output ($p = 0.00002$). The details of this bootstrap test are given in the Appendix.

For example, for the pair of flies whose egg-laying data are shown in Fig. 1b,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 about 130 eggs, a loss of 9.6%, as the longer lived fly of Fig. 1b would lose more in egg output than the shorter lived fly of Fig. 1c would gain as a consequence of the hypothetical exchange of lifetimes.

Predicting Subsequent Mortality From Early Patterns of Egg-Laying. We fit a Cox proportional hazards model for mortality after day 25 with the function π as a time-varying predictor, obtaining a highly significant ($p < .001$) relative risk function of $\exp(-1.37\pi(x))$ (Cox (1972); Anderson 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 to 30 to form low, medium and high risk groups of 177 flies each, we found observed death rates of 14/177, 26/177 and 61/177 respectively ($p < .005$). This demonstrates highly significant prediction of subsequent mortality from early reproductive patterns, solely based on the rate of exhaustion of reproductive potential.

In accordance with other medfly experiments (Müller et al. (1997b)), hazard rates rise rapidly, reach a shoulder, and then rise further (Fig. 1b-d). It is noteworthy that the hazard regression model with π as predictor of mortality was better than

alternative models, according to the Akaike information criterion (see Appendix), and also in terms of prediction error. Therefore, it appears that the remaining egg-laying potential function π is 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.

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 aging. 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 number of eggs produced. For example, a high reproduction rate with slowly declining reproductive potential is associated with longer lifespan according to our findings. In contrast, the classical cost of reproduction hypothesis would associate high reproduction rates with shortened lifespans.

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 establish 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 aging of the organism. In this sense, the reproductive clock is synchronized with an individual's biological age as contrasted to the chronological age.

Reproductive decline thus serves as an indicator not only of reproductive exhaustion and gonadal aging, but also of senescence. Individual medflies experience age-specific fecundity and mortality trajectories which are linked in such a way that flies generally are enabled to lay most of their potential eggs before death. Flies that exhaust their egg-laying potential fast 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 to flies with a faster advancing reproductive clock who tend to live shorter with rapidly declining daily egg-laying yields. One is tempted 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 the 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 favorable to reproduction, medflies can switch between radically different egg-laying and mortality trajectories (Vaupel et al. (1998)).

APPENDIX: STATISTICAL METHODS

Bootstrap Test via Resampling from Lifetimes. We construct a bootstrap sample of size 531 by sampling randomly with replacement from the observed 531 lifetimes. Denote the bootstrap sample of lifetimes by $X_i^*, i = 1, \dots, 531$. Then record $Q = \frac{1}{531} \sum_{i=1}^{531} \pi_i(X_i^*)$. To obtain π_i , use the fitted parameters β_0, β_1 for the i -th fly and insert as argument the i -th bootstrap sample lifetime X_i^* . So for each of the 531 flies, the remaining reproductive potential is calculated for an assumed lifetime X_i^* , which is determined according to the bootstrap sample. The bootstrap sampling process is repeated 50,000 times, and the empirical distribution of the 50,000 values of Q thus obtained defines the quantile of the observed average remaining potential, $\pi_{obs} = \frac{1}{531} \sum_{i=1}^{531} \pi_i(X_i)$ within this distribution, X_i being the actually observed 531 lifetimes. This quantile and thus the p-value was .0008.

Bootstrap Test via Exchanging Lifetimes in Random Pairs. We construct 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 compute the average difference in remaining reproductive potentials when the flies in each of the pairs exchange their lifetimes, $X_{i,1}, X_{i,2}$, while retaining their reproductive dynamics $\pi_{i,1}(x), \pi_{i,2}(x)$:

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

The relevant quantile and thus the p-value is the quantile of 0 within the empirical distribution of D^* from 100,000 bootstrap samples, and it was found to be 0.00002.

Akaike 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.

ACKNOWLEDGEMENTS

This research was supported by the National Science Foundation (H.G.M.), the National Institute of Aging (J.R.C., H.G.M. and J.W.V.), and the Max Planck Society (H.G.M., D.W., and J.W.V.)

REFERENCES

- Abrams, P. & Ludwig, D. 1995 Optimality theory, Gompertz' Law and the disposable soma theory of senescence *Evolution* **49**, 1055-1066
- Akaike, H. 1973 Information Theory and an Extension of the Maximum Likelihood Principle. In: *Second International Symposium of Information Theory and Control*, E.B.N. Petrov and F. Csaki, eds. (Akademia Kiado, Budapest) pp. 267-281
- Andersen, P.K., Borgan, O., Gill, R.D. & Keiding, N. 1993 *Statistical Methods Based on Counting Processes* (Springer, New York).
- Bell, G. 1984 Measuring the cost of reproduction. The correlation structure of the life tables of five freshwater invertebrates. *Evolution*, **38** 300-313
- Bell, G. & V. Koufopanou 1986 The cost of reproduction. Oxford Surveys in Evolutionary Biology, Vol.3, ed. R. Dawkins & M. Ridley. Oxford, Oxford University Press, 83-131
- Carey, J., Krainacker, D.A. & Vargas, R.I. 1986 Life history response of female Mediterranean fruit flies, *Ceratitis capitata*, to periods of host deprivation. *Entomol. exp. appl.* **42**, 159-167
- Carey, J., Liedo, P., Müller, H.G., Wang, J.L. & Chiou, J.M. 1998a Relationship of age patterns of fecundity to mortality, longevity and lifetime reproduction in a large cohort of Mediterranean fruit fly females. *J. Gerontol.: Biol. Sciences* **53A**, B245-B251
- Carey, J., Liedo, P., Müller, H.G. & Wang, J.L. 1998b A simple graphical technique for displaying individual fertility data and cohort survival: case study of 1,000 Mediterranean fruit fly females. *Functional Ecology* **12** 359-363
- Carey, J., Liedo, P., Müller, H.G., Wang, J.L. & Vaupel, J. 1998c Dual modes of ageing in Mediterranean fruit fly females. *Science* **281**, 996-998
- Chapman, T., Miyatake, T., Smith, H. & Partridge, L. 1998 Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. *Proceedings of the Royal Society of London Series B - Biological Sciences* **265** 1879-1894.

- Cox, D.R. 1972 Regression models and lifetables (with discussion). *J. Roy. Statist. Soc. B* **34**, 187-220
- Efron, B. & Tibshirani, R.J. 1993 *An Introduction to the Bootstrap*. (Chapman and Hall, London)
- Giesel, J. T. 1976 Reproductive strategies as adaptations to life in temporally heterogeneous environments. *Annual Review of Ecology and Systematics* **7**, 57-79
- Kirkwood, T.B.L. & Rose, M.R. 1991 Evolution of senescence: Late survival sacrificed for reproduction. *Philos. Trans. Roy. Statist. Soc. London B* **332**, 15-24
- Klein, J.P. & Moeschberger, M.L. 1997 *Survival Analysis Techniques for Censored and Truncated Data*. (Springer, New York).
- Manly, B.F.J. 1997 *Randomization, Bootstrap and Monte Carlo Methods in Biology*. (Chapman and Hall, London).
- Maynard Smith, J. 1958 The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura*. *J. Exp. Biol.*, **35** 832-842
- Minchella, D.J. & Loverde, P.T. 1981 A cost of increased early reproduction in the snail *Biomphalaria glabrata*. *Am. Naturalist* **118** 876-881
- Müller, H.G., Wang, J.L. & Capra, W.B. 1997a From lifetables to hazard rates: The transformation approach. *Biometrika* **84**, 881-892
- Müller, H.G., Wang, J.L., Capra, W.B., Liedo, P. & Carey 1997b Early mortality surge in protein-deprived females causes reversal of male-female life expectancy relation in Mediterranean Fruit Flies. *Proc. Nat. Acad. Sci. USA* **94** 2762-2765
- Partridge, L. 1987 Is accelerated senescence a cost of reproduction? *Functional Ecology* **1**, 317-320
- Partridge, L. & Barton, N.H. 1993 Optimality, mutation and the nature of ageing. *Nature* **362**, 305-311
- Partridge, L. & Farquhar, M. 1981 Sexual activity reduces lifespan of male fruit flies. *Nature* **294**, 580-582
- Sgro, C.M. & Partridge, L.W. 1999 A delayed wave of death from reproduction in *Drosophila*. *Science* **286**, 2521-2524

- Vaupel, J.W., Carey, J.R., Christensen, K., Johnson, T.E., Yashin, A.I., Holm, N.V., Iachine, I.A., Kannisto, V., Khazaeli, A.A., Liedo, P., Longo, V.D., Yi, Z., Manton, K.G. & Curtsinger, J.W. 1998 Biodemographic trajectories of longevity. *Science* **280**, 855-890
- Wagner, G.P., & Altenberg, L. 1996 Complex adaptations and the evolution of evolvability. *Evolution* **50** 967-976
- Westendorp, R.G.J. & Kirkwood, T.B.L. 1999 Human longevity at the cost of reproductive success. *Nature* **396**, 743-746
- Williams, G.C. 1957 Pleiotropy, natural selection and the evolution of senescence. *Evolution* **11**, 398-411

Figure 1 Trajectories of fecundity and mortality. Trajectories of fecundity are fitted to data from peak to day 25 (red solid) by nonlinear least squares and predicted thereafter (red dashed). Age at death is indicated by X . **a.** Trajectory fitted to average counts of daily eggs for the sample of 531 medflies. **b.** Daily egg counts, reproductive trajectory, and predicted smoothed (Müller, Wang & Capra (1997a)) individual hazard function (green) for fly with life span at 10% quantile. **c.** Same for fly at 50% quantile. **d.** For fly at 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 colored horizontal bar for each fly. Taken together, the bars provide the empirical survival function. The colors within each bar indicate 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.