

UC Davis

UC Davis Previously Published Works

Title

Distinctive egg-laying patterns in terminal versus non-terminal periods in three fruit fly species

Permalink

<https://escholarship.org/uc/item/00f689mw>

Authors

Meng, Xiang
Hu, Junjie
Plant, Richard E
et al.

Publication Date

2021-03-01

DOI

10.1016/j.exger.2020.111201

Peer reviewed



Published in final edited form as:

Exp Gerontol. 2021 March ; 145: 111201. doi:10.1016/j.exger.2020.111201.

Distinctive Egg-laying Patterns in Terminal versus Non-terminal Periods in Three Fruit Fly Species

Xiang Meng^{1,2}, Junjie Hu², Richard E. Plant³, Tim E. Carpenter⁴, James R. Carey^{5,6,*}

¹Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangdong Academy of Science, Guangzhou 510260, China.

²College of Life Science, Guangzhou University, Guangzhou 510006, China.

³Department of Plant Sciences and Biological and Agricultural Engineering, University of California, Davis 95616, USA

⁴Department of Medicine and Epidemiology, University of California, Davis 95616, USA

⁵Department of Entomology, University of California, Davis 95616, USA;

⁶Center for the Economic and Demography of Aging, University of California, Berkeley 94720, USA.

Abstract

The specific objective of this study was to use a logistic regression model for determining the degree to which egg laying patterns of individual females at the end of life (i.e., terminal segments) in each of three different fruit fly species could be distinguished from the egg-laying patterns over a similar period in mid-life (i.e., non-terminal segments). Extracting data from large-scale databases for 11-day terminal and 11-day non-terminal segments in the vinegar fly (*Drosophila melanogaster*), the Mexican fruit fly (*Anastrepha ludens*) and the Mediterranean fruit fly (*Ceratitidis capitata*) and organizing the model's results in a 2 x 2 contingency table, we found that: (1) daily egg-laying patterns in fruit flies can be used to distinguish terminal from non-terminal periods; (2) the overall performance metrics such as precision, accuracy, false positives and true negatives depended heavily on species; (3) differentiating between terminal and non-terminal segments is more difficult when flies die at younger ages; and (4) among the three species the best performing metrics including accuracy and precision were those produced using data on *D. melanogaster*. We conclude that, although the reliability of the prediction of whether a segment occurred at the end of life is relatively high for most species, it does not follow precisely

*Corresponding author: jrcarey@ucdavis.edu.

Author Statements:

Xiang Meng: Conceptualization, co-writing original draft, data collation and analysis. Junjie Hu: Conceptualization, co-writing original draft preparation; Richard Plant: formal analysis, reviewing and editing. Tim Carpenter: formal analysis, reviewing and editing. James Carey: Conceptualization; visualization, formal analysis, writing, funding acquisition.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

predicting remaining life will also be highly reliable since classifying an end of life period is a fundamentally different challenge than is predicting an exact day of death.

Keywords

Time-to-death; insect mortality; reproduction; biodemography; Mediterranean fruit fly; *Ceratitis capitata*; Mexican fruit fly; *Anastrepha ludens*; *Drosophila melanogaster*

1. Introduction

Accurate predictions of time-to-death (also known as thanatological age estimates; Riffe and others 2016) are important in biomedicine to allow patients to better consider their future, for health professionals to make more informed medical decisions, and for family members to have realistic expectations (Siontis and others 2011). Consequently a robust literature exists in the medical sciences concerned with predicting survival time of persons suffering from one or more of any number of different fatal diseases (de Beaufort and van de Vathorst 2016; deHaas and others 2019; Glare and others 2003; Harris and others 2014; Siontis and others 2011; Stearns and Norton 2004). This literature extends to the veterinary sciences and basic biology, as well to papers on predicting survival times for terminally-ill pets including dogs (Beer and others 2013; Goggs and others 2015), cats (Chakrabarti and others 2012; Payne and others 2015) and laboratory rodents (Trammell and Toth 2011).

One of the common threads across this thanatological research on humans, pets and rodents is that the predictions are informed, not only by extensive knowledge of the causes, origins and natural courses of the underlying diseases but, at least for humans, also by access to deep databases containing the outcome of tens of thousands, if not millions, of previous cases at different disease stages that end in the individual's death. Remarkably, even with access to this extensive information on disease progress and outcomes, the vast majority of models in biomedicine used for predicting survival times are both imprecise and inaccurate, with short-term survival usually overestimated and long-term survival often underestimated (Bartholomai and Frieboes 2018; Glare and others 2003; Grady and Berkowitz 2011). The inaccuracy of sophisticated models designed to predict human survival time that are based on extraordinary amounts of data and extensive biomedical information on both the disease and the patient lays bare the challenges of predicting survival time in non-human species for which virtually none of this information available.

Inasmuch as there is no practical need for developing models for predicting time to death in the overwhelming majority of non-domesticated organisms, it is unsurprising that the literature in this area is extraordinarily scarce. With the exception of the papers by Rauser, Mueller and their colleagues in which these researchers classify *Drosophila melanogaster* females according to whether or not they were in a death spiral stage (Mueller and others 2007; Mueller and others 2009; Rauser and others 2005; Rauser and others 2003; Rauser and others 2006), and the paper by Papadopoulos and his colleagues (Papadopoulos and others 2002) who showed that supine behavior (temporarily upside-down) was predictive of impending death in medflies, we are aware of no other papers that are expressly concerned with predicting the timing of death in fruit flies or other non-domestic group of organisms.

However, there are two groups of studies whose results are related to our research. The first group involves end-of-life egg laying patterns in the context of aging including: (1) Novoseltsev and his colleagues (Novoseltsev and others 2005; Novoseltsev and others 2004; Novoseltsev and others 2003) on the senescent stage of *D. melanogaster* and the medfly as exponentially-decreasing rate of reproduction, (2) Curtsinger and his colleagues (Curtsinger 2015; Curtsinger 2016; Curtsinger 2018; Khazaeli and Curtsinger 2010) on *working* (i.e., actively ovipositing with low but accelerating mortality) versus *retired* (i.e., terminal stage with limited fecundity and constant mortality) who characterized the end-phase degree of “roughness” of individual egg laying using the fractal concept of lacunarity (for additional perspectives see Le Bourg and Moreau 2014); and (3) Rogina and her colleagues (Rogina and others 2007) who, by manipulating the timing of mating in *D. melanogaster* females, discovered reproductive patterns that were conditional on when females mated. All experiments in her and her colleague’s studies revealed characteristics suggesting that longer-lived flies passed through three stages, the last of which they labeled “declining-terminal”.

The second group of studies related to our work, albeit more tangentially, includes research concerned with the timing of reproduction relative to their death. One study representative of this group involves research concerned with the length of remaining life in individual flies relative to others based on the rate of decrease in reproduction after the peak. For example, Müller and his colleagues (Müller and others 2001) showed that the exponential rate of decrease in egg laying by female medflies predicted the remaining life spans of individuals. The other area within this second group involves research on the cost of reproduction in which increments of reproduction in early life result in decrements in survival later in life including papers by Harshman and Zera (2006) on mechanisms (e.g., effects on immunity and metabolism) and modeling papers by Mangel and Heimpel (1998), Fletcher and his co-workers (Fletcher and others 1994) and Rosenheim (1999) concerned with foraging strategies relative to both remaining reproduction and lifespans.

There are at least three reasons why developing predictive models of impending death or that can be used to distinguish terminal periods (as we do here) in non-human species such as fruit flies is potentially important. *First*, accurate predictive models can affirm patterns associated with the transition from early stages of aging to the late stages in which the manifestations of increasing frailty are catastrophic, which is to say, ends in death. This same “staging” process might have the potential to be used to identify parse other stages of the aging process. *Second*, predictive models for death could be used in intervention trials in which flies showing early signs of impending death could be identified and subjected to treatments designed to test death-postponing interventions. *Third*, the model-building required to identify different patterns of reproduction that identify impending death in flies, in some form, may be relevant to the model-building process predicting the timing of death in humans. Indeed the model-building process itself might shed light on the classification process involved in separating the non-dying and dying as well as in calibrating survival time (Glare and others 2003).

In light of the paucity of information on end-of-life patterns of life history phenomena in non-human species as well as the potential importance to basic biology of these types of

studies, the overarching goal of our study was to determine whether it was possible to distinguish across three fruit fly species a set of general patterns of egg laying in individual females who were near death (i.e., terminal segments) from the egg-laying patterns in individual who were not near death (i.e., non-terminal segments). Our specific aims were to: (1) Visualize and summarize the egg-laying patterns in individuals from all species with respect to both their chronological and thanatological ages; (2) create subsets of these data in containing egg laying sequences from both the end-of-life (terminal segment) and mid-life (non-terminal segments); and (3) test hypotheses that the patterns of egg laying between the two categories of egg-laying segments have distinct patterns.

2. Material and Methods

Data sources

Individual-level daily egg laying data for three fruit fly species (Carey and others 2005a; Carey and others 1998a; Carey and others 1998b; Carey and Roach 2020; Novoseltsev and others 2005; Novoseltsev and others 2003) were used for the analysis for the two tephritid fruit fly species including the Mediterranean fruit fly (*Ceratitis capitata*), commonly known as the Medfly (n=1,000), the Mexican fruit fly (*Anastrepha ludens*), commonly known as the Mexfly (n=1,131), and the vinegar fly, *Drosophila melanogaster* (n=666). Husbandry details for each species are described in the above-cited papers. In brief rearing conditions were 25-27° C and 50-75% RH, and 12:12 L:D for the two tephritid species and 10:14 L:D for *D. melanogaster*. Whereas the two tephritid species were fed a 3:1 sugar-yeast hydrolysate diet, *D. melanogaster* females were fed a standard agar-gelled *Drosophila* food medium. Eggs were collected from mesh at one end of the individual cages for the tephritid females and from the food medium for *D. melanogaster* (Arking and others 2002).

These species were chosen primarily for the availability of databases on individual-level lifetime egg laying. However, they were also chosen because they allowed for three levels of comparison to test the robustness of our discoveries—between two dipteran families (*Drosophilidae* vs *Tephritidae*), between two tephritid genera (*Ceratitis* vs *Anastrepha*), and among three different species.

2.1 Data analysis

2.1.1 Parsing, configuring and summarizing—Reproductive data were excluded for the first 10 days of adult life (i.e., 11 days counting day of eclosion, age 0) for each of the species, a period during which all individuals matured and developed their first eggs. The remaining data for each fly were then parsed into 1-of-2 segment categories: (1) *Terminal segment* consisting of the sequence of daily reproduction from 11 days before death to and including the day of death. There were 873, 1,071 and 425 terminal segments derived from individual female Medflies, the Mexflies and *D. melanogaster*, respectively; and (2) *Midlife segments* of the same length. The midlife segments were created from contiguous 11-day series of individual flies that started after maturation (i.e., > day 10) and ended 11 days before the fly died. This selection criteria thus excluded individual flies that lived less than 32 days (i.e., 10 days maturation + 11 day midlife + 11 day terminal = 32 days). This segmentation process was repeated until the number of remaining days to the terminal

segment was less than 11. The remaining days were ignored. The 11-day egg-laying sequences were chosen (i.e., 10 days of potential egg laying + 1 day of death) primarily because the results of preliminary investigations revealed that 11-days was the shortest period of egg laying that contained patterns that yielded consistently high performance metrics across all three species. This period was also chosen because longer egg-laying periods (e.g., 12 to 15 days) would have substantially reduced the number of segments in each of the databases.

We computed two metrics from all of the 11-day egg laying sequences for all three species and the terminal and mid-life segments: 1) *Total eggs (TE)*. This metric was chosen based on the observation that most flies laid fewer eggs at the end of life; and 2) *egg-laying ratio (ER)*. This metric was based on the observation that the relative rate of egg laying in most flies decreased at the end of life. This metric was computed as the ratio of the number of eggs produced from 11 to 6 days prior to death to the number of eggs produced from days 5 to 0 prior to death. This ratio specified both a direction (e.g., decreasing egg production if >1) and a magnitude (e.g., 3-fold) of change as an individual fly approached death. Egg ratio was assigned a value of $ER=0$ if no eggs were laid in either time interval and a value of $ER=5.0$ if eggs were laid in the first 6 days but none in the last 5 days—a pattern that suggests rapid egg-laying decline to zero but that results in a zero in the denominator (and thus an undefined ratio). This value was chosen because it was at the mid-range of the highest ER-values when eggs were laid in both first and last segments. This ER value was high enough to serve as a major change metric between the first and last egg-laying sub-segments but not so high to be the overriding drivers of the statistical outcomes.

2.1.2 Concept visualization—Fig. 1 shows the egg laying patterns of selected individual medfly females at 10 different life table deciles. With the exception of female #5, the egg laying patterns during the terminal segments were consistent with the hypothesis that rate of egg laying near the end of each of their lives was both low (i.e., few in number) and decreasing (i.e., egg ratio $\gg 1.0$). However, the egg-laying patterns that characterize the end of female's life are also sometimes observed at times when they are not approaching death. Indeed, there are also a number of 11-day midlife sequences of some flies that are indistinguishable from these same patterns in the terminal phases. For example, egg laying rates decrease in fly #5 from days 20 to 30 and in fly #8 from days 30 to 40 days. Although these decreasing egg-laying patterns usually predict impending death, both of these flies another 11 days beyond these ages. Similar egg-laying trends are also evident during the midlife of fly #9. Fly #10 produced very few eggs for a 40-day period from ages 20 through 60 days. Thus this visual inspection of egg laying in 10 different individuals reveals the statistical challenge of classifying egg-laying sequences as either terminal or mid-life.

2.1.3 Reproduction metrics—Three summary metrics for age-specific reproduction (Carey and Roach 2020) were computed for each species in both chronological and thanatological time including *gross reproductive rate*, denoted GRR, defined as the number of eggs produced by a female that lived to the oldest age will lay, and *net reproductive rate*, denoted R_0 , and defined as the average number of eggs the average females will lay in her lifetime. Formulae for these metrics are given as:

$$GRR = \sum_{\alpha}^{\beta} m_x \quad (1)$$

$$R_0 = \sum_{\alpha}^{\beta} l_x m_x \quad (2)$$

where l_x denotes the probability of survival from birth to age x , m_x denotes the average number of eggs produced by a female age x , and α and β denote the ages of first and last reproduction, respectively. The third metric, denoted T , is the *average age of parenthood* computed as

$$T = \frac{\sum_{\alpha}^{\beta} x l_x m_x}{\sum_{\alpha}^{\beta} l_x m_x} \quad (3)$$

Although computing all three of these parameters is straightforward in both chronological or thanatological age, the values for and interpretations of GRR and T differ between the two age categories. Consider the following hypothetical case for clarifying the differences. Suppose that in chronological time, the age-specific egg laying in four female fruit flies was identical for the ages each were alive with reproductive peaks at 50 eggs/day on day 20. However, they each die at different ages 20 days apart—i.e., at ages 20, 40, 60 and 80 days. When their reproductive schedules are considered in thanatological ages, which is to say, relative to their age of death rather than their age of birth ($x=0$), then their reproductive peaks are in thanatological time correspond to ages 0, 20, 40 and 60 days (i.e., time to death). Although R_0 remains the same since every female still produces the same lifetime number of eggs, the values of both GRR and T will in the vast majority of cases be different because the timing of reproduction is relative to age of death rather than to age of birth (i.e., eclosion). Whereas day 20 was the average age of peak reproduction in chronological time for the hypothetical example, it is day 30 in thanatological time [i.e., $(0 + 20 + 40 + 60)/4$].

2.1.4 Contingency table—A 2x2 contingency table that classifies prediction is shown in Fig. 2 where the numbers along the major diagonal represent the correct decisions made, and the numbers in the other diagonal represent the errors (Fawcett 2006; Tharwat 2018). If the instance is positive (i.e., terminal segment) and the instance is a positive, it is counted as a true positive. However, if it is classified as a negative it is a false negative. If the instance is negative (i.e., midlife segment) and the instance is a negative, it is counted as a true negative. However, if it is classified as a positive it is a false positive. This contingency table forms the basis for a number of common metrics given below.

2.2 Logistic regression model

We used logistic regression to produce a probability that an 11-day sequence of egg laying was either the terminal sequence (=1) or not (=0). We define p as the probability that the outcome is 1, with the multiple logistic regression model written as

$$\hat{p} = \frac{\exp(b_0 + b_1X_1 + b_2X_2)}{1 + \exp(b_0 + b_1X_1 + b_2X_2)} \quad (4)$$

where \hat{p} is the expected probability that this was the terminal segment with the independent variables corresponding to the parameters b_1 , and b_2 as total eggs (TE) and egg ratio (ER), respectively. The model was applied to the data in two forms. The first, referred to as *uncensored*, considered all of the data, and the second, referred to as *censored*, removed from the model those data records in which no eggs were laid in the terminal 11-day segment—i.e., cases in which within-segment egg-laying patterns were non-existent.

The multiple logistic regression model is sometimes written differently. In the following form, the outcome is the expected log of the odds that the outcome is present,

$$\ln\left(\frac{\hat{p}}{1 - \hat{p}}\right) = \exp(b_0 + b_1X_1 + b_2X_2) \quad (5)$$

The coefficients derived from the model (e.g., b_1) indicate the change in the expected log odds relative to a one unit change in X_1 , holding all other predictors constant. Therefore, the antilog of an estimated regression coefficient, $\exp(b_i)$, produces an odds ratio.

2.3 Classification Performance

2.3.1 Accuracy and error rate—The *Fraction Correct (FC)*, also referred to as the *accuracy* metric, equals the fraction of both midlife and terminal segments that were correctly classified.

$$FC = \frac{(TP + TN)}{(TP + TN + FP + FN)} \quad (6)$$

where TP, TN, FP and FN denote true positives, true negatives, false positives, and false negatives, respectively. The complement of FC is the *Fraction Incorrect (FI)* and equals the fraction of all segments that were incorrectly classified.

$$FI = \frac{(FP + FN)}{(TP + TN + FP + FN)} \quad (7)$$

This metric is also known as the *misclassification rate*.

2.3.2 Sensitivity and specificity—The *True Positive Rate (TPR)*, also called *sensitivity*, is the proportion of terminal sequences that are correctly classified. Thus:

$$TPR = \frac{TP}{TP + FN} \quad (8)$$

Its counterpart, the *True Negative Rate (TNR)*, also called *specificity*, is the proportion of mid-life sequences that are correctly classified and is given by.

$$TNR = \frac{TN}{FP + TN} \quad (9)$$

2.3.3 False positive and false negative rates—The *False Positive Rate (FPR)*, also called *false alarm rate*, is the probability that a midlife sequence will be incorrectly classified as a terminal segment.

$$FPR = \frac{FP}{TN + FP} \quad (10)$$

False Negative Rate (FNR), is the proportion of terminal segments that were classified as midlife segments. This metric complements the sensitivity measure, TPR.

$$FNR = \frac{FN}{TP + FN} \quad (11)$$

2.3.4 Predictive values—Predictive values (positive and negative) reflect the performance of the prediction. The *Positive Predictive Value (PPV)*, also known as the *precision metric*, equal the proportion of all segments classified as terminal that are truly terminal.

$$PPV = \frac{TP}{TP + FP} \quad (12)$$

Its complement, the *False Discovery Rate (FDR)*, equals the proportion of all segments that were classified as terminal that were actually midlife segments.

$$\begin{aligned} FDR &= \frac{FP}{TP + FP} \\ &= (1 - PPV) \end{aligned} \quad (13)$$

Negative Predictive Value (NPV) is the proportion of all segments that were classified as midlife that were classified correctly.

$$NPV = \frac{TN}{TN + FN} \quad (14)$$

Its complement, the *False Omission Rate (FOR)*, is the proportion of all segments that were classified as midlife that were incorrectly classified.

$$\begin{aligned} FOR &= \frac{FN}{TN + FN} \\ &= (1 - NPV) \end{aligned} \quad (15)$$

This measure complements both the PPV and the FDR.

Likelihood ratios

The likelihood ratios combine both sensitivity and specificity—not all positive results are true positive and, likewise, not all negative results are true negatives. The *Positive Likelihood* (LR+) is the ratio of the *True Positive Rate* to the *True Negative Rate*. It measures how much the odds of the terminal segment classification increases when the prediction is positive.

$$LR + = \frac{TPR}{FPR} \quad (16)$$

The Negative Likelihood (LR−) is the ratio of the False Positive Rate to the *False Negative Rate*. This rate measures how much the odds of the likelihood of the terminal segment classification decreases when the prediction is negative.

$$LR - = \frac{FNR}{TNR} \quad (17)$$

The *Diagnostic Odds Ratio* (DOR) is the ratio of the odds of the probability that the terminal segment being correctly classified relative to it being incorrectly classified relative to the odds of the probability that the segment is terminal relative to the odds of the segment being positive but not being a terminal segment.

$$DOR = \frac{LR +}{LR -} \quad (18)$$

3. Results

3.1 Lifetime Reproductive Patterns

Event-history reproductive charts plotted in both chronological and thanatological time for all three fruit fly species are presented in Fig. 3. Several aspects of these graphs merit comment. *First*, the chronological plots of egg laying patterns for all species reveal the familiar progression starting at eclosion from the pre-reproductive, maturation period, followed by a periods of high reproduction with relatively high levels of intra-individual and inter-species variability. This period, in turn, is followed by a period of tapering off, the length of which depends largely on an individual's lifespan. The low levels of egg laying are evident at older ages in all species but is most striking in the oldest *D. melanogaster*. *Second*, the event-history plots in thanatological time reveal visually the repositioning of reproduction that occurs when the schedule is normalized with respect death rather than birth (eclosion). This shift is especially evident in diagonal band of high reproduction that tracks to the left of the cohort survival the curve. In these cases (lower row of graphs) the most advanced ages in thanatological time correspond the very youngest and thus most fecund ages in chronological time. *Third*, patterns of egg laying near death as seen in all

three species and for both time frames differ between short-lived and long-lived individuals. This is outcome of the differences in the underlying “causes” of death at young and old ages. Increasing frailty due to old age is the most likely “cause” of death in the longest lived individuals. This accounts for the progressive decrease in egg production at older ages. However, increasing frailty due to aging is an unlikely “cause” of death for flies that die young and at ages when they are at or near their peak in egg production. Thus no single egg laying pattern or combination of patterns will likely ever apply to all flies regardless of age or cause of death.

3.2 Gross and Net Reproduction

Comparisons of the average reproductive rates and timing for all three species plotted with respect to both chronological and thanatological ages are given in the different panels shown in Fig. 4. Because the number of eggs laid by the average female in her lifetime is the same regardless of whether the eggs are summed from birth to death or from death to birth, net reproductive rates, R_0 , will be the same regardless of whether it is considered in chronological or thanatological time. But as noted in Methods section, this not the case for gross reproductive rate (GRR) computations. For example, the 50% greater value of GRR for thanatological age ($\approx 2,020$) relative to the value of this metric for chronological age ($\approx 1,332$) in *D. melanogaster* is the result of a subset of extremely long-lived individuals who both matured early and produced many eggs at young ages. When re-plotted these young ages in chronological time represent the “old” ages in thanatological time. Thus individuals who are both long-lived and highly fecund at young chronological ages represent a large fraction of the small number at the tail end of the “death” cohort. Differences in the values of the mean age of parenthood, T , across species for chronological versus thanatological ages revealed that it was 8 days closer to birth than to death in *D. melanogaster*, but slightly over 4 days closer to death than to birth in the Mexfly and nearly equidistant from birth and death in the Medfly (i.e., 21.6 vs 20.3 days).

3.3 Independent Variables: Total eggs and egg ratio

The means and frequency distributions of the two independent variables we use in the regression model for each of the fly species are shown in the series of plots contained in Fig. 5. These graphs anticipate the outcome of the modelling results by revealing the differences between the metrics in the midlife segments relative to the terminal segments. There were striking differences in the metrics for each of the two categories of segments in *D. melanogaster* with nearly 5-fold fewer eggs and 3-fold greater average egg ratio in the terminal segments. The signs of the differences in the mean and overall distributions for the two tephritid species were similar but the magnitudes of the differences were much less. We thus anticipate more favorable performance metrics for distinguishing between terminal and mid-life egg laying patterns in *D. melanogaster* than in the two tephritid species.

3.4 Logistic Regression Model

The logistic regression model yields three general results. *First*, the model’s overall performance supports the concept that the egg-laying patterns of total eggs (TE) and egg ratio (ER) as individual flies approached death are, in the majority of cases, distinctly different from those patterns over an 11-day sequence in middle of their lives. This was

evident in the performance metric (Table 1) of fraction correct (FC)—i.e., the FC-value for all species exceeded 0.64 using all data and exceeded 0.73 using only the segments in which flies laid 25 eggs or greater (censored). *Second*, with a minor difference for several of the *D. melanogaster* metrics, the performance of the regression model was more favorable when applied to the censored data than with use of the uncensored data. The reason for the differences was because there were 11-day egg-laying sequences in which few or no eggs were produced or that egg laying was declining in midlife. These are the patterns for the independent variables that were associated with and thus predictive of the terminal segments. These midlife patterns occurred more in the two tephritid species than in *D. melanogaster* and thus helps explain the higher performance levels for the regression model in this species. *Third*, the performance metrics for *D. melanogaster* were extraordinarily high relative to those for the two tephritids as well as absolute. One metric especially stands out—positive predictive value (PPV) for *D. melanogaster* was nearly 95% (i.e., (0.9395)). In other words, given that an 11-day egg-laying segment of this species is classified as terminal, there is nearly a 95% chance that this classification is correct. The value of this performance metric in this fruit fly species contrasts to that for the values from data on the tephritid species where it ranged between around 66% to just under 75% depending on species and whether the database was complete or censored.

4. Discussion

We believe that there were a number of important results from this study. The first was the revelation that daily egg-laying patterns (i.e., TE; ER) in fruit flies can be used to distinguish periods (i.e., 11 days) in a female fly's life that end in death from those that do not (i.e., terminal vs non-terminal segments). Most of the previous analyses and models concerned with individual-level egg laying in fruit flies at or near the end of life focused primarily on classification of end-of-life stage such as senescent, terminal or retired stage. Our approach differed in that we made specific predictions that could be quantified according to performance metrics.

A second important result was that the overall performance metrics such as precision, accuracy and false positives and negatives were heavily species-dependent. This was because of between-species differences in the consistency of egg laying patterns in both the midlife and terminal segments. Among the three species the best performing metrics including accuracy and precision were those produced using data on *D. melanogaster*.

A third important outcome of our study was that it shed important light on the challenges inherent in predicting the timing of death. As we noted earlier, one reason for some of the low-performing metrics was that the egg-laying patterns indicative of flies that are at the end of their lives are also present in midlife. Consequently the segments in which these occur are misclassified as terminal rather than midlife. Another problem of misclassification occurred when flies either died at the height of their egg laying (i.e., when younger) or very old flies produced larger numbers of eggs close to their day of death. In other words, patterns of egg laying that were more common in midlife than at or near the end of life. These types of misclassifications were rare in *D. melanogaster* but occurred around one third of the time in

the Mexfly as was reflected in the much higher rates of false negatives for this species relative to the other two.

A fourth important and what we consider to be the overarching outcome of our study was “*proof-of-principle*”—patterns of reproduction at the individual level contain information that can be used to predict impending death in fruit flies. This general finding is important since it suggests that there are likely identifiable individual-level age-specific life history data ranging from information on respiratory, metabolic and locomotor activity (Avanesian and others 2009; Lane and others 2014; Williams and others 2004; Zou and others 2000) to sleep, circadian, and mating patterns (Chiu and others 2013; Priest and others 2008; Shaw and others 2000) that can be used singly or in various combinations to predict the timing of death, not only in fruit flies, but in a range of other organisms including in humans. Indeed, the egg laying patterns we used to identify impending death may be the rudimentary equivalents of clinical prediction rules (CPR) in humans (Grady and Berkowitz 2011; Reilly and Evans 2006; Siontis and others 2011) used to calibrate the timing of deaths across age groups and between sexes.

A unique strength of our study was in its multi-level comparisons involving two different fruit fly families (i.e., Drosophilidae vs Tephritidae), two different genera within one of these families (i.e., *Anastrepha* sp vs *Ceratitis* sp) and three different species overall (i.e., *D. melanogaster*, Medfly and Mexfly). Our results demonstrated (1) that the same independent variables could be used across families, genera and species to classify egg-laying segments (i.e., robust); but that (2) the performance metrics such as accuracy and precision were family-, genus- and species-dependent.

A caveat that applies to all studies like ours involving contingency tables is that performance metrics including PPV and NPV depend upon the relative numbers of positive and negative cases. Because the proportion of negatives influences the number of both false positives and true negatives, as the proportion of negatives increases, PPV decreases and NPV increases. For example, in disease epidemiology the if the prevalence of infection were low, the PPV might be very small (e.g., <1%) but will be much larger (e.g., 80%) in a population with moderate or high prevalence. So the fraction of true positives relative to false positives will be lower (and thus PPV lower) in the former (low prevalence) hypothetical scenario than in the latter (high prevalence) but in both cases the TPR remains the same.

This sample size-dependent relationship for some performance metrics in the contingency table was one of the primary reasons we used equal numbers of mid life and terminal segments for each fruit fly species rather than the number of mid-life segments possible for each species. For example, after a 10-day maturation period, flies that live 25, 50 and 100 days each have only a single 11-day terminal segment but 4, 29 and 68 possible mid-life 11-day segments, respectively. Because both the cohort sizes and life expectancies differed among the three fly species, the relative numbers of possible mid-life versus terminal 11-day segments also differed. Thus using different relative numbers of mid-life versus terminal segments for each species rather than equal numbers would have confounded comparisons. This perspective underscores the caution required in the interpretation of contingency table performance metrics such as sensitivity and specificity where one is much higher than the

other. Indeed, this is the case for the Medfly and Mexfly in which sensitivity ranges from 40 to 60% (uncensored) for both but specificity is substantially higher for both species—i.e., 90 and 70%, respectively. This we conclude that our findings are robust but that the specific values of the performance metrics are subject aspects of the sample including size and details such as segment length. An entirely different aspect of this large concept and one we don't address in this study is that of distinguishing between mid-life and terminal segments when, for example, age-specific reproductive patterns are altered by changing food quality modifying availability (Carey and others 2008; Carey and others 2002; Carey and others 2005b).

We believe that research on the classification and timing of death in non-human species has the potential to provide important insights into approaches on similar research in humans. This includes research in contexts ranging from estimations of time to death after withdrawal of life-sustaining treatment in patients (He and others 2015) including for organ transplants (Puts and others 2011), in nursing homes where 50% die within 3 years and over 30% need palliative care within a year (Hjaltadóttir and others 2011), in hospice end-of-life care where one of the key criteria for entry and Medicare eligibility is that the patient has 6 months or less to live if the disease takes its natural course (Harris and others 2014), and for physicians and their patients who are considering euthanasia (de Beaufort and van de Vathorst 2016) for what is referred to as “dying on time” for dementia patients who may not wish to continuing living in advanced stages of any number of dementia-related diseases, but the symptoms conflict with the due care criteria (i.e., must be able to confirm the request for euthanasia at the time of death (deHaas and others 2019).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research funded in part by a grant from the Center for the Economics and Demography of Aging, UC Berkeley. Youth Scholarship Foundation of Guangdong Institute of Applied Biological Resources (GIABR-qlx201701); GDAS Special Project of Science and Technology Development (2018GDASCX-0107). Because they sponsor our study abroad.

References Cited

- Arking R; Novoselteva J; Hwangbo D-S; Novoseltev V; Lane M Different age-specific demographic profiles are generated in the same normal-lived *Drosophila* strain by different longevity stimuli. *Journal of Gerontology: Biological Sciences*. 57A:B390–B398; 2002
- Avanesian A; Khodayari B; Felgner JS; Jafari M Lamotrigine extends lifespan but compromises health span in *Drosophila melanogaster*. *Biogerontology*. 11:45; 2009 [PubMed: 19430925]
- Bartholomai JA; Frieboes HB Lung Cancer Survival Prediction via Machine Learning Regression, Classification, and Statistical Techniques. 2018 IEEE International Symposium on Signal Processing and Information Technology (ISSPIT); 2018
- Beer KA; Syring RS; Drobatz KJ Evaluation of plasma lactate concentration and base excess at the time of hospital admission as predictors of gastric necrosis and outcome and correlation between those variables in dogs with gastric dilatation-volvulus: 78 cases (2004-2009). *Journal of the American Veterinary Medicine Association*. 242:54–58; 2013

- Carey JR; Harshman L; Liedo P; Müller H-G; Wang J-L; Zhang Z Longevity-fertility trade-offs in the tephritid fruit fly, *Anastrepha ludens*, across dietary-restriction gradients *Aging Cell*. 7:470–477; 2008 [PubMed: 18346215]
- Carey JR; Liedo P; Harshman L; Liu X; Müller H-G; Partridge L; Wang J-L Food pulses increase longevity and induce cyclical egg production in Mediterranean fruit flies. *Functional Ecology*. 16:313–325; 2002
- Carey JR; Liedo P; Mueller H-G; Wang J-L; Senturk D; Harshman L Biodemography of a long-lived tephritid: Reproduction and longevity in a large cohort of Mexican fruit flies, *Anastrepha ludens*. *Experimental Gerontology*. 40:793–800; 2005a [PubMed: 16154309]
- Carey JR; Liedo P; Müller H-G; Wang J-L; Chiou J-M Relationship of age patterns of fecundity to mortality, longevity, and lifetime reproduction in a large cohort of Mediterranean fruit fly females. *Journal of Gerontology: Biological Sciences*. 53A:B245–B251; 1998a
- Carey JR; Liedo P; Müller H-G; Wang J-L; Vaupel JW A simple graphical technique for displaying individual fertility data and cohort survival: case study of 1000 Mediterranean fruit fly females. *Functional Ecology*. 12:359–363; 1998b
- Carey JR; Liedo P; Müller H-G; Wang J-L; Zhang Y; Harshman L Stochastic dietary restriction using a Markov-chain feeding protocol elicits complex, life history response in medflies. *Aging Cell*. 4:31–39; 2005b [PubMed: 15659211]
- Carey JR; Roach DA *Biodemography: An Introduction to Concepts and Methods*. Princeton: Princeton University Press; 2020
- Chakrabarti S; Syme HM; Elliott J Clinicopathological Variables Predicting Progression of Azotemia in Cats with Chronic Kidney Disease. *Journal of Veterinary Internal Medicine*. 26:275–281; 2012 [PubMed: 22269057]
- Chiu JC; Kaub K; Zou S; Liedo P; Altamirano-Robles LA; Ingram D; Carey JR Deleterious effect of suboptimal diet on sleep-wake cycle in *Anastrepha ludens* manifests itself with age. *Scientific Reports*. 3 : 1773 | DOI: 10.1038/srep01773; 2013 [PubMed: 23639915]
- Curtsinger JW On the analysis and interpretation of late-life fecundity in *Drosophila melanogaster*. *Experimental Gerontology*. 72:22–28; 2015 [PubMed: 26344177]
- Curtsinger JW Retired flies, hidden plateaus, and the evolution of senescence in *Drosophila melanogaster*. *Evolution*; 2016
- Curtsinger JW Reproductive Homeostasis and Senescence in *Drosophila melanogaster*. *The Journals of Gerontology: Series A*. 74:1533–1538; 2018
- de Beaufort ID; van de Vathorst S Dementia and assisted suicide and euthanasia. *Journal of Neurology*. 263:1463–1467; 2016 [PubMed: 27017340]
- deHaas M; Hignett S; Jun TG Dying "on time" in dementia. International MinD Conference Designing with and for People with Dementia, Wellbeing, Empowerment and Happiness. [file:///C:/Users/Jim/AppData/Local/Packages/Microsoft.MicrosoftEdge_8wekyb3d8bbwe/TempState/Downloads/Hignett_de%20Haas%202019%20MinD-DyingOnTimeinDementia-final%20\(1\).pdf](file:///C:/Users/Jim/AppData/Local/Packages/Microsoft.MicrosoftEdge_8wekyb3d8bbwe/TempState/Downloads/Hignett_de%20Haas%202019%20MinD-DyingOnTimeinDementia-final%20(1).pdf); 2019
- Fawcett T An introduction to ROC analysis. *Pattern Recognition Letters*. 27:861–874; 2006
- Fletcher JP; Hughes JP; Harvey IF Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. *Proceedings of the Royal Society of London Series B: Biological Sciences*. 258:163–167; 1994 [PubMed: 7838854]
- Glare P; Virik K; Jones M; Hudson M; Eychmuller S; Simes J; Christakis N A systematic review of physicians' survival predictions in terminally ill cancer patients. *British Medical Journal*. 327:195; 2003 [PubMed: 12881260]
- Goggs R; Dennis SG; Di Bella A; Humm KR; McLauchlan G; Mooney C; Ridyard A; Tappin S; Walker D; Warman S; Whitley NT; Brodbelt DC; Chan DL Predicting Outcome in dogs with Primary Immune-Mediated Hemolytic Anemia: Results of a Multicenter Case Registry. *Journal of Veterinary Internal Medicine*. 29:1603–1610; 2015 [PubMed: 26473338]
- Grady D; Berkowitz SA Why Is a Good Clinical Prediction Rule So Hard to Find? *Archives of Internal Medicine*. 171:1701–1702; 2011 [PubMed: 22025427]

- Harris PS; Stalam T; Ache KA; Harrold JE; Craig T; Teno J; Smither E; Dougherty M; Casarett D Can Hospices Predict which Patients Will Die within Six Months? *Journal of Palliative Medicine*. 17:894–898; 2014 [PubMed: 24922330]
- Harshman LG; Zera AJ The cost of reproduction: The devil in the details. *Trends in Ecology and Evolution*. 22:80–86; 2006 [PubMed: 17056152]
- He X; Xu G; Liang W; Liu B; Xu Y; Luan Z; Lu Y; Ko DSC; Manyalich M; Schroder PM; Guo Z Nomogram for Predicting Time to Death After Withdrawal of Life-Sustaining Treatment in Patients With Devastating Neurological Injury. *American Journal of Transplantation*. 15:2136–2142; 2015 [PubMed: 25810114]
- Hjaltadóttir I; Hallberg IR; Ekwall AK; Nyberg P Predicting mortality of residents at admission to nursing home: A longitudinal cohort study. *BMC Health Services Research*. 11:86; 2011 [PubMed: 21507213]
- Khazaeli AA; Curtsinger JW Post-reproductive survival and the Faulkner Effect in *Drosophila melanogaster*. *Drosophila Information Service*. 93:154–155; 2010
- Lane SJ; Frankino WA; Elekonich MM; Roberts SP The effects of age and lifetime flight behavior on flight capacity in *Drosophila melanogaster*. *The Journal of Experimental Biology*. 217:1437–1443; 2014 [PubMed: 24790098]
- Le Bourg E; Moreau M Individual late-life fecundity plateaus do exist in *Drosophila melanogaster* and are very common at old age. *Experimental Gerontology*. 55:102–106; 2014 [PubMed: 24735899]
- Mangel M; Heimpel GE Reproductive senescence and dynamic oviposition behaviour in insects. *Evolutionary Ecology*. 12:871–879; 1998
- Mueller LD; Rauser CL; Rose MR An evolutionary heterogeneity model of late-life fecundity in *Drosophila*. *Biogerontology*. 8:147–161; 2007 [PubMed: 17028930]
- Mueller LD; Shahrestani P; Rauser CL Predicting death in female *Drosophila*. *Experimental Gerontology*. 44:766–772; 2009 [PubMed: 19799992]
- Müller HG; Carey JR; Wu D; Vaupel JW Reproductive potential determines longevity of female Mediterranean fruit flies. *Proceedings of the Royal Society, London B*. 268:445–450; 2001
- Novoseltsev VN; Arking R; Carey JR; Novoseltseva JA; Yashin AI Individual fecundity and senescence in *Drosophila* and medfly. *Journal of Gerontology: Biological Sciences*. 60A:953–962; 2005
- Novoseltsev VN; Carey JR; Novoseltseva JA; Papapopoulos NT; Blay S; Yashin AI Systemic mechanisms of individual reproductive life history in female medflies. *Mechanisms of Ageing and Development*. 125:77–87; 2004 [PubMed: 14706240]
- Novoseltsev VN; Novoseltseva JA; Boyko SI; Yashin AI What Fecundity Patterns Indicate About Aging and Longevity: Insights From *Drosophila* Studies. *The Journals of Gerontology: Series A*. 58:B484–B494; 2003
- Papadopoulos NT; Carey JR; Katsoyannos BI; Kouloussis NA; Müller H-G; Liu X Supine behaviour predicts time-to-death in male Mediterranean fruit flies. *Proceedings of the Royal Society of London: Biological Sciences*. 269:1633–1637; 2002
- Payne JR; Borgeat K; Brodbelt DC; Connolly DJ; Luis Fuentes V Risk factors associated with sudden death vs. congestive heart failure or arterial thromboembolism in cats with hypertrophic cardiomyopathy. *Journal of Veterinary Cardiology*. 17:S318–S328; 2015 [PubMed: 26776589]
- Priest N; Galloway L; Roach DA. Mating Frequency and Inclusive Fitness in *Drosophila melanogaster*. *The American Naturalist*. 171:10–21; 2008
- Puts MTE; Monette J; Girre V; Pepe C; Monette M; Assouline S; Panasci L; Basik M; Miller WH; Batist G; Wolfson C; Bergman H Are frailty markers useful for predicting treatment toxicity and mortality in older newly diagnosed cancer patients? Results from a prospective pilot study. *Critical Reviews in Oncology/Hematology*. 78:138–149; 2011 [PubMed: 20444620]
- Rauser CL; Abdel-Aal Y; Shieh JA; Suen CW; Mueller LD; Rose MR Lifelong heterogeneity in fecundity is insufficient to explain late-life fecundity plateaus in *Drosophila melanogaster*. *Experimental Gerontology*. 40:660–670; 2005 [PubMed: 16085380]
- Rauser CL; Mueller LD; Rose MR Aging, fertility, and immortality. *Experimental Gerontology*. 38:27–33; 2003 [PubMed: 12543258]

- Rauser CL; Tierney JJ; Gunion SM; Covarrubias GM; Mueller LD; Rose MR Evolution of late-life fecundity in *Drosophila melanogaster*. *Journal of Evolutionary Biology*. 19:289–301; 2006 [PubMed: 16405599]
- Reilly BM; Evans AT Translating Clinical Research into Clinical Practice: Impact of Using Prediction Rules To Make Decisions. *Annals of Internal Medicine*. 144:201–209; 2006 [PubMed: 16461965]
- Riffe T; Chung PH; Spikjker J; MacInnes J Time-to-Death Patterns in Markers of Age and Dependency. *Vienna Yearbook of Population Research*. 14:229–254; 2016
- Rogina B; Wolverson T; Bross TG; Chen K; Müller H-G; Carey JR Distinct biological epochs in the reproductive life of female *Drosophila melanogaster*. *Mechanisms of Aging and Development*. 128:477–485; 2007
- Rosenheim JA Characterizing the cost of oviposition in insects: a dynamic model. *Evolutionary Ecology*. 13:141; 1999
- Shaw PJ; Cirelli C; Greenspan RJ; Tononi G Correlates of Sleep and Waking in *Drosophila melanogaster*. *Science*. 287:1834–1837; 2000 [PubMed: 10710313]
- Siontis GCM; Tzoulaki I; Ioannidis JPA Predicting Death: An Empirical Evaluation of Predictive Tools for Mortality. *Archives of Internal Medicine*. 171:1721–1726; 2011 [PubMed: 21788535]
- Stearns SC; Norton EC Time to include time to death? the future of health care expenditure predictions. *Health economics*. 13:315–327; 2004 [PubMed: 15067670]
- Tharwat A Classification assessment methods. *Applied Computing and Informatics*. 10.1016/j.aci.2018.08.003 2018
- Trammell RA; Toth LA Markers for Predicting Death as an Outcome for Mice Used in Infectious Disease Research. *Comparative Medicine*. 61:492–498; 2011 [PubMed: 22330575]
- Williams AE; Rose MR; Bradley TJ. The respiratory pattern in *Drosophila melanogaster* selected for desiccation resistance Is not associated with the observed evolution of decreased locomotory activity. *Physiological and Biochemical Zoology*. 77:10–17; 2004 [PubMed: 15057713]
- Zou S; Meadows S; Sharp L; Jan LY; Jan YN Genome-wide study of aging and oxidative stress response in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences*. 97:13726–13731; 2000

Highlights

- Egg-laying patterns in three fruit fly species revealed impending death
- Precision, specificity and accuracy were highly species-specific
- Accuracy in *Drosophila melanogaster* exceeded 0.96
- Egg laying variability in two tephritids substantially reduced predictability

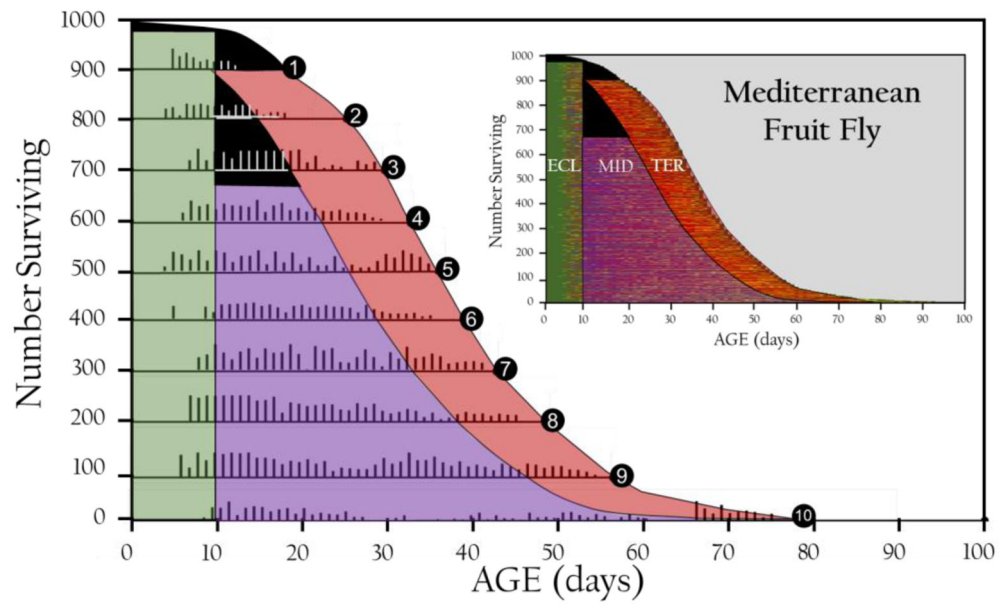


Figure 1.

Schematic of the life courses of single medfly females (see Carey et al. 1998) depicted as horizontal lines that were selected at each of 10 different survival levels out of the initial 1,000 flies. The vertical ticks of varying lengths along each female life-line represent the relative number of egg produced at each age. The areas with different color shadings in both the main graph and the inset indicate the sampling structure for analysis including ECL (green area) indicates the 10-day fly maturation period in which no samples were used; MID (purple) indicates the midlife age classes after the fly's maturation periods and ending before the 11-day period prior to their deaths; and TER (red) indicates the 11-day egg laying period before each fly's death.

		True Class	
		Positive	Negative
Hypothesized Class	True	True Positives (Terminal Segments)	False Positives (Terminal Segments)
	False	False Negatives (Midlife Segments)	True Negatives (Midlife Segments)

Figure 2.

The 2 x 2 contingency table for classifying different 11-day segments of fruit fly egg laying into either terminal (1) or midlife (0). The output of the predicted class is true or false.

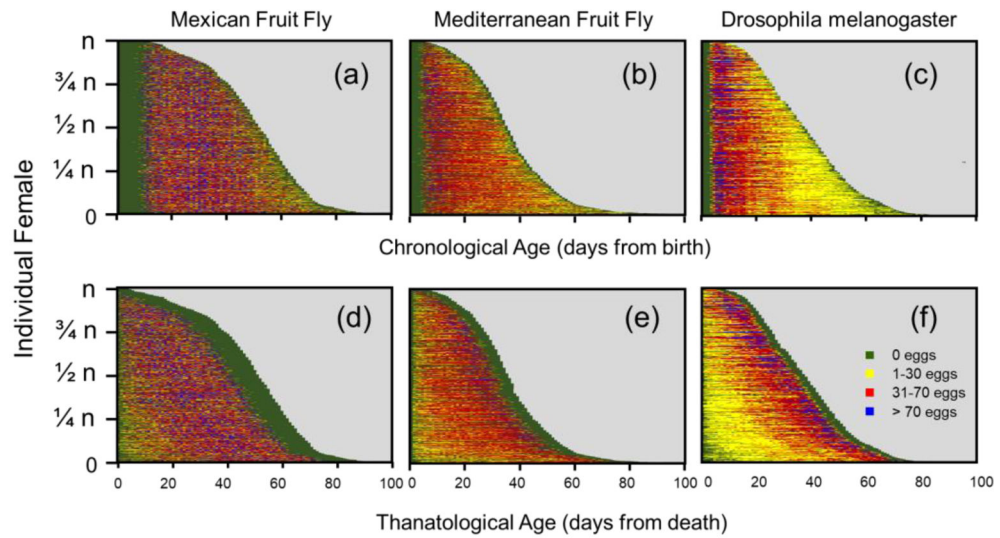


Figure 3.

Event history charts depicting individual-level daily reproduction in large cohorts of three fruit fly species according to either chronology age (top row) or thanatological age (bottom row). The life course of individual females is depicted as a single horizontal line divided into segments by day (age) and color-coded according to the number of eggs laid. The y-axis scales differ by species number where the initial numbers, n , are 1131, 1,000 and 666, for the Mexfly, the Medfly and *D. melanogaster*, respectively (graphic technique after Carey and others 1998b).

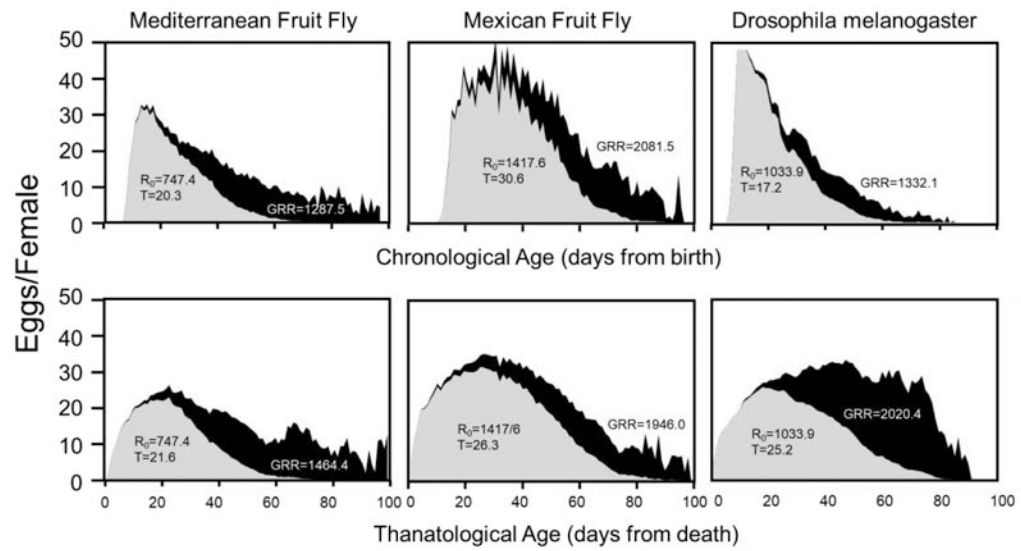


Figure 4.

Age-specific gross and net reproduction for three fruit fly species plotted according to chronological age (top row) and thanatological age (bottom row). Values shown for gross (GRR) and net (R_0) reproductive rates and mean age of reproduction (T).

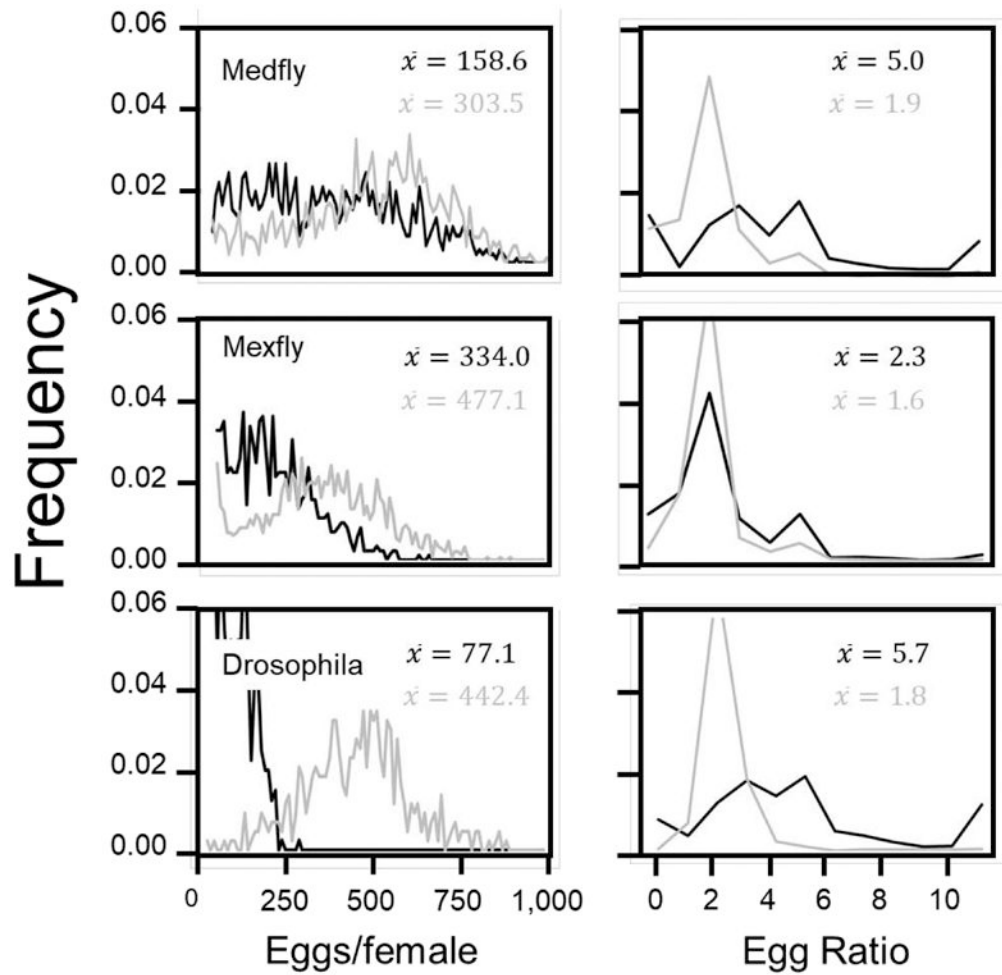


Figure 5.

Frequency distribution of two metrics that were derived from the 11-day sequences in either the terminal segment or the midlife segments in three fruit fly species: (1) the 11-day total eggs/female (left column); and (2) the ratio of eggs laid in the first 6 days of the segment to the last 5 days (middle column); Black curves and font correspond to the terminal segments and the gray to the midlife segments. Mean value shown for each metric by species.

Classification performance metrics for three fruit fly species for complete and censored 11-day segments of reproductive data in two classes—terminal and midlife segments.

Table 1.

	Notation	Medfly (<i>C. capitata</i>)		Mexfly (<i>A. ludens</i>)		<i>D. melanogaster</i>	
		All	Censored	All	Censored	All	Censored
Accuracy and error rate							
Fraction correct	FC	0.7238	0.8387	0.6422	0.7314	0.9612	0.9367
Fraction incorrect	FI	0.2762	0.1613	0.3578	0.2686	0.0388	0.0633
Sensitivity and specificity							
True positive rate	TPR	0.4433	0.7856	0.5987	0.5862	0.9859	0.9318
True negative rate	TNR	0.8942	0.8664	0.6857	0.8216	0.9365	0.9432
False positive and false negative							
False positive rate	FPR	0.1058	0.1336	0.3143	0.1784	0.0635	0.0568
False negative rate	FNR	0.5567	0.2144	0.4013	0.4138	0.0141	0.0682
Predictive values							
Positive predictive value	PPV	0.7180	0.7544	0.6557	0.6712	0.9395	0.9565
False discovery rate (1-PPV)	FDR	0.2820	0.2456	0.3443	0.3288	0.0605	0.0435
Negative predictive value	NPV	0.7256	0.8855	0.6308	0.7617	0.9851	0.9116
False omission rate (1-NPV)	FOR	0.2744	0.1145	0.3692	0.2383	0.0149	0.0884
Likelihood ratios							
Positive likelihood	LR+	2.62	6.59	1.78	2.82	63.26	10.82
Negative likelihood	LR-	0.39	0.28	0.55	0.43	0.06	0.05
Diagnostic odds	DO	6.73	23.76	3.25	6.52	1029.40	226.83