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Supine behaviour predicts the time to death in male Mediterranean fruitflies (*Ceratitis capitata*)

Nikos T. Papadopoulos[1,2], James R. Carey[2,3*], Byron I. Katsoyannos[1], Nikos A. Kouloussis[1], Hans-Georg Müller[4] and Xueli Liu[4]

[1]*Laboratory of Applied Zoology and Parasitology, Department of Agriculture, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece*
[2]*Department of Entomology, and* [4]*Department of Statistics, University of California, Davis, CA 95616, USA*
[3]*Center for the Economics and Demography of Aging, University of California, Berkeley, CA 94720, USA*

Over 97% of the 203 male medflies monitored in a lifetime study of their behaviour exhibited what we term supine behaviour (temporary upside-down orientation) starting an average of 16.1 days prior to their death (mean lifespan of 61.7 days). Supine onset increased the mortality risk by 39.5-fold and a unit increase in supine level increased mortality by 26.3%. The discovery that behavioural traits in insects can be used as biomarkers of their health and to predict their time to death has important implications regarding research on morbidity dynamics, behavioural neuroethology and gerontology, and the interpretation of longevity extension in model organisms.

Keywords: morbidity dynamics; biomarker of ageing; upside-down behaviour; *Ceratitis capitata*; Tephritidae

1. INTRODUCTION

A biomarker of ageing is a behavioural or biological parameter of an organism that either alone or in some multivariate composite will better predict the functional capacity or mortality risk at some late age than will chronological age (Markowska & Breckler 1999). Behaviour biomarkers of ageing are important because behaviour changes with ageing and thus behaviour itself can be used as an index of ageing, and any intervention to alter the chronological course of ageing must be assessed behaviourally because quality of life as well as longevity must be considered in the evaluation of any intervention (Baker & Sprott 1988). Due to their rapid generation time and low cost (relative to mammalian models such as rodents or primates) understanding behavioural biomarkers in fruitflies has important implications regarding research designed to understand morbidity dynamics, behavioural neuroethology and gerontology, and the interpretation of longevity extension in model organisms.

While monitoring the behaviour of male Mediterranean fruitflies (medfly) (*Ceratitis capitata*) throughout their lives, we discovered a behavioural trait that is unique to older, geriatric flies in general but especially to individuals that are gradually approaching death. We term this trait supine behaviour in accordance with the upside-down position of the temporarily immobile flies (figure 1). Supine males lie on their backs at the bottom of their cage appearing dead or moribund. But these flies are very much alive and moderately robust as becomes evident when they right themselves either spontaneously or after gentle prodding and initiate walking, eating and wing-fanning (Prokopy & Hendrichs 1979) behaviours, some of which are indistinguishable from that of normal flies.

Inasmuch as any upside-down adult insect is dysfunctional, it follows that the health of medfly males that we observed spending progressively greater amounts of time in the supine position was both poor and declining relative to the health of flies that were capable of remaining upright. Due to the fact that virtually nothing is known about the nature of chronic conditions (illnesses, infirmity; dementia) in insects, because no viable animal model systems exist for studying morbidity dynamics (e.g. determinants, age of onset, persistence and association with mortality), and because of the importance of understanding morbidity incidence and prevalence in human populations (Crimmins *et al.* 1994, 1996; Manton *et al.* 1997; Manton & Land 2000), we initiated studies designed to address and test the following questions regarding supine behaviour in medfly males. When do flies first begin to exhibit supine behaviour? Is supine behaviour sporadic or progressive? What is the age and frequency distribution of this behaviour for both cohorts and individuals? Is supine behaviour 'reversible' or is it a behavioural marker of impending death?

2. MATERIAL AND METHODS

We studied supine behaviour in 203 male Mediterranean fruitflies derived from a wild-caught colony (from infested hosts collected in Thessaloniki, Greece in autumn 1999) by placing individual one-day-old males in 5 cm × 12 cm × 7.5 cm plastic transparent cages with adult food (a protein hydrolysate–sugar mixture) and water and maintaining them throughout their lives at 25 ± 2 °C, 65 ± 5% relative humidity and 14 L : 10 D cycle with a photo phase starting at 06.00. Supine and other behaviours (calling, walking, resting and feeding) were recorded for each fly in 10 min intervals over a 2 h period from 12.00 to 14.00 each day until death. Thus, the daily supine number for each fly could range from 0 to 12 (i.e. 2 h period at six observations per hour).

* Author for correspondence (jrcarey@ucdavis.edu).

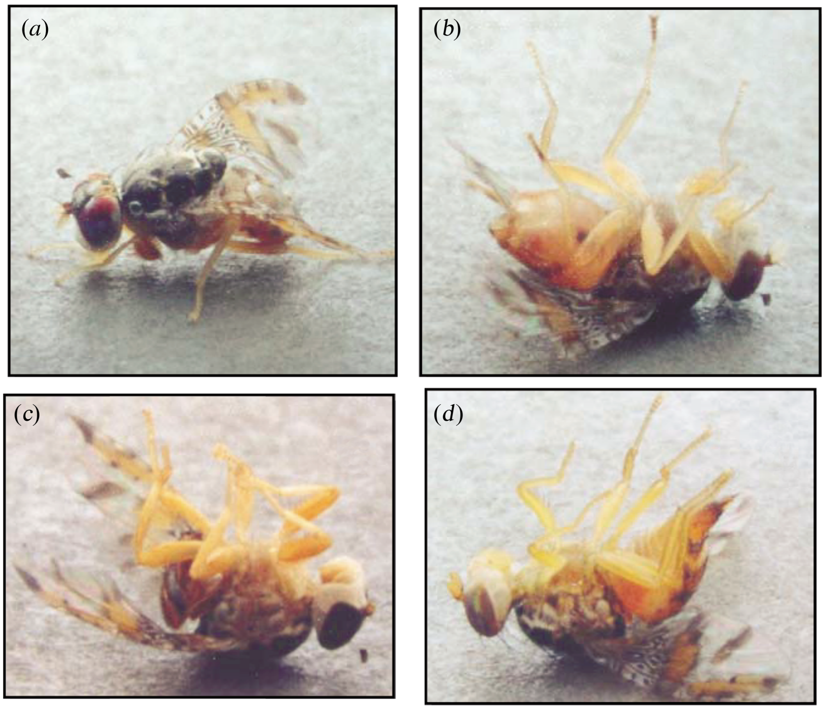

Figure 1. Mediterranean fruitfly: (*a*) male in normal resting position, (*b*) male in supine position, (*c*) recently dead male, and (*d*) female in supine position (i.e. showing that this behaviour is exhibited by both sexes). The abdomens of dead medflies (*c*) are usually shrunken (including the recently dead) and they are typically on their back or more frequently on their sides with their legs either partially or fully retracted. By contrast, supine flies (*b,d*) have plump abdomens with no signs of desiccation, can be observed slowly moving or pumping their proboscis and are nearly always found on the cage floor completely upside down on their backs with their legs extended. When supine flies are disturbed they begin moving their legs, use their wings and legs to become upright and then begin walking slowly (their wings usually appear droopy when resting or walking). Their feeding behaviour appears to be normal and in rare instances males exhibit sexual calling although it is much less intense than in normal males.

The model using the daily supine level $X(t)$ recorded with the age t as the time-dependent covariate is given by

$$\lambda(t) = \lambda_0(t)\exp\{\beta X(t)\},$$

where $\lambda(t)$ and $\lambda_0(t)$ denote the hazard and baseline hazard rates, respectively. We tested the null hypothesis H_0: $\beta = 0$ and rejected it with $p < 0.001$.

3. RESULTS

An event-history chart (Carey *et al.* 1998) showing the age patterns of the supine behaviour for each of the 203 males (figure 2) reveals a distinct association between individual lifespan and both the age of onset and the intensity of this behaviour. The band depicting supine behaviour shows that in many flies it begins to occur about two to three weeks prior to their death and therefore the period of its occurrence closely follows the cohort survival (l_x) schedule. Supine behaviour seldom occurred in very young flies (less than 25 days) but frequently occurred in flies that were over 50 days old—ages at which the mortality rate began to increase substantially. The event-history chart (figure 2) reveals four general properties of the medfly supine behaviour: (i) *persistent*—occurs on subsequent days after onset; (ii) *progressive*—intensity increases with age; (iii) *predictive*—onset and intensity is a strong indicator of impending death; and (iv) *universal*—nearly all male medflies exhibit this behaviour regardless of their age at death.

Smoothed plots of the relationships between onset and age progression of supine behaviour relative to the time of death for each of the 203 male medflies (figure 3) reveals that there is heavy clustering of the supine number near the time of death. Most of the supine behaviour began between 10 and 15 days prior to death with the highest concentrations occurring five to seven days prior to death. We fitted Cox proportional hazards regression models (Klein & Moeschberger 1997) with time-varying predictors to the data shown in figure 3 to test for the significance of both the onset (pre-supine to supine) and the level of supine behaviour with respect to the mortality (hazard) rate. Using the first day of supine onset we define the time-dependent covariate, $z(t)$, as $z(t) = 0$ if the fly is in pre-supine status and $z(t) = 1$ when the fly has reached supine status. The onset of supine behaviour is a highly significant predictor of mortality ($p < 0.0001$) and increases mortality risk considerably more than the level of supine behaviour. The model showed that a fly's mortality risk increased by 39.5-fold once it began experiencing supine behaviour. The level of supine behaviour is also a significant predictor of mortality and for every increase in supine level by 1.0 the hazard rate increases by 26.3%. The estimated value of β was 0.233 57. The numbers provided are for models that have just one predictor. Fitting a model with the onset and level of supine behaviour as predictors indicates that both are highly significant with mortality risk ratios of 17.59 and 1.18 for the onset and level of supine behaviour, respectively.

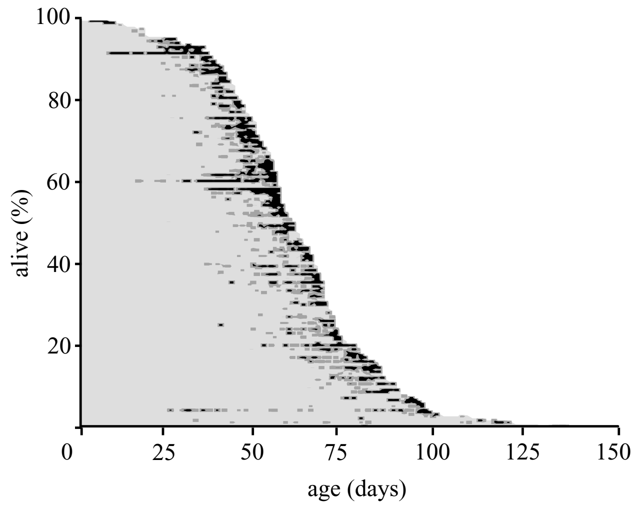

Figure 2. Event-history charts for supine behaviour in 203 male medflies relative to cohort survival (Carey *et al.* 1998). Each individual is represented by a horizontal 'line' proportional to his lifespan. Each day an individual displayed supine behaviour it is coded according to the number exhibited—light grey indicates no supine observations, dark grey indicates one to six supine observations and black indicates seven to twelve supine observations.

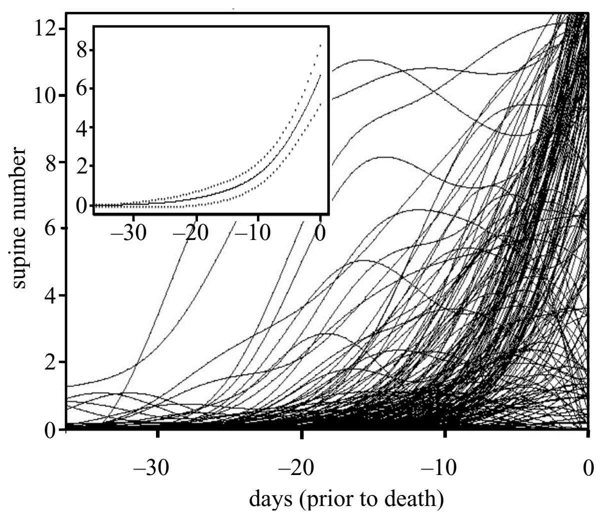

Figure 3. Smoothed plots for the supine number recorded in the 2 h observation period of each of 203 males aligned relative to time of death. Inset, mean supine number for the male medfly cohort. Dashed lines indicate ±2 s.d.

The impact on mortality risk of the onset of supine behaviour is illustrated in figure 4, that shows the remaining life expectancy at each age for all flies (middle curve) as well as flies disaggregated according to whether they had exhibited supine behaviour (lower curve) or not (upper curve). Three aspects of this figure merit special comment. First, striking differences occurred in the remaining life of young flies (less than 20 days) that had not exhibited supine behaviour and young flies that were experiencing supine behaviour—over 50–60 days of life remaining for the former (non-supine) but only 20 days or less for the latter (supine). Second, the remaining life expectancy of a non-supine fly between 25 and 70 days of age remains constant at about 35 life-days. However, once it enters the supine state its life expectancy immediately drops by 20 days to *ca.* 15 days. Third, the remaining life of flies that initiated the supine behaviour is also remarkably constant, ranging from slightly over 20 days for flies that are 10–30 days old to *ca.* 16–18 days for very young flies and flies older than 30 days.

We used the age at which a male medfly first exhibited supine behaviour to demarcate the end of the 'active' period of a fly's life to construct a cohort schedule of 'active life' (Katz *et al.* 1983; Manton & Land 2000). This schedule combined with the conventional survival (l_x) schedule, was used to estimate the age distribution and prevalence of inactive life by taking the difference at each age between the inactive and total life schedules (figure 5). The total and active life expectancies were computed as 61.7 and 45.6 days, respectively (Carey 2001) and thus the average male experienced 16.1 days or 26.1% of its 61.7 day lifetime partially in the supine state. The distribution of inactive life for the cohort varied with age (figure 5 inset) with the greatest prevalence occurring between 30 and 50 days and the least occurring at the younger and older ages. This measure of inactive life provides information about the dimensions of health and morbidity for a fly other than death that can be used as a model system for actuarial analysis as a health-status measure.

4. DISCUSSION

The current findings are important for several reasons. First, the use of supine behaviour as a proxy for health status and impending death in a medfly cohort provides insights into questions regarding morbidity dynamics (Fries 1980, 2000; Vita *et al.* 1998). The constancy of life expectancy once flies entered the supine state regardless of age of entry indicates that virtually all individuals undergo a transition period of declining health prior to death; that morbidity is a natural stage of the ageing process and its compression may have limits. Thus, time to supine may serve as an additional end-point of interest in different experiments concerned with health and vitality (e.g. feeding, mating and reproduction). The importance of insects as model systems to study human diseases and to design new therapeutic strategies for treating them has recently been suggested (Helfand 2002). For example, Auluck *et al.* (2002) demonstrated the value of the fruit fly (*Drosophila melanogaster*) for understanding Parkinson's disease, a neurodegenerative disorder of ageing.

Second, supine behaviour as a proxy for deteriorating health and fitness may be important in ecological contexts because it is morbidity that determines vulnerability and thus the age trajectory of mortality. This may explain why the cause of death structure in nature is often only weakly related to population dynamics (Ricklefs 1979). If increased vulnerability due to ageing effectively represents the underlying 'cause' of death in nature, then it follows that the immediate cause of death (predator) may be capricious and thus have little relevance to population regulation.

Third, the observation that flies commonly spend part of each day lying upside down over a period of several days or weeks prior to dying, raises not only the technical issue regarding the possibility that the recorded age of death is incorrect, but a related conceptual one as well—

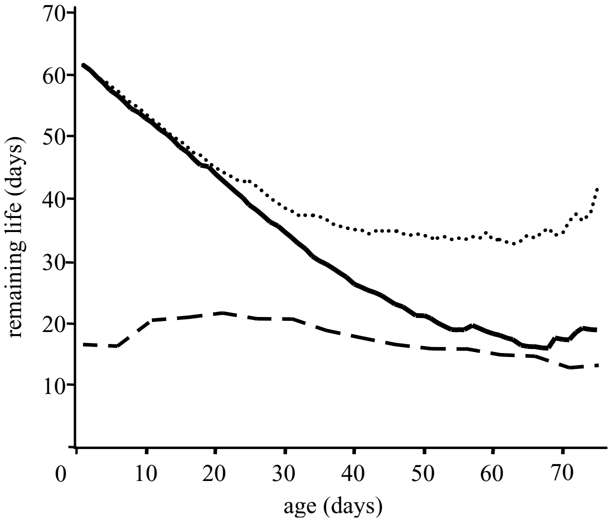

Figure 4. Schedules of remaining life expectancy (days) for male medflies for the cohort as a whole (centre line) as well for males both prior to (top line) and after (bottom line) the onset of supine behaviour.

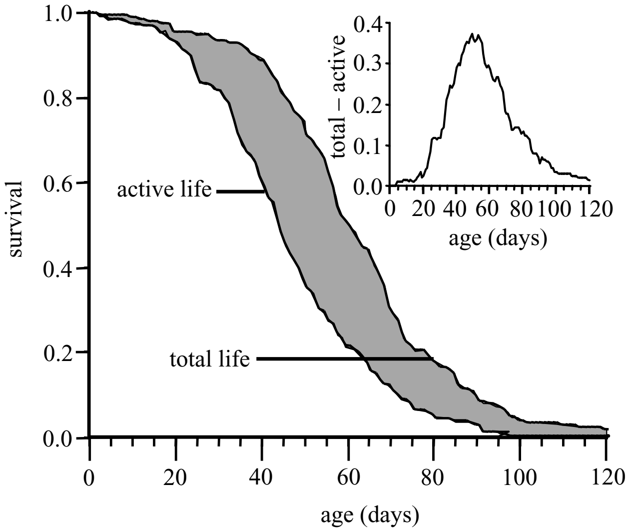

Figure 5. Schedules of active life and total survival for the male medfly cohort. The age at which active life for an individual ended was defined as the age at which supine behaviour was first observed. Inactive life was thus defined as all life-days beyond this onset age. The inset shows the within-cohort distribution of life-days that the average fly experienced in the inactive stage computed as the difference between total and active life (shaded area in the main figure).

age patterns of mortality and morbidity can become confounded when unhealthy (supine) flies that are capable of living many more days are mistaken for dead ones. This does not present a serious analytical problem when comparing the rates of actuarial ageing if the schedules of active and total life are approximately parallel or if the incidence of supine flies being mistaken for dead flies is random. However, the determination of mortality patterns can be substantially affected if the mistakes in the determination of age of death are non-random or if the length of the morbidity (inactive) period is shorter at young and old ages.

Fourth, if the underlying cause(s) of the supine behaviour are neurological and central in origin as opposed to declines in the structure and function of the peripheral nervous system or of the skeletal musculature, then medflies and presumably other insect species that exhibit this behaviour could be used as models for studying the onset and progression of neurological diseases, dementia and brain ageing (Price *et al.* 1999; Scheibel 1999) and, in turn, for elucidating the discourse between individual behaviour and the neural structures that underlie these behaviours. The results may be especially pertinent to emerging fields of evolutionary neurobiology and behavioural neuroethology (Francis 1995; Neuweiler 1999) as they relate to behavioural aspects of gerontology.

In general, we believe that the results reported here open up new opportunities for research on age-specific changes in behaviour and the relevance of these changes to ageing and longevity. In particular, the findings raise the possibility of identifying a suite of behaviours comparable to the activities of daily living in humans (Ramey *et al.* 1992) that could be used as disability indices for studying the antecedents of the diseases and infirmities that occur in the end-phase of life in model organisms.

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