Adult sex ratio effects on male survivorship of *Drosophila melanogaster* Meigen (Diptera, Drosophilidae)

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ABSTRACT. Adult sex ratio effects on male survivorship of *Drosophila melanogaster* Meigen (Diptera, Drosophilidae). The behavioral biology has a central role in evolutionary biology mainly because the antagonistic relations that occur in the sexual reproduction. One involves the effect of reproduction on the future life expectation. In this scenario, changes in male operational sex ratio could lead to an increase in mortality due to costs associated with excessive courtship and mating displays. Thus, this work experimentally altered the male sex ratio of *Drosophila melanogaster* Meigen, 1830, to determine its impact on mortality. The results indicated that mortality increases as the sex ratio changes, including modifications in the survivorship curve type and in the curve concavity, measured by entropy.

KEYWORDS. Adult sex ratio; entropy; survivor.

RESUMO. Efeito da razão sexual de adultos na curva de sobrevivência de machos de *Drosophila melanogaster* Meigen (Diptera, Drosophilidae). A biologia comportamental tem um papel central na biologia evolutiva principalmente pelas relações antagônicas que ocorrem na reprodução sexuada. Uma destas relações envolve o efeito da reprodução sobre a expectativa de vida futura. Neste cenário, alterações na razão sexual operacional de machos podem levar a um aumento na mortalidade por causa dos custos associados com o excesso de displays de corte e cópulas. Neste sentido este trabalho alterou experimentalmente a razão sexual em machos de *Drosophila melanogaster* Meigen, 1830, para determinar os efeitos em termos de mortalidade. Os resultados indicam que a mortalidade aumenta a medida que a razão sexual se enviesa incluindo alterações no tipo de curva de sobrevivência e da concavidade da curva, medida pela entropia.

PALAVRAS-CHAVE. Entropia; razão sexual de adultos; sobrevivência.

Behavioral ecology is one of the core topics in evolutionary biology manly due to the variety of behaviors and tactics associated with mating, as well as the fitness consequences linked to these strategies (Savalli 2001). One of these fitness consequences is a trade-off involving reproduction and longevity, which has been supported by evolutionary models as well as by empirical evidences (Tatar 2001). This trade off involves the harm males can inflicted on females due to sexual behavior (Arnqvist & Nilsson 2000) and, for males, the costs associated with courtship displays (Cordts & Partridge 1996), production of nuptial gifts and competing for mating (Whigby & Chapman 2004). When females make a higher investment than males in reproduction, such as in insects, there is a tendency of females to become more selective in mate choice, implying a selection on male ability to compete for mating opportunities (Kokko & Jennions 2008). One way to quantify the mating opportunities is to use the concept of operational sex ratio (OSR), which is defined as the proportion of sexually mature males to receptive females (Emlen & Oring 1977) or, more operationally, through the use of adult sex ratio (ASR), which quantifies the number of males and females in an arena (Kokko & Jennions 2008) . The rationale behind these concepts is that deviations from a 1: 1 sex ratio will lead to a competition for mates where the biased sex will tend to compete for access to mating (Kvarnemo & Ahnesjö 1996; Markow 2000).

Drosophila melanogaster Meigen, 1830, have a biased OSR in natural conditions (Markow 2000) and, in laboratory experiments, responds to such condition with an increase of male courtship display and mating rate (Whigby & Chapman 2004). As a consequence of the trade-off involving reproduction and longevity, the increase in courtship display and mating rate reduces life span (Cordts & Partridge 1996), although the global fitness is positively related to reproductive rate (Lessells 2006).

This features set up a scenario were premating competition should be expected under a male biased adult sex ratio. Thus, considering that courtship is a costly behavior, it could be hypothesized that in arenas where there is a male biased operational sex ratio the male longevity will tend to become lower with an increasing departure of adult sex ratio from 1:1. Based on this assumption, this paper attempts to test if longevity decreases with an increment in male biased sex ratio.

To test these hypothesis newly hatched *D. melanogaster* adults (virgins), from a Canton-S lineage, were placed in 200 ml vials containing 50 ml of banana-agar culture medium. Three types of crosses (treatments) were set up to test the sex ratio effect on male survivorship: Treatment 1 – 1:1 male/female sex ratio, with 14 males and 14 females; Treatment 2 – 2:1 male/female sex ratio, with 14 males and 7 females; Treatment 3 – 7:1 male/female sex ratio, with 14 males

and 2 females. All crosses were run at 25 ± 1 °C under a photoperiod of 12:12 (L:D) h. The flies were transferred once a week to new vials containing culture medium. The vials were checked to count and sex the dead flies every day until the last individual of the original set.

As failure-time data are skewed and our sample sizes are small we choose to fit a distribution to each cross as a way to describe mortality patterns (Lee & Wang 2003). The Weibull distribution were choose because fitted the data well (χ2 = 0,29; df = 2 and 38, P = 0,87), represents the mortality risk as a power of time (Crawley 2005) acting in an additive way (Ricklefs & Scheverlein 2002) and can be compared with standard survivorship curves (Pinder *et al.* 1978; Crawley 2005). Also, the fact that mortality risk are additive to an initial mortality parameter (m0) implies that initial mortality can be zero (Ricklefs & Scheverlein 2002) as occurred in our experimental design. The mean survival time (mean Weibull) were used for comparisons with entropy values. The R package (R Development Core Team 2009) were used for fitting a Weibull distribution to the data thought the survival package (Therneau & Lumley 2009).

The mortality data were used to make life tables and survivorship curves according to Carey (1993). The survivorship patterns can be graphically represented by three types of curves. The type I has a rectangular distribution and describes the situation were the individuals reach the maximum physiological longevity of the species. The type II describes a mortality that is age-independent. The type III is characterized by high early mortality and a raise of life expectation with time for the survivors (Demetrius 1978). However, this patterns shows only a general shape of the survivorship curve, making the comparisons highly empiric as the majority of curves fit between two of these patterns. Thus, the entropy can be a quantitative estimator of the survivorship curve and, therefore, enables a more detailed comparison among the survivorship patterns of both sexes or different species (Moura & Bonatto 1999; Fernandes *et al.* 2003). The entropy was calculated by the formula:

$$H = \sum_{x=0}^{w} e_x d_x / e_0 \qquad (1)$$

were: H is the entropy, e_x represents the life expectancy in the age x; d_x is the proportion of individuals that died between the ages x and $x+1$; e_o is the life expectancy in the initial age; w is the maximum age (in days) achieved by the last survivor and; $\sum e_x d_x$ is the sum of the weighted mean of life expectancy in all ages (Moura & Bonatto 1999; Fernandes *et al.* 2003).

The three treatments had a decrease in mean survival time (Table I, Fig. 1). *Drosophila melanogaster* males from the first treatment (1:1 sex ratio male/female) had an average mean survival higher than the second (2:1 sex ratio male female) and the third treatment (7:1 sex ratio males/females). The Weibull parameter scale (α) measures how hazard (risk of death) relates to time (Crawley 2005) in such a way that when α < 1, mortality occurs at earlier ages. All the estimates derived from the fitted models (Table I) indicated that hazard decrease with age but at different rates, reaching almost an exponential decay (α = 1) on treatment 3. This indicates that

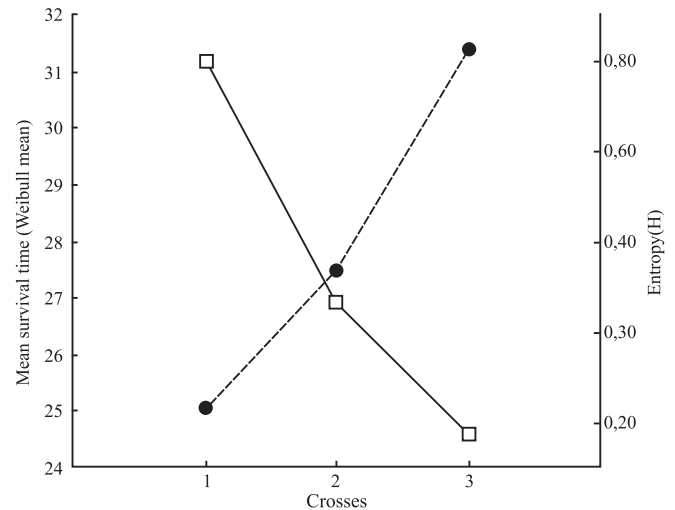Fig. 1. Mean survival time (□) in days and entropy levels (●) of adult *Drosophila melanogaster* M. males at each treatment.

mortality risk occurs at a higher rate when sex ratio is biased (Fig. 2).

The temporal distribution of longevity can be analyzed through the use of survivorship curves and entropy values of each treatment (Fig. 3). Regarding the shape of the survivorship curves, the first two treatments (1:1 and 2:1 male/female sex ratio) showed survivorship curves intermediary between type I and II and the third treatment (7:1 male/female sex ratio) had a curve intermediary between type II and III, showing more mortality in the first days (Fig. 3). The quantification of survivorship curve made through the entropy values showed a rise following the bias in adult sex ratio from 0,216 to 0,610 (Table I, Fig. 3).

When adult operational sex ratio skews from unity it is expected an increase in intrasexual competition to mating
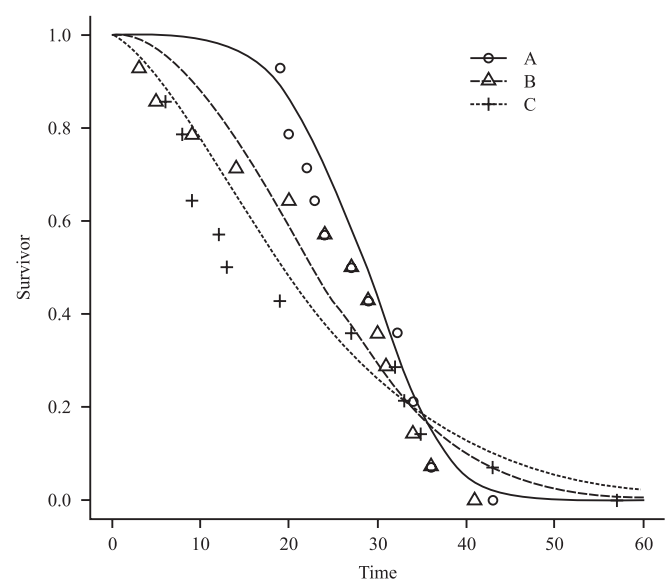

Fig. 2. Observed (points) and fitted (Weibull distribution) survival curves for *Drosophila melanogaster* M. males from the sex ratio treatments: 1:1 male/female sex ratio (A), 2:1 male/female (B) and 7:1 male/female (C).
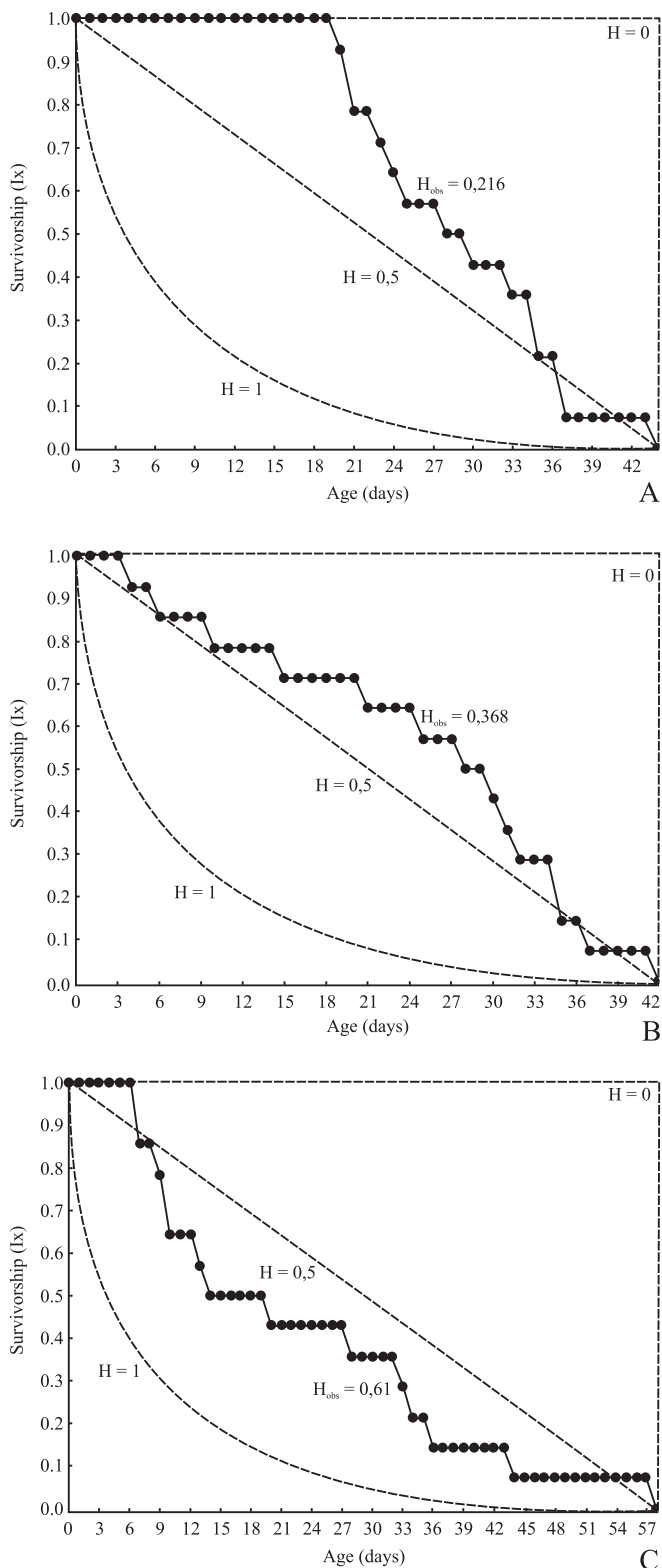
Table I. Mean survival time (Weibull mean), scale (Weibull parameter), type of survivorship curve and entropy values of *Drosophila melanogaster* M. males in each sex ratio (male/female) treatment.

Treatments	Mean survival time	Scale	Survivorship curve type	Entropy (H)
1:1 sex ratio	31,18	0,231	Between type I and II	0,216
2:1 sex ratio	26,92	0,476	Between type I and II	0,368
7:1 sex ratio	24,60	0,666	Between type II and III	0,610

Partridge 1996). Within this framework, our results showed that an experimentally induced sex ratio departure from unity produced as response a decrease in mean survival time (Table I).

Furthermore, our results showed that the type and concavity of the survivorship curve were affected by a male bias in adult sex ratio.

Within a sperm competition framework, there is an expectation that some males will perform better than others, therefore mating more frequently (Kokko & Jennions 2008). This pattern will create a large variance in mating rate due to the choosiness of females, which could be translated to a large variance in mortality distribution, leading to a result similar found here. In general, sperm competition occurs in male biased sex ratio species (Emlen & Oring 1977) as experimentally induced in this work. At least two factors could be responsible for decreasing longevity in a scenario like the induced in the experiments realized: mating competition (Cordts & Partridge 1996; Whigby & Chapman 2004; Reuter *et al.* 2008) and/or frequency of courtship behaviours (Friberg & Arnqvist 2003; Reuter *et al.* 2008). Although we could not disentangle these effects, there is an effect of adult sex ratio on male survivorship that probably was mediated by reproductive behavior.

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Fig. 3. Survivorship curves (solid line) and entropy values of adult *Drosophila melanogaster* M. males from the sex ratio treatments: 1:1 male/female sex ratio (A), 2:1 male/female (B) and 7:1 male/female (C). Theoretical entropy values are shown as dashed lines (H=0, H=0.5 and H=1).

access (Kvarnemo & Ahnesjö 1996; Kokko & Jennions 2008) and as can be expected, such competition have a cost that could be translated into an increase in mortality (Cordts &

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