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TECHNICAL REPORT

Starvation resistance in *Drosophila melanogaster*: testing for a possible 'cannibalism' bias

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Summary

1. Starvation resistance is often measured in physiological studies with *Drosophila* and other insects. One common method involves measuring time until death of groups of insects. This method assumes that survivors are not obtaining nutrition from their vial mates that die early. However, because some *Drosophila* larvae can scavenge carcasses, this group protocol might inadvertently lead to a 'cannibalism' bias.

2. We evaluated whether starvation resistance of *Drosophila melanogaster* was increased if fly carcasses were available from the beginning of the experiment. We used a mixed-model ANOVA to assess the direct and interactive effects of isofemale line, sex and immediate access to carcasses.

3. Males survived starvation longer than females, despite the smaller size of males. Isofemale lines differed significantly in resistance. Immediate access to fly carcasses had no impact on resistance.

4. These results suggest that starving adult flies do not gain measurable benefits from access to carcasses. Consequently, this experiment seemingly validates a widely used method of measuring starvation resistance.

Key-words: Experimental methods, isofemale line

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Introduction

Starvation resistance is a commonly measured trait in studies with *Drosophila*. It is directly relevant to general issues of the evolution of stress resistance (Hoffmann & Parsons 1991) as well as to studies of direct and correlated responses to artificial selection (Chippendale, Chu & Rose 1996), of geographical clines in stress resistance (Karan *et al*. 1998; Hoffmann & Harshman 1999; Robinson, Zwaan & Partridge 2000), and of its physiological (Harshman, Hoffmann & Clark 1999) and genetic (Harbison & Mackay 2002) bases.

Starvation resistance is often assayed by placing groups of flies in a vial or bottle giving them access to water but not to food, and then monitoring times until death (Chippendale *et al*. 1996; Harshman *et al*. 1999). In our own experiments using this group protocol with *Drosophila subobscura* (R. B. Huey *et al.*, unpublished data), we noticed that the distributions of death times of individual flies within a vial were often suggestively left skewed; specifically, a few flies lived much longer than did other flies in about a third of all vials. Because *D. melanogaster* larvae can scavenge on dead fly carcasses (Gregg *et al*. 1990), we wondered whether such 'last-to-die' adult flies might be either cannibalizing their dead vial mates or perhaps feeding on microorganisms growing on carcasses. Were either the case, this group protocol would overestimate true starvation resistance.

To evaluate whether a 'cannibalism' bias does occur, we tested whether the median starvation resistance of flies would be increased if fly carcasses were accessible to test flies from the very beginning of an experiment. Control flies would gain access to fly carcasses only after their cohorts began to die (~24 h). Thus, although flies in both experimental and control treatments eventually had access to carcasses, only the experimentals had access from the initiation of starvation. To implement this design, we used an isofemale line approach, such that each line was represented in both experimental and control vials; and we separated males and females as well. We can thereby determine whether starvation resistance is influenced by sex, by the presence of carcasses, whether it has a genetic component in our stocks, and also whether isofemale lines (or sex) differ in their response to prolonged access to dead vial mates (i.e. a isofemale line – or sex – by 'feeding' interaction).

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Materials and methods

We used 16 isofemale lines of *D. melanogaster* collected by M. Frazier in Wenatchee, WA, in July, 2001. Lines were maintained at 25 °C (12L:12D) and at controlled density until mid-October 2001. We then set up two vials of males and two of females, each with about 10 flies (3–5 days of age), for each isofemale line. Vials contained moist sponges, but no media. We 'seeded' one vial for each sex with several dead flies (killed by heat shock), checked the vials every 6 h (06.00, 12.00, 18.00 and 24.00 hours), and then recorded the median time of death. In early April 2003, we repeated the entire experiment with the same isofemale lines: thus we could determine whether results were robust to block effects.

Our experimental design allows examination of two fixed effects (immediate access to dead flies ('food') and sex) and of two random effects (block and isofemale line), and the interactions. To compute statistics we used R (Ihaka & Gentleman 1996) and the R-package 'nlme' for a mixed-model ANOVA.

Results

Our mixed-model analysis evaluates whether starvation resistance was influenced by sex, food and block (as fixed effects) and by isofemale line (as a random effect). Including block as a random factor did not improve the fit of the model (likelihood ratio 0·66, P = 0·41), and so we combined the two blocks. The interaction between food and sex was insignificant (P = 0·28).

Sex had a substantial effect on starvation resistance: males had significantly longer starvation times on average than did females (P < 0·0001, Fig. 1), despite their smaller body size. However, presence of dead flies as a potential source of food had no effect (P = 0·96, Fig. 1). Including isofemale line greatly improved the fit of the model to the data over a model that excluded this random effect (likelihood ratio = 46·68, P < 0·0001), suggesting that these isofemale lines harbour significant genetic variation for starvation resistance.

Discussion

Our experiment shows that median death time of starving flies was not extended if flies were given immediate access to dead flies. Thus, even though larval *Drosophila* can be 'facultative carnivores' (Gregg *et al*. 1990), adults cannot – or at least do not – derive a measurable survival benefit from cannibalism. Consequently, our experiment seemingly validates a commonly used method of assessing starvation resistance (see Introduction).

Because many published experiments have used group protocols for estimating starvation resistance in *Drosophila* (see Introduction), our results are decidedly reassuring. Nevertheless, group protocols might still generate biased estimates of resistance. For one thing, individuals in groups will interact socially; and the intensity of such interactions may influence starvation

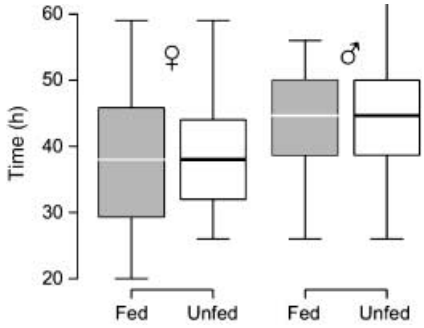

Fig. 1. Effects of sex and treatment on starvation resistance of *D. melanogaster*. Plotted are the median, interquartiles (boxes) and ranges.

times. Moreover, social interactions might help explain the relatively long lives of 'last to die' flies (see Introduction). As flies die off, fewer flies will of course be interacting, such that these late survivors might be spending less energy on interactions and hence survive relatively longer. This potential social bias can be evaluated by comparing survival times of flies kept singly *vs* in groups. Should a social bias prove to exist, then an appropriate experimental protocol might involve monitoring death times of flies kept individually in small vials rather than of groups of flies. Such a design would also increase power, as many individuals can easily be monitored. However, set-up time will increase substantially.

Isofemale lines differed significantly in starvation resistance, suggesting that starvation resistance is probably heritable in our stock of flies. Of course, the realized heritability of starvation resistance is already well established in *D. melanogaster*, as resistance responds rapidly both to direct and correlated selection (Chippendale *et al*. 1996; Harshman *et al*. 1999) and shows geographical clines in several species (Karan *et al*. 1998; Hoffmann & Harshman 1999; Robinson *et al*. 2000).

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