SURVIVAL AND FITNESS VARIATION IN *DROSOPHILA PSEUDOOBSCURA*: EFFECTS OF TEMPERATURE STRESS, SOCIAL GROUP, MATING STATUS, AND MATE PREFERENCE

by

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(Under the direction of Wyatt W. Anderson and Patricia Adair Gowaty)

ABSTRACT

Survival variation is partly a function of quality differences among individuals, variation in reproductive activities, social group exposure, and rearing temperature. Similarly, fitness variation partly reflects differences in survival, the number and quality of mates, and the amount of energy available and devoted to reproduction. In these studies we examined survival variation in *Drosophila pseudoobscura* related to the length of exposure to a mild heat stress, social group composition, mating status, mate preference, and preference status of potential mates. We also examined fitness variation in *D. pseudoobscura* associated with mate preference and length of time with mate.

In the first experiment we tested survival effects of two mild heat stress conditions (exposed for one-week or lifetime) and three social groups (alone, single sex groups, and mixed sex groups). We found that survival significantly decreased with lifetime exposure to a mild heat stress. Individuals alone had the highest survival, individuals in mixed sex groups had the lowest survival, and females outlived males in both stress treatments and all social groups. We also found that survival differences among groups increased under lifetime exposure to a mild heat stress, making this stress an experimental tool for detecting subtle survival differences among treatment groups, and justifying our use of this heat stress in the subsequent experiments.

The next two studies examined fitness and survival variation associated with mate preference and length of time with mate. Male and female choosers chose between two potential mates (discriminatees) in arenas that minimized preference constraints. Female choosers with their preferred male for 21 days had increased fecundity, while preferred females with males for one day had increased net fitness and their offspring had higher egg-to-adult viability. Pairs together for 21 days had increased total offspring and egg-to-adult offspring viability compared to those with their mate for one day. While there were no survival differences for female choosers mated with their preferred or non-preferred male, males had significantly increased survival when with their preferred female for one day. There were no survival differences between mated or unmated preferred and non-preferred discriminatees.

Finally, we examined survival cost of reproducing in males and females by comparing the survival of virgins, flies that had a single copulation, and flies that had multiple copulations. We found that females with multiple copulations had significantly decreased survival compared to both virgins and females with a single copulation, while males did not experience any survival cost of reproducing.

INDEX WORDS: *Drosophila pseudoobscura*, mate choice, survival, fitness, temperature stress, costs of mating

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEWS

Drosophila pseudoobscura

Distribution and Basic Ecology

Drosophila pseudoobscura are native to temperate areas of the western United States and Mexico, with an isolated population in Bogota, Columbia (Lakovaara and Saura 1982). They breed where they feed, in decaying fruit and plant matter, with a large part of their nutrition coming from yeast in both larval and adult stages. Known breeding sites include cacti and agave plants, sap fluxes of oaks and other trees, as well as vineyards near humans (Powell 1997).

They are one of the most mobile *Drosophila* species (Grossfield 1978), with an average movement of 100 to 300 meters per day in forests, and reaching several kilometers per day in less suitable habitat like deserts (Powell 1997). They have an estimated daily survival probability of 0.91 in the wild (Dobzhansky and Wright 1947), and females typically outlive males in the lab (Taylor and Condra 1980; Taylor et al. 1981).

Reproduction and life history

Females are larger than males and reach sexual maturity at three days old, compared to one day for males (Markow 1996). When seven day old virgins were paired together in a vial, their latency to copulation was 56.47 ± 4.64 sec ($\mu \pm$ S. E.; $N = 315$), and their copulation duration was 7.07 ± 0.10 min ($\mu \pm$ S. E.; $N = 310$). Previous work found that males transferred approximately 25,000 sperm in a single mating, capable of producing about 350 adult offspring (Snook 1995). However, females mate with multiple males in both laboratory (Dobzhansky and Spassky 1967) and wild (Anderson 1974) populations, needing an average of one to two days between matings (Markow 1996).

D. pseudoobscura females begin laying fertilized eggs within hours after mating, and some can lay fertilized eggs from a single mating for at least 25 days (pers. obs.). However, the adult offspring emerging from eggs laid by singly mated compared to multiply mated females begins to drop off at day six of egg-laying because of sperm depletion (Fig. 1.1). At room temperature (approximately $21 \pm 1^{\circ}$ C), most eggs hatch within two days, followed by three larval stages and pupation. For vials of eggs kept at room temperature, time from eggs laid to first emerging adults in a vial is 18.98 ± 0.04 days ($\mu \pm$ S. E.; $N = 5155$), with most adults emerging in the first two days (Fig. 1.2).

The beginning of *Drosophila pseudoobscura* as a model organism

The use of *D. pseudoobscura* for evolutionary studies began with Theodosius Dobzhansky, a Russian immigrant who came to the United States in 1927 to work with T. H. Morgan at Columbia University, the center of *Drosophila* studies at that time. Dobzhansky then followed Morgan to California Institute of Technology, where he became increasingly interested in studying evolution. He recognized that *D. melanogaster* was ill-suited for evolutionary work because of its close relationship with humans. When he learned of a local *Drosophila* species that had wild, free-living populations with little human contact, he chose this organism, *D. pseudoobscura*, for his studies on evolution in natural populations (Powell 1997). Since then, *D. pseudoobscura* has become one of the most studied *Drosophila* species, and as a testament to their importance, only the second fruit fly species, after *D. melanogaster*, for which scientists sequenced the entire genome (Richards et al. 2005).

Circadian Rhythms in *Drosophila*

We kept flies in our mate choice experiment (see below) in an unlit incubator. Therefore, this section explores research on the effects of darkness on circadian rhythms. Circadian rhythms are daily, endogenous cycles that regulate various physiological functions and behaviors. Clock rhythms are entrained, or synchronized to 24-hour periods, by daily light and temperature cycles in nature. In constant light and/or temperature in laboratory settings, however, some rhythms remain ‘free-running’ (i.e. running while free of environmental cues) on cycles close to 24 hours, while others become arrhythmic (Pittendrigh 1960). In *Drosophila*, circadian rhythms control development, mating, oviposition, sperm release, and activity, with some known effects of unnatural laboratory settings, like constant temperature and light, on these rhythms.

Development rhythms

The most studied developmental circadian rhythm is eclosion rhythm. In *Drosophila*, eclosion peaks shortly after the dark to light transition (Prasad and Yoshi 2003). While *D. melanogaster* maintains an eclosion rhythm in constant darkness (DD) (Paranjpe et al. 2004), in *D. pseudoobscura* eclosion becomes aperiodic in constant light (LL) or DD conditions (Engelmann 1966; Zimmerman 1969). However, a rhythm can be initiated by a transition from either light to dark or dark to light (Engelmann 1966; Winfree 1972) or just from a change in light intensity (Chandrashekaran and Loher 1969a; Chandrashekaran and Loher 1969b). Under constant temperature conditions, eclosion rhythm is free-running in *D. pseudoobscura* (Zimmerman et al. 1968), but an introduction of temperature cycling resets the clock (Maier 1973).

Mating and reproductive rhythms

Drosophila vary in their ability to mate in darkness, with *D. pseudoobscura* considered light-independent, meaning mating frequency does not decrease in darkness (Mayr and Dobzhansky 1945; Wallace and Dobzhansky 1946). In *D. melanogaster*, there is a daily mating rhythm, with more mating during the day than night, and this rhythm is maintained in DD conditions, suggesting endogenous control (Sakai and Ishida 2001). There is also a daily rhythm to courtship in many *Drosophila* species (Hardelan 1972). In addition, light condition affects latency to copulation in *D. mercatorum*, becoming highly variable under LL but stable in 12-h light: 12-h dark (LD) or DD conditions.

Oviposition rhythm for females is under endogenous control in *D. melanogaster*, with the rhythm maintained in DD or LL conditions (Paranjpe et al. 2004; Sheeba et al. 2001). However, the total number of eggs laid is significantly decreased when females are in DD versus in LD or LL (Sheeba et al. 2000).

Male release of sperm to the testes is also under circadian control. In gypsy moths, rhythmicity is maintained in LD and DD conditions (Giebultowicz et al. 1989), but is disrupted in LL, affecting sperm release and resulting in sterility (Giebultowicz et al. 1990). Similarly, males mutant for genes controlling clock periodicity in *D. melanogaster* released fewer sperm and had fewer offspring (Beaver et al. 2002).

Activity and longevity

There is a daily activity rhythm in *D. pseudoobscura*, with a peak of activity at the onset of light and a second peak at the end of the light period in LD conditions (Engelmann 1966). While a rhythm remains in DD conditions, the first peak of activity disappears, leaving only the second peak (Engelmann 1966). Thus flies kept in DD are less active. This same paper also reports no longevity costs from disruption of rhythms with changing light conditions. In addition, there was no survival difference in *D. melanogaster* between flies kept in LD and DD conditions, but flies kept in LL conditions had significantly decreased survival compared with LD and DD (Sheeba et al. 2001).

Possible effects of darkness on adult *D. pseudoobscura*

Summarizing the information on *D. pseudoobscura* above, being held in darkness does not affect mating frequency or longevity but does decrease activity. Generalizing from *D. melanogaster*, *D. pseudoobscura* females might have decreased fecundity in darkness, but male sperm release will not likely be affected. Since in the mate choice experiment below (Chapter 3), we kept flies in an unlit incubator from age 7 until death, the two main possible effects of this darkness are decreased activity and fecundity. Since all flies experienced the same environment, and since rhythmicity for mating and reproductive functions and behaviors most likely remained in darkness, as all the studies above suggest, the results from that experiment should still be generalizable to other organisms as well.

While circadian rhythms for a wide variety of functions and behaviors remain free-running in constant conditions, ideally all organismal laboratory experiments would occur in incubators with natural cycles of light and temperature. No studies that I am aware of have reared flies in incubators that mimic daily temperature fluctuations, and temperature is sometimes more important that light in entraining circadian rhythms (Liu et al. 1998). In addition, twilight and dawn periods are rarely, or never, mimicked in incubators, and these periods also play a role in entraining rhythms (Chandrashekaran and Loher 1969a; Chandrashekaran and Loher 1969b).

Temperature Effects on Survival in *Drosophila*

Early *Drosophila* researchers realized that rearing temperature in the lab affected survival, with increasing temperatures decreasing survival (Loeb and Northrop 1917). Alpatov and Pearl (1929) proposed a “rate of living” hypothesis that aging increased as temperature increased (Fig. 1.3a). However, work in *D. subobscura* did not support this, with researchers reporting that flies kept at 30° C for the first half of their life, then at 20° C thereafter, had the same survival probability as flies always kept at 20° C (Clark and Maynard-Smith 1961a; Clark and Maynard-Smith 1961b). They proposed a “threshold” hypothesis that it is more difficult to maintain vitality at higher temperatures, and the threshold below which an organism dies is higher in high temperatures (Fig. 1.3b). Thus, while the aging process early in life is independent of temperature, the dying process is not, and organisms decreasing in vitality will die sooner at increased temperatures. This explained their results in *D. subobscura*, and subsequent data in some *Drosophila* species have been consistent with their hypothesis, while other studies have not been consistent with either the rate of living or threshold hypotheses (see Lamb 1978). Sex differences in response to temperature, often with females surviving heat stress better because of its sterilizing effects, have confounded the results of many studies (Hollingsworth 1970).

In addition to the effects of constant higher temperatures on survival, many researchers have studied the survival effects of short-term exposure to very high temperatures in *D. melanogaster* and a few other *Drosophila* species. In nature, individuals will likely experience short-term exposure to very high temperatures (between 37° C and 40° C), and selection should favor both behavioral and physiological responses to this stress (Loeschcke et al. 1994). Most laboratory studies have investigated the release of heat shock proteins and their effects on survival and other fitness measures (e.g. Dahlgaard et al. 1998; Krebs and Loeschcke 1994). In *D. melanogaster*, less than two hours exposure to 37° C decreases survival, mating frequency, and fecundity for females (Krebs and Loeschcke 1994). There is both inter- (Krebs 1999) and intra-specific (Dahlgaard et al. 1998) variation in the concentration of heat shock proteins following heat shock, but this variation is not correlated with survival, and females typically survive the stress better than males (Dahlgaard et al. 1998).

There is no research on the effects of heat stress on *D. pseudoobscura* survival. However, cold temperature resistance in this species is known, with adults in laboratory studies living longest at 5° C (compared to up to 16° C), and females able to survive well at -3° C (Crumpacker and Markinkovic 1967), probably because in nature females can overwinter for many months, and hold viable sperm until the next spring (Collett and Jarman 2001)! Since they are a temperate and desert species, in nature *D. pseudoobscura* experience large daily fluctuations in temperature and could therefore be sensitive to constant exposure to increased temperatures.

Mate Choice

Mate choice models

While Darwin (1871) was the first to discuss female preferences for male traits, he did not offer an explanation for the origin of preferences. Fisher (1930) filled in this gap by describing a system where there is a variable, heritable male trait that gives a survival advantage to males, along with heritable variation in females who favor this trait in their mates. For females that prefer the male trait, they will have sons with the trait, conferring higher survival of sons, and also daughters who favor the trait and therefore have higher survival in their offspring. Males with this trait can therefore have both higher survival and higher mating success via female choice. Even if the survival advantage of the trait for males disappears over time, or even if the trait becomes associated with decreased survival, it can still be maintained once female preference for the trait has spread in the population.

Williams (1966) further articulated Fisher's hypothesis by generalizing it to any male trait that indicates fitness. If fitness is heritable, males should be selected to advertise their fitness, while females should be selected to assess it (Williams (1975) later doubted that fitness was heritable enough to maintain female preferences). Hypotheses where males advertise fitness and female preferences respond to it became know as 'indicator' models (Andersson 1994).

Two important indicator models emerged after Williams: Zahavi's (1975) handicap hypothesis, and Hamilton and Zuk's (1982) hypothesis based on host-parasite interactions. Zahavi (1975) proposed that advertised and energetically expensive male traits are honest signals of high quality because only healthy males would be capable of producing them. Females should therefore prefer males with these elaborate traits, possibly leading to further exaggeration of the traits, even if that trait decreases male survival. Similarly, Hamilton and Zuk (1982) hypothesized that bright colors in birds are indicators of high quality because only birds with fewer parasites can produce bright colors.

The mate choice models discussed so far are all based on female preference for good genes that indicate absolute quality of males. However, there is an alternative good genes model where preference is for complementary genes, meaning genes that increase offspring heterozygosity (Brown 1997) and/or lead to maximum immune diversity that will be able to keep up with quickly evolving parasites (Wedekind 1999). Rather than having all females want a few best males as the models above predict, in this hypothesis mate choice is self-referential, so the best mate for one individual in the population is not the best male for all individuals.

Potential benefits of choice

Anisogamy theory (Parker 1972) and parental investment theory (Trivers 1972) predict that the sex investing more, in terms of gametes or parental investment, will be choosier while the other sex will be more indiscriminate. Since species with higher female gametic and parental investment are common, female choice has been the focus of most mate choice studies, although most of these studies look for the male trait mediating preference. So, what benefits do the female's get by choosing? Females can receive direct fitness benefits that increase their reproductive output or survival, including increased fecundity, paternal investment, and access to resources (Andersson 1994). They might also receive the indirect benefit of increased offspring survival (Andersson 1994) from either increased paternal investment or good genes (absolute quality or complementary).

Evidence for direct and indirect benefits to females exists in many species (Andersson 1994). However, many mate choice studies find only small or non-significant effects of mate preference on fitness (Alatalo et al. 1998). Yet if costs to choosing are very low, as could occur with intense male-male competition or high variance in male signals (Alatalo et al. 1998), or a male-biased operational sex ratio that decreases female search costs (Clutton-Brock and Parker 1992), choice could be advantageous, and still potentially evolutionarily significant, even if benefits were low or inconsistent.

Because males can differentially affect females in mating because of variation in body size (Pitnick and Garcia-Gonzalez 2002), ejaculate toxicity (Civetta and Clark 2000; Sawby and Hughes 2001a), or ejaculate quality (Wedell and Sandberg 1995), there is potential for female preference to respond to that variation as well. However, survival predictions regarding mate choice are not straightforward. There is empirical evidence for three distinct survival consequences for females mating with preferred mates: 1) mating with preferred males increases female survival because in doing so females avoid costly male manipulation (Gowaty 1997; Moore et al. 2003); 2) mating with preferred males decreases survival while also increasing other fitness measures (Cordero and Eberhard 2003); or 3) mating with preferred males decreases a female's survival while simultaneously decreasing other fitness measures (Friberg and Arnqvist 2003).

One possibility for these discrepancies is differences in how studies determine mate preference. Many studies, including ones that have reported that preferred males harm females (e.g. Friberg & Arnqvist 2003), use a phenotypic trait in males, such as large body size, as a proxy for being a preferred male, rather than allowing individual females to choose males. Indeed, social and ecological constraints on the expression of choice are predicted to have significant effects on breeder and offspring fitness (Gowaty 1997). In nature, and in some experimental studies, females will be constrained in the expression of their mating preference because of social factors like male-male competition or male aggression towards females, or ecological factors affecting available mates or breeding sites. Minimizing those constraints in determining female, and male, choice is crucial in experimental studies attempting to understand the fitness and survival consequences of mate preference.

While less studied, males also choose in many species, and benefit from choice, including male *D. pseudoobscura* (Gowaty et al. 2002; Gowaty et al. 2003b). Early hypotheses about male choice predicted that males should be choosy when there was high paternal investment (Trivers 1972) or expensive ejaculates (Dewsbury 1982; Petrie 1983). Subsequent work, however, predicted that males could benefit from choosing, even when they only invested sperm, if there was high variation among females (Gwynne 1991; Hubbell and Johnson 1987; Johnstone et al. 1996; Owens and Thompson 1994; Parker 1983) or if there were low search costs for males, like in systems where females benefit from remating (Bonduriansky 2001). In addition, in species where there is sperm competition, like in many insect species, males could engage in cryptic male choice by varying their copulation length, ejaculate size, or nuptial gift, when present, while still seeming indiscriminate in mating (Bonduriansky 2001; Parker 1970).

Studies on the survival consequences to males mating with preferred or non-preferred females are lacking. However, males could potentially adjust their courtship, the most expensive of the mating process for *D. melanogaster* (Cordts and Partridge

1996), copulation length, and ejaculate size or quality in response to the preference status of the female, all of which might affect their longevity.

Compensation theory and evidence

Unavoidably coupled with the above discussion about benefits to mate choice is a discussion about fitness costs to mating with non-preferred mates. Given social and ecological constraints on the expression of mate preference, non-preferred matings are inevitable for some individuals. For instance, females might mate with non-preferred males because of social constraints like forced copulation or mate guarding or ecological constraints like low population density that increases search costs.

Since there can be negative fitness consequences of non-preferred matings, there should be selection on individuals constrained to mate with a non-preferred mate to compensate for the fitness costs, especially offspring viability costs (Gowaty 1997; Gowaty and Buschhaus 1998). If a non-preferred mating results in decreased offspring viability, compensation theory predicts that females would respond by laying more eggs or having more offspring. While this is opposite of the direct benefits hypotheses mentioned above that predict increased fecundity in preferred matings, it makes sense relative to compensation. If offspring are less viable in non-preferred matings, females in these matings should have more offspring in order to keep up with females in preferred matings. Indeed, there is evidence for offspring viability benefits to unconstrained mate preference in mallards (Bluhm and Gowaty 2004b), house mice (Drickamer et al. 2000; Drickamer et al. 2003; Gowaty et al. 2003a), and *D. pseudoobscura* (Anderson et al.

MS), along with evidence for viability compensation in non-preferred matings in mallards (Bluhm and Gowaty 2004a) and *D. pseudoobscura* (Anderson et al. MS).

Costs of Reproducing

The connection between surviving and reproducing is a well-documented relationship that fits into two main theoretical backgrounds: life history theory (e.g. (Stearns 1976), which is concerned with predicting optimal fitness trade-offs in a given environment; and the evolution of senescence (e.g. (Williams 1957), which attempts to explain the evolutionary reasons for and consequences of mortality. Since fitness is a function of both age-specific fertility and mortality, one of the most evolutionarily important trade-offs involves the costs of reproducing on longevity.

Studies in *Drosophila* reveal many costs for females, including energetic costs of egg production, (Lamb 1964; Maynard-Smith 1958; Partridge and Mangel 1999) and copulation (Chapman et al. 1996; Fowler and Partridge 1989), costs of receiving sperm and accessory fluid (Chapman 1992; Chapman et al. 1993; Chapman et al. 1995; Lung et al. 2002), and a reduction in stress resistance from mating (Salmon et al. 2001).

In addition to costs of reproducing related to the number of matings females have, there can be variation in costs of mating depending on variation in their mates. For instance, males can differentially harm females based on: male body size (Pitnick and Garcia-Gonzalez 2002), with larger males harming females more than smaller ones in *D. melanogaster*; ejaculate quality (Wedell and Sandberg 1995), with larger males giving more nuptial gifts in bushcrickets; or ejaculate toxicity (Civetta and Clark 2000; Sawby and Hughes 2001b), with toxicity associated with male genotype in *D. melanogaster*. As discussed above, female preference could respond to this variation, and could serve to decrease their costs of mating in some cases (Gowaty 1997; Moore et al. 2003).

Because males generally invest less in reproduction, costs of mating for males are lower than those for females. However, in *D. melanogaster* there are energetic costs of courtship (Cordts and Partridge 1996; Partridge and Fowler 1990; Partridge et al. 1987) and copulation (Partridge and Andrews 1985; Partridge and Farquhar 1981; Prowse and Partridge 1997). While there have been no studies examining how variation in females might affect a male's cost of mating, it is easy to imagine ways this could occur. When attempting to mate with less receptive females that mated recently or are simply uninterested in the courting male, males might intensify courtship. Similarly, males could vary copulation length or sperm and seminal fluid transfer to females in response to the female's mating status (virgin, mated) or preference status (preferred, non-preferred).

Chapter Outline

Chapter 2

This study tested the survival effects of two exposures (one week or lifetime) to a mild heat stress of 26° C on individuals in three social group treatments (alone, in single sex groups, or in mixed sex groups). The results of this study justified my use of the temperature stress in the subsequent studies.

Chapter 3

Here we examined the fitness and survival consequences of being with preferred or non-preferred mates. We measured preference for both male and female choosers in preference arenas, kept the chooser with their preferred or non-preferred mate for one day or 21 days, and measured total number of eggs laid in 25 days, total number of adult offspring emerging, egg-to-adult offspring viability, and adult survival.

Chapter 4

Using unmated preferred or non-preferred flies from the above experiment, plus doing additional preference tests where we kept both the preferred and non-preferred individuals, we analyzed survival differences in unmated preferred and non-preferred discriminatees.

Chapter 5

From the mate choice experiment above, we had three mating treatments: virgin, with mate for one day, or with mate for 21 days. This allowed us to analyze survival costs of reproducing for both males and females, comparing survival of virgins, flies that had a single copulation (with mate for one day), and flies that had multiple copulations (with mate for 21 days).

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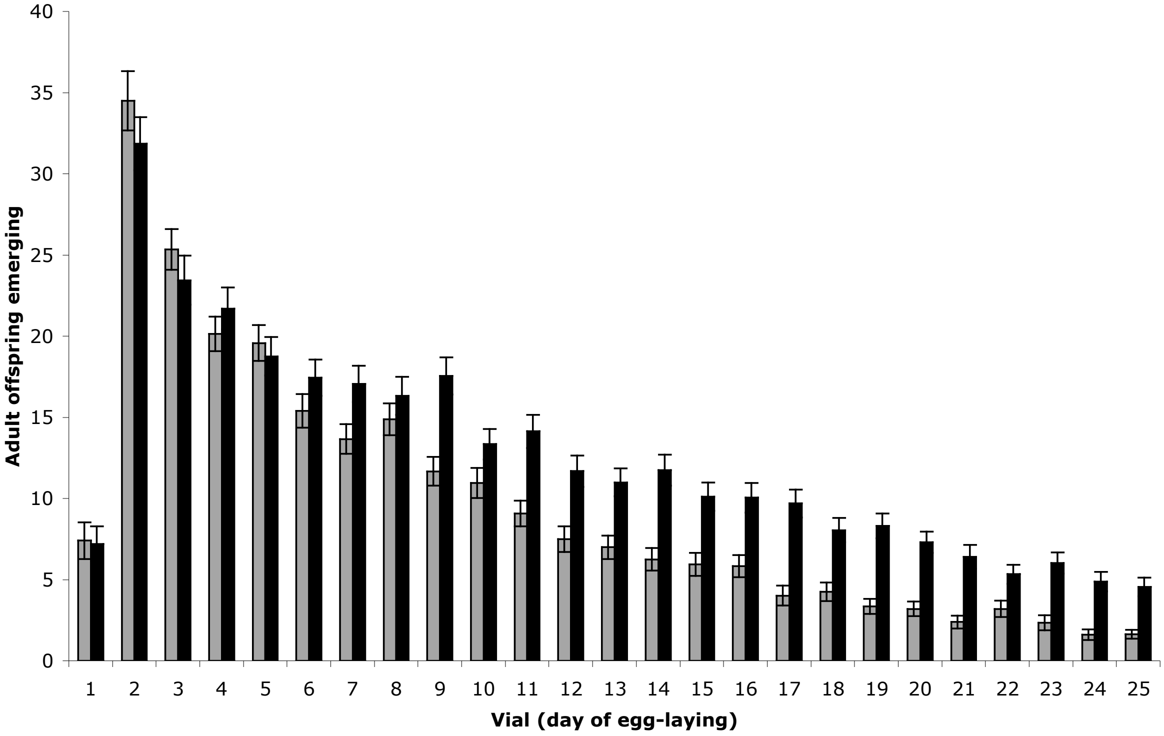
Figure 1.3. (a) Pearl's "rate of living" hypothesis. (b) Clarke and Maynard Smith's "threshold" hypothesis. L = survival at low temperature; H = survival at high temperature; y = length of dying phase at high temperature; z = length of dying phase at low temperature. (Taken from Lamb 1978, pg. 62).

Figure 1.1


Adult offspring emerging
0
5
10
15
20
25
30
35
40
1
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4
5
6
7
8
9
10
11
12
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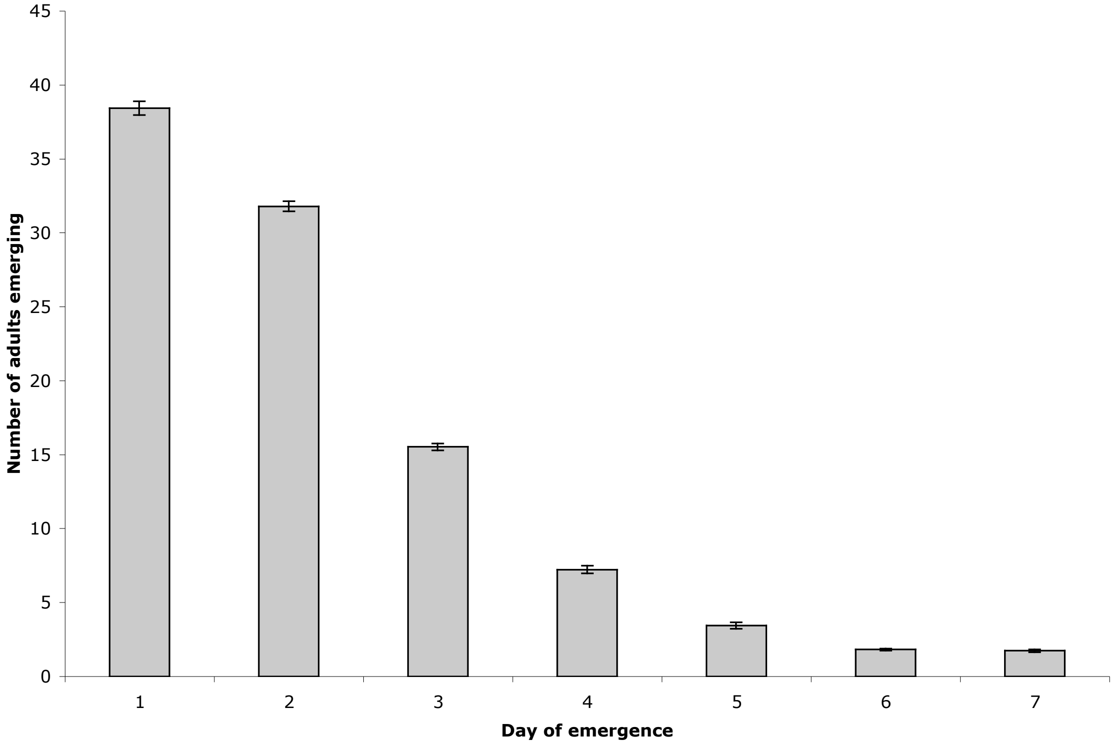
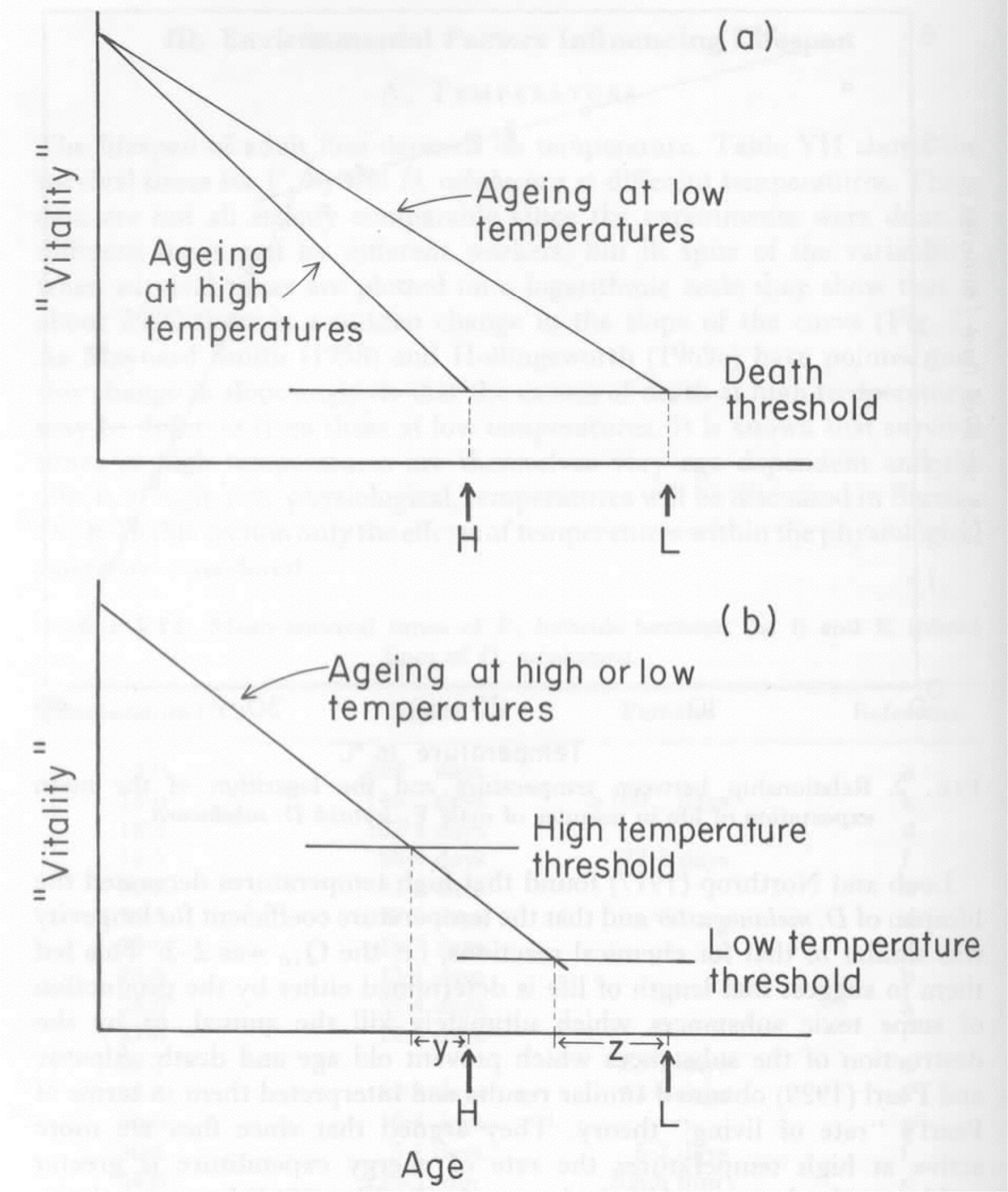
25
Vial (day of egg-laying)

Figure 1.2



45
40
35
30
25
20
15
10
5
0
Number of adults emerging
1
2
3
4
5
6
7
Day of emergence

Figure 1.3


(a)
"Vitality"
Ageing at low temperatures
Ageing at high temperatures
Death threshold
H
L
(b)
"Vitality"
Ageing at high or low temperatures
High temperature threshold
Low temperature threshold
y
z
H
L
Age

CHAPTER 2

SURVIVAL EFFECTS OF MILD HEAT STRESS AND SOCIAL GROUP COMPOSITION IN *DROSOPHILA PSEUDOOBSCURA*[1]

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[1] Lebow, E. T., P. A. Gowaty, Y. K. Kim, and W. W. Anderson. To be submitted to *Journal of Insect Behavior*.

Abstract

The survival effects of mild heat stress on *Drosophila pseudoobscura* are unknown. In this study we examined the effects of both a short-term and long-term mild heat stress of 26° C on survival in three social group treatments: alone, in single sex groups, and in mixed sex groups. As predicted, individuals alone lived longest, individuals in the mixed sex groups had the shortest longevity, and the long-term stress treatment significantly decreased survival in all social groups. Additionally, females outlived males in all social groups and under both stress conditions. We also found that survival differences increased among social group treatments in the long-term stress treatment, making this mild heat stress a potential experimental tool for detecting subtle survival differences among treatment groups.

Key words: heat stress, survival, social group, *Drosophila pseudoobscura*

Introduction

The effects of elevated temperature on longevity in *Drosophila* are well-studied. In *D. subobscura* and *D. melanogaster*, constant exposure to increased temperature decreases longevity (Lamb 1978), while short-term elevated temperature exposure can actually increase longevity in *D. melanogaster* (Minois 2000). Unlike tropical *D. melanogaster*, *D. pseudoobscura* live in temperate climates with a larger range in daily temperatures and might therefore be more susceptible to decreased survival under a constant heat stress. While *D. pseudoobscura* adults can tolerate cold temperatures (Crumpacker and Marinkovic 1967; Marinkovic et al. 1969), research on the survival effects of increased temperature in this species is lacking.

In this study we compare the effects of two exposures to a mild heat stress of 26° C, one-week and lifetime, on the longevity of *D. pseudoobscura*, with the prediction that flies in the lifetime stress will have significantly decreased survival. Because stressing animals could reveal subtle survival differences among experimental groups, we tested the effects of each heat stress in three social group treatments: alone, in same sex groups, and in mixed sex groups. We predicted that: 1) individuals alone will outlive those with same sex conspecifics because of the costs of same sex interactions, and 2) individuals with opposite sex flies will have the lowest survival because of associated survival costs of reproduction. There is evidence that mated animals are less resistant to stress (Salmon et al. 2001), and we imagine that the costs of same sex interactions might also increase in stressed conditions. Thus, we make the additional prediction that survival differences among social groups, especially between individuals alone versus those with conspecifics, will increase in the lifetime stress treatment.

Methods

Breeding

Wyatt Anderson collected *Drosophila pseudoobscura* in Mesa Verde, CO in the summer of 1995 and established isofemale lines in Athens, GA. We used eight isofemale lines to establish a population cage in July 1997 with twenty half-pint glass bottles, each containing approximately 30 ml of food composed of yeast, cornmeal, agar, molasses, and a small amount of propionic acid to suppress the growth of mold. The oldest bottle was rotated out of the cage and replaced with a fresh one on Monday, Wednesday, and Friday of each week.

In June 2002, approximately 60 generations after starting the cage, we added four fresh bottles to the population cage. We removed these bottles one week later. To reduce the density of developing flies in each bottle, we divided each one into three new bottles by scooping off the top layer of food containing eggs and larvae. When adults began emerging, approximately 16 days after we removed the bottles from the cage, we collected virgin flies every eight hours, twice a day, and sexed them under CO_2. We maintained up to ten flies per vial, each vial containing approximately 5 ml of food, and aged flies for seven days.

Treatment groups

We began the experiment when flies were seven days old. Males and females were placed in one of three social group treatments: 1) alone in a vial (A, *N* = 30 per sex); 2) with five other same sex individuals (SS, *N* = 30 bottles per sex) in a bottle; or 3) with two same sex and three opposite sex individuals in a bottle (MS, *N* = 35 bottles total). Bottles were more than six times the size of vials, and contained approximately six times as much food, controlling for density and food availability. There were two stress treatments for each social group: 1) in a 26° C incubator from age 7 to age 14, and then at room temperature (about 21° C) until death (one-week stress); or 2) in a 26° C incubator from age 7 until death (lifetime stress). All flies had light 24 hours a day. However, the flies at room temperature had dimmed light about 12 hours a day, while the flies in the incubator had constantly bright light. We transferred all flies weekly to new vials or bottles, and recorded longevity daily.

Analyses

We performed a Cox proportional hazards analysis (Cox 1972) using sex, social group, stress treatment, plus all interaction terms as covariates on survival. Like a multivariate ANOVA, this regression method provides a way to analyze the effects of multiple predictor variables on survival simultaneously.

For each sex and stress treatment, we analyzed the effects of social group on longevity in two ways: 1) by comparing the mean age of death using one-way ANOVAs, and 2) by comparing Kaplan-Meier generated survival curves using both Log-Rank and Wilcoxon tests, one weighing all survival times equally, and one with a greater weight to shorter survival times, respectively. For individuals in single sex bottles, we used the mean age of death of all six flies in one bottle for our analyses. For individuals in mixed sex bottles, we used the mean age of death for the three males and the mean age of death for the three females for analysis. We used JMP 5.1© for all statistical analyses. We set statistical significance at $P \leq 0.05$.

Results

The proportional hazards analysis revealed significant effects of sex, social group, and stress treatment on mean age of death (sex: $X^2_{df=1} = 65.18$, $P < 0.0001$; social group: $X^2_{df=2} = 103.67$, $P < 0.0001$; stress treatment: $X^2_{df=1} = 84.56$, $P < 0.0001$; Table 2.1), with females living longer than males, individuals alone surviving best and those in the two sex groups surviving worst, and with individuals in the 1-week stress outliving those in the lifetime stress treatments. Among the interaction terms, there was a significant effect of stress level times treatment group ($X^2_{df=2} = 9.30$, $P = 0.01$), but not of stress level times sex ($X^2_{df=1} = 0.46$, $P = 0.50$), social group times sex ($X^2_{df=2} = 0.43$, $P = 0.81$), or sex times social group times stress treatment ($X^2_{df=2} = 3.78$, $P = 0.15$).

For females in the one-week stress treatment, there were significant differences in mean age at death among social groups ($F_{2,91} = 10.67$, $P < 0.001$). In the subsequent least significant difference (LSD) analysis, both A and SS females lived significantly longer than MS females, but there was not a significant difference between A and SS females. The survival curve analysis had the same results, with A and SS females not having significantly different survival, but both living significantly longer than MS females (Fig.

2.1a, Table 2.1). For females in the lifetime stress treatment, there were also significant differences in mean age at death among social group treatments ($F_{2,91} = 20.59$, $P < 0.001$). There was not a significant survival difference between SS and MS females in the LSD analysis, while A females lived significantly longer than both groups. In the survival curve analysis, however, there were significant differences between each pair of curves (Fig. 2.1b, Table 2.1)

For males in the one-week stress treatment, there were no significant differences among social groups in either the mean age at death ($F_{2, 90} = 0.64$, $P = 0.53$) or in the survival curves (Fig. 2.2a, Table 2.1). For males in the lifetime stress treatment there were significant differences among all groups for both mean age at death ($F_{2,92} = 17.53$, $P < 0.0001$) and for the survival curves (Fig. 2.2b, Table 2.1).

Females significantly outlived males in both stress treatments and in all social group treatments (Fig. 2.3, Table 2.2).

Discussion

The prediction that individuals alone would outlive those with same-sex conspecifics was supported only in the lifetime stress treatments. In addition, flies alone responded significantly better to the lifetime stress treatment than flies with conspecifics, as indicated by the significant interaction term between social group and temperature stress in the Cox proportional hazards analysis. Because we controlled for density and food availability, flies with same-sex conspecifics probably did not have competition for food or space in the one-week stress treatment and their survival therefore did not significantly decrease. In the lifetime stress treatment, however, food quality decreased more quickly because of high temperatures, and by the end of the week food at 26° C was typically moldier than the food at room temperature. Therefore, there was likely an additional stress of poor food quality on flies at higher temperatures. A previous study in this species found that nutritional stress increased survival differences for singly versus multiply mated flies (Turner and Anderson 1983), and this could also be the case in this study that nutritional stress increased survival costs to being with others versus being alone. Food competition might have also increased as food quality decreased in the lifetime stress treatment, perhaps explaining why flies alone, and thus absent of competition, responded significantly better to the lifetime heat stress than flies with conspecifics. Any future studies should better control for food quality, such as changing the food more often under increased temperature, to divorce the effects of food quality and competition from other experimental variables.

Those with opposite sex flies had the lowest survival. In the one-week stress treatment, there was a significant survival cost for females in mixed sex groups, but not for males. For female *Drosophila,* there are well-known survival costs of reproducing, mostly attributable to exposure to males (Partridge and Fowler 1990) and receipt of accessory gland proteins (Chapman et al. 1995). In addition, a previous study showed that stress susceptibility increased for mated female *D. melanogaster* (Salmon et al. 2001), so mated females, who have a higher energy expenditure in mating than males, could be more susceptible to this short-term stress. In *D. pseudoobscura*, males do not experience as great a survival cost to reproduction as females (Lebow et al., MS) and therefore did not have significantly decreased survival in the mixed sex groups in the one-week stress treatment.

However, being with opposite sex flies in the lifetime stress treatment had significant survival costs for males as well as females. A previous study found that males in three *Drosophila* species decreased courtship and mating as temperature increased (Patton et. al 2001), so increased survival costs to being with females for males in our lifetime stress treatment is unlikely due to increased mating effort, the most costly act in the reproductive process for males in *D. melanogaster* (Cordts and Partridge 1996). However, the temperature stress could have revealed subtle survival costs to reproduction for males that do not appear in more benign environments.

Females outlived males in all stress and social group treatments. Previous studies in *D. pseudoobscura* have shown that selection for longer life is stronger for females, and this typically results in females living longer than males (Taylor and Condra 1980, Taylor et al. 1981). However, a previous study in our lab found that males outlived females at room temperature (unpublished data). Indeed, which sex outlives the other depends on breeding condition in *D. subobscura* (Maynard-Smith 1959) and *D. melanogaster* (Gonzales 1923), and also on temperature (Lamb 1978). In *D. melanogaster*, some studies have found that females survived longer than males when exposed to short-term extreme temperature stress (Dahlgaard et al. 1998, Krebs & Loeschcke 1994), while others have found the opposite (Lansing et al. 2000). Since we used a mild heat stress in our study, and since the sex difference in longevity was highly significant in both stress treatments, suggesting that the result is not simply due to females responding better to stress, we conclude that our result is consistent with previous research showing stronger selection on male longevity, resulting in females outliving males.

The lifetime temperature stress effectively decreased longevity, as predicted, and also increased survival differences among social group treatments. We posit that the lifetime temperature stress magnified the survival differences among individuals in different social treatments in the less stressful environment. Therefore, exposing *D. pseudoobscura* to stress can be an effective way of revealing subtle but potentially biologically significant differences, and might be incorporated into future studies for this effect.

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Table 2.1. Summary of mean survival (days) by sex, stress treatment (1 week or lifetime), and social group (A = alone, MS = mixed sex, SS = single sex), with Log-rank and Wilcoxon tests comparing differences in survival curves between groups.

	Stress	**Social Group**	**N**	**Mean ± SD**	**Groups Compared**	**Test**	**X^2**	**P**
Female	1 week	A	30	75.67	A/SS	Log-Rank	1.41	0.23
				±6.84		Wilcoxon	0.01	0.92
		MS	35	56.29	MS/A	Log-Rank	15.92	< 0.0001
				±6.33		Wilcoxon	13.77	0.0002
		SS	29	73.94	SS/MS	Log-Rank	13.66	0.0002
				±6.95		Wilcoxon	16.94	< 0.0001
	Lifetime	A	30	63.57	A/SS	Log-Rank	30.36	< 0.0001
				±5.37		Wilcoxon	17.67	< 0.0001
		MS	34	40.13	MS/A	Log-Rank	36.72	< 0.0001
				±5.05		Wilcoxon	23.43	< 0.0001
		SS	30	47.40	SS/MS	Log-Rank	4.81	0.03
				±5.37		Wilcoxon	7.00	0.01
Male	1 week	A	28	48.18	A/SS	Log-Rank	1.54	0.21
				±8.12		Wilcoxon	0.002	0.96
		MS	35	43.02	MS/A	Log-Rank	3.47	0.06
				±7.26		Wilcoxon	0.51	0.48
		SS	30	48.35	SS/MS	Log-Rank	0.85	0.36
				±7.84		Wilcoxon	2.47	0.12
	Lifetime	A	30	48.47	A/SS	Log-Rank	16.57	< 0.0001
				±5.20		Wilcoxon	8.74	0.003
		MS	35	27.35	MS/A	Log-Rank	17.33	< 0.0001
				±4.81		Wilcoxon	9.97	0.002
		SS	30	37.15	SS/MS	Log-Rank	4.31	0.04
				±5.20		Wilcoxon	9.98	0.002

Table 2.2. Summary of analyses comparing male and female survival by stress treatment and social group.

Grouping	Sex	*N*	Mean ± SD	Test	X^2	*P*
1 week	F	94	67.92 ± 2.14	Log-Rank	32.98	< 0.0001
	M	93	46.29 ± 2.23	Wilcoxon	45.64	< 0.0001
Lifetime	F	94	49.93 ± 1.82	Log-Rank	21.68	< 0.0001
	M	95	37.11 ± 1.71	Wilcoxon	24.56	< 0.0001
Alone	F	60	69.62 ± 3.12	Log-Rank	17.63	< 0.0001
	M	58	48.33 ± 3.39	Wilcoxon	23.11	< 0.0001
Mixed sex	F	69	48.33 ± 1.91	Log-Rank	16.18	< 0.0001
	M	70	35.19 ± 1.90	Wilcoxon	23.96	< 0.0001
Single sex	F	59	60.45 ± 2.28	Log-Rank	30.28	< 0.0001
	M	60	42.75 ± 1.82	Wilcoxon	31.35	< 0.0001

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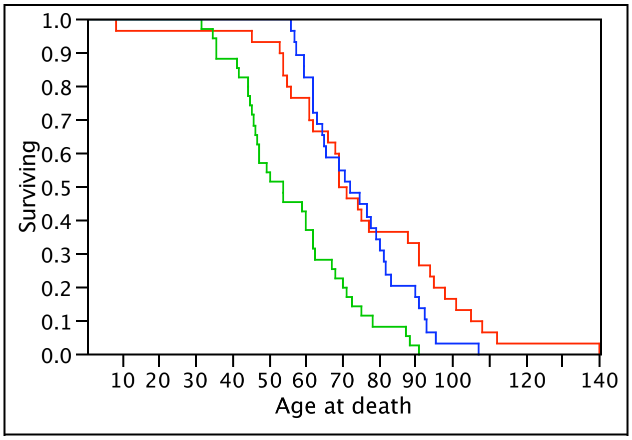
Figure 2.1. Survival curves of females by social group (red = alone, blue = single sex groups of six, green = mixed sex groups of three females and three males) in (a) one-week and (b) lifetime stress treatments.

Figure 2.2. Survival curves of males by social group (red=alone, blue=single sex groups of six, green= mixed sexes groups of three females and three males) in (a) one-week and (b) lifetime stress treatments.

Figure 2.3. Survival curves comparing male and female survival by (a) stress treatment (one-week or lifetime) and (b) social group (A, SS, or MS).

Figure 2.1

(a)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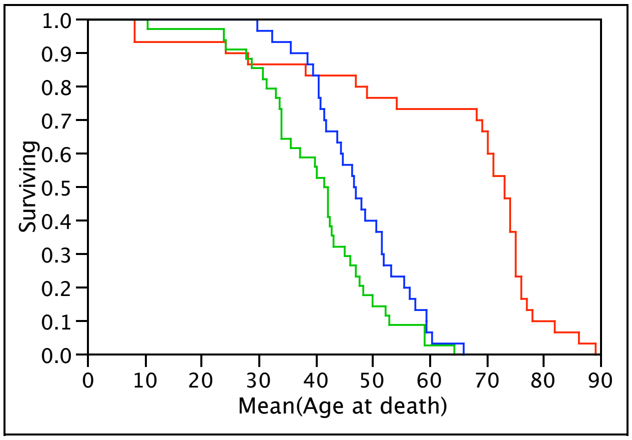
Surviving
10 20 30 40 50 60 70 80 90 100
120
140
Age at death

(b)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Surviving
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50
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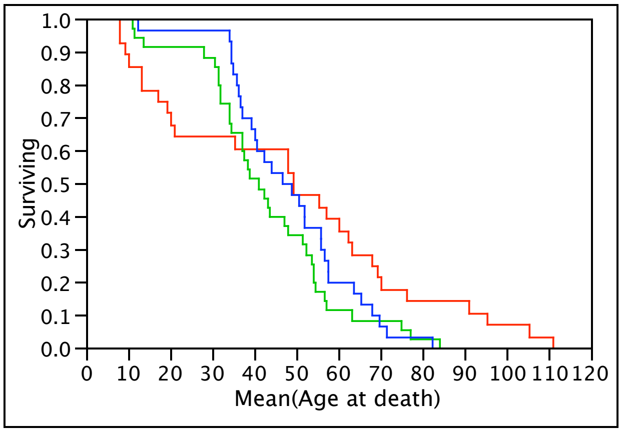
90
Mean(Age at death)

Figure 2.2

(a)


Surviving
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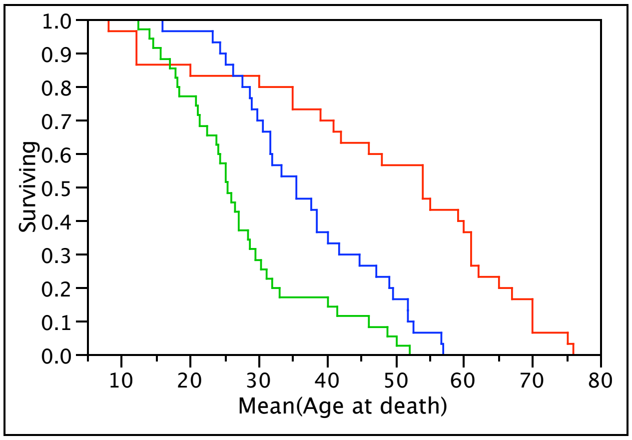
90
100
110
120
Mean(Age at death)

(b)


Surviving
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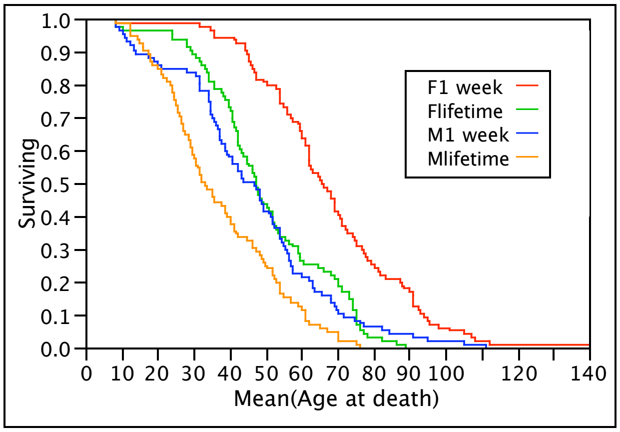
80
Mean(Age at death)

Figure 2.3

(a)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0.4
0.3
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0.0
Surviving
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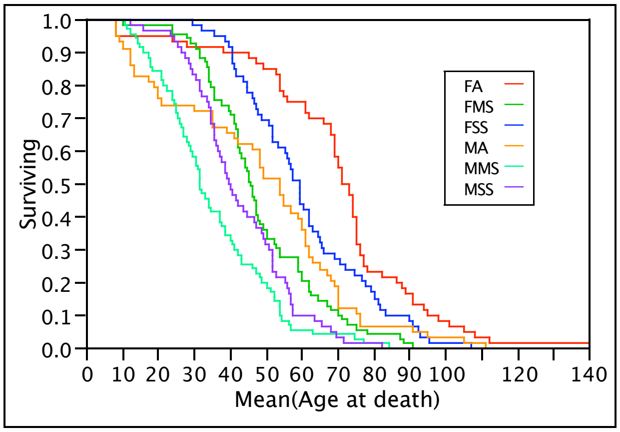
30
40
50
60
70
80
90
100
120
140
Mean(Age at death)
F1 week
Flifetime
M1 week
Mlifetime

(b)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Surviving
0
10
20
30
40
50
60
70
80
90
100
120
140
Mean(Age at death)
FA
FMS
FSS
MA
MMS
MSS

CHAPTER 3

MALES BENEFIT FROM MATING WITH PREFERRED FEMALES IN *DROSOPHILA PSEUDOOBSCURA*[1]

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[1] Lebow, E. T., P. A. Gowaty, Y. K. Kim, and W. W. Anderson. To be submitted to *Evolution*.

Abstract

Mate choice can increase fitness for a chooser and its offspring, and also decrease costs of mating. While male mate choice is documented in many insect species, few studies have looked at male and female choice simultaneously. In this study we determined mate preference for both male and female choosers of *Drosophila pseudoobscura* in individual preference arenas that minimize preference constraints. We paired choosers with their preferred (P) or non-preferred (NP) mate and kept a pair together for either one day or 21 days under mild heat stress conditions. We did not find significant fitness differences between P and NP matings in ANOVAs. However, examining demographic differences between P and NP over 25 days of egg-laying revealed that females with their preferred male for 21 days had higher LxMx than females with their non-preferred male for 21 days, and preferred females with males for one day had higher net fitness and their offspring had higher egg-to-adult viability than males with their non-preferred female for one day. In addition, males with their preferred female for one day had significantly higher survival than males with non-preferred females, while there were no survival benefits for females with their preferred male.

Key words*:* male choice, female choice, survival, fitness, cost of mating, *Drosophila pseudoobscura*

Introduction

Being choosy in mating is thought to evolve when the benefits to the chooser outweigh the costs of choosing (Andersson 1994). Benefits can include direct fitness benefits, like increased fecundity or parental investment (Andersson 1994; Kirkpatrick and Ryan 1991), or indirect benefits such as increased offspring quality (Hamilton and Zuk 1982; Williams 1966; Zahavi 1975) or attractiveness (Fisher 1930). While traditional mate choice models predict that females with their preferred male will have increased fecundity, compensation theory predicts that females with their *non-preferred* mate will have increased fecundity to compensate for decreased offspring viability (Gowaty 1997). If fecundity compensation occurs, then females should have decreased survival in non-preferred matings because of the survival costs of egg production (Lamb 1964; Maynard-Smith 1958).

In species in which females invest more than males in offspring, anisogamy theory (Bateman 1948) and parental investment theory (Trivers 1972) predict that females are more selective in mating while males are indiscriminate. Therefore, female choice and the male traits females choose for have dominated the literature. However, males across many taxa also choose and benefit from choice. Traditional predictions about male choice were that males would benefit from choosing when they had a high parental investment in offspring (Trivers 1972) or expensive ejaculates (Dewsbury 1982; Petrie 1983). Subsequent work, however, predicted that males could benefit from choosing, even when they only invested sperm, if there was high variation among females (Gwynne 1991; Hubbell and Johnson 1987; Owens and Thompson 1994; Parker

1983) or if there were low search costs for males, such as in systems where females benefit from remating (Bonduriansky 2001). Most predictions regarding male mate choice are that males choose females with high fecundity (Bonduriansky 2001). There have been no studies finding survival benefits for males mating with their preferred females, though we imagine that males could vary courtship duration and intensity, copulation length, or sperm transfer in response to the preference status of the female, all of which could affect their longevity.

While there is clearly female and male choice occurring simultaneously in many species, as predicted by theoretical models (Johnstone et al. 1996; Parker 1983) and observed in *Drosophila* (Gowaty et al. 2003), few empirical studies have looked at male choice simultaneously with female choice. In this study we investigated the fitness and survival consequences of both female and male choice in *D. pseudoobscura*. We kept individuals with their preferred or non-preferred mate for either one day or 21 days under mild heat stress conditions, a stress that magnifies differences among experimental groups (Lebow et al. MS). The 21 days together treatment was included in order to examine the effects of long-term enforced monogamy with a non-preferred mate. We did not measure the phenotypic traits mediating preference of either males or females. Rather, we were interested in the fitness and survival consequences of mate preferences independent of the cues mediating preference.

Methods

Study subjects

WWA collected *D. pseudoobscura* in Mesa Verde, CO in the summer of 1995 and established isofemale lines in Athens, GA. He used eight isofemale lines to make a population cage in July 1997 with 20 half-pint glass bottles of food composed of yeast, agar, cornmeal, molasses, and propionic acid as a preservative. In March 2004, we added four glass bottles to the cage and removed them one week later. To reduce the density of eggs and larvae in a bottle, we divided the food from each bottle into three fresh bottles. We performed this breeding protocol weekly for the duration of the experiment. Once adults began emerging from bottles, we collected virgin males and females twice a day under CO_2 and kept them in vials of up to 10 individuals of the same sex until age seven.

Preference tests

At age seven days, we placed flies into arenas (Fig. 3.1) and measured the time a single focal fly (chooser) spent near two flies of the opposite sex (discriminatees). The arenas were made from tygon tubing with a long corridor where the chooser fly could walk, and two cells, each containing one discriminatee, that are separated from the main corridor by a screen. The chooser can see and smell the discriminatees, but the discriminatees cannot see each other because of white tape placed on the side of each cell. This method eliminates intrasexual behavioral competition and intersexual coercion.

We performed two 20-minute preference tests for each chooser, with approximately eight hours between tests. For each test we introduced the chooser into the center of the arena and recorded the total time the chooser spent in each third of the arena- two areas on each end near the discriminatees plus the area in between. We determined preference in test 1 based on the following behavioral criteria, where T_A is time spent in the third of the arena near discriminatee A and T_B is total time spent near discriminatee B: 1) T_A or $T_B > 9$ minutes; and 2) T_A or $T_B > 60\% (T_A + T_B)$. If these criteria were met in test 1, then we re-tested the flies in the afternoon of the same day with the position of the discriminatees reversed in the arena. Between tests, we soaked arenas in an unscented soapy solution for 30 minutes, rinsed them in water, and dried them in a drying oven. Passing test 2 included passing the two above criteria plus the additional criterion of choosing the same fly chosen in test 1. We performed male and female preference tests daily, Monday through Friday, from March 2004 to December 2004.

Groups

If the chooser met all criteria we paired it with either its preferred (P) or non-preferred (NP) mate and recorded the time to copulation and the duration of copulation.

We kept the pair together for either one day or 21 days. Therefore, for each sex, we had four experimental groups: with P for one day (P1), with P for 21 days (P21), with NP for one day (NP1), and with NP for 21 days (NP21). To control for environmental variance, we rubberbanded together vials from a P pair with an NP pair of the same age and length of time with mate. All flies were under a mild heat stress of 26°

C in an unlit incubator except for approximately one hour a day when they were transferred to new food at room temperature. *Drosophila pseudoobscura* is considered a "light independent" species whose frequency of mating does not decrease in darkness, meaning flies together for 21 days would continue mating in the incubator (Mayr and Dobzhansky 1945; Wallace and Dobzhansky 1946).

All flies were transferred daily for 25 days, then bi-weekly after that until death. Vials past day 25 were checked daily for any deaths. For the first 25 days, we counted the number of eggs laid daily. We kept these vials of eggs at room temperature, again rubberbanding together a P with an NP vial of the same day of egg-laying. We monitored all vials of eggs for emerging adult offspring. Once adults began emerging, we counted emerging adults daily for seven days. Therefore, we recorded adult survival data and data on three fitness measures: total eggs laid in 25 days of egg-laying, total adult offspring emerging from those eggs, and the egg-to-adult viability of offspring.

Arena behavior and sample sizes

We performed 668 first tests with the female as the chooser. Of these, 432, or 64.67%, passed test 1. Twenty-four could not be retested in test 2 because we lost or injured one of the flies. Of the 408 retested, 163 passed test 2, meaning that 25.31% of females passed all criteria. This gave a sample size of $N \geq 40$ for each group (P1, NP1, P21, NP21).

We performed 617 first tests with the male as the chooser. Of these, 417, or 67.59%, passed test 1. Twenty-one could not be retested. Of the 396 that were retested, 163 passed test 2, meaning that 27.35% of males passed all criteria. This gave a sample size of $N \geq 40$ for each group.

Fitness and survival analyses

ANOVAs

We did multiple ANOVAs with three independent variables- sex of chooser, mated with P or NP, and together for one day or 21 days- plus all interaction terms, on three measure of fitness: total number of eggs laid, total number of adult offspring, and egg-to-adult viability of offspring over 25 days of egg-laying.

Fitness over time

Because we recorded fitness measures and survival daily, we were able to examine demographic differences between P and NP matings in each time treatment for male and female choosers separately, including LxMx (fecundity at age x multiplied by the female's survival probability at age x), LxProdx (net fitness, or adult offspring produced at age x multiplied by the female's survival probability at age x) and egg-to-adult offspring viability over time. We graphed each fitness component cumulatively by female age, calculating the cumulative LxMx, LxProdx, and egg-to-adult offspring viability at each female age for 25 days of egg-laying.

Survival

We compared survival of choosers mated with P or NP partners in each time treatment. We also compared survival of the P and NP mated discriminatees. We used the Kaplan-Meier method to generate survival curves, and then compared those curves using a Log-rank test, which puts equal weight on all survival times, and a Wilcoxon test, which puts heavier weight on shorter survival times. We used the JMP 5.1© statistical package for all analyses.

Results

Comparison of male and female chooser arena behavior

There was not a significant difference between the percentage of female and male choosers passing test 1 (Likelihood ratio X^2= 1.22, $P = 0.27$, $N = 1285$) or passing all criteria (Likelihood ratio X^2= 0.60, $P = 0.74$, $N = 1285$). Females that met all criteria spent an average of 32.54 ± 0.36 (SE) min over both tests with their preferred male, while males that met all criteria spent an average of 34.02 ± 0.37 (SE) min with their preferred female over both tests, significantly longer than females spent with their preferred male ($F_{1,324} = 8.30$, $P = 0.004$).

Copulation latency and duration

For female choosers, there were no significant differences between P and NP matings either in copulation latency (P: $\mu \pm SE = 48.80 \pm 9.37$ sec; NP: $\mu \pm SE = 63.20 \pm 9.53$ sec; $F_{1,157} = 1.15$, $P = 0.29$) or copulation duration (P: $\mu \pm SE = 411.15 \pm 10.98$ sec; NP: $\mu \pm SE = 420.01 \pm 11.20$ sec; $F_{1,154} = 0.32$, $P = 0.57$).

For male choosers, there were no significant differences between P and NP matings in latency to copulation (P: $\mu \pm SE = 60.62 \pm 9.10$ sec; NP: $\mu \pm SE = 53.39 \pm$

9.10 sec; $F_{1,155} = 0.32$, $P = 0.58$) or copulation duration (P: $\mu \pm SE = 434.31 \pm 12.34$ sec; NP: $\mu \pm SE = 432.12 \pm 12.26$ sec; $F_{1,154} = 0.02$, $P = 0.90$).

Fitness

*ANOVA*s

We did not find significant differences between the sexes or between P and NP matings in any of the three measures of fitness (Figs. 3.2, 3.3; Table 3.1), nor did we find significant fecundity differences between one day and 21 day treatments. There were, however, significant differences between one day and 21 days pairs, with flies together for 21 days having higher numbers of total adult offspring (Fig. 3.2b; Fig 3.3b) and their offspring having higher egg-to-adult viability (Fig. 3.2c; Fig. 3.3c; Table 3.1).

Fitness over time

For female choosers, cumulative LxMx is higher in P matings for both time treatments (Fig. 3.4a). For male choosers, however, it is not consistent, with P greater than NP when mates were together for one day, but NP greater than P when mates were together for 21 days (Fig. 3.4b).

Female choosers with their P male for 21 days had higher LxProdx, but there was not a difference between P and NP females with their mate for one day (Fig. 3.5a). For male choosers, again, the data are not consistent, with females preferred by males having higher LxProdx in the one day treatment, but slightly lower LxProdx in the 21 days treatment (Fig. 3.5b).

Cumulative egg-to-adult offspring viability did not differ between P and NP matings for either time treatment (Fig. 3.6a). For male choosers, however, offspring from P matings had higher egg-to-adult viability when mates were together for 1 day, while there was no difference in the 21 days treatment (Fig. 3.6b).

Survival

Choosers

There were no survival differences between female choosers with their P or NP mate in either time treatment (Figs. 3.7a and 3.7b; Table 3.2). There was, however, significantly higher survival for males with their P females for one day, but no survival difference between male choosers with their P or NP female for 21 days (Figs. 3.7c and 3.7d; Table 3.2).

Mated discriminatees

There were no significant survival differences between mated male P and NP discriminatees in either time treatment (P1/NP1: Log-rank $P = 0.32$, Wilcoxon $P = 0.57$; P21/NP21: Log-rank $P = 0.93$, Wilcoxon $P = 0.86$; Table 3.2). There were no significant differences between mated female P and NP discriminatees in either time treatment (P1/NP1: Log-rank $P = 0.74$, Wilcoxon $P = 0.31$; P21/NP21: Log-rank $P = 0.32$, Wilcoxon $P = 0.28$; Table 3.2).

Discussion

<u>Fitness and survival effects of mate preference</u>

We did not find significant differences between P and NP matings in the ANOVAs. For fecundity, direct benefits hypotheses predict increased fecundity in females mating with preferred males (Andersson 1994; Kirkpatrick and Ryan 1991). Our ANOVA results do not support this prediction, nor do they find evidence for fecundity compensation in non-preferred matings (Gowaty 1997). However, females with their P male in both time treatments had higher cumulative LxMx (Fig. 3.4a), suggesting that there might be increased fecundity in P matings. However, the LxMx data were not consistent between time treatments for male choosers (Fig. 3.4b) This finding is not consistent with many hypotheses that male choice is mostly for females with higher fecundity (Bonduriansky 2001).

There were no significant differences between P and NP in total adult offspring or egg-to-adult offspring viability in the ANOVA. However, females with their P male for 21 days had higher cumulative net fitness in the demographic curves (Fig. 3.5a), as did P females with the male chooser for one day (Fig. 3.5b). Similarly, offspring from males with their P female for one day had a higher cumulative egg-to-adult viability than males with their NP female for one day (Fig. 3.6b). So while the ANOVA did not show significant benefits to P matings for these two measures of fitness, the demographic data are suggestive of a net fitness and egg-to-adult offspring viability benefit for males with their P female for one day, as well as a net fitness benefit to females with their P male for 21 days.

Both total adult offspring and egg-to-adult offspring viability were significantly higher when pairs were together for 21 days. This is likely due to sperm depletion of females kept with their mate for only one day. For those females, both productivity and egg-to-adult viability began to drop off before the end of 25 days of egg-laying (see Figs. 3.5 and 3.6).

We did not separate out fertilized from unfertilized eggs in this study, and females with males for only one day likely ran out of sperm before the end of egg counting, possibly confounding the egg-to-adult viability results with variation in the amount of sperm transferred in a single mating. A previous study in our lab found that males delivered significantly less sperm in P matings than NP matings, when either the male or the female was the chooser (unpublished). If females in this study similarly received less sperm from preferred males, in order to for us to detect a viability benefit in P matings there would have had to be a very strong egg-to-adult viability benefit in fertilized eggs to overcome the possible increased number of unfertilized eggs, due to sperm depletion, that were included in the egg-to-adult offspring viability calculation. Future studies, therefore, should attempt to include only fertilized eggs in calculating egg-to-adult viability.

In a previous mate choice study in this lab, performed in 1998, both female and male choosers mating with preferred mates had offspring with significantly higher egg-to-adult viability (Anderson et al. MS). Our failure to repeat this result in the ANOVAs could be because of increased inbreeding in the population cage, or because of decreased variation among potential mates, since our study used flies that had been in the population cage six years longer than the flies from the previous study. However, females and males still showed choosy behavior in this study, with approximately 25 percent passing our criteria, and there are benefits to mate preference revealed in the demographic data, including an egg-to-adult offspring viability benefit for male choosers.

Previous work has been inconsistent on the survival effects of mate preference. There is evidence that mating with preferred mates increases female survival because in doing do females avoid costly male manipulation (Gowaty 1997; Moore et al. 2003). However, there is also evidence that mating with preferred males decreases female survival while simultaneously increasing other fitness measure (Cordero and Eberhard 2003). Our results are not consistent with either since there were no significant survival effects for females with their P male. Males, on the other hand, had a significant survival benefit to being with a P female versus an NP female for one day, but there were no correlated differences in either latency to first copulation or copulation duration between P and NP matings. We did not, however, measure courtship duration, a different measure than latency to copulation. We also only observed the flies immediately after pairing. It could be that there was variation in length or intensity of courtship initially and/or during the one day together. Courtship is the costliest mating behavior for males in terms of survival in *D. melanogaster* (Cordts and Partridge 1996), and males with NP females could be intensifying courtship. It is also possible that there was cryptic male choice occurring during copulation, since, as mentioned above, males in this species delivered significantly less sperm when mated with P females versus NP females in a previous study. If sperm production is costly, this factor could have contributed to the survival benefit. This is the first study we're aware of to find a survival benefit to mate preference for males.

There were no survival differences between P and NP discriminatees of either sex. This result is not consistent with mate choice models that predict mate choice is for absolute quality differences between mates (Andersson 1994). Since longevity is a measure of quality (Trivers 1972), mate choice for absolute quality would result in preferred individuals living longer than non-preferred individuals. However, alternative mate choice models predict that mate choice could be more for complementary genes, such as genes that increase offspring heterozygosity (Brown 1997) or immune diversity (Wedekind 1999). Unlike mate choice for absolute quality, mate choice for complimentary genes would be self-referential and not correlated with survival variation of potential mates. Therefore, not finding survival differences between P and NP discriminatees suggests that mate choice is not for absolute quality differences between individuals in this species, and could be for complementarity.

Choosy males

Previous empirical evidence for male mate choice in *Drosophila* involved choice for either virgin females to decrease sperm competition, or female size or nutritional status, two factors correlated with fecundity (reviewed in Bonduriansky 2001). In this study, however, all females were virgins and we did not find evidence for male preference for increased fecundity. Rather, males with their preferred female for one day had significantly increased survival, and seemed to choose females who had higher net fitness and with whom their offspring had higher egg-to-adult offspring viability (Figs. 3.5b and 3.6b).

We also found that males spent significantly more time with their preferred mate than female choosers. The fact that males had stronger preferences than females could be the result of higher variation in females than in males, one of the main components affecting choosiness (Hubbell and Johnson 1987; Owens and Thompson 1994). Examining the genetic variation in the population cage, and whether it is higher in females than males, could address this question.

Another factor affecting choosy behavior in males is female remating rate (Bonduriansky 2001). Males should be choosier when search costs are low, such as when more females are available for mating. In species where females benefit from remating, such as when insects give nuptial gifts, male search costs are predicted to be lower since more females should be interested in mating. In *D. pseudoobscura*, search costs for males should be high since mating is costly to females, a few matings are probably enough to fertilize all of a female's eggs, and males do not give nuptial gifts. However, a recent study in our lab found that polyandrous females have offspring with significantly higher egg-to-adult viability (unpublished). This fact could lower male search costs since females might be more willing to re-mate even if they already have enough sperm to fertilize their eggs.

The fact that we could not perform statistical analyses on the demographic curves limits our conclusions. However, it is clear from examining them that there are interesting, potentially biologically significant, differences over time that are masked in the ANOVAs. Therefore, future studies could include similar demographic data, along with suggestions for appropriate statistical tests for analyzing the curves.

Acknowledgements

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Table 3.1. Summary of ANOVA results testing the effect of three independent variables- sex of chooser, being with P or NP mates, being with a mate for 1 day or 21 days- plus all interaction terms, on three measures of fitness.

	P-value							
Fitness measure	Overall model	Length of time w/mate	With P or NP	Chooser sex	Sex * with P/NP	Sex * length of time w/mate	With P/NP * Length of time w/mate	Sex * with P/NP * length of time w/mate
Eggs	0.71	0.20	0.98	0.57	0.40	0.63	0.78	0.21
Adult offspring	0.002	< 0.0001	0.47	0.52	0.99	0.74	0.79	0.23
Percent eggs becoming adults	0.006	0.0002	0.29	0.68	0.42	0.11	0.42	0.31

Table 3.2. Summary of mean survival by sex, preference test role (chooser/discriminatee), preference status (P/NP), and length of time with mate (1 day/21 days) for mated flies.

	N	Mean longevity	± SE
Females choosers			
P1	41	68.41 days	4.21
NP1	40	71.13	4.27
P21	40	60.25	4.26
NP21	39	64.00	3.72
Male choosers			
P1	39	73.05	3.42
NP1	35	63.03	3.24
P21	37	61.87	3.16
NP21	41	64.71	3.05
Female discriminatees			
P1	40	67.45	4.17
NP1	40	58.53	5.25
P21	39	58.49	4.23
NP21	40	64.80	3.85
Male discriminatees			
P1	39	67.36	2.53
NP1	39	69.92	3.01
P21	39	64.18	3.27
NP21	39	63.72	3.33

List of Figures

Figure 3.1. Photograph of the preference arena showing the long corridor (A) where the choosers walks, and the two cells holding the discriminatees (B). Note the visible lines drawn on the tube dividing the arena into three equal areas.

Figure 3.2. Mean number of eggs laid (A), number of adult offspring (B), and egg-to-adult offspring viability (C) over 25 days of egg-laying for female choosers with their P or NP mate in the one day (solid line) and 21 day (dashed line) treatments, with standard error bars.

Figure 3.3. Mean number of eggs laid (A), number of adult offspring (B), and egg-to-adult offspring viability (C) over 25 days of egg-laying for male choosers with their P or NP mate in the one day (solid line) and 21 day (dashed line) treatments, with standard error bars.

Figure 3.4. Cumulative LxMx by female age for each group (P1, NP1, P21, NP21) for female (A) and male (B) choosers.

Figure 3.5. Cumulative LxProdx by female age for each group (P1, NP1, P21, NP21) for female (A) and male (B) choosers.

Figure 3.6. Cumulative egg-to-adult offspring viability by female age for each group (P1, NP1, P21, NP21) for female (A) and male (B) choosers.

Figure 3.7. Survival curves for female (A, B) and male (C, D) choosers with their preferred (black line) or non-preferred (gray line) mate for either one day (A, C) or 21 days (B, D). Neither pair of curves for females is significantly different (P1/NP1: Log-rank $P = 0.51$, Wilcoxon $P = 0.53$; P21/NP21: Log-rank $P = 0.73$, Wilcoxon $P = 0.74$). For males, the one day curves are significantly different (P1/NP1: Log-rank $P = 0.009$, Wilcoxon $P = 0.008$), while the 21 day curves are not (P21/NP21: Log-rank $P = 0.32$, Wilcoxon $P = 0.28$).

Figure 3.1

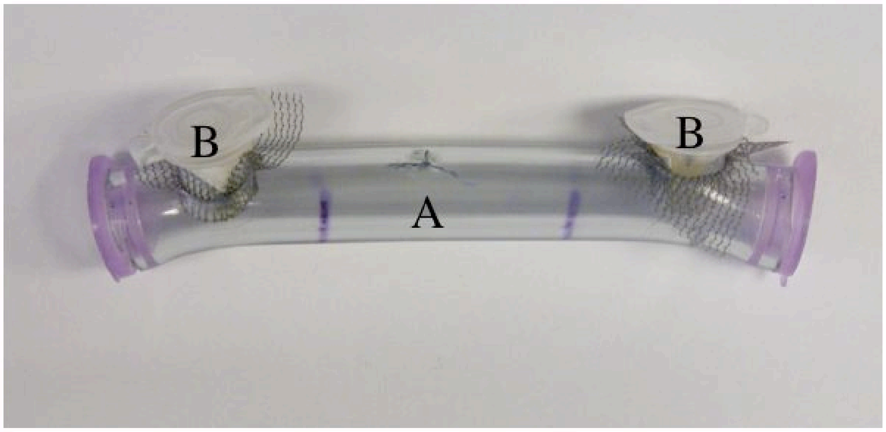


Figure 3.2

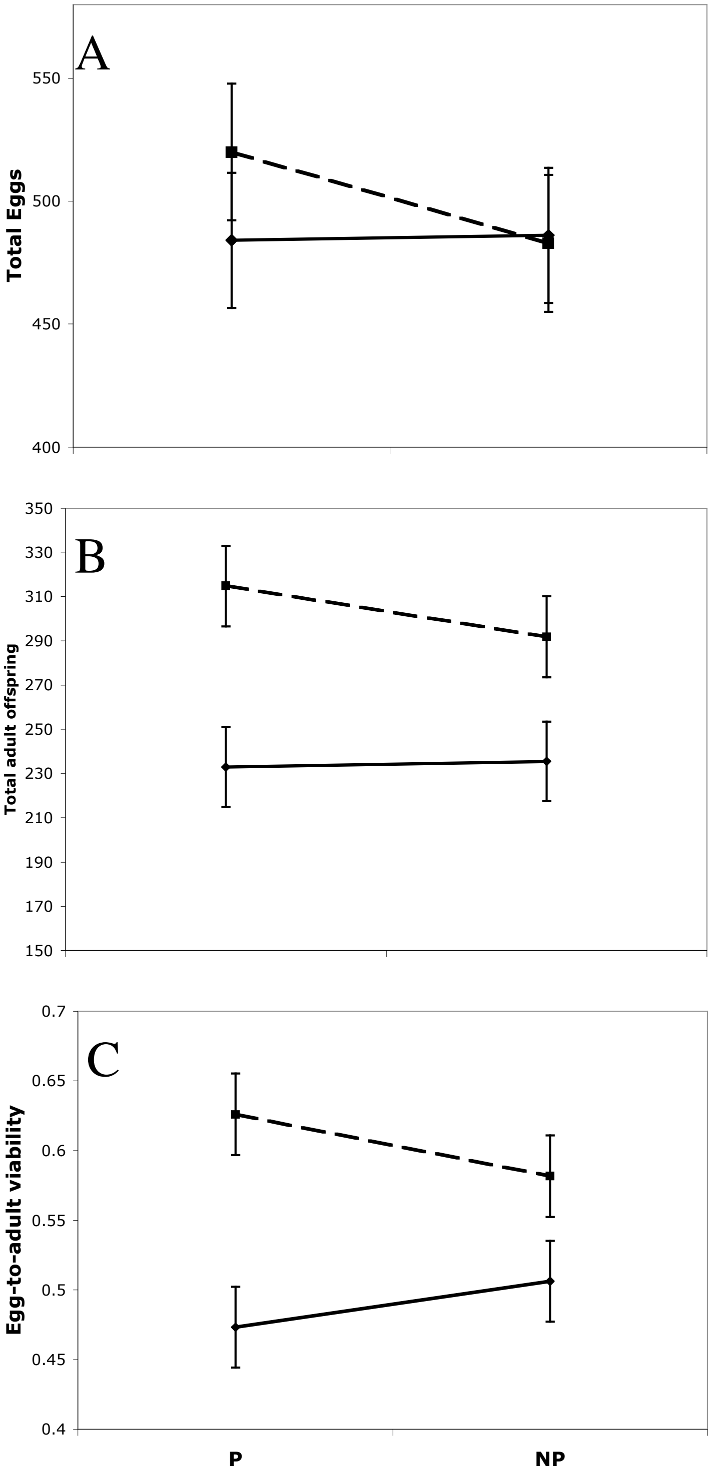

A
Total Eggs
550
500
450
400
B
Total adult offspring
350
330
310
290
270
250
230
210
190
170
150
C
Egg-to-adult viability
0.7
0.65
0.6
0.55
0.5
0.45
0.4
P
NP

Figure 3.3

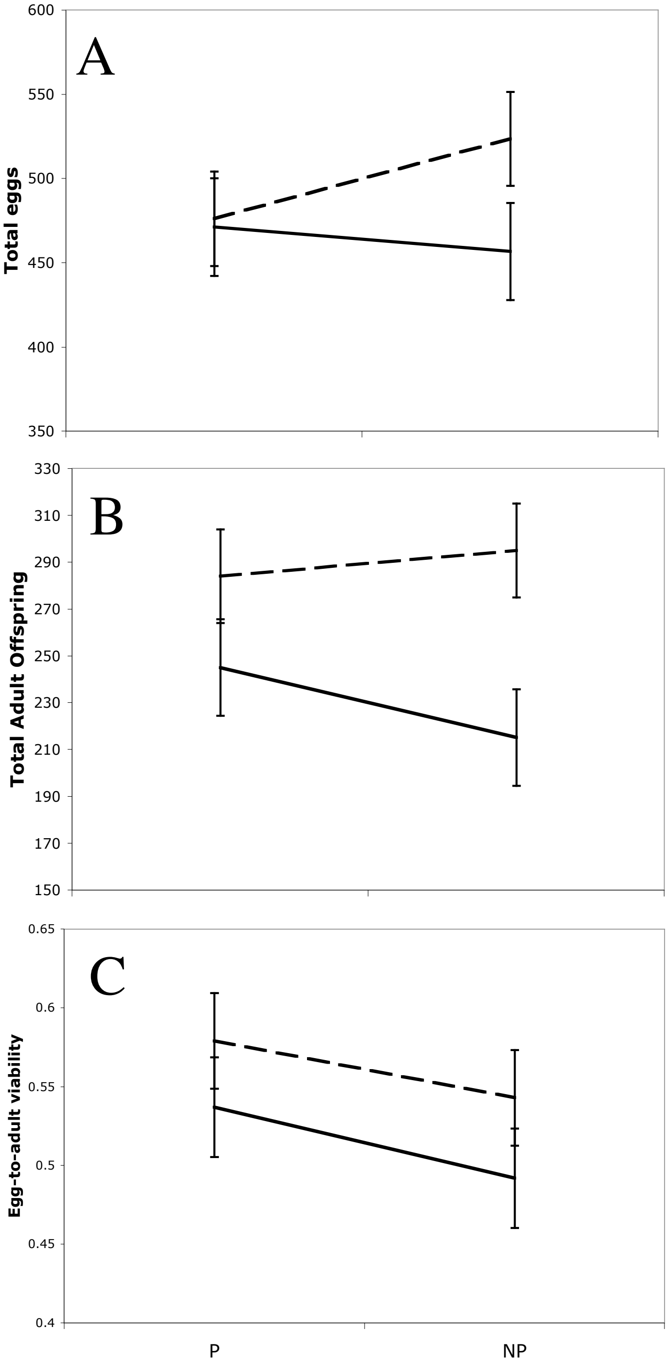

A
600
550
500
450
400
350
Total eggs
B
330
310
290
270
250
230
210
190
170
150
Total Adult Offspring
C
0.65
0.6
0.55
0.5
0.45
0.4
Egg-to-adult viability
P
NP

Figure 3.4

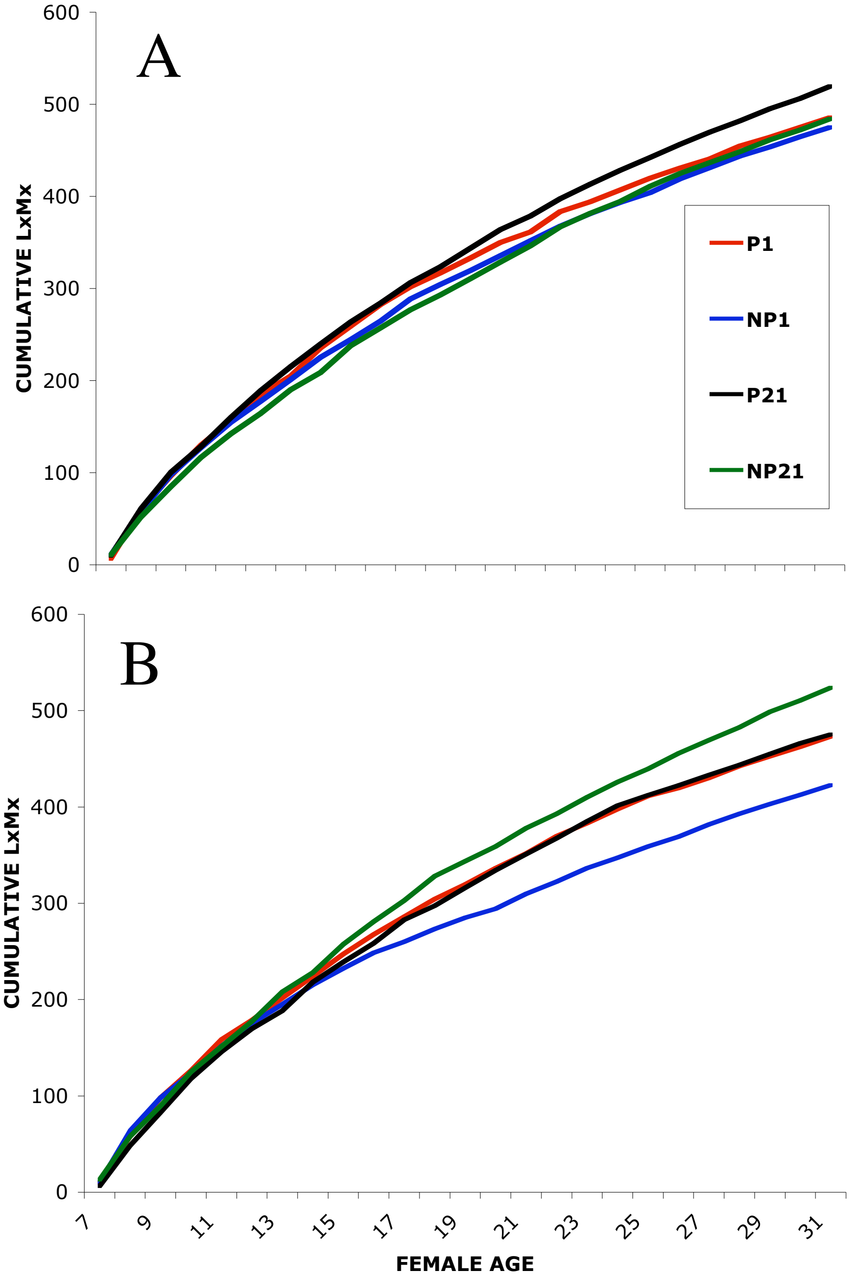

A
B
600
500
400
300
200
100
0
CUMULATIVE LxMx
P1
NP1
P21
NP21
7
9
11
13
15
17
19
21
23
25
27
29
31
FEMALE AGE

Figure 3.5

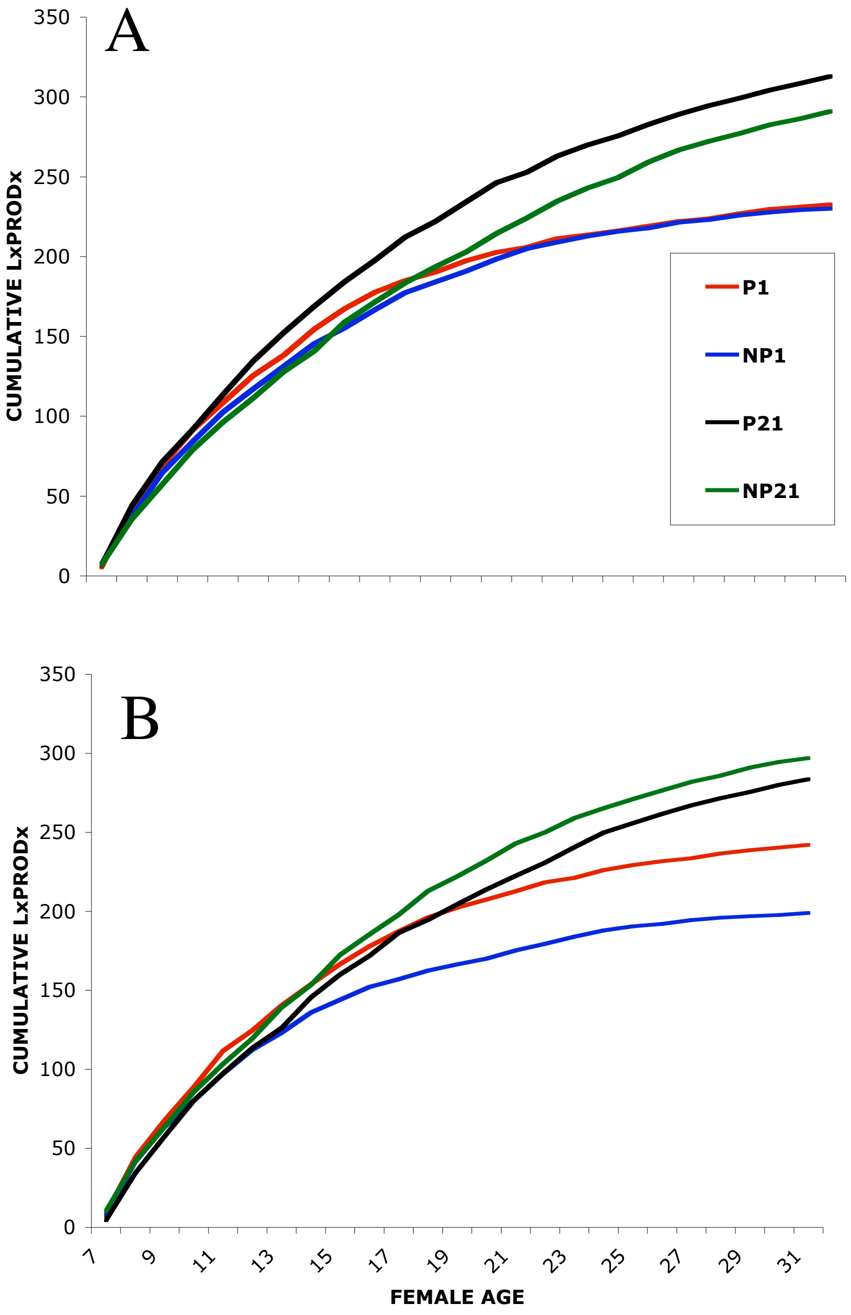

A
B
CUMULATIVE LxPRODx
0
50
100
150
200
250
300
350
P1
NP1
P21
NP21
FEMALE AGE
7
9
11
13
15
17
19
21
23
25
27
29
31

Figure 3.6

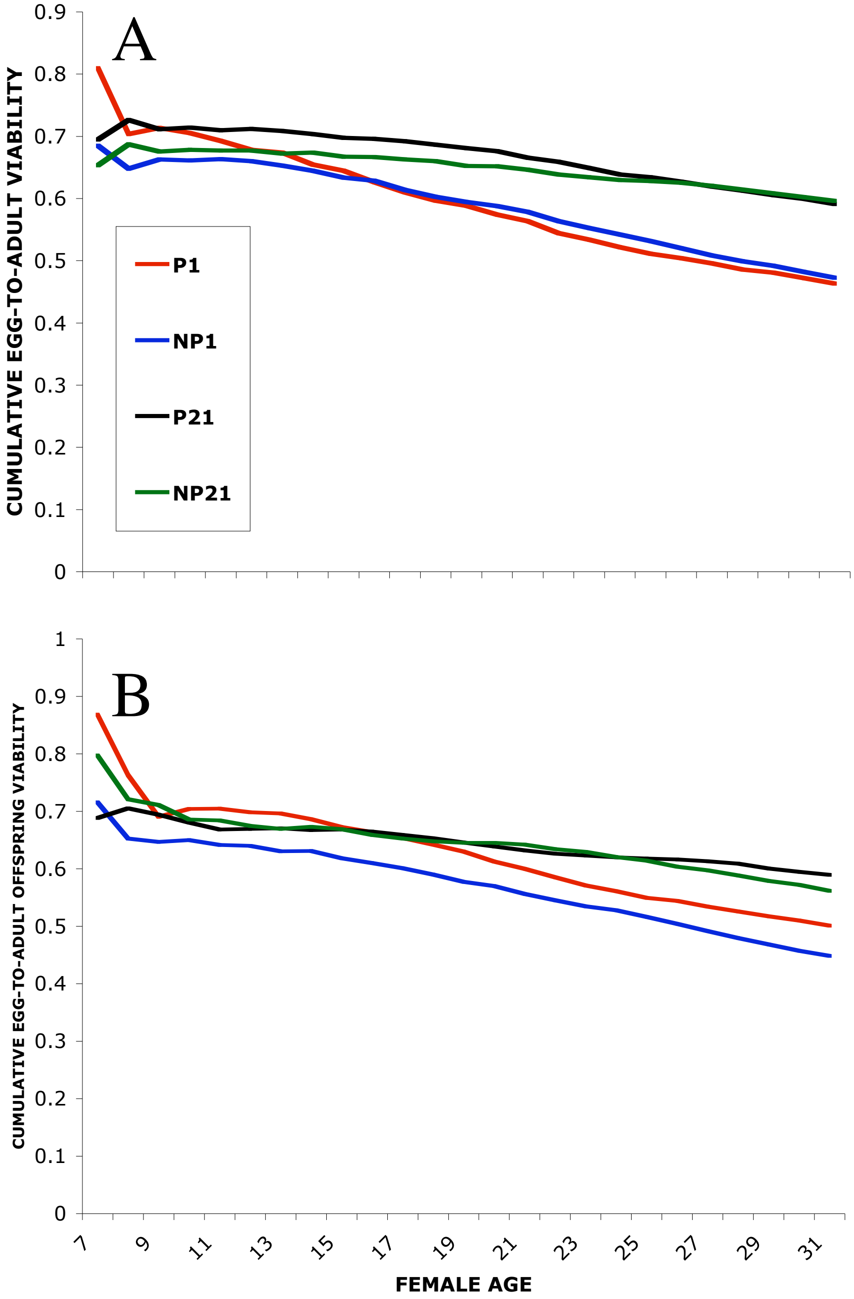
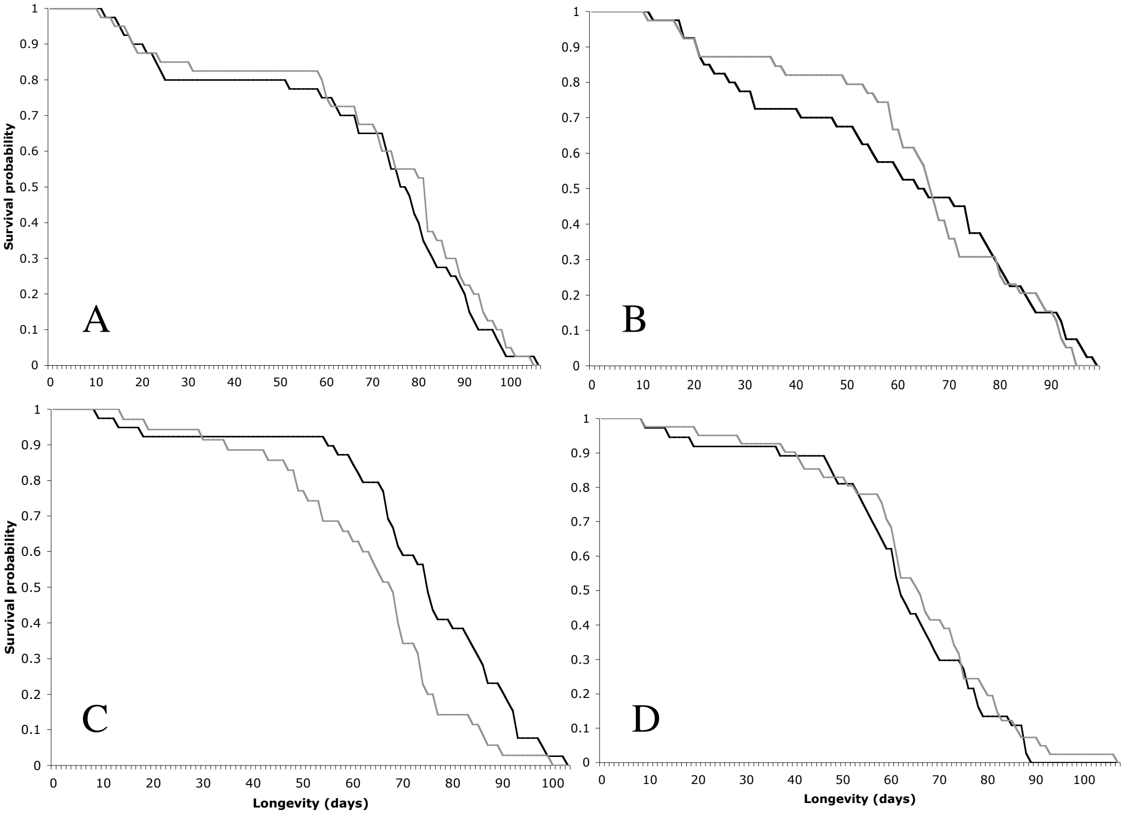

A
CUMULATIVE EGG-TO-ADULT VIABILITY
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
P1
NP1
P21
NP21
B
CUMULATIVE EGG-TO-ADULT OFFSPRING VIABILITY
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
7
9
11
13
15
17
19
21
23
25
27
29
31
FEMALE AGE

Figure 3.7


A
B
C
D
Survival probability
Survival probability
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
10
20
30
40
50
60
70
80
90
100
Longevity (days)
Longevity (days)

CHAPTER 4

NO SURVIVAL DIFFERENCES BETWEEN PREFERRED AND NON-PREFERRED INDIVIDUALS IN *DROSOPHILA PSEUDOOBSCURA*[1]

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[1] Lebow, E. T., P. A. Gowaty, Y. K. Kim, and W. W. Anderson. To be submitted to *American Naturalist*.

Abstract

Traditional mate choice models predict that all females in a population will prefer the highest quality males. Since longevity is a measure of quality, preferred individuals should therefore live longer than non-preferred ones. We tested this prediction by comparing survival of unmated male and female *Drosophila pseudoobscura* that were either preferred or non-preferred by the opposite sex. We determined mate preference in individual preference arenas that eliminated intrasexual behavioral competition and intersexual coercion, where one fly chooses between two flies of the opposite sex. There were no significant survival differences between preferred or non-preferred flies of either sex, rejecting the prediction of some mate choice models that choice is for absolute quality.

Key words: mate choice, good genes, complementarity, *Drosophila pseudoobscura*

Introduction

Researchers studying sexual selection by female choice have been primarily interested in the male traits that are attractive to females, plus what benefits the females receive from choosing (Andersson 1994). In indicator models of mate choice, males advertise their quality and females assess it, preferring males that signal high quality and could pass high quality genes to their offspring (Hamilton and Zuk 1982; Williams 1966; Zahavi 1975). These models assume that mate choice is for absolute quality of a mate, meaning all females in a population will prefer the same, highest quality males. Alternatively, mate choice could be self-referential, meaning that different females will prefer different males because of interaction effects between parental genes on offspring (Trivers 1972).

If mate choice is for absolute quality, then preferred mates should outlive non-preferred mates since longevity is an indicator of quality (Trivers 1972). Thus to test for absolute quality differences between alternative potential mates, we examined the survival differences between preferred and non-preferred individuals in male and female *Drosophila pseudoobscura*. Because males also choose in this species (Gowaty et al. 2003), and receive benefits from mating with females they prefer (Anderson et al. MS; Lebow et al. MS-a), we tested survival differences in females that are preferred or non-preferred by males as well as vice versa. Finding significant survival differences between preferred and non-preferred potential mates would support the hypothesis of mate choice for absolute quality genes. However, finding no survival differences between preferred and non-preferred individuals would reject that hypothesis, but would be consistent with self-referential mate choice models that predict the best mate for one individual is not necessarily the best mate for another.

Methods

Breeding

Wyatt Anderson collected *Drosophila pseudoobscura* in Mesa Verde, CO in the summer of 1995 and established isofemale lines in Athens, GA. We used eight isofemale lines to establish a population cage in July 1997 with twenty half-pint glass bottles, each containing approximately 30 ml of food composed of yeast, cornmeal, agar, molasses, and a small amount of propionic acid to prevent the growth of mold. The oldest bottle was rotated out of the cage and replaced with a fresh one on Monday, Wednesday, and Friday of each week.

In February 2004, approximately 90 generations after starting the cage, we sampled it by adding four fresh bottles to the population cage. We removed these bottles one week later. To reduce the density of developing flies in each bottle, we divided each one into three new bottles by scooping off the top of food containing eggs and larvae. When adults began emerging, approximately 16 days later, we collected virgin flies every eight hours, twice a day, and sexed them under CO_2. We maintained up to 10 flies per vial and aged them for seven days.

Preference tests

At age seven days, we placed flies into arenas (Fig. 4.1) and measured the time one focal fly (chooser) spends near two flies of the opposite sex (discriminatees). The arenas were tygon tubing with a long corridor where the chooser fly can walk, and two cells, each containing one discriminatee, that are separated from the main corridor by a screen. The chooser can see and smell the discriminatees, but the discriminatees cannot see each other because of white tape placed on the side of each cell. This method eliminates the effects of intrasexual behavioral competition and intersexual coercion on preference behavior.

We performed two 20-minute preference tests for each chooser, with approximately eight hours between tests. For each test we recorded the total time the chooser spent in each third of the arena- two areas on each end near the discriminatees plus the area in between. We determined preference in test 1 based on the following behavioral criteria, where T_A is time spent in the third of the arena near discriminatee A and T_B is total time spent near discriminatee B: 1) T_A or T_B $>$ 9 minutes; and 2) T_A or T_B $> 60\% (T_A + T_B)$. If these criteria were met in test 1, then we re-tested the flies in the afternoon with the position of the discriminatees reversed in the arena. Between tests, we soaked arenas in an unscented soapy solution for 30 minutes, rinsed them in water, and then dried them in a drying oven. Passing test 2 included passing the two above criteria plus the additional criterion of choosing the same fly chosen in test 1. We performed male and female preference tests daily, Monday thru Friday, from March 2004 to March 2005.

Unpaired discriminatees

From each preference tests we mated the chooser with either its preferred or non-preferred mate to analyze the fitness and longevity consequences of mate preference (see Lebow et al. MSa). From each test, we had either a P or NP discriminatee that was not mated with the chooser. From the 163 female choice tests that met all choice criteria we had 159 unmated male discriminatees, and from 163 male choice tests we had 158 unmated female discriminatees (four male and five female discriminatees were lost or injured following the second test and we did not include them in the analyses). We held flies in a 26° C unlit incubator, a mild heat stress condition that magnifies survival differences among experimental groups (Lebow et al. MSb). We transferred all flies daily for 25 days then bi-weekly after that, and recorded longevity daily. To control for environmental variance, we kept one P with one NP of the same sex and age in separate vials that were rubberbanded together so they experienced similar environmental conditions until death.

Paired discriminatees

In addition to keeping one unmated discriminatee from each preference test described above (either P or NP), we did additional preference tests but held both the P and NP discriminatees as virgins until death. This provided an additional experimental control because each set of paired P and NP discriminatees were discriminated by the same chooser. We did an additional 217 first tests with the female as the chooser, of which 48, or 22.1%, passed all choice criteria. We did 178 additional first tests with the male as the chooser, yielding 48, or 27%, that passed all choice criteria. When all criteria were met, we kept P and NP discriminatees from the same test in separate vials that were rubberbanded together, transferred them daily for 25 days, bi-weekly after that, and recorded their longevity. We again held all flies in a 26° C unlit incubator.

Sample sizes

Unpaired discriminatees

Of the 159 unpaired male discriminatees, we did not collect longevity data for eight of them due to loss or accidental injury or death during transfers, leaving 151 for analysis, 74 P and 77 NP. Of the 158 unpaired female discriminatees, we lost longevity data on four for the above reasons, leaving 154 for analysis, 76 P and 78 NP.

Paired discriminatees

Of the 48 pairs of unmated male discriminatees, we lost or injured 16 individuals from 14 pairs, leaving 39 P, 41 NP, and 34 pairs for analysis. Of the 48 pairs of unmated female discriminatees, we lost or injured 11 individuals from nine pairs, leaving 44 P, 43 NP, and 39 pairs for analysis.

Analyses

We compared survival means of paired and unpaired P and NP discriminatees of each sex using ANOVAs. We performed a power analysis to calculate the probability of correctly rejecting the null hypothesis, and also the minimum survival difference between P and NP discrminatees (in days) that would be needed to detect a significant difference with an ANOVA.

We also generated Kaplan-Meier survival curves of P and NP discriminatees by sex. We compared the survival curves using two tests: a Wilcoxon tests that puts greater weight on short survival times, and a Log-rank test that puts equal weight on all survival times. Additionally, we used paired t-tests to analyze the difference, P minus NP, in longevity of paired P and NP discriminatees.

Results

ANOVAs and power

There were no significant differences between P and NP discriminatees for either sex (males P: μ ± SE = 67.44 ± 1.59; males NP: μ ± SE = 68.76 ± 1.72; females P: μ ± SE = 79.07 ± 2.11; females NP: μ ± SE = 76.46 ± 2.22; Table 4.1). The probability of a type II error, or incorrectly accepting the null hypothesis, in the retrospective power analysis is 0.91 for males and 0.86 for females (see discussion, however) and there would have needed to be a minimum difference between P and NP of 4.63 days for males and 6.04 days for females to reject our null hypothesis of no differences between P and NP (Table 4.1). In addition, we would have needed a sample size of N = 1283 for female discriminatees, and N = 2811 for male discriminatees to detect a significant survival difference given our observed means and variances.

Survival curves

There were no significant differences in survival between P and NP discriminatees for either males or females (Fig. 4.2).

Paired t-tests

The distribution of survival differences in days (P – NP) for males fit a normal distribution ($W = 0.94$; $P = 0.06$), while the distribution for females did not ($W = 0.93$; $P = 0.02$). Therefore, we did a paired t-test for males and a Wilcoxon signed-rank test for females. The mean difference, P minus NP, was not significantly different from zero either for male and female unmated discriminatees in a one-tailed test testing P > NP (Fig. 4.3).

Discussion

There were no significant survival differences between unmated preferred and non-preferred discriminatees of either sex, suggesting that absolute quality differences between P and NP discriminatees do not exist. These results are not consistent with the prediction of traditional mate choice models that preference is for absolute quality differences between potential mates (Andersson 1994).

Since we did not find a significant survival differences between P and NP for either sex, we performed a retrospective power analysis to determine if our test was powerful enough to detect significant differences. While the power we report is low for both male and female tests (Table 4.1), this is inevitably the case since we used our observed data to calculate it, including our observed effect size (2.61 days, or the difference between the mean longevity of P and NP), which we already know did not produce a significant difference (Hayes and Steidl 1997; Rotenberry and Wiens 1985; Thomas 1997). The more meaningful number, then, is the minimum effect size at which we would have detected a significant difference given the high variability in our data (Table 4.1). Since for males the 4.63 days minimum effect size refers to when *non-preferred* males would have significantly greater longevity than *preferred* males (since NP longevity is slightly higher than P longevity), this number is meaningless in terms of our hypotheses that there would either be no survival difference between P and NP or that P would survive significantly longer than NP. For females then, the question is if a survival difference of less than 6.04 days would also have been biologically meaningful; if so, then our test was not powerful enough to detect a biologically meaningful difference, in this case a difference that reflects absolute quality differences between females. It is possible that a difference of six days or less would have biological significance. However, given the high variability in our data and the necessary sample size of $N = 1283$ to detect a significant difference with our observed effect size (2.61 days), empirically finding a significant difference is outside the realm of experimental possibility.

The second concern is if we are indeed measuring preference in our arenas. The arenas were designed to eliminate intrasexual competition and intersexual coercion, allowing for unconstrained mate preference. Previous studies using this exact arena in *D. pseudoobscura* found males and females mating with their preferred mates had offspring with higher egg-to-adult viability(Anderson et al. MS), and also net fitness, offspring viability, and survival benefits for males mating with preferred females (Lebow et al. MSa). Using similarly designed arenas, researchers have also found benefits to mating with preferred mates in cockroaches (Moore et al. 2003), mallards (Bluhm and Gowaty

2004), and mice (Drickamer et al. 2000; Drickamer et al. 2003). Based on these results, we feel confident that the arenas are capturing mate preference.

While not finding survival differences between preferred and non-preferred potential mates is not consistent with many mate choice models, there are alternative mate choice models based on complementarity, where offspring receive parental genes that complement each other (Trivers 1972), possibly by increasing heterozygosity (Brown 1997) or increasing immunocompetence via immune diversity (Wedekind 1999). In these models, the best mate for one individual is not necessarily the best mate for another because the benefits of mate choice depend on the interaction between the male and female, and thus these models do not predict survival differences between preferred and non-preferred individuals.

There is evidence in mammals (Egid and Brown 1989; Ober et al. 1997; Potts et al. 1991; Yamazaki et al. 1976) and fishes (Landry et al. 2001) that pre-copulatory mate choice for complementarity exists, mainly associated with olfactory detection of MHC dissimilarity. Insects also have diverse immune loci, and there is recent evidence that their immune system is much more complex than previously thought, capable of producing thousands of receptors (Watson et al. 2005). There could be selection for detecting immune complementarity in potential mates, possibly through olfaction, an important courtship component in *Drosophila* (Ehrman and Kim 1998). Subsequent genetic studies could address this.

Our data are consistent with a previous study in the lab that also found no survival differences between preferred and non-preferred unmated flies (unpublished data). In that study the flies were at room temperature, and perhaps in too ideal of conditions to detect subtly survival differences. Since we still did not find significant survival differences between preferred and non-preferred individuals under a mild heat stress condition in this study, we conclude that mate choice in this system is not for intrinsic quality differences that would be correlated with survival differences between preferred and non-preferred flies. We cannot conclude, however, that mate preference is for complementarity. While our data are consistent with complementarity hypotheses, future studies directly testing this using genetic markers would be necessary to make that conclusion.

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Table 4.1. Summary of mean longevity and ANOVA results comparing survival of P and NP unmated discriminatees by sex. The last column, minimum effect size, is the smallest difference between P and NP that would have given a $P \leq 0.05$, allowing us to reject the null hypothesis.

Sex	Preference status	N	Mean longevity (days)	± SE	F	*P*	1 – ß (power)	Minimun effect size (days)
M	P	113	67.44	1.59	0.32	0.58	0.09	4.63
	NP	118	68.76	1.72				
F	P	120	79.07	2.11	0.72	0.40	0.14	6.04
	NP	121	76.46	2.22				

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Figure 4.1. Photograph of the preference arena showing the long corridor (A) where the chooser walks, and the two cells holding the discriminatees (B). Also note the visible lines drawn on the tube dividing the arena into three equal areas.

Figure 4.2. Survival curves for A) male and B) female unmated discriminatees by their preference status- P (black line) or NP (gray line). There are no significant differences between P and NP in either graph (males: Log-rank $P = 0.33$, Wilcoxon $P = 0.26$; females: Log-rank $P = 0.32$, Wilcoxon $P = 0.56$).

Figure 4.3. Distribution of differences in longevity (P-NP) for A) male and B) female pairs. Neither mean difference is significantly different from zero using a one-tailed test (testing $P > NP$) in a paired t-test for males ($t = -0.62$; $N = 34$; $P = 0.73$), and a Wilcoxon signed-rank tests for females ($W = -91.00$; $N = 39$; $P = 0.90$).

Figure 4.1

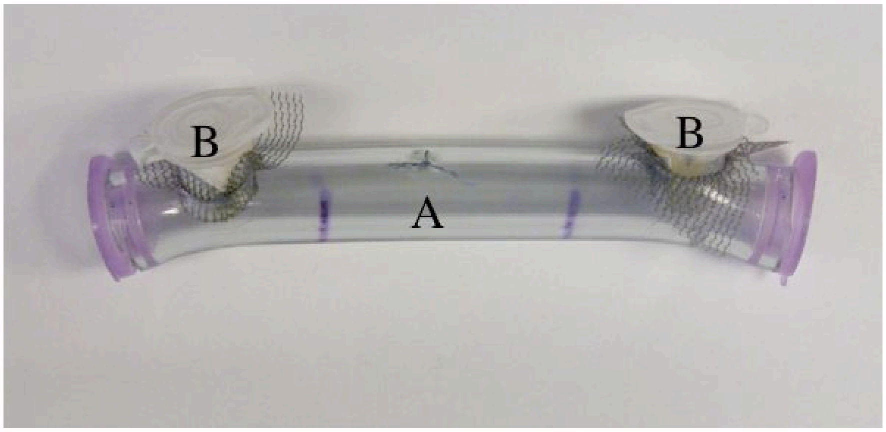


Figure 4.2

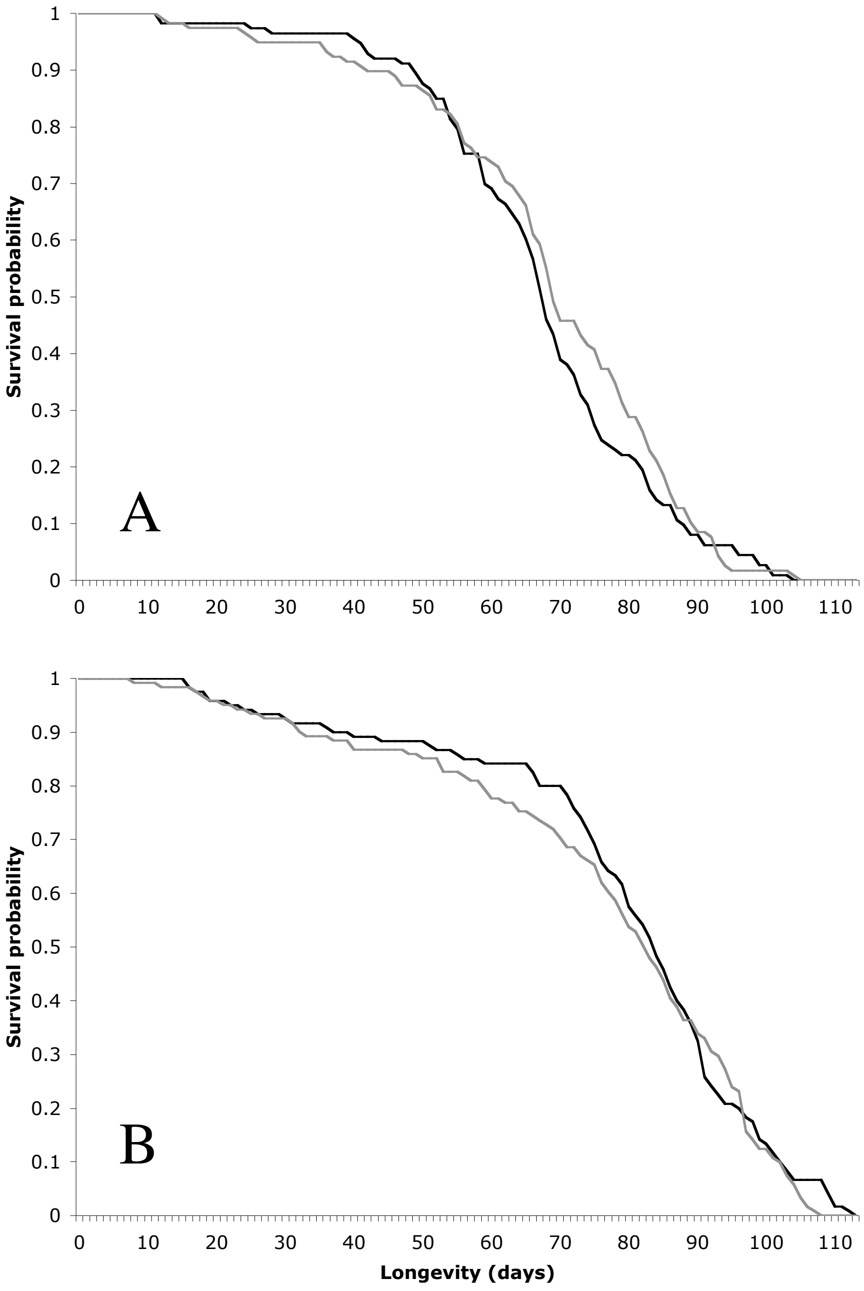
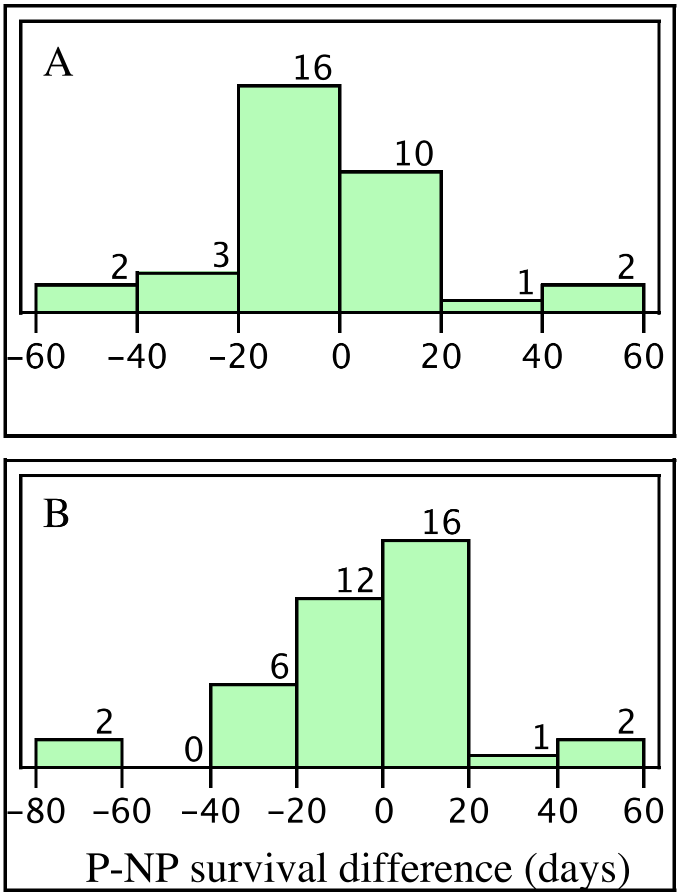

A
B
Survival probability
Survival probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
100
110
Longevity (days)

Figure 4.3


A
2
3
16
10
1
2
−60
−40
−20
0
20
40
60
B
2
0
6
12
16
1
2
−80
−60
−40
−20
0
20
40
60
P-NP survival difference (days)

CHAPTER 5

SURVIVAL COSTS OF REPRODUCING FOR FEMALES AND MALES IN *DROSOPHILA PSEUDOOBSCURA*[1]

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[1] Lebow, E. T. P. A. Gowaty, Y. K. Kim, and W. W. Anderson. To be submitted to *Evolution*.

Abstract

While cost of reproducing is well-studied in *Drosophila melanogaster*, little is known about it in *D. pseudoobscura*. We studied variation in survival as an estimate of the costs of reproduction for both females and males in *D. pseudoobscura*, comparing the survival of virgins, flies with mates for one day (single copulation), and flies with mates for 21 days (multiple copulations). We also examined fitness differences associated with costs of mating, such as egg-laying, as well as benefits of multiple copulations, like increased productivity and egg-to-adult offspring viability, between females with a single or multiple copulations. There was no difference in survival between virgins and females with a single copulation, but survival was significantly decreased for females with multiple copulations compared with both virgins and females with a single copulation. While females with multiple copulations did not have increased fecundity, they had higher productivity (total adult offspring) and their offspring had higher egg-to-adult viability. We did not find any significant survival costs of reproducing for males.

Key words*:* cost of reproduction, cost of mating, *Drosophila pseudoobscura*, survival

Introduction

The cost of reproduction represents an energetic trade-off between having offspring and surviving. This relationship is well documented in *Drosophila* (Chapman et al. 1998), with studies isolating different aspects of reproduction for females, such as costs of egg production (Maynard-Smith 1958, Lamb 1964, Partridge et al. 1987), receipt of sperm and accessory gland proteins (Chapman 1992, Chapman et al. 1993, Chapman et al. 1995, Lung et al. 2002), and energetic costs of copulation (Chapman et al. 1996, Fowler and Partridge 1989). Costs for males include energetic costs of courtship (Cordts and Partridge 1996, Partridge and Fowler 1990, Partridge et al. 1987) and copulation (Partridge and Andrew 1985, Partridge and Farquhar 1981, Prowse and Partridge 1997).

Few studies, however, have looked at costs of reproducing in *D. pseudoobscura*. Previous work in this species reported that females with multiple copulations have higher fecundity (Beckenbach 1978) and productivity (Pruzan-Hotchkiss et al. 1981; Turner and Anderson 1983) than singly mated females. Turner and Anderson (1981) also looked at survival, and reported that females with multiple copulations had decreased survival over singly mated ones. No studies in this species, however, have looked at survival differences between mated and virgin females, nor have any examined the cost of mating in males.

In this study we examine costs of reproducing in both male and female *D. pseudoobscura* by comparing the survival of three groups within each sex: virgins, with mate for one day, and with mate for 21 days. All flies were under a mild heat stress, a stress that magnifies differences among experimental groups and thus increases the likelihood of detecting survival costs to reproducing (Lebow et al. MSa). Because egg production is thought to contribute to costs of reproduction, and because potential fitness benefits to mating multiply are relevant to considering any survival costs, we also measured differences in fecundity, productivity, and egg-to-adult offspring viability between females kept with their mate for one day and 21 days.

Methods

Wyatt Anderson collected *Drosophila pseudoobscura* in Mesa Verde, CO in the summer of 1995 and established isofemale lines in Athens, GA. We used eight isofemale lines to establish a population cage in July 1997 with twenty half-pint glass bottles, each containing approximately 30 ml of food composed of yeast, cornmeal, agar, molasses, and a small amount of propionic acid to prevent the growth of mold. The oldest bottle was rotated out of the cage and replaced with a fresh one on Monday, Wednesday, and Friday of each week.

In February 2004, approximately 90 generations after starting the cage, we added four fresh bottles to the population cage. One week later, we removed these bottles, and divided each one into three new bottles by scooping off the top of food containing eggs and larvae. When adults began emerging, approximately 16 days later, we collected virgin flies every eight hours, twice a day, and sexed them under CO_2. We maintained up to 10 flies per vial and aged them for seven days.

We began the experiment at age seven days. We first determined mate preference for both males and females to examine the fitness and longevity consequences of mate preference in separate analyses. In these tests, one chooser fly was able to choose between two flies of the opposite sex in a preference arena (see Lebow et al., MSb for detailed methods). If the fly had a preference, we paired the chooser with either its preferred or non-preferred mate in a vial and observed copulation. After copulation, the pair was kept together for either one day or 21 days. In addition to mated flies, we also kept all unmated flies from the preference tests.

After pairing, all flies were maintained under a mild heat stress of 26° C in an unlit incubator except for approximately one hour a day when they were transferred to new food at room temperature. *Drosophila pseudoobscura* is considered a "light independent" species that does not require light for interspecific mate discrimination or mating (Mayr and Dobzhansky 1945; Wallace and Dobzhansky 1946), and thus the flies kept together for 21 days could continue to mate in the incubator.

All flies were transferred daily for 25 days, then bi-weekly after that until death. Vials past day 25 were checked daily for any deaths. For the first 25 days, we counted the number of eggs laid daily for mated females. We kept these vials of eggs at room temperature. We monitored all vials of eggs for emerging adult offspring. Once adults began emerging, we counted emerging adults for seven days. Therefore, we collected adult survival data and data on three fitness measures for mated flies: total number of eggs laid in 25 days of egg-laying, total number of adult offspring emerging from those eggs, and the egg-to-adult survival of offspring. The experiment ran from March to December 2004.

Analyses

Adult Survival

We compared survival curves of unmated flies, flies with mate for one day, and flies with mate for 21 days, for both males and females. We used the Kaplan-Meier method to generate survival curves, and then compared those curves using a Log-rank and Wilcoxon test. While Log-rank tests weigh all survival times equally, a Wilcoxon test puts heavier weight on early deaths. We did a Bonferroni correction on the 16 tests we ran, resulting in a significance level of $P \leq 0.003$.

Fitness

We analyzed fitness differences for flies with their mate for one day versus 21 days using one-way ANOVAs for three measures of fitness: eggs laid in 25 days, total adult offspring from those eggs, and egg-to-adult offspring survival.

Results

Female survival

There was a significant effect of virginity and the length of time with mate on female survival (Fig. 5.1a; Table 5.1). There was no significant decrease in survival between females with males for one day and virgin females, but survival significantly decreased for females with males for 21 days compared with virgin females. For mated females, females with males for 21 days had significantly lower survival than females with their mate for one day, but this was not significant early in life, as indicated by the non-significant Wilcoxon test.

Male survival

There was not a significant effect of virginity and length of time with mate on male survival (Fig. 5.1b; Table 5.1). Survival did not differ between males with females for one day and virgin males, nor did it differ between virgins and males with females for 21 days, or between males with females for one day and 21 days.

Fitness

There was no significant difference in the number of eggs laid between females with their mate for one day and 21 days (one day: $\mu \pm \mathrm{SE} = 475.35 \pm 14.17$ eggs, $N = 157$; 21 days: $\mu \pm \mathrm{SE} = 500.63 \pm 14.04$; $N = 160$; $\mathrm{F} = 1.61$; $P = 0.21$; Fig. 5.2a). However, productivity (one day: $\mu \pm \mathrm{SE} = 232.10 \pm 9.73$ adult offspring, $N = 157$; 21 days: $\mu \pm \mathrm{SE} = 296.55 \pm 9.64$; $N = 160$; $\mathrm{F} = 22.15$; $P < 0.0001$; Fig. 5.2b) and egg-to-adult offspring viability (one day: $\mu \pm \mathrm{SE} = 0.50 \pm 0.02$ egg-to-adult viability, $N = 155$; 21 days: $\mu \pm \mathrm{SE} = 0.58 \pm 0.02$; $N = 158$; $\mathrm{F} = 14.65$; $P < 0.0001$; Fig. 5.2c), were both significantly higher for females with their mate for 21 days.

Discussion

Virgin females did not have a significant survival advantage over females who were with a male for one day. Since *D. pseudoobscura* rarely remate in 24 hours

(Markow 1996), it is likely that this single copulation and short exposure to males is not taxing enough to females to decrease their survival. However, there was a survival cost to females with their mate for 21 days, with both virgins and females with males for one day living significantly longer than females with males for 21 days, consistent with a previous study in *D. pseudoobscura* that also found significantly decreased survival for females with lifetime access to males compared to singly mated females (Turner and Anderson 1983).

While the survival costs of mating for female fruit flies was initially attributed to the costs of egg production in *D. subobscura* (Lamb 1964; Maynard-Smith 1958), subsequent studies on the effects of reproduction in *D. melanogaster* found that variation in egg-laying did not fully explain survival differences (Fowler and Partridge 1989; Partridge et al. 1987). In *D. pseudoobscura*, unlike *D. melanogaster*, we did not find a significant difference in the number of eggs laid between females with males for one day or 21 days, unlike a previous study in this species (Beckenbach 1978), meaning that variation in egg production does not explain the survival differences we observed. Because females in this study were constantly with the male for 21 days, survival costs could have been associated more with exposure to males (e.g. Partridge and Fowler 1990) or receipt of accessory gland proteins (e.g. Chapman et al. 1995), as in *D. melanogaster*. While there was a survival cost to multiple copulations for females, they also experienced significant fitness benefits in productivity, consistent with previous work (Pruzan-Hotchkiss et al. 1981; Turner and Anderson 1981), and egg-to-adult offspring viability, possibly making the benefits to multiple copulations outweigh the survival cost.

There was no significant cost of mating for males. Previous studies have found a survival cost of mating for male *D. melanogaster* given regular access to virgins (Partridge and Farquhar 1981), mainly attributable to the costs of courtship (Cordts and Partridge 1996). However, survival costs were not observed when males were given access to females for seven days (Prowse and Partridge 1997). Three weeks of access to females, as in this study, could also not be long enough to detect a mating cost for males in this species. Additionally, a study in *D. melanogaster* found that once reproductive activity ceased, male longevity returned to that of virgin males (Partridge and Andrews 1985). Because mating access stopped after 21 days in our study, this could also explain the lack of a significant survival cost to mating for males.

Research in *D. melanogaster* has become sophisticated at isolating the survival costs of different aspects of reproduction, and future studies in *D. pseudoobscura* could also determine where the costs of reproducing for females lies. There is reason to expect that specific costs might be different for *D. pseudoobscura* than for *D. melanogaster* females. First, there are interspecific differences in the effects of multiple copulations on egg-laying, with many studies in *D. melanogaster* finding significantly increased fecundity with increased matings (e.g. Wolfner 1997), while this study found no such effect. There also appear to be interspecific differences in benefits to multiple copulations, with *D. melanogaster* not receiving productivity benefits from multiple copulations (Fowler 1973) like those found in *D. pseudoobscura* in this paper and the others referenced above. Both of these facts could decrease a female's costs of reproducing in *D. pseudoobscura* and possibly affect which aspects of reproduction are costliest to her. Future studies on males in this species could also determine at what point, if ever, males begin to experience a decrease in survival from reproducing.

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Table 5.1: Summary of adult male and female survival by sex, length of time with mate (virgin, with mate for one day, with mate for 21 days) and analyses of Kaplan-Meier generated survival curves for differences between and among groups.

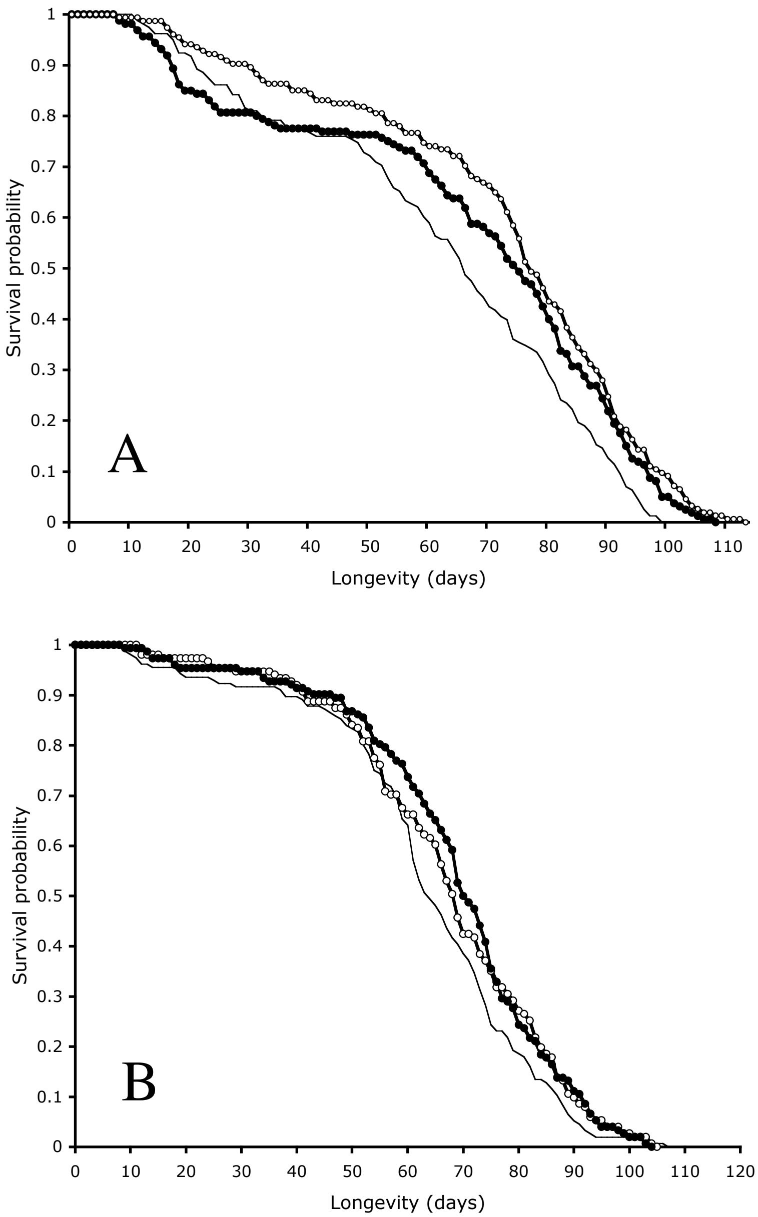
Sex	**Length of time w/ mate**	**N**	**Survival Mean ± SE**	**Groups Compared**	**Test**	**X^2**	**P value**
F	Virgin	154	72.05 ± 2.03	Virgin & one day	Log-Rank	2.30	0.13
					Wilcoxon	2.56	0.11
	24 hours	160	66.34 ± 2.27	One day & 21 days	Log-Rank	9.27	0.002
					Wilcoxon	4.21	0.04
	21 days	158	61.89 ± 2.00	21 days & virgin	Log-Rank	19.52	< 0.0001
					Wilcoxon	14.77	0.0001
	Combined	472	66.71 ± 1.23	All 3 female groups	Log-Rank	20.42	< 0.0001
					Wilcoxon	14.38	0.0008
M	Virgin	151	67.42 ± 1.57	Virgin & one day	Log-Rank	0.07	0.80
					Wilcoxon	0.73	0.39
	24 hours	152	68.69 ± 1.55	One day & 21 days	Log-Rank	4.96	0.03
					Wilcoxon	6.94	0.01
	21 days	156	63.65 ± 1.59	21 days & virgin	Log-Rank	3.04	0.08
					Wilcoxon	2.62	0.11
	Combined	459	66.56 ± 0.91	All 3 male groups	Log-Rank	5.39	0.07
					Wilcoxon	7.00	0.03

List of Figures

Figure 5.1. Survival curves of female (A) and male (B) longevity by virginity and length of time with mate. Open circle line is for virgins, closed circle is for one day with mate, and straight line is for 21 days with mate.

Figure 5.2. Total eggs (a), total adult offspring (b), and percentage of eggs becoming adults (c) by length of time with mate (one day or 21 days).

Figure 5.1


A
Survival probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
100
110
Longevity (days)
B
Survival probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
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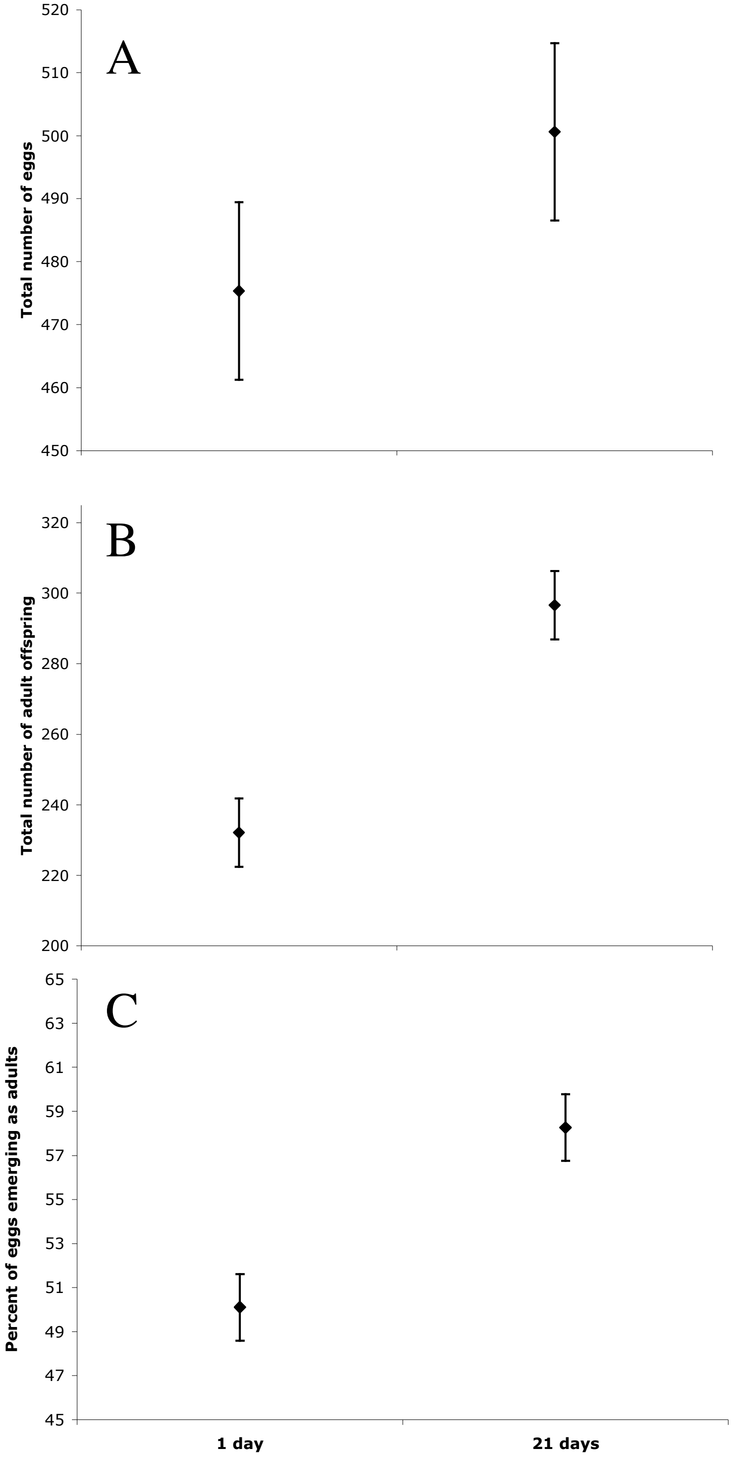
20
30
40
50
60
70
80
90
100
110
120
Longevity (days)

Figure 5.2


A
Total number of eggs
520
510
500
490
480
470
460
450
B
Total number of adult offspring
320
300
280
260
240
220
200
C
Percent of eggs emerging as adults
65
63
61
59
57
55
53
51
49
47
45
1 day
21 days

CHAPTER 6

CONCLUSIONS

These experiments explained variation in survival in *Drosophila pseudoobscura* associated with mild heat stress, social group composition, mating preferences, and mating status, as well as fitness variation in this species associated with mating preference and length of time with mate.

Chapter 2 demonstrated that a lifetime mild heat stress both decreases survival in *D. pseudoobscura* and increases differences among social group treatments compared to a one-week heat stress. Individuals alone handled the lifetime stress best, and females outlived males in both stress treatments and all social groups.

The experiments presented in chapters 3 and 4 tested mate choice hypotheses about fitness and survival benefits to mate preference and survival differences between discriminatees. Consistent with direct benefits hypotheses, females with their preferred male for 21 days had increased fecundity. However, there were no productivity, egg-to-adult offspring viability, or survival benefits to females with their preferred mate for either one day or 21 days. Male choosers, on the other hand, chose females who had higher net fitness and with whom they had offspring with higher egg-to-adult viability when paired for one day. Male choosers also received survival benefits when they were with their preferred female for one day. Individuals that were preferred or non-preferred by the opposite sex did not have significantly decreased survival, rejecting predictions of mate choice hypotheses based on absolute quality, but consistent with mate choice hypotheses based of complementarity.

Finally, the analyses presented in chapter 5 showed costs of reproducing for females and males. This was the first study to compare survival of virgins to mated females in *D. pseudoobscura*, as well as the first study in this species to look for a costs of reproducing to males. We found that females with multiple copulations had decreased survival over virgins and singly mated females, while there was no survival difference between virgin and singly mated females. Unlike females, however, males did not experience any survival costs to reproducing.