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Ejaculate-derived nutritional contribution and female reproductive success in *Drosophila mojavensis* (Patterson and Crow)

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Abstract. Paternal investment in offspring has been shown to occur in many insect species, but its impact on reproductive fitness is often difficult to demonstrate. Males of the cactophilic fruit fly species, *Drosophila mojavensis* (Patterson and Crow), provide an ejaculate donation to females which is incorporated into their developing oocytes. Male size is uncorrelated with donation size in this species. The amount of this donation is depleted with successive matings, although male fertility remains constant. Males are observed to court preferentially females whose ovaries contain mature oocytes, thus ensuring the rapid utilization of their own sperm and their paternity.

Females allowed unlimited access to dietary yeast exhibit high levels of fecundity, but nutritionally deprived females are not able to manufacture enough oocytes to utilize the sperm from a single mating. In the absence of dietary yeast, early female fecundity is enhanced by male ejaculate donations. However, the ejaculate donation does not appear to influence total lifetime fecundity in the absence of continued remating. Females of this species remate frequently. In natural populations, during periods of dietary stress, nutrients derived from repeated matings may constitute an important resource for total lifetime fecundity of females.

Key-words: Drosophilia, nutrition, oviposition, size

Introduction

The evolutionary significance of male investment in offspring has been the subject of many theoretical and empirical papers (Trivers, 1972; Borgia, 1979; Thornhill & Alcock, 1983). In several insect species, males have been shown to provide a nutrient benefit to females in the form of substances transferred in the ejaculate (Boggs & Gilbert, 1979; Boggs & Watt, 1981). It has been possible, in larger insects having a defined spermatophore, to demonstrate a relationship between the amount of male donation and subsequent egg development in recipient females (Gwynne, 1981, 1983; Butlin, Woodhatch & Hewitt, 1987).

A large number of *Drosophila* species also have been found to pass an ejaculate donation to females (Markow & Ankney, 1984, 1988). Although male-derived substances have been found in female somatic tissues and ovarian oocytes of these species, ejaculate donations have not yet been demonstrated to increase female fitness in any *Drosophila* species. A nutritional explanation for the observation by Turner & Anderson (1983) that starved but frequently mated *D. pseudoobscura* females show greater productivity than starved females which mated only occasionally, was suggested when it was discovered that *D. pseudoobscura* seminal substances are detectable in ovarian oocytes (Markow & Ankney, 1988). Ejaculate donation in *Drosophila* species is firmly associated with the appearance of a copulatory plug (Markow & Ankney, 1988), suggesting a relationship between the donation, parental investment and reproductive fitness in this genus.

The relatively large quantity of male donation in *D. mojavensis* (Patterson and Crow) makes this species an attractive candidate for efforts to understand both its genetic basis and its influence on fitness. The ecology of this cactophilic, Sonoran Desert endemic species has been under intensive study (Barker & Starmer, 1982). *Drosophila mojavensis* females remate daily, creating the opportunity for cuckoldry. Markow (1988) demonstrated that despite strong sperm precedence favouring the last male to mate, those progeny sired by the first male after subsequent matings actually receive ejaculate substances from their later-mating 'stepfathers'. This curious situ-

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ation provides a new twist to predictions about parental investment adaptations which demand that the contribution of these ejaculate donations to *D. mojavensis* reproductive fitness be defined.

The present study was undertaken to assess the relative importance of ejaculate-derived nutrients and dietary resources to female reproductive success in female *D. mojavensis*. The influence of the major adult dietary resource, live yeast, on adult size, egg production and male mating behaviour was first determined. We also examined the relationship between male fertility and male donation over repeated matings in order to design appropriate experiments on the role of the donation for female productivity.

Materials and methods

Flies

Adult male and female *Drosophila mojavensis*, strain A900 (Santa Rosa mountains, Arizona), were separated under light ether anaesthesia 6 h after eclosing and placed in shell vials with culture medium, either with or without added live yeast (*Saccaromyces cereviciae* (Meyen ex Hansen)). Flies were transferred to fresh vials (yeasted or unyeasted) every 3 days. Females were held for 6 days and males for 10 prior to testing.

Dietary yeast and adult size

Adult size was analysed by recording both wet and dry weights and comparing these to thorax length, a measure of pre-imaginal growth that is correlated with other size characters such as wing length (Robertson & Reeve, 1952). Wet weights were measured on a Cahn 25 electrobalance to 0·001 mg immediately after anaesthetizing the flies. Each fly was subsequently measured for thorax length, dried at 60–65 °C for 72 h in a desiccator, and weighed again.

Dietary yeast and female reproduction

The effect of live yeast on oviposition was determined by transferring once-mated females daily to fresh vials and counting eggs laid. Three groups of females were used. Group 1 females were held with live yeast until mated on day six and then placed in vials with live yeast for oviposition. Group 2 females were also held with live yeast but were placed in vials without added yeast after mating. Group 3 females were held in vials without added yeast before and after mating.

Dietary yeast and female attractiveness

The influence of female feeding on male courtship behaviour was determined by placing a single virgin male in an observation vial containing two females, one that had access to yeast, the other having not had access to yeast. The first female to be courted by the male was recorded. Females were subsequently removed and their ovaries were dissected in insect Ringer solution. The number of mature oocytes (King, 1970) per ovariole was determined.

Male size, donation and fertility over sequential mating

Radiolabelled males were obtained by placing 50 eggs in a shell vial containing 1·5 g of standard cornmeal molasses medium to which 70 μCi of a mixture of 14C amino acids (ICN10147) had been added. Male flies were separated upon eclosing and stored five flies per culture vials when they were mated to 6-day-old virgin females. The culture medium in the storage vials was prepared without radioactive label, but a drop of live yeast suspension, prepared with 14C amino acids had been added to the surface of the medium to allow feeding by the maturing adult males.

The amount of radioactivity passed by a male was determined by scintillation counting (Markow & Ankney, 1988). A single reproductive tract of a mated female was placed in a scintillation vial containing 100 μl of Scintigest tissue solubilizer and crushed with a glass rod. Tissues were allowed to digest for 24 h at 50 °C at which time 2·5 μl of glacial acetic acid was added to neutralize the solution. Five millilitres of Scinti Verse I scintillation fluid was added and each vial was allowed to sit for an additional 24 h at 24 °C before counting. Counts per minute were converted to decompositions per minute (DPM) following a standard quench curve. Following their first mating, the thorax lengths of 10 males were measured and their bodies prepared for counting as described above. The proportion of total available male counts transferred, as well as the relationship between donation size and male thorax length were then calculated.

At 9 days of age, males were sequentially mated to six different virgin females. Females had been maintained in vials seeded with fresh live yeast prior to mating. After each copulation was completed, females were separated into two groups: in one group females were dissected immediately and their reproductive tracts prepared for scintill-

Table 1. Average radiolabel passed and progeny produced at each of six successive matings by *D. mojavensis* males (DPM) (n = 10 males mating^{-1}).

Mating number	Mean ± SE DPM	Mean ± SE progeny
1	202·76 ± 18·87	68·9 ± 8·1
2	261·29 ± 19·55	84·4 ± 7·6
3	170·86 ± 17·31	62·7 ± 8·4
4	89·20 ± 15·63	59·7 ± 6·8
5	99·19 ± 16·66	63·2 ± 7·3
6	81·69 ± 15·86	58·9 ± 9·9

ation counting. In the other group, females were placed in individual food vials and transferred daily to allow counting of progeny resulting from successive male matings.

Male donation and female reproduction

Adult females were stored without dietary live yeast for 6 days prior to being mated for 3 successive mornings to males from one of two categories. All males were 9 days old. Category A males were virgins. Category B males had been mated three times in rapid succession immediately before being placed with the virgin females. Therefore, at the time of mating to the female in question, each male had a normal amount of sperm, but a donation potential equivalent to males on their fourth mating (see Table 1). Because adult males had been provided with additional dietary label during maturation, this decrease in ejaculate label was attributed to a depletion of accessory gland material and not to a depletion of label accumulated during the larval period. Females from the two groups (mated to category A or B males) were remated to the same type of male for 3 successive mornings, after which daily egg counts were recorded until females died.

Results

Dietary yeast and adult size

Access to yeast resulted in significantly greater female weight. Regression of both wet weight and dry weight on thorax lengths of males and females respectively are shown (Fig. 1a and b). Thorax lengths were identical (1·02 mm) between yeast-fed and yeast-starved flies, yet there was no overlap for the groups when body size was determined by weight. The effect of adult dietary live yeast on male size was not significant. The correlations among thorax length, wet and dry weights (Table 2) indicate that all are good estimators of the others both with and without yeast.

Dietary yeast and female reproduction

Egg production over 10 days after copulation was highly dependent on the availability of live yeast (Fig. 2). Females mating once, but never provided with live yeast, averaged only 16·2 eggs per female over 10 days while those continuously supplied with yeast laid an average 130 eggs. Standard errors were high because some females never laid

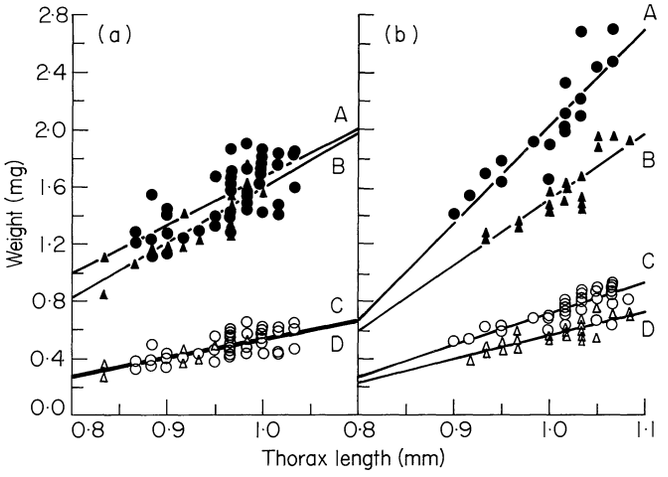

Fig. 1. (a) Regression of wet weight (A and B, closed symbols) and dry weight (C and D, open symbols) on thorax length for males held in vials with live yeast (A and C, circles) and without (B and D, triangles). (b) Regression of wet weight (A and B, closed symbols) and dry weight (C and D, open symbols) on thorax length for females held in vials with live yeast (A and C, circles) and without (B and D, triangles).

Table 2. Correlations of three measures of size, thorax length (THL), wet weight (WWT), and dry weight (DWT) for males and females held with (a) and without (b) live yeast.

(a) With yeast.

	Males		
Females	THL	WWT	DWT
THL		0·714 (n = 51)	0·678 (n = 51)
WWT	0·862 (n = 20)		0·96 (n = 51)
DWT	0·875 (n = 38)	0·965 (n = 40)	

(b) Without yeast.

	Males		
Females	THL	WWT	DWT
THL		0·889 (n = 19)	0·854 (n = 19)
WWT	0·889 (n = 20)		0·990 (n = 19)
DWT	0·861 (n = 40)	0·971 (n = 20)	

any eggs at all, a result consistently observed in our work with several strains of *D. mojavensis*. Yeast-fed females were identical in the proportion (65%) of females ovipositing on media with or without yeast, but only 45% of females never receiving yeast oviposited within the 10 days the experiment was conducted. The daily pattern of oviposition also differed among treatments. Group 2 females, held on yeast only until mating, continued to oviposit at a high rate until day four while in group 1, high oviposition levels continued until day nine. Those females never given live yeast laid 60% of their eggs after day five. Clearly adult nutrition dictates the reproductive potential of mated females.

Dietary yeast and female attractiveness

When 77 different males were presented with a choice of females, the yeast-fed females were courted first in 56 cases ($P < 0·01$). Dissections of females held with and without live yeast revealed that 6-day-old yeast-fed females averaged 3·1 ± 0·65 mature oocytes per ovariole while those without live yeast had only 0·89 ± 0·40 ($P <$ 0·0001).

Male size, ejaculate donation, and fertility over sequential matings

Because *Drosophila* individuals do not pass a spermatophore that can be weighed, the amount of ejaculate relative to male size must be determined by some other means. The total amount of label in a male was highly correlated with his thorax length (r = 0·88, P = 0·0008), suggesting that total radioactivity is a good indicator of male size. No correlation existed between the absolute amount of ejaculate transferred and male size, either as total male label (r = 0·32, P = 0·36) or male thorax length (r = 0·31, P = 0·38). The average percentage of a male's total size passed as ejaculate was 2·86 ± 0·002. Larger, more radioactive males did not pass greater amounts to females. Therefore, we found a negative correlation between the proportion of a male's counts transferred and male size either by thorax length (r = −0·61, P = 0·006) or by total radioactivity (r = −0·78, P = 0·007).

Male fertility does not decrease over six successive matings to yeast-fed females (Table 1). This is true despite the corresponding decrease in labelled ejaculate provided to the female. A curious feature of these data is the increase in both progeny and donation on the second mating. By the fourth mating, the amount of label transferred to females is about one-third that of the average of the first and second matings.

Male donation and female reproduction

The discovery that the number of sperm passed

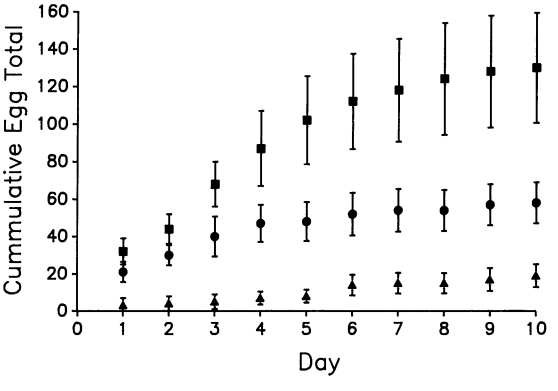

Fig. 2. Cumulative egg totals for females, once mated at 6 days of age. Group 1 females (squares) were held on yeast and eggs were collected from vials with added live yeast. Group 2 females (circles) were held on yeast and eggs were collected from vials without added yeast. Group 3 females (triangles) were held without live yeast and eggs were collected from vials without added yeast.

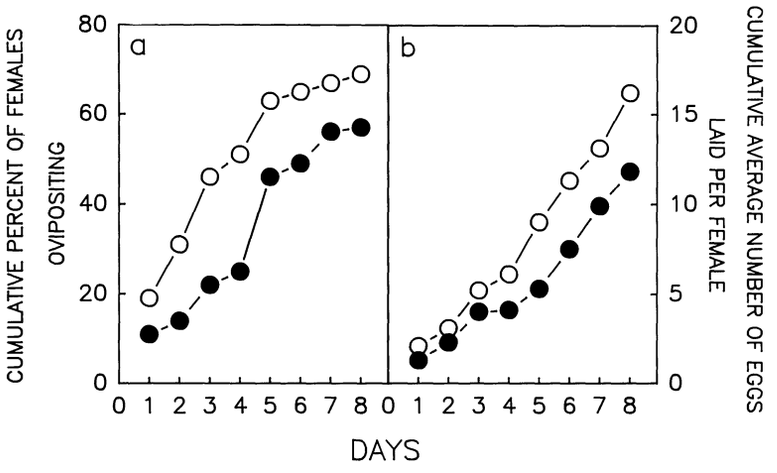

Fig. 3. (a) Cumulative per cent of females ovipositing over 8 days. Open circles represent females mated to virgin males; closed circles represent females mated to depleted males. (b) Cumulative average number of eggs laid per female over 8 days. Open circles represent females mated to virgin males; closed circles represent females mated to depleted males.

remains constant over successive matings while the ejaculate components are diminished provides a means of experimentally testing the importance of the donation for female reproduction. Females displayed a reproductive advantage when they received larger ejaculates than females receiving equal amounts of sperm but a comparatively reduced ejaculate. Oviposition for 8 days after mating by females mated to category A and category B males is presented in Fig. 3. The cumulative proportion of females ovipositing is shown in Fig. 3a and the cumulative average number of eggs laid by females is shown in Fig. 3b. The data from the three replications were pooled and oviposition by females mated to two categories of males were compared by the Komolgorov–Smirnov two-sample test (Siegel, 1956). For cumulative proportion of females ovipositing over 8 days, $\chi^2 = 47{\cdot}096$, at d.f. 2, $P < 0{\cdot}0001$. For cumulative average number of eggs laid, $\chi^2 = 149{\cdot}677$, $P = 0{\cdot}0001$. Females mating to males providing a greater donation show an early reproductive advantage by both measures. However, when lifetime oviposition rates are compared, it is clear that females mated to category B males eventually manufacture enough eggs ($\bar{x} = 30{\cdot}8 \pm 4{\cdot}9$) to catch up to category A mated females ($\bar{x} = 31{\cdot}5 \pm 6{\cdot}4$) before death. The average ages at which females in the two groups died (Category A $\bar{x} = 100{\cdot}1 \pm 20{\cdot}4$ and Category B $\bar{x} = 98{\cdot}7 \pm 24{\cdot}3$) were not significantly different.

Discussion

Availability of dietary yeasts for adult flies makes a large difference in the weight attained by females. The restriction of the yeast effect to females is obviously related to the production and accumulation of eggs. Sang & King (1961) reported that in *D. melanogaster*, protein supply was the most critical factor for oogenesis, but was unnecessary for continued adult survival. The ready supply of mature oocytes in well-fed females suggests that male *D. mojavensis*, if provided with a choice, should preferentially court and mate with these females. Our results support this prediction, indicating that male choice operates in this species and operates in a way that maximizes male fitness.

Dietary yeast enables females to utilize their entire sperm supply for fertilization. Following a single insemination, females with an unrestricted source of dietary yeast laid approximately eight times as many eggs over 10 days as did yeast-deprived females. In the present study, females receiving yeast both before and after mating deposited about 130 eggs female^{-1} in 10 days. This number of eggs is larger than the total lifetime progeny number reported previously (Markow, 1982). We attribute this difference to an artefact produced by the abundance of yeast in this treatment. Females with continuous access to yeast often deposited eggs right in the yeast itself. We have noticed in this experiment and others that when yeast-dependent stimulation of oviposition occurs, many of the eggs are not fertile. Egg counts, hatch rates and adult progeny counts are more equally matched (mean eggs or progeny = 65) when *D. mojavensis* oviposits on media without excessive amounts of live yeast on the surface (Markow, 1982). When females have mated once

and have oviposited about 65 fertile eggs, their receptacles are empty, indicating that all sperm have been used.

In the absence of dietary yeast, females receiving a larger ejaculate have an early reproductive advantage, even though they can deposit the same number of fertile eggs as females mated to ejaculate-depleted males. If females are receiving more nutrients with which to make eggs from category A males, why is this not reflected in a greater total fecundity? In *Drosophila*, the ejaculate contains a protein that increases oviposition (Chen, 1984) and it is possible that more of this protein is transferred by virgin males. However, the boost in fecundity is not detected unless females have experienced dietary stress, making it unlikely that the difference between males is due to differences in their ability to stimulate egg laying. It is also possible that while total fecundity was similar, egg composition may have differed between the two groups as a function of differential donation levels. Dietary nutrients may be of a different composition and take longer to metabolize than ejaculate nutrients before they can be utilized in oogenesis. Such differences might be reflected in delayed hatching or other developmental parameters. Sang & King (1961) have previously shown that protein deprivation of females results in a delayed development time of offspring from their eggs.

In nature, *D. mojavensis* feeds and breeds on necrotic cactus tissue (Heed, 1978, 1982) decayed by various species of bacteria and yeasts (Starmer & Fogleman, 1986). The concentrations of natural yeasts in these tissues have been found to vary over two orders of magnitude (Fogleman, Starmer & Heed, 1981), suggesting that at certain times they may be a limiting resource for females. With respect to larval feeding, a significant effect on development time and thorax length exists when yeast bicultures are contrasted with monocultures (Starmer & Fogleman, 1986).

While adult *Drosophila* will feed on any yeast provided, offspring development time may vary slightly with the species of yeast on which parental flies had fed as adults (Sang, 1956). This subtle difference in adult nutrition influences progeny development as well as the onset of oviposition. Starmer & Fogleman (1986) have identified 19 different yeast species present in the organ pipe cactus, *Stenocereus thurberi*. Only six, however, are present in more than 20% of the necrotic arms tested and the concentration of these yeast species varies. This variability may be compensated for by nutritional contributions from other sources such as the male ejaculate.

Several observations suggest that flies in natural populations suffer some degree of nutritional stress. First of all, field-caught flies tend to be smaller than those reared in the laboratory (Krebs, 1989). Second, the necrotic tissue of cacti utilized by *D. mojavensis* does not in itself provide an adequate carbohydrate source to support adult flies (Brazner, Aberdeen & Starmer, 1984). In addition to diet, females appear to have evolved an alternative means of meeting the great nutritional requirements of oogensis. The importance of ejaculate-derived substances for female fecundity is illustrated by the greater earlier productivity of females mated to males with undepleted ejaculates. Both the percentage of females ovipositing and the cumulative number of eggs produced over a 10 day period indicated a marked early advantage for females mating to males passing relatively more protein. Since *D. mojavensis* females remate frequently (Markow, 1982), this advantage may assume considerable significance under conditions of meagre dietary resources.

Acknowledgments

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