Larval rearing environment affects several post-copulatory traits in Drosophila melanogaster

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In Drosophila melanogaster, accessory gland proteins (Acps) that a male transfers during mating affect his reproductive success by altering the female's behaviour and physiology. To test the role of male condition in the expression of Acps, we manipulated the pre-adult environment and examined adult males for relative transcript abundance of nine Acps, and for post-copulatory traits that Acps influence. Larval culture density had no effect on any measured trait. Larval nutrient availability impacted the number of sperm transferred and stored, the male's ability to induce refractoriness in his mate, but relative transcript abundance of only a single Acp (Acp36DE). Reduced male body size due to low yeast levels affected sperm competition. Our data indicate that some female-mediated post-copulatory traits (induced refractoriness and sperm transfer and storage) might be influenced by the male's developmental environment, but relative expression of most Acps and some traits they influence (P1) are not.

Keywords: accessory gland proteins; sperm competition; sperm storage; transcript levels; body size

1. INTRODUCTION

In Drosophila melanogaster, accessory gland proteins (Acps) that males transfer to females during mating impact traits associated with post-copulatory sexual selection by modulating the behaviour and physiology of mated females. Acps regulate egg laying and oviposition, affect sperm storage, reduce a female's receptivity to remating and associate with variation in sperm competition phenotypes (reviewed in Wolfner 2002; Kubi 2003; Wolfner et al. 2005; Wong & Wolfner 2006).

If Acps are more costly to produce than other non-sexually selected traits, we predicted that they may exhibit a relative reduction in expression under stress.
(d) Sperm transfer and storage
Three-day-old virgin marker females (on bw) were mated to three- or four-day-old males reared on 100, 50 or 10% yeast and frozen in liquid nitrogen immediately after copulation (to estimate sperm transferred) or after 8 hours (to estimate sperm stored) and stored at −80°C. Uteri (for sperm transfer) and seminal receptacles (for stored sperm) were dissected in 50% acetic acid and incubated in orcein stain (2% orcein and 0.25% carmine dissolved in 60% acetic acid) for 1 hour and then mounted on a slide containing a drop of acetic acid, covered with a cover slip and sealed with nail polish. Sperm were counted under 100× magnification light microscopy. Data were analysed using ANOVA.

(e) Body size
Four-day-old male flies were dried at 60°C for 24 hours in an oven and weighed on a Sartorius microbalance. We used linear regression to examine effects of body size on treatments. For PI′, refractoriness, sperm transfer and storage, we compared treatment means of body weight versus treatment means of each trait and used the residuals in ANOVA if r² was significant (p≤0.05).

3. RESULTS

(a) Relative Acp transcript abundance
RPL32 transcript abundance did not differ between treatments, but was positively correlated with transcript abundance of all nine Acp genes (r²=0.54–0.97). Larval culture density had no effect on the relative transcript abundance of any of the tested Acps, while larval nutrient availability affected the relative transcript abundance of Acp36DE (F₄,₉₄ = 4.658, p = 0.006). Separate one-way ANOVAs revealed that larval dextrose availability had no effect on the relative transcript abundance of Acp36DE, but males reared on 50% yeast had reduced relative levels of Acp36DE transcript when compared with controls (F₂,₁₅ = 8.0645, p = 0.004; figure 1a).

(b) Sperm competition phenotypes
Larval culture density did not affect any of the measured sperm competition phenotypes (N = 56 females; p>0.30 for all phenotypes). In contrast, nutrient availability during larval life affected PI′ (F₄,₉₄ = 3.19; p = 0.017; marginally significant after sequential Bonferroni correction) and female refractoriness (N = 119 females in five treatments; p = 0.015 from permutation test). Larval rearing did not affect female fecundity (F₄,₉₀ = 1.00; p = 0.41) nor did larval dextrose availability affect female productivity (F₂,₇₅ = 1.64; p = 0.20). Separate one-way ANOVAs revealed that dextrose levels in larval medium had no effect on PI′ (F₂,₄₆ = 1.30; p = 0.282), but yeast levels did (F₂,₆₂ = 3.89; p = 0.026; significant after Bonferroni correction). Males reared on 10% yeast sired a lower proportion of offspring when compared with controls (Tukey test, p = 0.023; figure 1b). Pairwise comparisons among all treatments revealed that female refractoriness was marginally elevated when mated to males reared on 10% dextrose (0.46 ± 0.10 s.e. versus 0.17 ± 0.08 s.e.; p = 0.03; figure 1c) and was marginally reduced when mated to males reared on 10% yeast (0.04 ± 0.04 s.e. versus 0.17 ± 0.08 s.e.; p = 0.05; figure 1c) when compared with controls. In addition, significant differences in refractoriness were detected between females mated to males reared on 10% dextrose versus males reared on either 10% yeast (0.46 ± 0.10 s.e. versus 0.04 ± 0.04 s.e.;...
We find little evidence that relative Acp transcript abundance depends on the male’s larval rearing environment. Larval yeast availability affected the relative transcript levels of only one of the nine tested Acps (Acp36DE). The correlation between the effects of larval environment and relative Acp36DE transcript levels was not linear as expected, but only reduced when males were reared on 50% yeast. Because females require Acp36DE for normal sperm storage (reviewed in Bloch Qazi & Wolfner 2003), we explored whether sperm storage was decreased when females mated to these males. We found that females mated to 50% yeast males store significantly more sperm than 10% yeast males and about the same numbers as control males. Thus, the decrease that we observed in relative Acp36DE transcript abundance when males were reared on 50% yeast does not suggest that these males transfer less Acp36DE to the female. We can currently offer no explanation as to why relative Acp36DE transcript levels were lower when yeast levels were only moderately reduced.

We note that while relative Acp transcript levels were unchanged in most treatments, protein levels may be affected. Thus, it will be of future interest to determine if the larval rearing environment affects the amount of Acp protein transferred to the female.

In summary, our experiments suggest that males’ abilities to sequester and allocate resources in response to the larval environment may ultimately affect variation in several traits involved in post-copulatory sexual selection.

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