Supplemental Data

Seminal Fluid Protein Allocation and Male Reproductive Success

Stuart Wigby, Laura K. Sirot, Jon R. Linklater, Norene Buehner, Federico C. F. Calboli, Amanda Bretman, Mariana F. Wolfner, and Tracey Chapman

Supplemental Results

Effects of Accessory Gland, Testis, and Body Size on Sperm Displacement Ability

An analysis including the selection regime and the size of accessory glands, testis and body (wing area) of individual males showed that accessory gland size and testis size both explained significant proportions of the variance in sperm displacement ability (accessory gland, $F_{1,56} = 8.55$, $p = 0.005$; testis, $F_{1,56} = 5.04$, $p = 0.029$). Both accessory gland and testis size were positively associated with sperm displacement ability. However, there was a significant 3-way interaction between testis, body size and selection regime ($F_{3,56} = 6.34$, $p = 0.0009$) suggesting that the effect of testis size was dependent upon body size and selection regime. Hence whilst there was no significant effect of accessory gland size selection on sperm displacement ability, male reproductive morphology may be important in sperm competition in general.

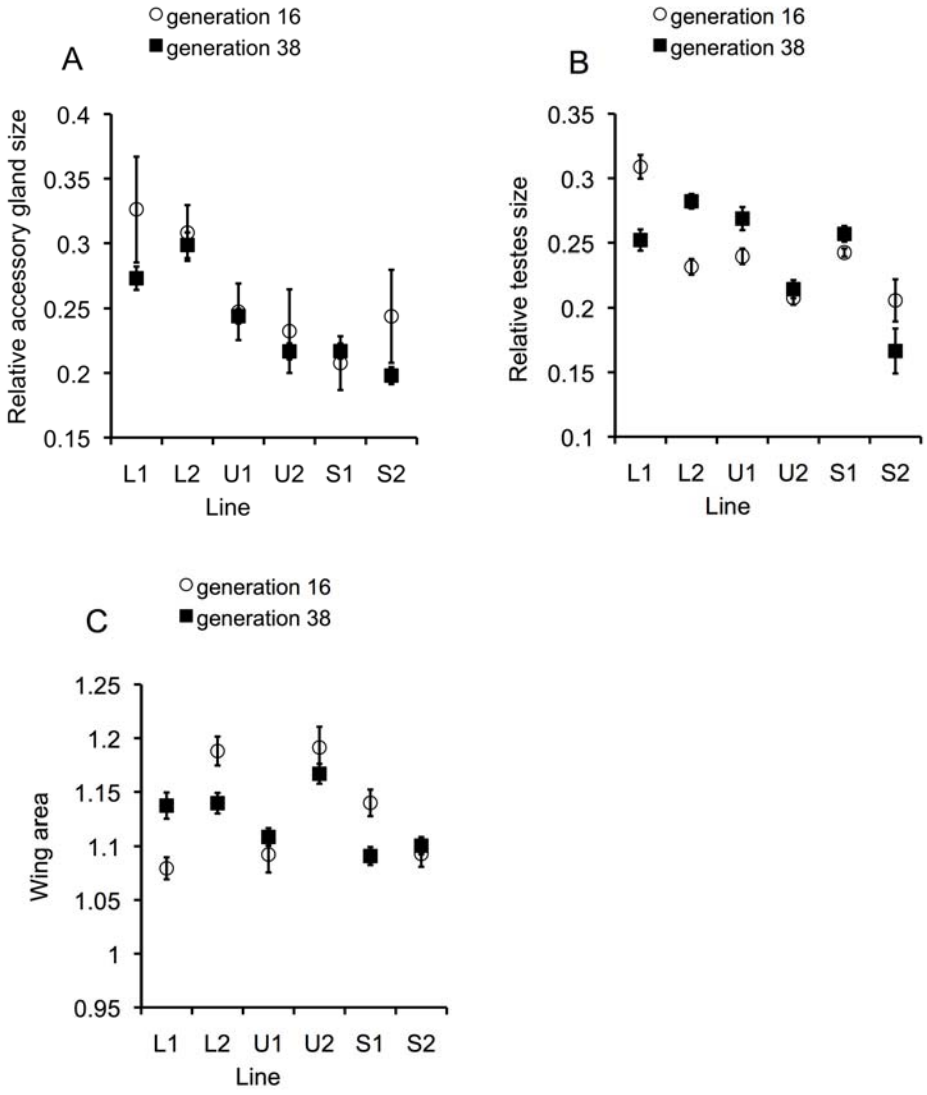
Supplemental Experimental Procedures

ELISAs for Sex Peptide and Ovulin

Frozen flies were placed on ice and dissected in 10% Dulbecco's Phosphate Buffering Solution (DPBS; 14mM NaCl; 0.2mM KCl; 0.1 mM KH_2PO_4; 0.7mM Na_2HPO_4) with protease inhibitors (Roche Complete protease inhibitor cocktail tablets; 1 tablet/50ml). The female lower reproductive tract or male accessory gland was placed in a microcentrifuge tube and ground for 30 sec in 20µl 10% DPBS with protease inhibitors. The pestle was rinsed into the extract with 200µl of 10% DPBS with protease inhibitors (bringing the final sample volume to 220µl) and the samples were stored on ice. Samples were mixed and 50µl of each sample was loaded onto each of four ELISA plates (BD Biosciences). Two replicate wells for each sample were tested (on separate plates) for each of ovulin and sex peptide. Plates were incubated overnight at 4ºC with shaking. The following day, the solution containing the samples was discarded and each well received 100µl of blocking solution (DPBS containing 0.05% Tween 20 and 5% non-fat dry milk). Plates were incubated for 1 hr with shaking at room temperature. Blocking solution was then replaced with 50µl of primary antibody diluted in blocking solution at 1:350 and 1:1000 for ovulin and sex peptide respectively (anti-ovulin antibody: [1]; sex peptide antiserum was generously provided

by E. Kubli, [2]), and wells were incubated for 1hr with shaking. Wells were washed with 0.05% Tween 20-DPBS (150µl/wash) and incubated for 1 hr with 50µl of horseradish peroxidase-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories, Inc.) diluted 1:2000 in blocking solution. The level of ovulin or sex peptide was detected through a reaction of the horseradish peroxidase with 100µl 3,3',5,5'-tetramethylbenzidine substrate (KPL, Gaithersburg, Md.) and the reaction was stopped by the addition of 100µl 1 M H_3PO_4 after the wells developed a deep blue colour or after 30 min. Optical density at 450nm (OD_{450}) was determined using a Molecular Devices kinetic microplate reader. Each plate also included a blank well initially loaded with 50µL of 10% DPBS. The OD_{450} value of the blank well was subtracted from the OD_{450} values of all of the other samples on the plate. OD_{450} values for the samples on one plate were then regressed against OD_{450} values of the same samples from the replicate plate. Points with residuals greater than three standard deviations were considered to have low repeatability and were removed. The OD_{450} values of the two replicate plates were averaged and converted to male accessory gland equivalents using a standard curve of male accessory glands.

Figure S1. Size of Reproductive Organs and Wings of Selection Line Males



(A) There was a significant divergence in relative accessory gland size (mean ± SE accessory gland size (mm^2)/ wing area (mm^2)) as a result of selection (selection effect, generation 16, $F_{2,3} = 71.4$, $p = 0.003$; generation 38 $F_{2,3} = 11.3$, $p = 0.04$). The relative accessory gland size of L males was significantly higher than that of both S and U males, which did not differ from one another (generation 16: L vs U, $z = 4.38$, $p < 0.0001$; L vs S, $z = 5.15$, $p < 0.0001$; U vs S, $z = 0.79$, $p = 0.71$; generation 38: L vs U, $z = 3.28$ $p < 0.003$; L vs S, $z = 4.62$, $p < 0.001$; U vs S, $z = 1.34$, $p = 0.37$).

(B) Relative testis size (mean ± SE testis size (mm^2)/ wing area (mm^2)) and (C) body size (mean ± SE wing area (mm^2)) did not differ between L, U and S males (all comparisons, $F_{2,3} < 2.06$, $p > 0.27$).

Supplemental References

1. Monsma, S.A., and Wolfner, M.F. (1988). Structure and expression of a Drosophila male accessory gland gene whose product resembles a peptide pheromone precursor. Genes Dev. *2*, 1063-1073.
2. Peng, J., Chen, S., Busser, S., Liu, H., Honegger, T., and Kubli, E. (2005). Gradual release of sperm bound sex-peptide controls female postmating behavior in Drosophila. Curr. Biol. *15*, 207-213.