Feeding-related traits are affected by dosage of the *foraging* gene in *Drosophila melanogaster*

Supplemental figure legends

Figure S1: *for^{HR}* alleles, and generation of the *for^{0}* and *for^{dup}* alleles.

A) Schematic of the episomal targeting construct, that likely failed to linearize, aligned to regions of homology at the *foraging* locus. **B)** Schematic of *for^{HR4}* and *for^{HR1}* alleles and the recombination event that generated the *for^{0}* and *for^{dup}* alleles. The episomal element integrated into the regions of homology at either end of the locus. This generated a duplication of the homology regions. The *loxP* sites were used to delete the *foraging* locus. The resulting *for^{0}* allele was a clean deletion with only *loxP* and *attP* sites remaining. The resulting *for^{dup}* allele contained two wild type copies of the *foraging* gene, as well as a 20kb segment between the *foraging* genes containing the duplicated homology regions in the *forHR1* and *forHR4* alleles.

Figure S2: Verification of the *for^{0}* null allele. A) Schematic of the *foraging* locus with the annotated homology arms (HA1, HA2, tan boxes) and primers that bind outside these homology regions (nullF, nullR, green triangles). These primers span a 45kb region in the wild type locus. **B)** Schematic of the *for^{0}* null allele with the homology arms and primers annotated. These primers span a 10.7kb region in the *for^{0}* allele. **C)** *in-silico* gel electrophoresis of undigested and digested PCR product spanning the *for^{0}* allele. **D)**

Gel electrophoresis of undigested and digested PCR product spanning the *for*^{0} allele. Restriction enzymes are labelled above the gels. The digestion patterns match the *in-silico* predictions. Sequencing of this 10.7kb PCR product confirmed the authenticity of the allele (data not shown).

Figure S3: Construction and verification of the *for*^{BAC} construct.

A) Schematic of the p(attlox) recombineering BAC. We redesigned the P[acman] BAC (Venken *et al.*, 2006) to include a *loxP* sequence and altered the syntony of the *attB* sequence and multiple cloning site. **B)** Homology arms flanking the *foraging* gene were cloned into the P(attlox) vector to make pGAP^{for}. These homology arms were designed to correspond to the breakpoints of the *for*^{0} null allele. **C)** CH321-64J02 BAC used as the source for the *foraging* sequence. This BAC contained a *copia* transposable element within the *foraging* gene. We removed the *copia* sequence using the galK selection technique (Warming *et al.*, 2005). **D)** Once the *copia* element was removed, pGAP^{for} was used to isolate the *foraging* gene from the CH321-64J02^{-copia} BAC to generate the P(attlox)^{for} BAC. **E)** *in-silico* and **F)** *in-vitro* gel electrophoresis of restriction digestions of the P(attlox)^{for} BAC.

Figure S4: Effects of hemizygous rover and sitter larvae on path length, food intake and fat levels.

A) Larval path length of heterozygous *for*^{R}/*for*^{0}, and *for*^{s}/*for*^{0} individuals ($t = 7.3133$, $df = 55.44$, $p = 1.108e-09$). **B)** Larval food intake of heterozygous *for*^{R}/*for*^{0}, *for*^{s}/*for*^{0}, and

homozygous *for^0* individuals (a.u., arbitrary fluorescence units; t = -5.1479, df = 122.8, p-value = 1.015e-06). **C)** Larval triglyceride levels of heterozygous *for^R/for^0*, *for^s/for^0* and homozygous *for^0* individuals (t = -3.4632, df = 29.43, p-value = 0.001656).

Figure S5: *foraging* gene dosage and allelic contributions to *foraging* gene expression in whole larvae

Expression of *foraging* mRNA of homozygous *for^R*, *for^s*, heterozygous *for^R/for^s* heterozygous, *for^R/for^0*, and *for^s/for^0*, whole larvae homogenates amplifying each promoter region and the common coding region. **A)** Schematic of the *foraging* (as in Figure 1). Promoter-specific regions targeted for qPCR identified by the upper vertex of the light blue triangles. **B)** *pr1*-specific ($F_{(4,10)}$=66.4, p=3.7e-7), **C)** *pr2*-specific ($F_{(4,10)}$=22.99, p=5.0e-5), **D)** *pr3* ($F_{(4,10)}$=25.28, p=3.3e-5), and **E)** *pr4*-specific ($F_{(4,10)}$=18.99, p=1.2e-4), and **F)** common coding ($F_{(4,10)}$=53.28, p=1.0e-6) expression of regions of *foraging* quantified RT-qPCR. Due to the gene structure, *pr3* is not specific and amplifies a sub-set of *pr1* and *pr2*, as well. **B-F)** Individual data points are plotted (n=3/genotype; triangle, square, circle) with a bar representing the mean of the 3 samples. All the ΔΔCt's were calculated relative to the mean *for^s* ΔCt for the common coding region in **F**. (***, p<0.001; **, p<0.01; *, p<0.05; p-values are relative to *for^s*).