

FIGURE S1. A schematic of the protocol used to decompose each nearly genome-wide hemiclone (including the X and the two major autosomes, II and III) into separate X- and autosomal-hemiclones. Hexagons depict individuals, and rectangles within hexagons depict chromosomes (sex chromosomes at the left position, autosome-II at the middle position, and autosome-III at the right position). Black rectangles represent “target” chromosomes and white rectangles represent chromosomes randomly derived from the base LHM population. The compound X chromosome (hereafter “DX”) is C(1)DX y f and is symbolized by a chevron, while translocations of autosomes II and III are symbolized by rectangles spanning the two autosomal chromosome positions. The two types of autosomal translocations used were T(2;3) rdgC st pp bwD (designated “CG” and expressing a dominant brown-eye maker; solid shaded rectangle) and T(2;3) rdgC st pp (“TSP”; hatched rectangle). The former type of autosomal translocation was maintained in stocks either combined with compound X chromosomes (“DX-CG” flies), or with sex chromosomes obtained from the base LHM population (“DX-LHM” flies), while the latter type of autosomal translocation was paired with sex chromosomes obtained from the base LHM population (“LHM-TSP” flies). To produce X-hemiclones, X-II-III hemiclonal males were mated to virgin DX-LHM females (Step X-1). From the progeny, brown-eyed males were collected. These males, when mated to DX-CG females, permitted the target X to be clonally amplified and maintained indefinitely (X clonal amplification line; Step X-2). Males from an X clonal amplification line were mated to virgin LHM females to produce X-hemiclone females (Step X-3), or to DX-LHM females to produce X-hemiclone males (Step X-4). To produce hemiclones containing only the target autosomes II and III (A-hemiclones), X-II-III hemiclonal males were mated to virgin LHM-TSP females (Step A-1a). From the progeny, red-eyed males were collected and mated to virgin DX-CG females in order to swap in the autosomal translocations containing the dominant brown-eyed bwD marker (CG; Step A-1b). From the progeny, brown-eyed males were collected. These males, when mated to virgin DX-CG females, permitted the autosomes II and III to be clonally amplified and maintained in definitely (Autosomes II-III clonal amplification line; Step A-2). Males from the Autosomes II-II clonal amplification line were mated to virgin LHM females to produce A-hemiclone females (Step A-3), or to DX-LHM females to produce A-hemiclone males (Step A-4).

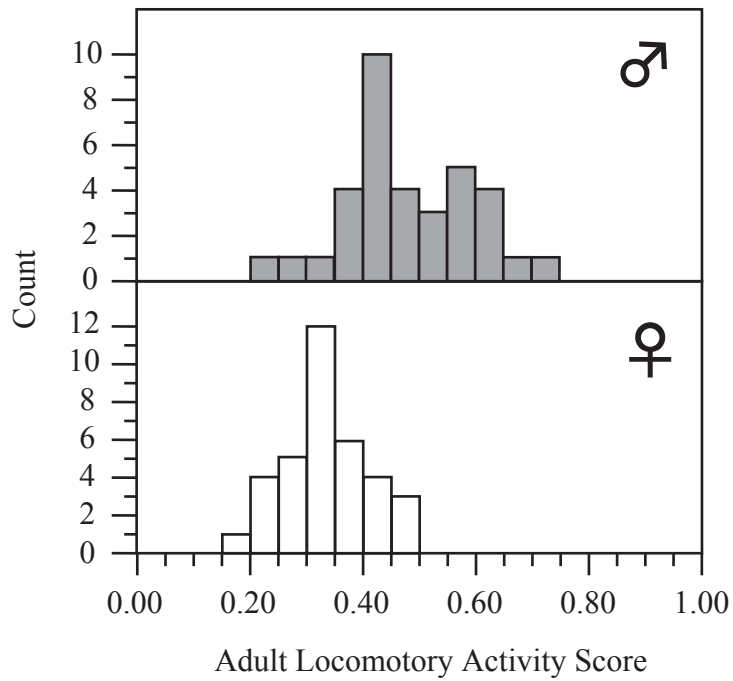


FIGURE S2. Frequency histogram of mean activity levels (measured as in figure 1) obtained for the same 35 hemiclones when expressed in males or females. Both distributions are approximately normally distributed (Shapiro-Wilks test; males  $W=0.98$ ,  $p=0.78$ ; females  $W=0.98$ ,  $p=0.87$ ).

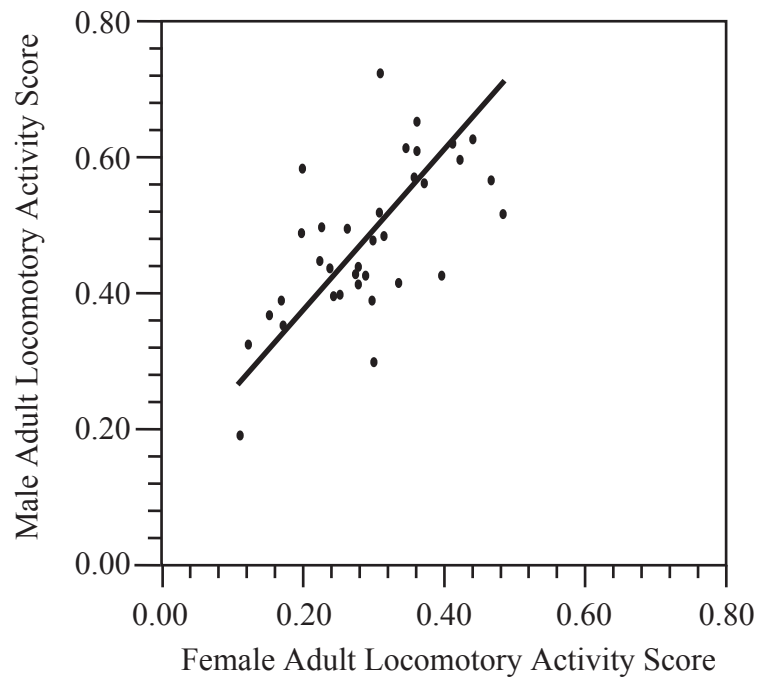


FIGURE S3. The phenotypic association between male and female adult locomotory activity (measured as in figure 1) from a sample of 35 nearly genome-wide hemiclones that were expressed in both sexes. The correlation between the sexes is positive ( $r=0.68$ ,  $d.f.= 34$ ,  $p<0.001$ ). The Reduced Major Axis regression is shown.