1 **Supplementary material for "Rapid evolution of the inter-sexual genetic correlation for**

2 **fitness in *Drosophila melanogaster*"**

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7 *Supplementary methods*

8 *Fitness measurements for the LH_M-UCL population*

9 **Female fitness assay:** We created females carrying the target haplotypes in a LH_M-UCL background by crossing

10 10 hemiclone carrier males with 15 LH_M females. These parental flies were transferred into new vials every day

11 for three consecutive days and egg density was standardised to 150-200 in each vial, as in the LH_M rearing

12 regime. Virgin hemiclone females emerging from these crosses were collected on a single day, corresponding

13 to 9-11 days after egg laying, depending on the vial of origin. The following day, we set up 'adult competition'

14 vials containing 10 virgin target females, 20 virgin LH_M-*bw* competitor females and 30 virgin LH_M-*bw* males. Fly

15 density and yeast amount were doubled compared to rearing regime conditions of the stocks in order to

16 reduce sampling variance and obtain more repeatable fitness scores. Forty-eight hours later, target and

17 competitor females were anaesthetised using CO_2 and placed singly in new vials to lay eggs. After 19.5 hours

18 (1.5 hour more than the 18 hours egg-laying period to compensate for the post-anaesthesia recovery time),

19 females were removed and the vials were stored for offspring to complete their development. Once all of the

20 offspring had emerged, vials were frozen and the offspring counted. Raw female fitness scores were calculated

21 as the average number of progeny produced by each group of hemiclone females from the same 'adult

22 competition' vial of origin. Across the three blocks of female fitness assays that were performed in the UCL

23 population, we measured the fitness of a total of 30 individual females per hemiclone line.

24 **Male fitness assay:** To introduce the target haplotypes into a male LH_M-UCL background, 10 hemiclone carrier

25 males were crossed with around 30 females of a DX-LH_M stock. These DX-LH_M females carry an attached X in a

26   LH$_M$ background [6] and cause father-to-son transmission of the X chromosome. As with the female assays,

27   vials from these crosses were transferred into fresh vials for three consecutive days. Due to bearing the

28   compound X chromosome, around half of the eggs laid by DX-LH$_M$ females were not viable. Therefore, the egg

29   density of those vials was standardised at twice the normal density, i.e. 300-400 eggs per vial. Virgin males

30   carrying the target haplotype were collected on a single day, corresponding to 9-11 days after egg laying,

31   depending on the vial of origin. The following day, we set up the 'adult competition' vials consisting of 10

32   virgin target males, 20 virgin LH$_M$-*bw* competitor males and 30 virgin LH$_M$-*bw* females. The fly density and yeast

33   amount were again doubled compared to the LH$_M$ rearing regime to reduce sampling variance. Males and

34   females were left to interact for 66 hours, corresponding to the 48 hours of adult competition and 18 hours of

35   oviposition in the LH$_M$ rearing regime. Subsequently, females were isolated in vials containing yeast and let to

36   lay eggs for at least 30 hours. Females were then removed and offspring allowed to complete their

37   development. Vials containing the emerged offspring were frozen, after which offspring were classified by eye-

38   colour (wildtype or *bw*) and counted. Raw male fitness scores were calculated as the proportion of offspring

39   emerging from a vial that were sired by target hemiclone males (i.e., had wildtype eye-colour), averaged over

40   the 30 vials from a particular replicate assay. Over the three blocks of male fitness assays that were performed

41   in the LH$_M$-UCL population, we measured the fitness of a total of 30 individual males per hemiclone line.

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Supplementary tables

Table S1. ANOVA tables for analyses of fitness across laboratories. These analyses model sex-specific fitness measures obtained for the nine hemiclones assayed at UU and UCL as a function of Laboratory, Hemiclone and their interaction (see main text for details).

A. Females

Model term	df	Sum of Squares	Mean Squares	F ratio	P value
Laboratory	1	0.390	0.390	0.531	0.47
Hemiclone	8	67.130	8.391	11.418	<0.0001
Laboratory: Hemiclone	8	7.008	0.876	1.192	0.33
Residuals	45	33.071	0.735		

B. Males

Model term	df	Sum of Squares	Mean Squares	F ratio	P value
Laboratory	1	1.309	1.309	2.031	0.16
Hemiclone	8	58.477	7.310	11.339	<0.0001
Laboratory: Hemiclone	8	7.953	0.994	1.542	0.16
Residuals	63	40.614	0.645		

56 **Table S2. Basic information on sequencing data from LH$_M$-UU and LH$_M$-UCL.** Average sequencing depth was

57 calculated for covered sites after applying read and mapping quality thresholds (both set to 20, see Methods).

58 Assembly coverage is the percentage of the reference genome that is covered to a depth of at least one read.

Population	Individuals per pool	Average sequencing depth	Assembly coverage (%)
LH$_M$-UU	165 females	151.2	95.23%
LH$_M$-UCL	165 females	144.0	93.55%

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64 **Table S3. Chromosomal distribution of SNP loci.** SNP summary statistics for both experimental populations.

65 The table provides information on the number and percentage of SNPs called in both populations that were

66 covered to a depth of at least 100 sequencing reads (Total SNPs), the number of those SNPs with private

67 polymorphisms either in LH_M-UU (SNPs segregating only in LHm-UU) or in LH_M-UCL. The percentages of SNPs

68 located on each chromosome arm were tested for significant over- or under-representation relative to the

69 proportional contribution of those chromosome arms to the total genome size (provided in parentheses) using

70 one-sample Z-tests (P-values were Bonferroni-corrected for multiple testing). Percentages in *italics* differ

71 significantly from the expected value given in parentheses.

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| Chr | Total SNPs | | SNPs segregating only in LH_M-UU | SNPs segregating only in LH_M-UCL |
	count	percentage		
X	69,304	*10.15⁻* (18.62)	10,149	6,553
2L	156,414	*22.91⁺* (19.12)	15,649	13,017
2R	145,193	*21.26⁺* (17.57)	12,852	13,384
3L	155,915	*22.83⁺* (20.39)	22,738	13,381
3R	155,202	*22.73⁻* (23.18)	22,521	13,382
4	804	*0.12⁻* (1.12)	87	21
Autosomes	613,528	*89.85⁺* (81.38)	73,847	53,185
Total	682,832	100	83,996	59,738

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77   **Table S4. Inversion markers in LH$_M$-UU and LH$_M$-UCL.** Results of testing for the presence of marker alleles for

78   chromosomal inversions that were identified by Kapun et al. (2014). For each of the inversions, the table

79   shows the total number of markers identified by Kapun et al. ('Markers'), as well as for each population the

80   number of these marker loci that had coverage passing our quality filters ('Covered') and the number of those

81   had significant polymorphism ('Polymorphic'). Numbers in parentheses in the 'Polymorphic' column indicate

82   the number of loci at which the marker allele was found to be present in a given population.

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Inversion	Markers	UU		UCL	
		Covered	Polymorphic	Covered	Polymorphic
In(2L)t	16	13	0	15	0
In(2R)Ns	67	65	1 (0)	63	1 (0)
In(3L)P	73	69	0	70	0
In(3R)C	144	136	2 (0)	141	3 (1)
In(3R)K	4	4	0	4	0
In(3R)Mo	150	147	2 (1)	144	1 (0)
In(3R)Payne	19	17	0	16	0

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88 **Table S5. Results of a Gene Ontology analysis on genes with candidate SNPs.** The table, provided as a

89 separate Excel file, lists terms describing biological processes (Tab. S5A) and molecular functions (Tab. S5B)

90 significantly enriched among candidate genes, up to a false discovery rate of 5%.

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94 **Table S6. Results of a clustering analysis of Gene Ontology terms associated with candidate genes.** The table,

95 produced by DAVID's clustering function and provided as a separate Excel file, lists clusters of terms that are

96 related or redundant due to their hierarchical nesting and overlapping gene associations.

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Supplementary figures

100 **Figure S1**. **Male and female adult fitness across genotypes in the LH$_M$-UU population.** The figure shows

101 average standardised male and female fitness across 100 hemiclonal lines randomly extracted from LH$_M$-UU

102 (cf. Fig. 1 in Innocenti and Morrow 2010). The labelled blue diamonds and red squares show the fitness

103 estimates of the male beneficial/female detrimental and female beneficial/male detrimental hemiclones that

104 were also assayed at UCL (cf. Fig. 2 in the main text).
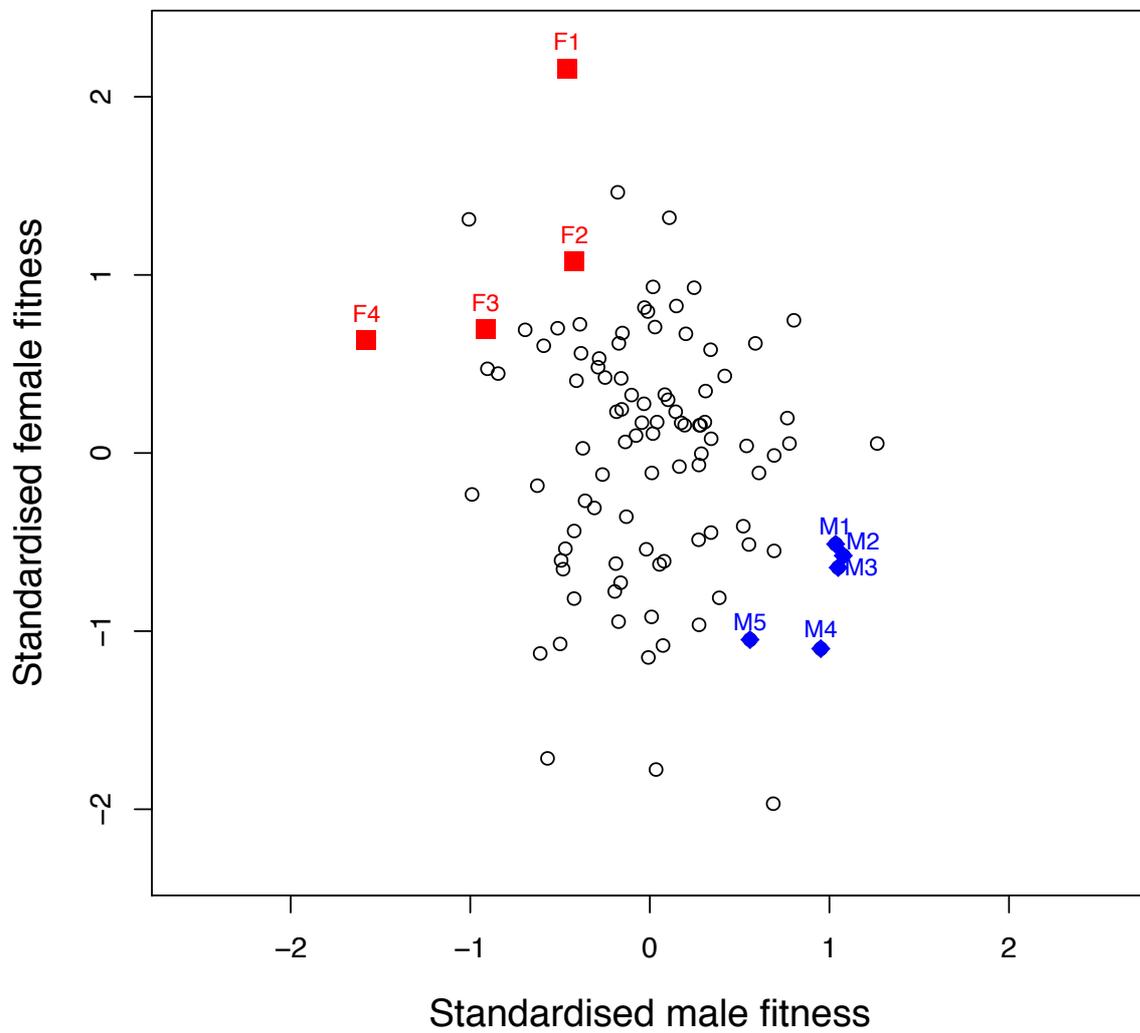
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107 **Figure S2. Relationship between fitness measures obtained across laboratories.** The scatterplots show mean

108 fitness values obtained for the set of nine reference genomes that were assayed at the University of Uppsala

109 (UU) and UCL (blue- and red-coloured points in Figs. 2 and S1). Black bars indicate standard errors of the mean.

110 Separate plots are shown for females (left panel) and males (right panel).

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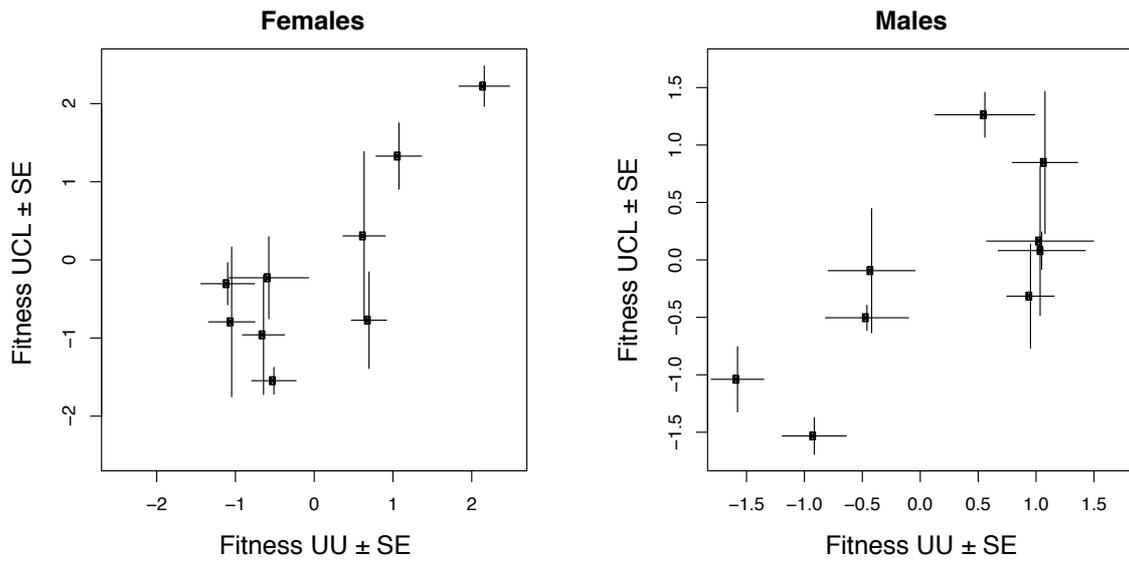
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113 **Figure S3. Difference in genetic polymorphism between LH$_M$-UU and LH$_M$-UCL.** The boxplots show the

114 distribution of the difference in expected heterozygosity, calculated as H$_{e,UU}$ - H$_{e,UCL}$, for the autosomes and the

115 X chromosome. Statistical results are provided in the main text.

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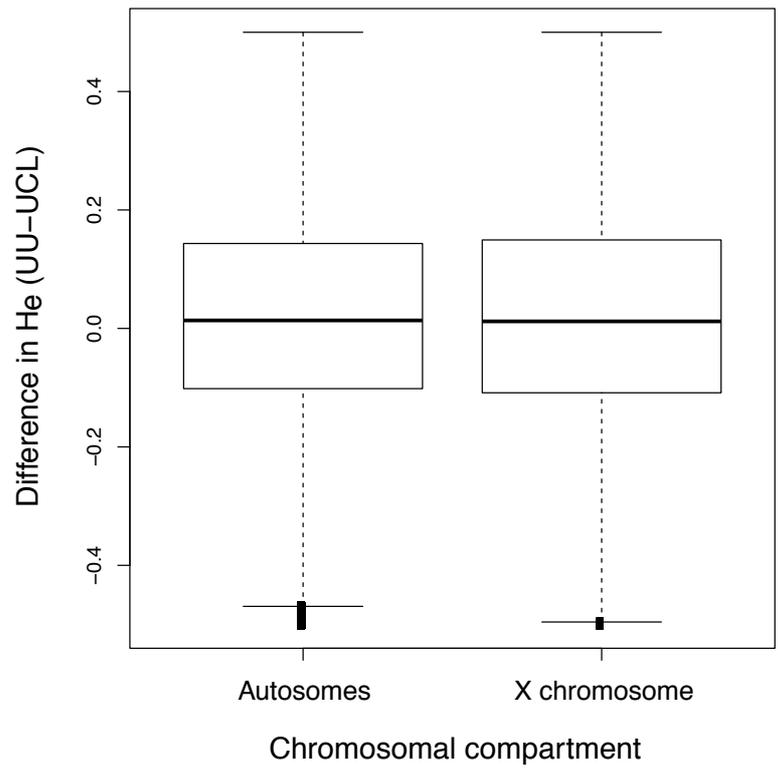
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118   **Figure S4. F$_{ST}$ at SNP loci along chromosome arms**. Loci with differentiation above the significance threshold

119   are represented in red.

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121   Due to its large size, the figure is provided as a separate file.
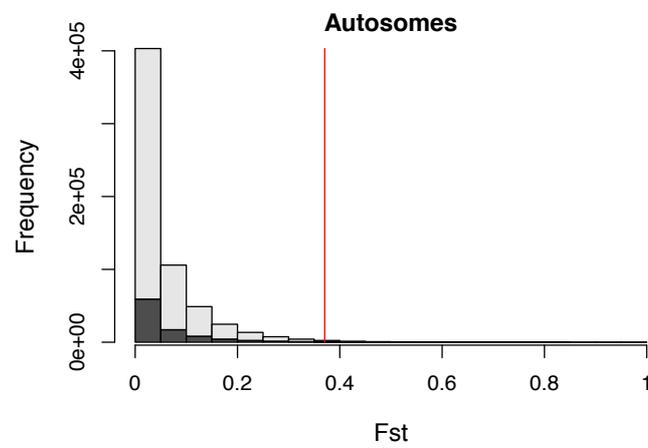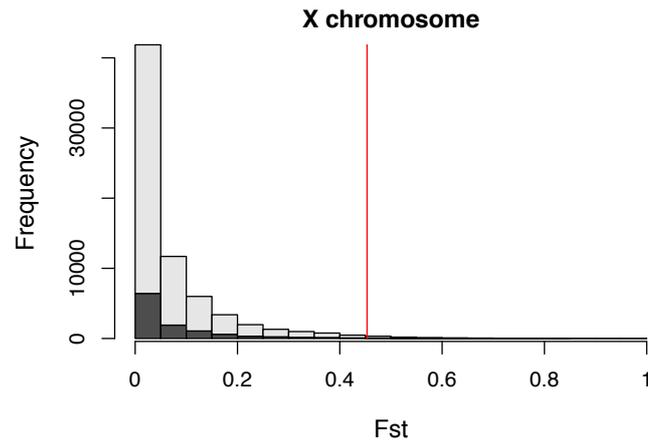
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127   **Figure S5. Distribution of F_{ST} among SNP loci.** The figure shows the distribution of F_{ST} for loci on the

128   autosomes (A) and the X chromosome (B). Synonymous SNPs are shown in dark grey, other SNPs in lighter
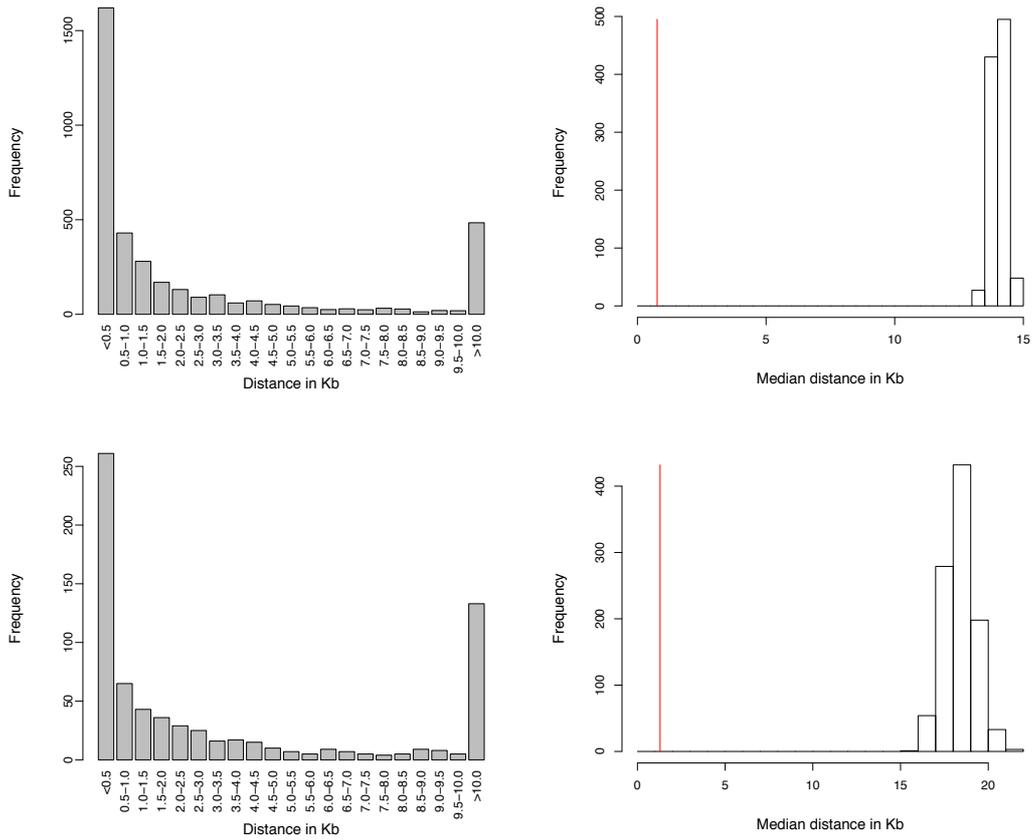
129   grey.

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135 **Figure S6. Distribution of distances between neighbouring candidate SNPs.** The figure shows the distribution

136 of observed distances between adjacent candidate SNPs on the autosomes (A) and the X chromosome (C). The

137 right-hand panels show the null distribution of median distances between randomly sampled SNP loci on the

138 autosomes (B) and the X chromosome (D). The red line in (C) and (D) indicates the observed median distance.

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