LETTER

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Genome-wide analysis of a long-term evolution experiment with *Drosophila*

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Experimental evolution systems allow the genomic study of adaptation, and so far this has been done primarily in asexual systems with small genomes, such as bacteria and yeast[1–3]. Here we present whole-genome resequencing data from *Drosophila melanogaster* populations that have experienced over 600 generations of laboratory selection for accelerated development. Flies in these selected populations develop from egg to adult ~20% faster than flies of ancestral control populations, and have evolved a number of other correlated phenotypes. On the basis of 688,520 intermediate-frequency, high-quality single nucleotide polymorphisms, we identify several dozen genomic regions that show strong allele frequency differentiation between a pooled sample of five replicate populations selected for accelerated development and pooled controls. On the basis of resequencing data from a single replicate population with accelerated development, as well as single nucleotide polymorphism data from individual flies from each replicate population, we infer little allele frequency differentiation between replicate populations within a selection treatment. Signatures of selection are qualitatively different than what has been observed in asexual species; in our sexual populations, adaptation is not associated with 'classic' sweeps whereby newly arising, unconditionally advantageous mutations become fixed. More parsimonious explanations include 'incomplete' sweep models, in which mutations have not had enough time to fix, and 'soft' sweep models, in which selection acts on pre-existing, common genetic variants. We conclude that, at least for life history characters such as development time, unconditionally advantageous alleles rarely arise, are associated with small net fitness gains or cannot fix because selection coefficients change over time.

Experimental evolution uses well-defined selection protocols to force phenotypic divergence[4,5]. Studies of experimentally evolved populations have identified mutations responsible for particular adaptations[6] and provided some general insights into the nature of adaptation in asexually reproducing populations[7]. Adaptation in these populations is driven by so-called classic selective sweeps, or the fixation of newly arising beneficial mutations and the genome-wide haplotypes associated with them. By contrast, an obligate sexually reproducing system can harbour a great deal of standing genetic variation on which selection can act. Standing variation is theoretically predicted to lead to rapid evolution in novel environments[8], and case studies of ecologically relevant genes bear out this prediction[9–11]. The idea that short-term evolution may act primarily on pre-existing intermediate-frequency genetic variants that are swept the remainder of the way to fixation has been termed a soft sweep[8,12] model.

We collected genome-wide resequence data for outbred, sexually reproducing, replicated populations of *D. melanogaster* selected for accelerated development and their matched control populations. Using the Illumina platform, we obtained short-read sequences from three genomic DNA libraries: a pooled sample of five replicate populations that have undergone sustained selection for accelerated development and early fertility for over 600 generations (ACO); a pooled sample of five replicate ancestral control populations, which experience no direct selection on development time (CO); and a single ACO replicate population (ACO_1). The ACO treatment has evolved strongly differentiated life history phenotypes relative to those of the CO treatment[13] (summarized in Fig. 1; see also Supplementary Fig. 1 for the history of the populations).

To identify single nucleotide polymorphisms (SNPs) significantly differentiated between the ACO and CO populations, we aligned reads to the reference genome of *Drosophila* and considered only those genomic positions at which there were two observed allelic states. After quality-filtering, we were left with 688,520 SNPs: approximately one SNP for every 175 base pairs (bp) on the 120-megabase (Mb) euchromatic genome (Methods). The average alignment depth at identified SNPs was ~20× in both the ACO and CO libraries (Supplementary Fig. 2), and ~10× in the ACO_1 library. For every SNP, we calculated $-\log_{10}(P)$ from a Fisher's exact test (L_{10}FET) for a difference in allele frequency between the ACO and CO libraries, as well as the ACO and ACO_1 libraries.

We examined each SNP to determine whether it encodes an amino-acid polymorphism, a segregating stop codon or a segregating interruption to a consensus splice junction (Supplementary Fig. 3). We

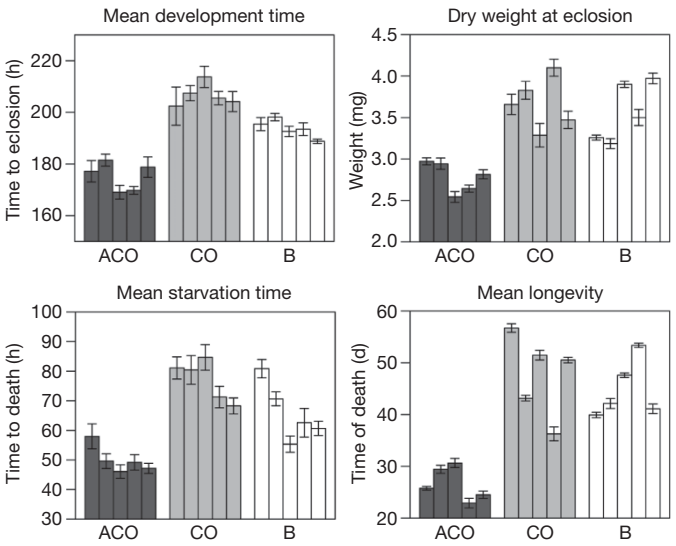

Figure 1 | Summary of phenotypic divergence in the selection treatments described in this study. Grey bars represent values measured in each of the five replicate populations in the ACO and CO treatments. Measures from the five baseline (B) replicate populations represent phenotypes typical of populations kept on two-week generation maintenance schedules. Only data for females are shown. Longevity and starvation resistance data were collected after at least 619 generations of ACO treatment, and both development time and dry weight data (dry weight values are mean masses of groups of ten females) were collected after 640 generations of ACO treatment. Error bars, s.e.m. for each replicate population.

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identified 37,185 non-synonymous SNPs, 190 segregating stop codons and 118 segregating splice variants. Of the ~37,000 putative non-synonymous SNPs, 662 SNPs in 506 genes are associated with an L_{10}FET score >4 (only 3.7 SNPs are expected to exceed this threshold by chance alone). These 662 SNPs are potential candidates for encoding the causative differences between the ACO and CO populations, to the extent that those differences are due to structural as opposed to regulatory variants (compare with ref. 14). We carried out a functional analysis of the 475 of these genes that have DAVID IDs (http://david.abcc.ncifcrf.gov/; ref. 15) and present the results for the functional categories that have a false-discovery rate of less than 10% for Swiss-Prot protein keywords, InterPro domains and all Gene Ontology biological processes (Supplementary Table 1). For the biological processes, there is an apparent excess of genes important in development; for example, the top ten categories are imaginal disc development, smoothened signalling pathway, larval development, wing disc development, larval development (*sensu* Amphibia), metamorphosis, organ morphogenesis, imaginal disc morphogenesis, organ development and regionalization. This is not an unexpected result, given the ACO selection treatment for short development time, but it indicates an important role for amino-acid polymorphisms in short-term phenotypic evolution. We have created custom tracks representing our data for the UCSC Genome Browser that allow a user to browse a region of interest and examine allele frequency divergence in that region along with functional annotations of segregating SNPs (see, for example, Supplementary Fig. 4).

Previous work suggests that linkage disequilibrium in individual ACO and CO replicate populations may extend anywhere from 20 to 100 kilobases[5] (kb). Strong linkage disequilibrium suggests that although the individual Fisher's exact tests on the SNPs of this study do not have a great deal of power to detect changes in allele frequency, a sliding-window analysis may have considerable power. We carried out a 100-kb genome-wide sliding-window analysis to identify regions diverged in allele frequency between the ACO and CO libraries and between the ACO and ACO_1 libraries (Fig. 2; see Methods for details including the definition of $L_{10}FET_{5\%Q}$). The sliding-window analysis identifies a large number of genomic regions showing significant divergence between the accelerated development populations and their matched controls (Fig. 2, black line), and very little evidence for divergence between a single replicate evolved population (ACO_1) and the pooled sample consisting of all five ACO populations (Fig. 2, grey line). We observe an apparent excess of diverged regions on the X chromosome relative to on the autosomes, an observation that might be expected if adaptation were driven by selection on initially rare recessive or partially recessive alleles. The sharpness of the peaks in Fig. 2 suggests that regions of the genome that have responded to experimental evolution are precisely identified, but in fact even the sharpest peaks tend to delineate ~50–100-kb regions (compare with Supplementary Fig. 5). We are unable to determine the extent to which additional sequencing coverage would offer increased resolution, or whether the levels and patterns of linkage disequilibrium in these populations are limiting. Regardless, it is apparent that allele frequencies in a large portion of the genome have been affected following selection on development time, suggesting a highly multigenic adaptive response.

Recent research on evolutionary genetics has focused on classic selective sweeps, which are evolutionary processes involving the fixation of newly arising beneficial mutations[16]. In a recombining region, a selected sweep is expected to reduce heterozygosity at SNPs flanking the selected site. Sliding-window plots (100 kb) of heterozygosity in ACO and CO lines suggest that there are indeed local losses of heterozygosity (Fig. 3, red and blue lines, respectively). This is the case particularly for the ACO populations, which have experienced more generations of stronger selection in their recent evolutionary history than the CO populations. Regions of reduced heterozygosity are strongly associated with regions of differentiated allele frequency (compare Figs 2 and 3; Supplementary Fig. 6). Notably, we observe

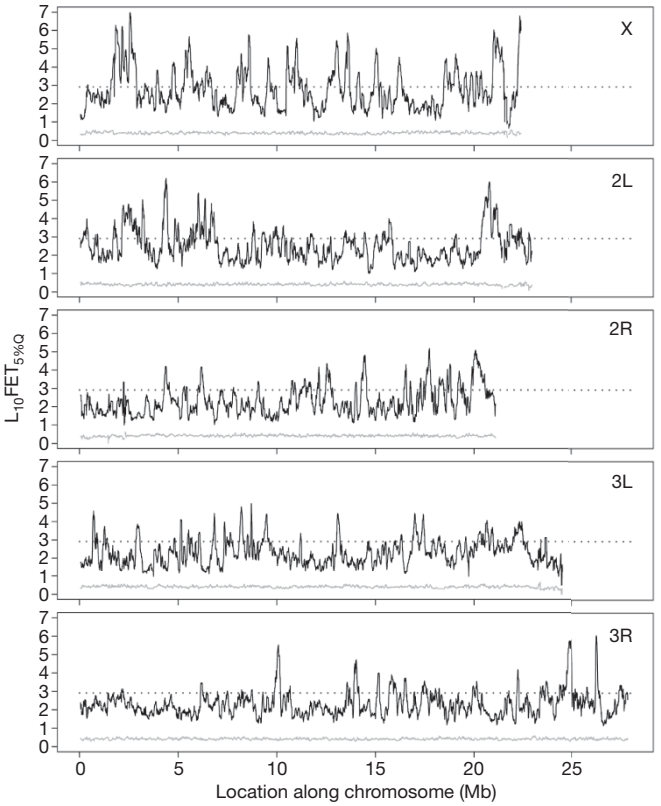

Figure 2 | Differentiation throughout the genome. Sliding-window analysis (100 kb) of differentiation in allele frequency between the ACO and CO populations: the solid black line depicts $L_{10}FET_{5\%Q}$ scores at 2-kb steps (Methods). The dotted line is the threshold that any given window has a 0.1% chance of exceeding relative to the genome-wide level of noise. The grey line depicts $L_{10}FET_{5\%Q}$ scores for a difference in allele frequency between ACO_1 and the ACO pooled sample. The five panels show the five major *D. melanogaster* chromosome arms (as indicated).

no location in the genome where heterozygosity is reduced to anywhere near zero, and this lack of evidence for a classic sweep is a feature of the data regardless of window size.

The ACO_1 sample and the ACO pool show very little evidence for allele frequency differentiation (Fig. 2, grey line). Similarly, the sliding-window analysis of heterozygosity in ACO_1 (Fig. 3, grey line) shows remarkable concordance with the reductions in heterozygosity in the ACO pool (Fig. 3, red line). To better assess allele frequency differences between replicate populations, we individually genotyped 35 females from the five replicate populations of each selection treatment at 30 loci at which the resequence data predicted significantly different allele frequencies. Replicate populations within a selection treatment have very similar allele frequencies (Fig. 4a), and individual genotypes are consistent with allele frequency estimates from the resequenced pooled libraries (Fig. 4b). We therefore conclude that the congruence in allele frequencies and patterns of heterozygosity between the ACO_1 and ACO libraries is unlikely to be some sort of artefact of sample preparation or data analysis.

We consider two possible explanations for the convergence of allele frequencies and heterozygosity levels between replicate populations. First, selection is acting on the same intermediate-frequency variants in each population. Under this scenario, convergence in allele frequencies is due to parallel evolution. Second, unwanted migration between replicate populations, even at very low levels, could explain observed similarities. Despite preventative measures in place to isolate replicate populations during routine maintenance, some degree of migration between the replicate populations within a selection treatment is probable (successful migration between treatments is not as likely, owing to the selection

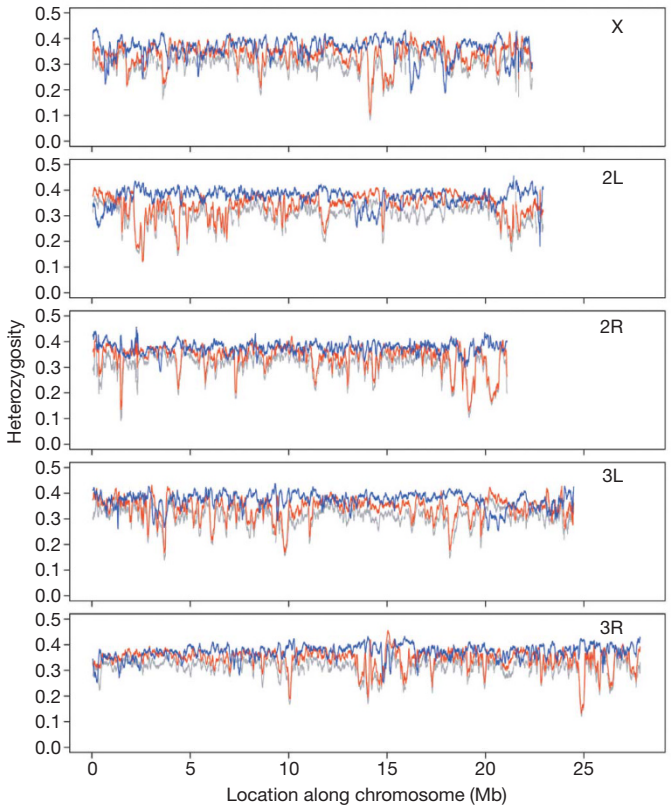

Figure 3 | Heterozygosity throughout the genome. Sliding-window analysis (100 kb) of heterozygosity in the CO pool (blue), the ACO pool (red) and ACO_1 (grey), with a 2-kb step size. The panels show the five major chromosome arms of *D. melanogaster*.

regimes effectively precluding the survival and reproduction of migrants). If migration is occurring, its rate must be low, as we have observed substantial and sustained phenotypic differences between replicate populations within selection treatments (compare with Fig. 1). A small amount of cross-contamination between replicate populations does not rule out our inference that classic selective sweeps have not occurred during the evolution of these populations. If classic sweeps are occurring in the presence of migration, the ACO pool should show regions of zero heterozygosity, because unconditionally beneficial alleles can move between populations, whereas in the absence of migration we expect to see regions of zero heterozygosity in a single replicate population. In fact, we see no evidence for sweeps in ACO_1 nor in the pool of all the populations with accelerated development.

There are several possible explanations for our failure to observe the signature of a classic sweep in these populations, despite strong selection. Classic sweeps may be occurring, but have had insufficient time to reach fixation. This explanation is consistent with observed data, but requires that newly arising beneficial alleles have small associated selection coefficients (Supplementary Fig. 7). Alternatively, selection in these lines may generally act on standing variation, and not new mutations. This soft sweep model predicts partial losses of heterozygosity flanking selected sites, provided that selection begins acting when mutations are at low frequencies[12,17], and this is consistent with our observed data. However, if a large fraction of the total adaptive response is due to loci fixed by means of soft sweeps, there should be insufficient genetic variation to allow reverse evolution in these populations. But forward experimental evolution can often be completely reversed with these populations[5], which suggests that any soft sweeps in our experiment are incomplete and/or of small effect (Supplementary Fig. 5). A third explanation is that the selection coefficients associated with newly arising mutations are not static but in fact decrease over time. This could be the case if initially rare selected alleles increase to frequencies where additional change is hindered, perhaps by linked deleterious alleles or antagonistic pleiotropy. Laboratory evolution experiments typically expose populations to novel environments in which focal traits respond quickly and then plateau at some new value (compare with refs 13, 18). Chevin and Hospital[19] recently modelled the trajectory of an initially rare beneficial allele that does not reach fixation because its selective advantage is inversely proportional to the distance to a new phenotypic optimum, and that optimum is reached, because of other loci, before the variant fixes. This model therefore has appeal in the context of experimental evolution, as it assumes populations generally reach a new phenotypic optimum before newly arising beneficial mutations of modest effect have had time to fix.

Our work provides a new perspective on the genetic basis of adaptation. Despite decades of sustained selection in relatively small, sexually reproducing laboratory populations, selection did not lead to the fixation of newly arising unconditionally advantageous alleles. This is notable because in wild populations we expect the strength of natural selection to be less intense and the environment unlikely to remain constant for ~600 generations. Consequently, the probability of fixation in wild populations should be even lower than its likelihood in these experiments. This suggests that selection does not readily expunge genetic variation in sexual populations, a finding which in turn should motivate efforts to discover why this is seemingly the case.

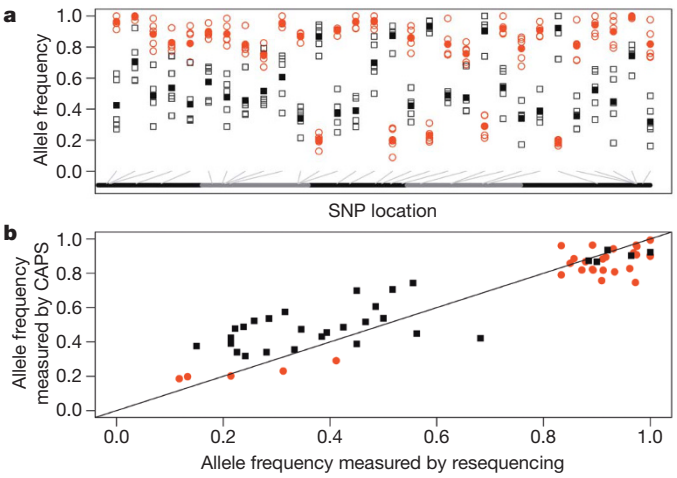

Figure 4 | Analysis of individual genotypes, measured by cleaved amplified polymorphic sequence (CAPS) techniques. a, Allele frequency estimates of the most common allele at 30 SNPs genotyped in 35 females per replicate population. Red circles represent ACO estimates and grey squares represent CO estimates. Open symbols are allele frequencies for ACO_1–ACO_5 and CO_1–CO_5, and filled symbols represent treatment means. Alternating black and grey bars designate the X, 2L, 2R, 3L, and 3R arms, respectively, with grey lines indicating SNP location. **b**, Scatter plot comparing allele frequency estimates at the same 30 SNPs obtained from the Illumina resequencing versus individual genotyping. Red circles represent ACO, black squares represent CO and the straight line represents a slope of unity.

METHODS SUMMARY

Experimental evolution system. The ACO_1–ACO_5 selection treatments are maintained on a 9–10-d cycle and the control treatments, CO_1–CO_5, are maintained on a 28-d cycle. The flies used for sequencing were collected after 605 generations (ACO) and 252 generations (CO) of selection.

Genome sequencing. DNA was extracted from 25 female flies collected from each of the ACO_1–ACO_5 and CO_1–CO_5 populations and pooled within selection treatments to make two Illumina paired-end libraries. We also created a library for the ACO_1 replicate population only. The pooled libraries were each run on four (unpaired 54-bp) lanes of an Illumina Genome Analyser II, and the ACO_1 library was run on a single (paired-end 36-bp) lane.

SNP identification and sliding-window analysis. We used MOSAIKALIGNER to align all of our sequences to the reference genome of *Drosophila*. We then used custom PERL scripts to count the number of single nucleotide mismatches at every position in the genome, as a function of selection treatment. Fisher's exact tests

were conducted at each SNP and in regions of the genome enriched for SNPs showing differences in allele frequency between the ACO and CO treatments (or ACO and ACO_1 treatments) identified using a 100-kb sliding-window analysis with a step size of 2 kb. We used the same sliding window and step size in the analysis of heterozygosity.

CAPS analysis. We used CAPS techniques to genotype panels of 35 individual flies from the ACO_1–ACO_5 and CO_1–CO_5 populations at 30 significant loci implicated by the resequencing data. Each of these 30 loci also had to harbour an allele-specific restriction endonuclease cleavage site.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions M.K.B., P.S. and J.P.D. performed the laboratory experiments. M.K.B., K.R.T. and A.D.L. analysed the data. M.K.B., M.R.R. and A.D.L. designed the project, and M.K.B., K.R.T., M.R.R. and A.D.L. wrote the manuscript.

Author Information The FASTQ files associated with this project have been deposited in GenBank's Short Read Archive under the study accession number SRP002024. Data and source code files to reproduce the analyses of this work are available on request from the authors. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to M.K.B. (burkem@uci.edu) or A.D.L. (tdlong@uci.edu).

METHODS

Evolutionary history of the laboratory system. This experimental system was founded in 1975 on a single outbred population sampled from South Amherst, Massachusetts, and sets of replicated populations experiencing different selection treatments have been maintained since 1980 under the specific conditions of large population size ($N > 1{,}000$) and discrete generations. The selection treatments used for this study are five replicate populations maintained on a 9–10-d cycle (ACO_1–ACO_5) and control populations maintained on a 28-d cycle (CO_1–CO_5). Both ACO and CO treatments share the same long-lived ancestor (O_1–O_5) (Supplementary Fig. 1). When compared to the populations of the CO treatment, the ACO populations feature a suite of significantly differentiated phenotypes, including shorter development time and reductions in pre-adult viability, longevity, adult body size and stressresistance[13,20,21] (Fig. 1). The flies used for sequencing were collected from the ACO populations after 605 generations of divergence from a CO ancestor population, and from the CO treatments after 252 generations of divergence from the same CO ancestor. The CO treatment does not feature stringent selection, as it entails no more than moderate selection for postponed reproduction, resulting in moderately increased development time and longevity. For a comparison of ACO and CO treatments with a standard 14-d maintenance treatment (labelled B (baseline) in the Rose laboratory), refer to Fig. 1.

Phenotype assays. Before measuring phenotypes, we handled all populations for two generations on a standard 14-d rearing schedule. The populations assayed have been maintained on banana/corn syrup/agar medium, developed in 8-dram glass culture vials and kept on a 24-h light cycle. All phenotype assays were conducted after flies were collected for Illumina sequencing; at least 600 generations of ACO selection had elapsed at the time of the assays.

Longevity assay: Approximately 3,500 individual flies from each replicate population were transferred from culture vials into large Plexiglas cages on day 14 post-oviposition. Fresh food was supplied to each cage every 2 d, and the dead flies in each cage were counted and sexed every 2–3 d until all flies in all cages had died.

Starvation resistance assay: Forty-eight individual flies were transferred from each population's culture vials into assay vials with no food and moist cotton balls 16–17 d post-oviposition. Flies were monitored every 4 h, and dead flies were sexed and counted at every check.

Development time assay: Within 3 h of oviposition, ~60 eggs were transferred to each of five 8-dram 'development' vials with fresh medium per replicate population (~300 individuals were assayed per replicate population, meaning ~1,500 individuals were assayed per selection treatment). Development vials were kept at 25 °C and were monitored until eclosion. On eclosion, adults were collected and sexed every 6 h. The assay was terminated on day 14 post-oviposition, when only one or two flies were eclosing by the time of each check.

Dry weight assay: Seventy individual females were flash-frozen on day 16 post-oviposition and then placed in a drying oven for 24 h. Flies were weighed in groups of ten, and these grouped masses were represented in units of milligrams (Fig. 1).

DNA and sequencing. DNA was extracted from pooled samples of 25 female flies collected from each of the ACO_1–ACO_5 and CO_1–CO_5 populations (Puregene DNA Extraction Kit, Qiagen). Equal molarities of each pooled sample were combined to produce 1.5 μg of genomic DNA from each selection treatment, to make two Illumina paired-end libraries using the standard library preparation protocol. The ACO_1 library was prepared from a different DNA sample, made from 100 females collected 25 generations after the pooled ACO sample was created. The ACO and CO pooled libraries were each run on four unpaired 54-bp lanes of an Illumina Genome Analyser II, and the ACO_1 library was run on a single PE36 lane. Clusters passing Bustard quality filters were saved as FASTQ files for use in subsequent bioinformatics steps.

SNP identification. FASTQ files were processed using the MOSAIKALIGNER set of tools (version 0.9.0891 of the MOSAIK Software Suite; http://bioinformatics.bc.edu/marthlab/Mosaik) as well as a collection of custom PERL scripts. MOSAIKBUILD and release 5.1 of the *D. melanogaster* genome were used to create a jump database with a hash size of 13. All reads were aligned to that reference genome using MOSAIKALIGNER with the following alignment parameters: -hs 13 -mm 4 -a all -m unique -mhp 100 -act 20 -km -pm. The resulting aligned reads were combined to create a single AXT file, with each read marked to indicate experimental treatment. A custom PERL script then parsed every position on the five major chromosome arms counting the number of 'A', 'C', 'G', 'T' or '-' alleles as a function of evolutionary treatment. MOSAIKALIGNER has the ability to align short indels relative to the reference sequence, thus allowing us to score sites with a deletion relative to the reference sequence as '-'. We did not consider positions that represent an insert relative to the reference sequence, as they present computational difficulties. We define the major allele at a site as the most frequently observed allelic state across both experimental treatments, the minor allele as the second most frequently observed allelic state and discard alleles not belonging to those two categories. Although tri-allelic SNPs can exist, our approach ignores such SNPs, as in Illumina GAII data sets rare third alleles are likely to be errors. To be considered an SNP, a site was required to have a total alignment depth of more than 15 and less than 150 (very high alignment depths are likely to be repetitive DNA). The site was also required to have an alignment depth of more than two in both experimental treatments, as well as a minor allele frequency of more than 2% using a binomial likelihood-ratio approach[22]. SNPs failing to meet these criteria were much more likely to be false positives. Several lines of evidence suggest that the vast majority of the quality-filtered SNPs are real: for instance, over 80% of them are also polymorphic in a collection of ~39 inbred strains from Raleigh, North Carolina (DPGP release 0.5); we observe very few SNPs encoding stop codon and/or disruptions of consensus splice junctions; and the validation rate for a subset of SNPs assayed in individual flies using CAPS was 100%.

Differentiated SNPs and sliding-window analysis. We carried out a Fisher's exact test at every SNP and refer to the negative base-ten logarithm of the resulting P value as an L_{10}FET score. Given that the average coverage in each pooled population was ~20×, individual Fisher's exact tests are unlikely to reach genome-wide statistical significance even with large allele frequency differences between populations (for example, fisher.test(matrix(c(15,5,5,15),ncol = 2))$ p.value = 0.3% in R). To identify regions of the genome enriched for SNPs showing differences in allele frequency between the ACO and CO treatments, we carried out a 100-kb sliding-window analysis with a step size of 2 kb on the quantile score that only 5% of the L_{10}FET scores exceeded ($L_{10}FET_{5\%Q}$; for each window in R we record quantile(λ,probs = 0.95), where λ is the list of L_{10}FET values for that window). Qualitatively, this statistic does not perform differently from the mean L_{10}FET score, but it has the feature of providing information with respect to SNPs in the significant tail of the distribution. It is difficult to determine whether the $L_{10}FET_{5\%Q}$ score for any given window is significantly greater than genome-wide background. Randomization or permutation tests commonly used to assess significance remove the autocorrelation in L_{10}FET scores due to linkage disequilibrium, and as a result tend to be liberal. Therefore, for each window we calculated a standard deviation on the $L_{10}FET_{5\%Q}$ score via 100 bootstrap replicate samples of $\lambda(\sigma_{L_{10}FET_{5\%Q}})$. In R, we then placed a conservative 99.9% upper bound on $L_{10}FET_{5\%Q}$ as median($L_{10}FET_{5\%Q}$) + qnorm(0.999) × quantile(v,probs = 0.75), where v is the list of $\sigma_{L_{10}FET_{5\%Q}}$ scores over all windows. As observed linkage disequilibrium in these populations tends to extend from 20 to 100 kb, using a marginal threshold of 0.1% implies a total number of peaks across the whole genome exceeding this threshold of fewer than ten.

For every identified position in the genome, we asked whether it was predicted to encode a non-synonymous polymorphism, a segregating stop or a segregating splice junction polymorphism. A MySQL database was created from the annotation of the *D. melanogaster* coding sequences. The database contains the locations of all canonical splice positions and the effect of every possible single nucleotide change in coding regions. We then cross-referenced this database with our list of significantly differentiated SNPs using MySQL queries. A Gene Ontology analysis was carried out using the DAVID website (http://david.abcc.ncifcrf.gov/; ref. 15) and the list of the unique gene names generated from the above query (Supplementary Table 1).

Genotyping individuals using CAPS. DNA was extracted from 35 individual female flies obtained from the ACO_1–ACO_5 and CO_1–CO_5 populations from the same generation as that used to create the pooled libraries. We designed 30 pairs of PCR primers to amplify ~300-bp amplicons at loci that contained a predicted SNP that was at significantly different frequencies in the ACO and CO treatments (L_{10}FET, >5.1) and encoded an allele-specific polymorphic restriction site. PCR and restriction reactions were carried out in 384-well plates, run on 3% agarose gels and visually scored. Details of the PCR and restriction reaction conditions can be found in Supplementary Table 2. To determine whether CAPS allele frequencies were significantly different in the ACO and CO selection treatments, we carried out paired t-tests (pairs of replicates have shared evolutionary history; compare with Supplementary Fig. 1) of arcsine-transformed allele frequency estimates. The SNPs examined using CAPS were all at significantly different allele frequencies in the ACO and CO treatments ($P < 0.025$). We expect the differences between the allele frequencies measured by resequencing to be slightly larger than the differences between allele frequencies measured by CAPS, owing to the 'winner's curse' phenomenon; this could explain why our r^2 value ($r^2 = 0.85$) is not closer to one (Fig. 4b).

Data availability. The FASTQ files associated with this project are deposited in GenBank's SRA under the accession numbers SRR036932–SRR036940. Two gzipped data files are available on request from the authors. The first includes a table with information on the 688,520 high-quality SNPs, and the source code and files needed to regenerate other necessary database tables to reproduce the analyses of this work. The second contains four tables that can be uploaded to the UCSC Genome Browser to create custom tracks representing the sliding-window $L_{10}FET_{5\%Q}$ scores,

all sites with an L_{10}FET score >5, all non-synonymous polymorphisms and all stop/splice variants.

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