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Direct observation of adaptive tracking on ecological time scales in *Drosophila*

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Abstract

INTRODUCTION: Adaptation is a foundational process in evolutionary biology that is central to human health and the conservation of biodiversity. Adaptive tracking, defined as continuous adaptation in response to rapid environmental change, is a potentially critical mechanism by which populations persist in changing environments. However, little is known about the pace, extent, and magnitude of adaptive tracking in response to natural temporal variation. This may be attributable to the historical perception that adaptation occurs slowly relative to environmental change as well as to difficulties in measurement. Replicated field experiments that directly measure phenotypic and genomic evolution in response to ongoing environmental change could provide the resolution necessary to answer fundamental questions about the process of adaptation, including those about adaptive tracking. If adaptive tracking does occur in populations experiencing rapid environmental change, it would alter our understanding of the importance of evolutionary change for ecological outcomes.

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P.S. designed research. S.R., N.J.B., J.H., and P.S. conducted the experiment. T.Y. and S.T. prepared sequencing libraries. S.M.R., S.I.G., D.A.P., and P.S. analyzed data and wrote and revised the manuscript.

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SUPPLEMENTARY MATERIALS
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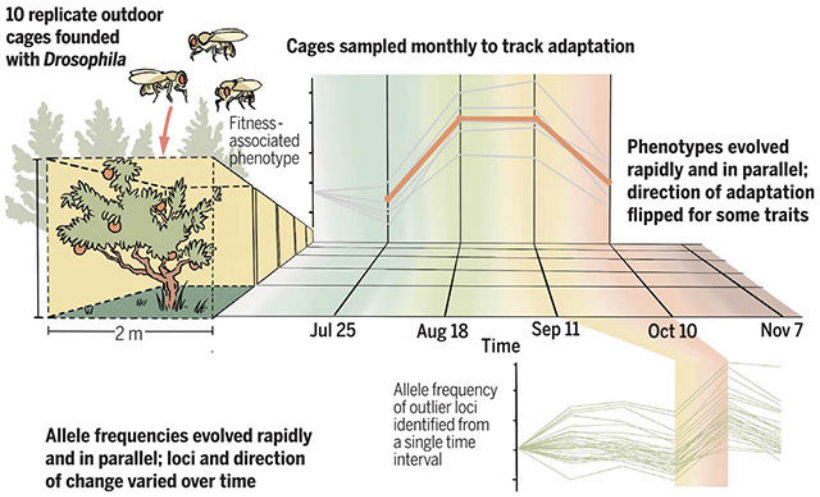
RATIONALE: The design of our field experiment focused on three key elements to study adaptive tracking in response to ongoing environmental change: (i) generating highly accurate measurements of phenotypic and genomic evolution, (ii) taking these measurements on a time scale similar to that of natural environmental change, and (iii) collecting measurements from independent replicate populations experiencing similar environmental conditions. Specifically, we directly measured phenotypic and genomic evolution in response to natural seasonal change across 10 independent replicate field populations, each comprising up to 100,000 *Drosophila melanogaster* individuals. We measured the evolution of heritable phenotypes related to stress tolerance and reproductive output under typical laboratory conditions (repeated common-garden rearing) over monthly intervals from July to November 2014. Individuals collected at the same intervals from each population were used for full-genome pooled sequencing to measure genomic change over time. We specifically assessed the degree of parallel phenotypic and genomic change across replicate populations, as any parallelism would be strong evidence of the deterministic evolutionary process of adaptation. We further looked for reversals in the direction of adaptation over time, which would demonstrate variation in the direction of natural selection (termed fluctuating selection) and would provide strong evidence for adaptive tracking.

RESULTS: We assayed phenotypic evolution of six fitness-associated phenotypes and genomic evolution at 1.9 million single-nucleotide polymorphic sites across the genome. We found clear evidence of parallel phenotypic and genomic evolution indicative of adaptive tracking. Phenotypic adaptation was pervasive and remarkably fast relative to measures of trait change from many prior studies. Moreover, the direction of evolution changed over time for development rate (a key component of reproductive output) and desiccation tolerance (an important trait in stress resistance). Similar patterns were observed in genomic data, with clear signatures of parallel allele frequency change across time intervals, including some that resulted from fluctuating selection. Genomic regions implicated in selection were found on all chromosomes, yet most were under selection over only a single time interval. At least 165 independent genomic regions were under selection during the experiment; allele frequencies at >60% of variant sites genome-wide were shaped in part by selection. Taken together, these data underscore that natural selection is capable of driving evolution in multiple fitness-associated phenotypes and much of the genome even over short time scales.

CONCLUSION: Our experiment shows the feasibility of observing adaptive tracking in response to environmental change in real time using replicated field experiments. Our data from this approach support a model of adaptive tracking in which populations adapt in response to continuous environmental change, with selection acting on multiple phenotypes and tens to hundreds of genetic variants. The pronounced action of fluctuating selection suggests that evolutionary rates may be systematically underestimated and that fluctuating selection could be an underappreciated mechanism that maintains diversity. Determining whether adaptive tracking is a general feature of natural populations, and elucidating the mechanisms by which it occurs, could be transformative for understanding the generation and maintenance of biodiversity.

Graphical Abstract



Adaptation tracks ongoing environmental change. We conducted a replicated longitudinal field experiment using outbred *Drosophila* populations to assess the phenotypic and genomic basis of adaptation in response to natural seasonal environmental change. We observed fast phenotypic adaptation in multiple phenotypes, parallel change in allele frequencies at dozens of independent loci, and reversals in the direction of adaptative evolution for some traits and loci over monthly time intervals.

Abstract

Direct observation of evolution in response to natural environmental change can resolve fundamental questions about adaptation, including its pace, temporal dynamics, and underlying phenotypic and genomic architecture. We tracked the evolution of fitness-associated phenotypes and allele frequencies genome-wide in 10 replicate field populations of **Drosophila melanogaster** over 10 generations from summer to late fall. Adaptation was evident over each sampling interval (one to four generations), with exceptionally rapid phenotypic adaptation and large allele frequency shifts at many independent loci. The direction and basis of the adaptive response shifted repeatedly over time, consistent with the action of strong and rapidly fluctuating selection. Overall, we found clear phenotypic and genomic evidence of adaptive tracking occurring contemporaneously with environmental change, thus demonstrating the temporally dynamic nature of adaptation.

Continuous adaptation in response to rapidly changing environmental conditions, termed adaptive tracking, could be a crucial mechanism by which populations respond to environmental change. Adaptive tracking has historically received little study because of the impression that adaptive evolutionary change is too slow to track complex and rapidly changing selection pressures in the wild (1). Moreover, theory suggests that variable and complex selective pressures should in general lead to the evolution of phenotypic plasticity or bet-hedging (2, 3). Yet multiple longitudinal field studies and experiments demonstrate that adaptation can indeed occur very rapidly at individual traits or loci in response to strong environmental perturbations (4–10). Whether this translates into populations undergoing adaptive tracking in response to multifarious ecological changes, when theory predicts

that pleiotropy (cases where a single gene affects multiple traits) should constrain natural selection and prevent adaptive tracking (11, 12), is unknown. If adaptive tracking does indeed occur in such situations, it would have broad implications for our understanding of the limits and pace of polygenic adaptation (13), the prevalence of fluctuating selection (14) and its role in the maintenance of genetic variation (15), and the importance of rapid adaptation in ecological outcomes (16).

Identification of adaptive tracking requires direct measurement of phenotypic and genotypic evolution across replicate field populations in response to ongoing natural environmental change. Ideally, an experimental system would provide (i) the means for highly accurate measurements of even subtle, heritable shifts in key independent fitness-related phenotypes and loci under selection; (ii) the ability to assay multiple replicate populations exhibiting some degree of ecological and environmental realism to detect parallel genetic and phenotypic changes indicative of adaptation (17); and (iii) high-resolution temporal sampling to quantify rapid fluctuations in the magnitude and direction of selection as environmental changes occur.

Here, we used field mesocosms to measure the extent, pace, repeatability, and genomic basis of adaptive tracking in *Drosophila melanogaster* within the naturally fluctuating, temperate environment of a single growing season in Pennsylvania, USA (10, 18, 19) (Fig. 1). The design precluded migration and allowed populations to expand to a large adult census size (on the order of 100,000 adults in each replicate at the maximum population size). To initiate the experiment, we derived an out-bred baseline population of *D. melanogaster* from a set of 80 inbred strains originally collected in the spring from Pennsylvania (table S1). On 15 July 2014, 10 replicate cages were each founded with 1000 individuals from the baseline population. All populations were fed every second day and tracked until the first hard frost on 7 November 2014. Specifically, at four time points we measured the evolution of six complex, fitness-associated phenotypes, focusing on those related to either reproductive output (fecundity, egg size, and development rate) or stress tolerance (starvation tolerance, chill coma recovery, and desiccation tolerance) (Fig. 1). To do so, we repeatedly collected and reared individuals from each field cage in standard laboratory conditions (i.e., multigeneration common garden) to distinguish evolution from phenotypic plasticity. All phenotypes were measured in the F_3 generation. We also tracked changes in allele frequencies genome-wide in each replicate using pooled sequencing at five time points. We employed haplotype-based allele frequency estimation (20) to generate highly accurate allele frequency trajectories. We assessed changes in allele frequency at individual sites and in clusters of linked sites to assess the magnitude and genetic architecture of adaptation.

Phenotypic patterns of adaptation and adaptive tracking

Population dynamics were largely consistent among the replicates; population size increased sharply during summer, peaked in early fall, and then declined steadily as minimum daily temperatures declined in late fall (Fig. 1). These population dynamics mimic the patterns observed in *D. melanogaster* populations (21) and many other multivoltine organisms inhabiting temperate natural environments, with summer exponential growth, high densities

in late summer to early fall, and late-fall population declines. Egg production showed a similar pattern (fig. S1), and overall recruitment from egg to adult was low (fig. S2). Similarity in the ecological conditions among replicate populations, including abiotic factors (fig. S3) and population dynamics (Fig. 1), suggests that similar selective landscapes may have driven parallel evolution across replicates.

Phenotypic evolution was rapid and parallel, but temporal patterns varied across traits. To measure phenotypic evolution, we sampled individuals from the founding population and ~2500 eggs from each cage at the first four time points (25 July, 18 August, 11 September, 10 October), reared them in common-garden laboratory conditions for three generations, and assayed phenotypes in the F_3 progeny (Fig. 1). For all six phenotypes, which are known to be polygenic and associated with fitness (22), we observed substantial trait evolution with an average of 23% change in the mean trait value for each cage across all phenotypes over each time interval. Variation in environmental parameters among cages did not implicate any individual factors as agents of selection (fig. S4); this may be attributable to the limited variation between cages or the complexity of the selective landscape. Prior experiments conducted in these mesocosms have found evidence of rapid adaptation in response to experimentally manipulated agents of divergent selection (10, 19).

All six phenotypes showed evidence of parallel evolution, indicative of adaptation, over time. Four of the six phenotypes evolved rapidly, repeatedly, and in a consistent direction across the duration of the experiment (fecundity, $F_{3,27} = 43.75$, $P < 0.0001$; egg size, $F_{3,27} = 11.5$, $P < 0.0001$; starvation, $F_{4,36} = 129.05$, $P < 0.0001$; chill coma recovery, $F_{4,36} = 197.75$, $P < 0.0001$) (Fig. 2). The magnitude of change was often substantial. For example, the average increase in fecundity was 61% over each monthly sampling interval across replicates, representing one to four overlapping generations. Desiccation tolerance and development rate also evolved rapidly and in parallel ($F_{4,36} = 86.66$, $P < 0.0001$, Fig. 2C; $F_{4,36} = 98.70$, $P < 0.0001$, Fig. 2F), but the direction of evolution varied over time. Fluctuations in the direction of evolution for these phenotypes had considerable effects on phenotypic trajectories; for desiccation tolerance, the amount of evolution measured over the whole experiment (founder to 10 October) was less than what was observed over the first interval (founder to 25 July). Identifying the fitness effects of any specific instance of phenotypic evolution is complicated by underlying correlations among traits, pleiotropy, and an unknown (and potentially temporally variable) phenotype-to-fitness map, but the pace and parallelism of phenotypic evolution are suggestive of strong links to fitness.

The pace of parallel trait evolution observed over the short time scales examined in this study was unusually fast. As expected, we observed rapid parallel evolution when outbred laboratory populations were introduced into the field enclosures and adapted to the field environment (founder $\rightarrow T_1$). However, we also observed evidence of rapid adaptation between intervals in the enclosures for all six phenotypes, with some showing reversals in the direction of evolution across intervals (Fig. 2, C and F). The rate of phenotypic adaptation, calculated in haldanes [phenotypic evolution in units of standard deviations of the trait per generation (23, 24)], was computed as a mean change across replicates for each phenotype over each interval and across the whole experiment (Fig. 2G). The rate of adaptation over the whole experiment ranged from moderate to extremely fast for different

traits (0 to 0.8 haldanes) (25). However, when calculated over each sampling interval, the rate of adaptation was often comparable to or faster than the pace of phenotypic change measured in any prior field study or experiment (Fig. 2G).

The pace, magnitude, and parallelism of the phenotypic evolution we observed is notable for three reasons: (i) The evolutionary rates were calculated on the basis of the phenotypic shifts of the F_3 progeny in common-garden conditions, thus excluding phenotypic plasticity as the driver of change. (ii) Because we focused only on the parallel phenotypic shifts across the cages, our estimates describe the rate of putatively adaptive phenotypic change. (iii) These patterns of rapid adaptation were observed for multiple fitness-associated phenotypes, each with a complex and likely distinct genetic architecture (26). Overall, our results show that strong and temporally variable natural selection can consistently drive rapid and polygenic adaptation of multiple fitness-associated phenotypes on the same time scale as the environmental change.

Genomic patterns of rapid adaptation and adaptive tracking

To investigate the genomic architecture underlying the observed rapid phenotypic adaptation, we performed whole-genome pooled sequencing of 100 randomly selected individuals from the baseline population and from each replicate population at five time points across the experiment (Fig. 1). Allele frequencies at 1.9 million biallelic sites were inferred for each sample via haplotype inference using HAF-pipe (20) at accuracy levels consistent with an “effective coverage” of >100× (fig. S5 and table S2) (27). This high-resolution dataset yielded strong evidence for rapid genome-wide evolution. Specifically, we observed that the genome-wide estimates of F_{ST} between the founder population and all five monthly time points (mean 0.0030 ± 0.0002 standard error) exceeded expected margins of error based on technical and biological replicates (0.00026 ± 0.000024 and 0.0018 ± 0.000048, respectively, $P<2\times10^{-8}$, t test; Fig. 3). Furthermore, divergence from the founder population changed significantly over time, both genome-wide ($P < 2.3 \times 10^{-5}$, Kruskal-Wallace P value for difference in means across time points) and for individual chromosomes ($P < 0.006$; fig. S6). Given the large population sizes (up to 10^5), it is unlikely that such substantial evolutionary change can be attributed solely to random genetic drift.

Further examination of the magnitude and direction of evolution across the 10 replicate cages showed substantial genomic adaptation, as defined by parallel, and thus deterministic, allele frequency shifts across replicate cages. To test for parallel shifts, we used a leave-one-out cross-validation approach. For each monthly time interval ($T_i \rightarrow T_{i+1}$, $i = 1, 2, 3, 4$), we used a generalized linear model (GLM) to identify sets of single-nucleotide polymorphisms (SNPs) whose frequency shifted significantly across the nine training cages, and then tested whether shifts at those SNPs in the 10th left-out cage exceeded shifts at randomly chosen matched control sites. Using this test, we found widespread parallel genomic adaptation for the first three sampling intervals (in 28 of 30 leave-one-out tests) (Fig. 4B). The pattern of parallelism was muted and evolution was more idiosyncratic in $T_4 \rightarrow T_5$. We also repeated the procedure for SNPs that shifted across the whole experiment ($T_1 \rightarrow T_5$) and found a similarly strong signal of parallel adaptation (10 of 10 tests). The magnitude of allele frequency shifts in each interval (2 to 8%) and over the whole experiment (2 to 5%)

corresponded to very strong effective selection strength at the most parallel sites of ~10 to 50% per monthly interval (one to four generations) (27). This pattern was largely repeated when analyzing sites from each chromosome individually (fig. S7). In simulated populations with the same demographics as the experimental populations, allele frequency shifts of this magnitude were consistently achieved with selection coefficients of ≤50% on alleles spanning a wide range of initial frequencies over similar time scales (table S3) (27). The pronounced parallel shifts in allele frequency across independent populations demonstrate the strong action of natural selection.

Our cross-validation analysis also yielded clear evidence of variation in the magnitude and direction of selection over time, consistent with the observed patterns of phenotypic evolution for some traits (Fig. 2). Specifically, the leave-one-out analysis and the time-series genomic data allowed us to examine the full trajectory of alleles detected at any specific time interval (T_{det}). We found that these alleles often shifted significantly more than alleles at control sites (Fig. 4B) at other time intervals; however, the nature of these shifts varied over time. In some left-out cages and at some time intervals, alleles shifted in a direction consistent with their behavior during T_{det} (orange points); however, in other cases the direction flipped, resulting in significant reverse shifts (blue points). Reverse shifts were strongest for sites with $T_{det} = T_3 \rightarrow T_4$ (August → September) during the time when populations expanded most rapidly and reached their maximum. These $T_3 \rightarrow T_4$ parallel sites showed consistent shifts in the opposite direction during the preceding interval ($T_2 \rightarrow T_3$, July → August) when the populations were still expanding. In many cages, these sites also shifted in the opposite direction during the subsequent ($T_4 \rightarrow T_5$, October → November) interval when population sizes were declining. These patterns likely reflect the action of rapidly fluctuating selection over the 4 months of the experiment.

With a complex and rapidly fluctuating selective landscape, adaptation occurs over multiple time scales simultaneously, with clear and distinct signatures of adaptation over each interval and over the whole duration of the experiment. Hence, inferred rates of adaptation are dependent on the time scale of sampling (13). Our results clearly illustrate the extent to which lower-resolution temporal sampling would obscure the inference of adaptive tracking. Although sites identified during individual time intervals often showed median shifts of >2% in a single month, the strongest parallel sites detected from lower-resolution sampling (i.e., sampling only at T_1 and T_5) showed smaller monotonic shifts at each interval (on average, 0.6% per month). Moreover, the magnitude of this discrepancy varied widely over time. Taken together, these results underscore the value of high-resolution temporal sampling in revealing the existence of both temporally variable and temporally consistent directional selective forces.

Identifying the genomic architecture and putative functions of causal loci

The number and genomic locations of causal loci involved in adaptation are central to understanding the mechanics of the adaptive process (28). To quantify the genomic architecture of adaptation, we examined the distribution of parallel sites across the genome and developed an algorithm to differentiate putatively independent targets of selection from the sites whose shifts could largely be ascribed to linkage disequilibrium and genomic

hitchhiking (genetic draft). We first fit allele frequencies from all 10 cages to a GLM and identified significantly parallel sites (fig. S8) at each time segment ($n = 4274$) and across the whole experiment ($n = 5036$), yielding 9310 significant shifts overall (Fig. 5A and table S4) (27). As expected from the leave-one-out analysis, the sets were largely nonoverlapping: The 9310 detected parallel shifts occurred at 9000 unique SNPs. Moreover, at each time interval and across the whole experiment, parallel sites were strongly clustered (empirical $P < 0.01$; fig. S9) and also showed significantly higher average linkage values than the matched control sites ($P < 10^{-16}$, paired t test; fig. S10) (27), which suggests that most parallel sites were merely linked to causal loci rather than being causal themselves.

We next identified the minimum number of independent genetic loci under selection using an algorithm that aggregated the parallel sites into clusters of linked sites (fig. S10) (27). This algorithm clustered 8214 parallel SNPs detected across all the time segments [~90% of all SNPs at a false discovery rate (FDR) of <0.05] into 165 unlinked independent clusters (Fig. 5A and table S5). These clusters were found on every chromosome and at every time segment, with an average of 4.5 clusters per chromosome per month. Simulations confirmed that although interference among multiple causal sites can temper shifts at any individual site, the number of clusters detected here still fell well within the realm of plausible selection landscapes. Specifically, when allele frequency trajectories for pairs, groups of 5, or groups of 10 selected loci were simulated simultaneously on the same chromosome, the majority (61.5%) of simulated selected sites required selection strengths no greater than $s = 0.5$ to achieve a minimum shift of 2% per monthly time segment, and the vast majority (80.2%) required selection strengths no greater than $s = 1$. Furthermore, although inversions can drive patterns of adaptation in *Drosophila* (29, 30), no inversion markers were found among the parallel sites, and only three of the 165 clusters were strongly linked to inversions with average $R^2 > 0.1$ (table S7 and fig. S11). Combining clusters from all time segments, 61% of all assayed SNPs and 62% of the genome were contained in at least one cluster, highlighting the pervasive impact of short-term adaptive evolution at tens to hundreds of independent selected sites on allele frequencies genome-wide.

The genomic distribution and frequency shifts of these clusters suggested rapid changes in the targets and direction of selection over time. Specifically, 36 of the 90 clusters (40%) identified at a specific monthly time interval did not overlap any clusters identified at other monthly intervals, which suggests that selection at these loci was limited to 1 month. Among the remaining 54 clusters, only 27 (50%) contained SNPs that were significantly linked to SNPs in the cluster they overlapped. These 27 clusters formed nine distinct "super-clusters" (Fig. 5A) with high internal linkage, representing genomic regions in which allele frequencies shifted significantly in multiple monthly intervals. Strikingly, in five of the six superclusters involving a cluster from $T_3 \rightarrow T_4$ linked to a cluster from $T_4 \rightarrow T_5$, 90% of SNPs flipped direction between months, and in the sixth cluster >80% flipped direction, together totaling 10,464 SNPs that flipped direction (fig. S12). A smaller majority of SNPs (67%) flipped in the supercluster formed by a cluster from $T_2 \rightarrow T_3$ linked to a cluster from $T_3 \rightarrow T_4$. Finally, in the two superclusters involving sets of linked clusters from three different time segments ($T_2 \rightarrow T_3$, $T_3 \rightarrow T_4$, $T_4 \rightarrow T_5$), together covering more than 5 Mb of chromosome arm 3L, most SNPs (72% and 85%, respectively) flipped direction twice. We further confirmed that similar dynamics characterized the full set of

putatively causal SNPs by choosing the SNP with the strongest parallelism *P* value in each cluster and examining its trajectory (Fig. 5B). Even though the initial frequencies of these marker SNPs (fig. S13) and the exact shape of their trajectories varied widely, we observed a consistent trend: Markers for the clusters identified at an individual monthly time interval often changed little during other months or even moved in the opposite direction (especially clusters identified at $T_3 \rightarrow T_4$), whereas markers for clusters identified across the whole experiment tended to shift evenly and monotonically over time. The analysis of overlapping clusters and marker SNPs revealed patterns similar to those seen in individual SNP-based analyses, together supporting an oligogenic and rapid adaptive response to momentary selection pressures that often results in strong and rapidly fluctuating selection.

We next tested whether the identified genomic targets of this rapidly fluctuating selection were associated with any specific phenotypic traits (31) or pathways. We specifically investigated the set of 111 genes–one per cluster–that overlapped with the cluster's top marker SNP. This set of genes is strongly enriched ($P < 0.001$ in all cases) for genes with a known phenotypic effect (85 genes), and more specifically for genes involved in behavior (27 genes), cell-to-cell signaling (34 genes), neuronal function (25 genes), synaptic function (14 genes), and the central nervous system (21 genes) (table S6). Many of these genes are crucial to core developmental and signaling pathways including the Wnt signaling pathway [genes *frizzled2* (the receptor of *wingless*), *armadillo* (β-catenin), *sgg* (GSK3), *flo2* (long-range Wnt signaling), *reck* (regulation of Wnt signaling), *huwe1* (negative regulation of Wnt signaling), and *tkv* (dpp/BMP signaling)]. Strikingly, one cluster marker SNP is found in *SNF4Aγ*, the gamma subunit of the central metabolic switch kinase AMPK (adenosine 5'-monophosphate–activated protein kinase). *SNF4Aγ* is one of two key genes previously found to be involved in adaptation to high temperature during experimental evolution of a sibling species, *D. simulans* (32). On balance, these patterns suggest that adaptive tracking in our outdoor mesocosms may be driven by shifts in the function of neuronal, metabolic, and development pathways that modulate sensing and regulatory processes and affect the way environmental cues are interpreted by the organism.

Discussion

The phenotypic and genomic patterns we observed are consistent with a form of adaptive tracking in which (i) populations adapt in response to continuous environmental shifts, (ii) parallel evolution is driven by strong selection on multiple phenotypes and on a substantial number (tens to hundreds) of strongly selected genetic variants, (iii) the identity of the phenotypes and variants under selection changes considerably over short time scales, and (iv) selection operates at multiple time scales, acting in a consistent direction across the whole experiment on some variants and phenotypes but rapidly fluctuating in direction and magnitude at others (33). This fluctuating selection leads to inferred rates of adaptation being slower when measured from the beginning to the end of the experiment as compared to single monthly intervals. The observed pattern that evolutionary rates are fastest when measured over shorter time scales may be driven by fluctuating selection (13, 34).

The pace, complex architecture of adaptation, and temporal evolution of some phenotypes in our field cages are generally consistent with prior observations of seasonal evolution in

natural temperate populations of *D. melanogaster* (21, 35–37). However, with additional temporal resolution and replication, we detected rapidly fluctuating patterns of adaptation that suggest that populations of *D. melanogaster* are continuously and adaptively tracking the environment; this is surprising but not implausible given the pace of environmental change (38). These patterns also imply that segregating functional variation is abundant and that much of the segregating variation in fitness is likely due to balancing selection (39), including temporally fluctuating selection that maintains genetic variation (14, 40, 41). The functional analysis of the genomic regions under selection further suggests that the rapid adaptation detected here is likely driven by modulation of high-level signaling pathways that feed into developmental and neuronal functions capable of modifying multiple phenotypes in a coordinated fashion. This may explain how selection can rapidly modify so many ostensibly unrelated phenotypes at the same time.

Our experiment shows that it is possible to observe adaptive tracking in real time, thereby providing a new lens to study the synchronous ecological and evolutionary dynamics of natural populations. We have focused here on *D. melanogaster*, but the environmental and organismal features that gave rise to adaptive tracking, such as the presence of strongly shifting environmental pressures on generational time scales, are likely common (7, 8, 42, 43). Understanding the complex interplay among environmental change, population dynamics, standing genetic variation, and trait architecture that dictates the extent of adaptive tracking is a considerable challenge. Determining whether adaptive tracking is a general feature of natural populations and defining the factors that shape the extent of adaptive tracking could be transformative in understanding the generation and maintenance of biodiversity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and materials availability:

We have deposited census and phenotypic evolution data in the Dryad digital repository (doi: 10.5061/dryad.9zw3r22gg). Sequenced founder lines can be found at NCBI Accession PRJNA722305. Sequencing data from evolved cages can be found at NCBI Accession PRJNA793595. Scripts for the genomic analysis and simulations can be found at https://doi.org/10.5281/zenodo.5839005 (44).

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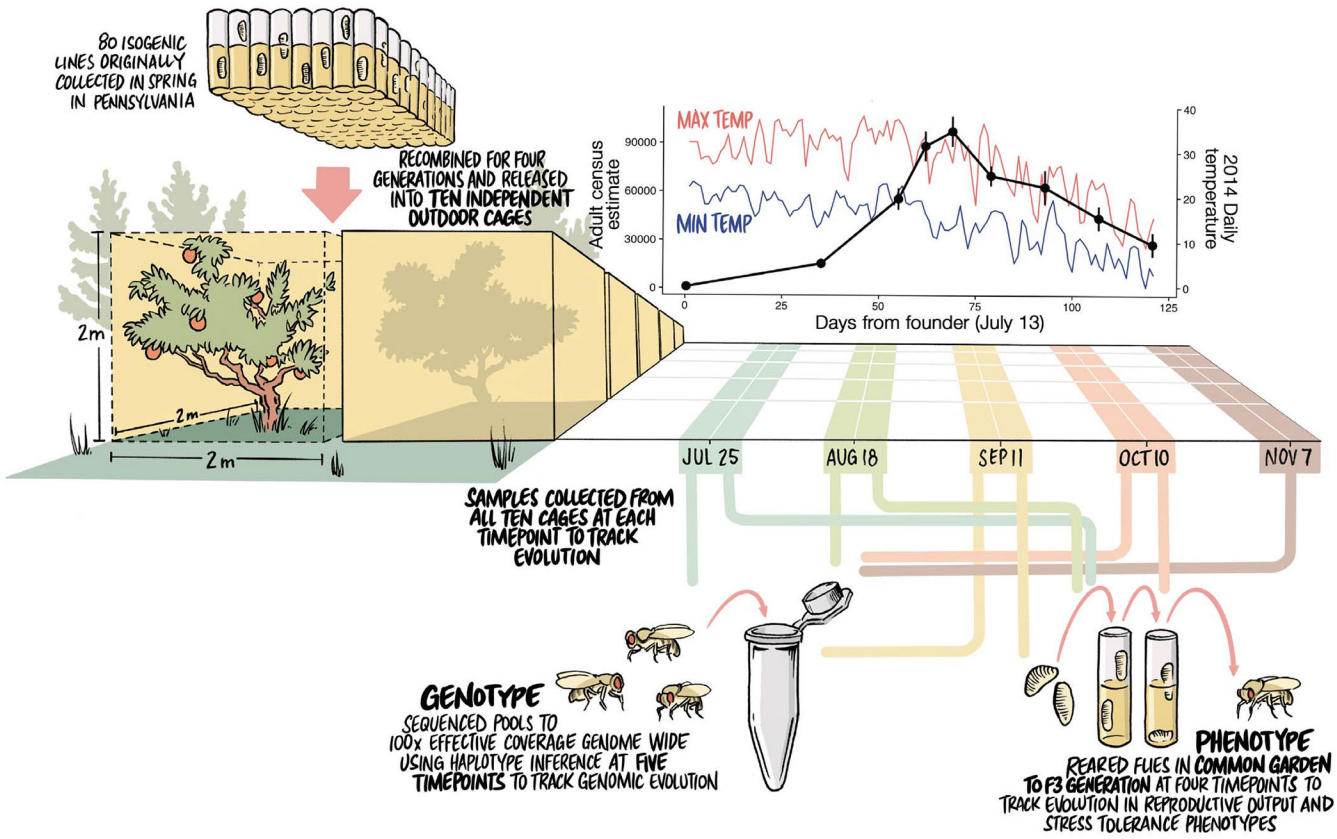

Fig. 1. Experimental arena, design, and population dynamics.

The experiment was designed to reflect ecological and evolutionary realism while testing for adaptation using replicate populations. Eighty inbred lines originally collected in spring from an orchard in Pennsylvania were recombined and expanded for four generations into a genetically diverse outbred population in the laboratory. From this outbred population, we used 500 males and 500 females to found each of 10 independent outdoor cages (2 m × 2 m × 2 m). We measured daily minimum and maximum temperatures (blue and red lines, respectively) and estimated adult population size of each replicate over 4 months of seasonal change (black line, mean; gray lines, per replicate). To study adaptation, we tracked phenotypic evolution by collecting eggs from each replicate, rearing them in common-garden laboratory conditions for three generations, and then measuring six fitness-associated phenotypes. We conducted this procedure on the founder population and at four subsequent time points to measure phenotypic evolution over time. To study adaptation at the genomic level, we sequenced pools of 100 females from each cage to >100× effective coverage at five time points and assessed changes in allele frequencies.

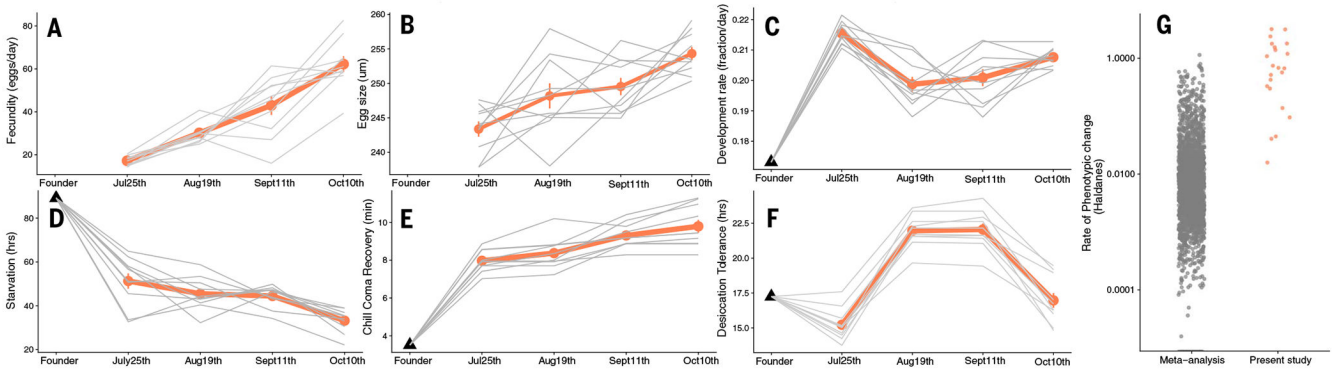

Fig. 2. Parallel evolution of stress tolerance traits, reproductive output traits, and comparison of the rate of adaptation.

(**A** to **F**) Trajectories of phenotypic evolution for reproductive-associated traits [(A) to (C)] and stress resistance traits [(D) to (F)] as measured after three generations of common-garden rearing. (A) Mean fecundity as number of eggs per female per day. (B) Mean egg size. (C) Development rate as the fraction of development to pupation completed in 1 day [1/(total hours/24)]. (D) Starvation tolerance as time to death by starvation. (E) Recovery time after chill coma. (F) Desiccation tolerance as time to death from desiccation. Black points are the mean phenotypes of the founding population, gray lines represent mean phenotypic trajectories of individual populations, and red lines are the mean of all cage means. (**G**) Comparison of the rates of adaptation from this experiment over individual intervals (red) to rates of phenotypic change from a prior meta-analysis (gray) (25).

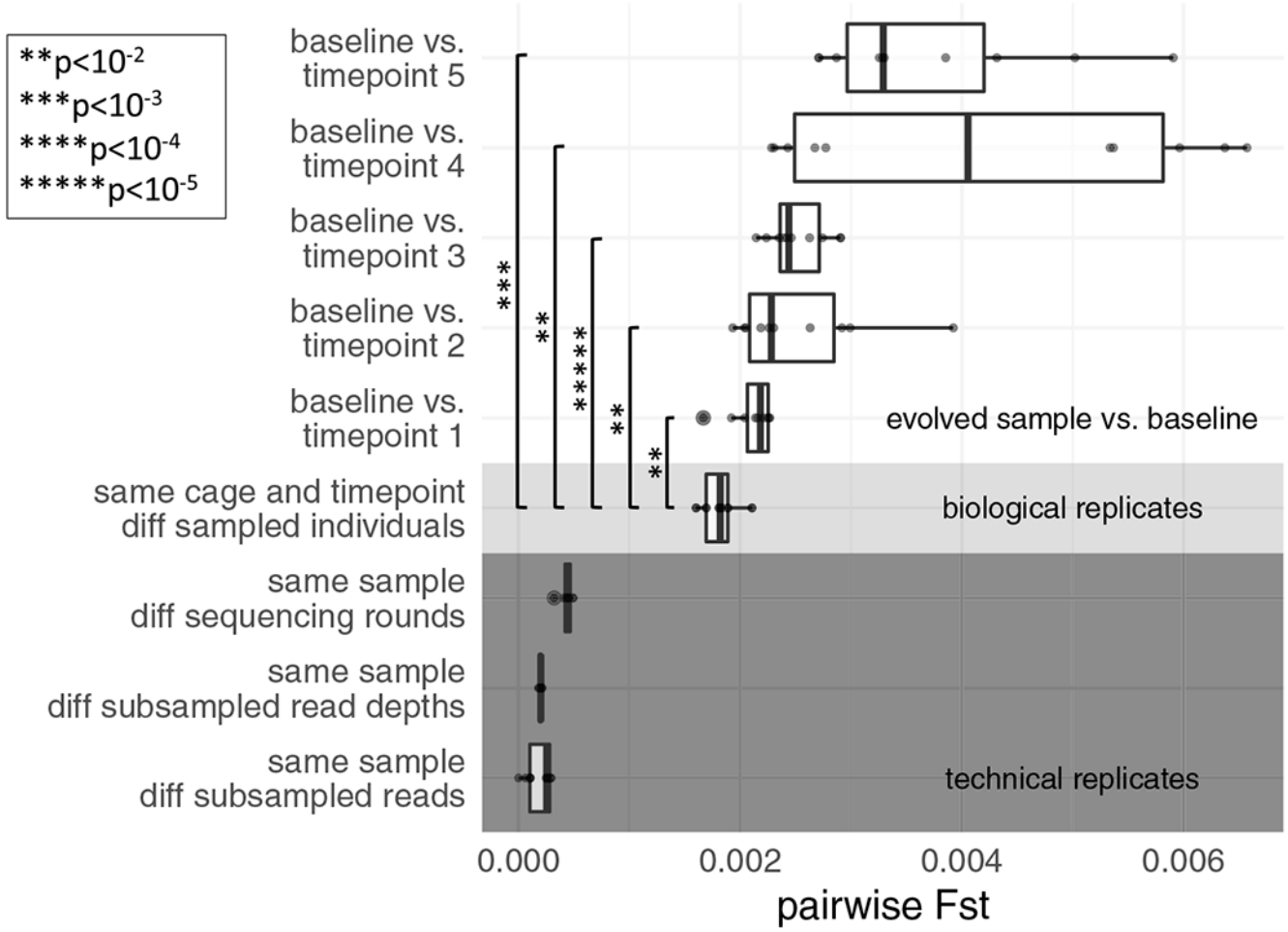

Fig. 3. Genome-wide evidence of evolutionary divergence.

Distributions of genome-wide mean pairwise F_{ST} values between technical replicates (dark gray; same flies, different reads), biological replicates (light gray; different flies, same time point), and experimental samples from different time points compared to baseline (white). Note that negligible F_{ST} values between pairs of technical replicates are consistent with extreme precision of haplotype-derived allele frequencies (HAFs), which suggests that the variance in allele frequency estimates for biological replicates is primarily driven by sampling of different individuals. Asterisks represent the significance of divergence over time relative to biological replicates (*t* test).

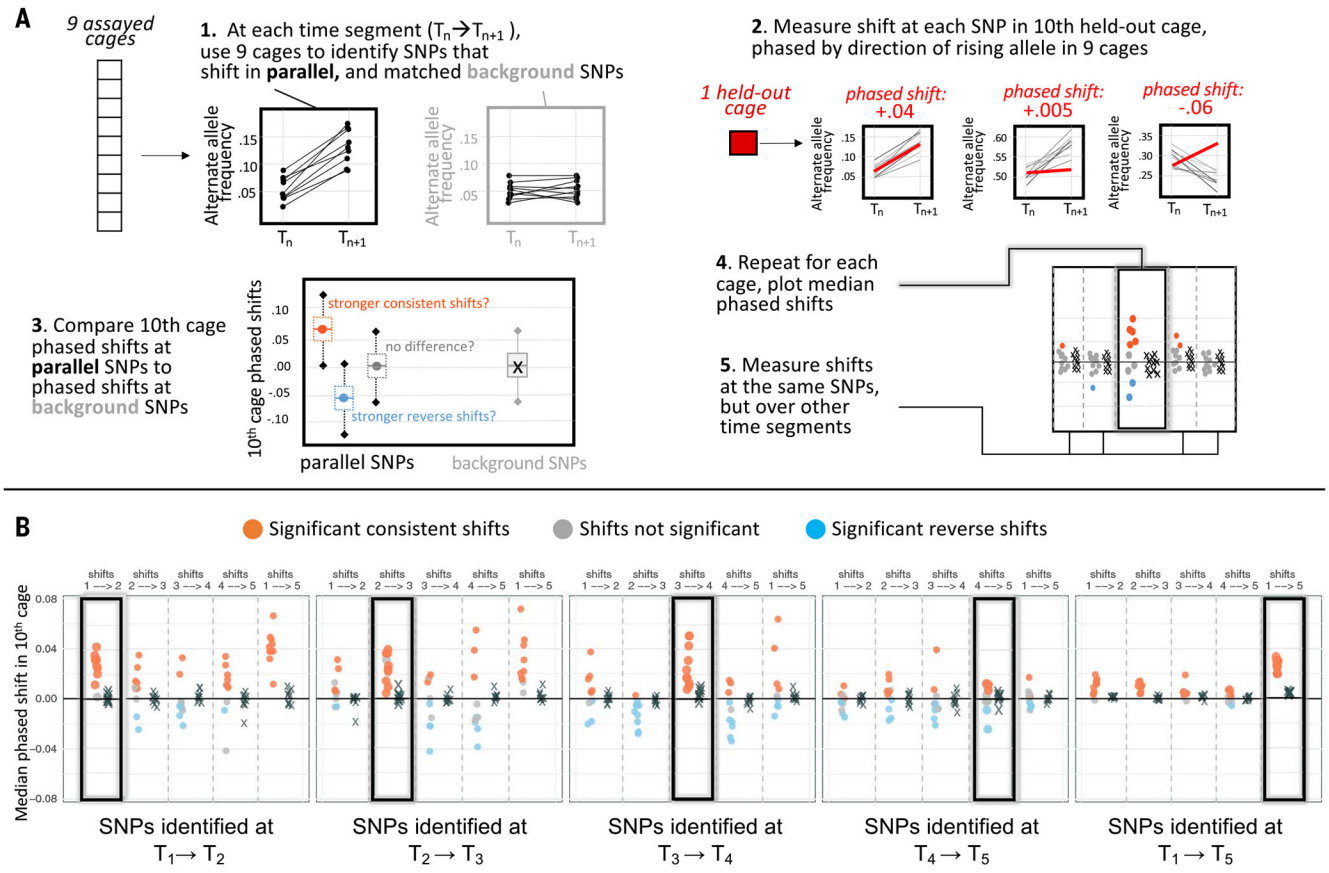

Fig. 4. Using genomic data to test for evolutionary parallelism indicative of adaptation.

(**A**) Graphical description of the leave-one-out 10-fold cross-validation process for significant sites. In each round, significantly parallel sites (FDR < 0.05, effect size > 2%) at each time segment were identified using nine of the 10 cages, then the shift at those sites in the 10th left-out cage was measured, after phasing such that positive values represent shifts in the same direction as the nine assayed cages and negative values represent shifts in the reverse direction. The set of phased shifts at parallel sites was compared to phased shifts at background sites matched for chromosome and initial frequency and assigned to one of three significance bins: consistent (orange) or reverse (blue), or no significant difference from background (gray). Shifts at these same sites over other time segments were also measured, phased, and assigned to significance bins. (**B**) The median shift for each set of parallel sites (circles) and background sites (x marks) is plotted for each left-out cage. Each block of five panels represents shifts at the same sets of sites, those identified as parallel at the time segment labeled below the block. Shifts measured at that same time segment are highlighted in the panel with a dark shadowed outline.

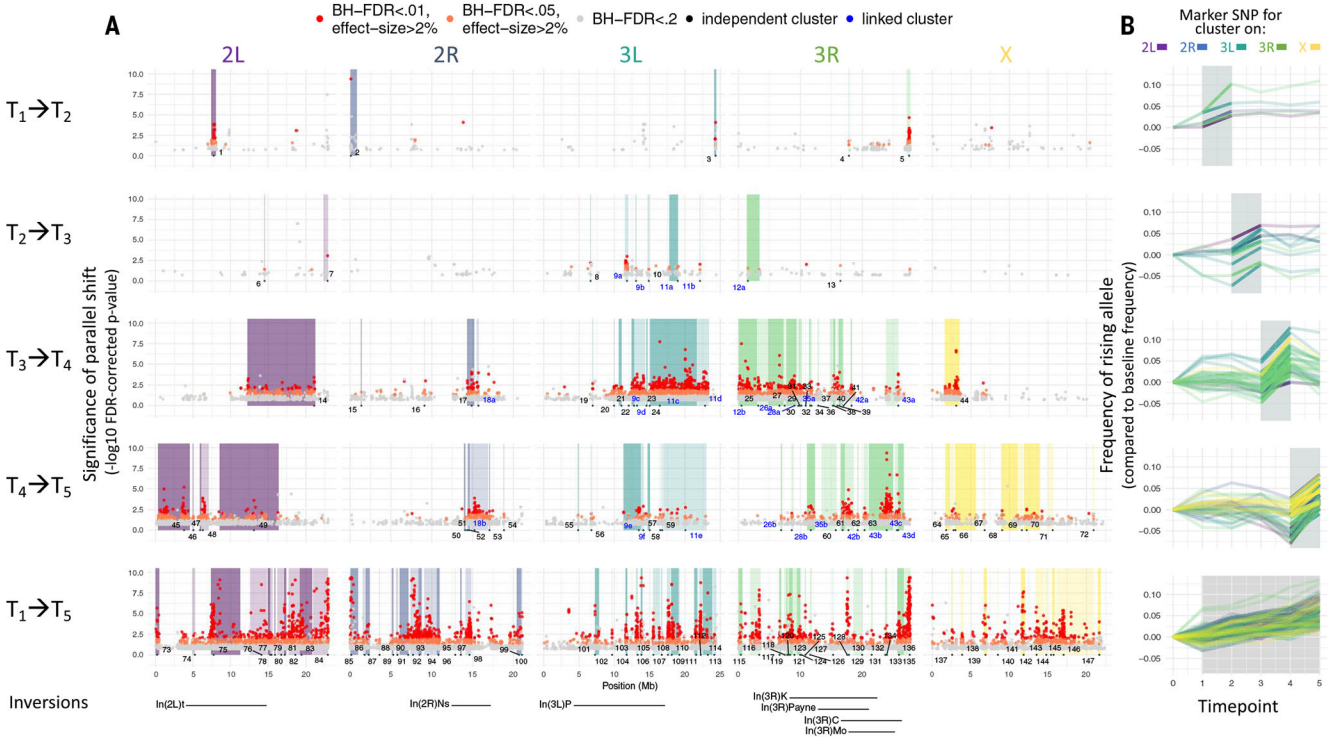

Fig. 5. The genomic architecture of parallel allele frequency change over time.

(**A**) Manhattan plot of sites with significant parallel allele frequency shifts over time in 10 replicate cages. Each dot shows the $-\log_{10}$ of the FDR-corrected *P* value (*y* axis) corresponding to the significance of the allele frequency shift at a given SNP position (*x* axis) over a given time segment of the experiment (rows). Only SNPs with an FDR of <0.2 are shown, and dots are colored according to three significance bins (top). Shaded areas indicate regions of the genome that are likely driven by the same causal site, as defined by a clustering algorithm accounting for SNP linkage. Each clustered genome block is identified by a number marking the position of the top parallel SNP. Clusters from different time segments that are significantly linked ("superclusters") are given the same number, labeled in blue. The positions of seven common chromosomal inversions are indicated below. (**B**) Allele frequency trajectories are shown for the top marker SNP from each cluster. Each trajectory is translated to show allele frequency change relative to initial frequency in the baseline population and phased to show the frequency of the rising allele at the time segment in which the cluster was identified. The time segment over which the SNPs were identified as outliers is shaded in gray.