Variation in Resource Environment Drives Adaptive Divergence in *Drosophila melanogaster*

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ABSTRACT

Natural populations often experience heterogeneity in the quality and abundance of environmentally acquired resources across both space and time, and this variation can influence population demographics and evolutionary dynamics. In this study, we directly manipulate resource quality in replicate populations of Drosophila melanogaster cultured in experimental mesocosms in the field. We found no significant effect of resource variability on demographic patterns. Furthermore, while resource variation altered the patterns of phenotypic and genomic evolution, this effect is secondary to population responses to seasonally fluctuating selective pressures. Seasonal adaptation was observed for all traits assayed and was highly polygenic; in contrast, adaptation to the resource environment was trait-specific and exhibited an oligogenic architecture. This illustrates the capacity of populations to adapt to an additional axis of variation (resource availability) without hindering the adaptive response to seasonal change. This ability for populations to simultaneously track and respond to variations in multiple environmental factors likely drives divergent or fluctuating selection, contributing to the maintenance of genetic and phenotypic variation in natural populations.

INTRODUCTION

Identifying the environmental factors that shape population demographics and evolutionary dynamics remains a fundamental goal in ecology and evolution. A major component of variation in natural habitats is the availability and quality of resources (e.g., water, light, nitrogen, food supply, etc.). Variation in the quality or availability of critical resources can impact the growth and productivity of populations (Blaustein *et al.* 2003; Gilg *et al.* 2003; Schindler 1974) and alter selection pressures that shift patterns of adaptive evolution (Grant & Grant 1989). As ecological and evolutionary processes can occur on the same rapid timescales (Hendry *et al.* 2007; Hendry & Kinnison 1999; Johnston & Selander 1964; Reznick *et al.* 1997), an open question is whether variation in environmental resources can simultaneously drive both ecological and evolutionary dynamics.

Environmental quality affects population demographics in various systems and contexts (Belovsky *et al.* 1999; Franken & Hik 2004; Griffen & Drake 2008; Hakoyama *et al.* 2000; J. S. Bancroft 2003; Klok & De Roos 1998; MacArthur 1984; Philippi *et al.* 1987; Pimm *et al.* 1988). Furthermore, the quality and availability of environmentally acquired resources are used as predictors when estimating the growth rate and carrying capacity of populations (Hobbs *et al.* 1982) and may also affect different components of fitness (Chatelain *et al.* 2021; Mitrovski & Hoffmann 2001). Resource-limited population growth is especially prevalent in systems where water or food supplies are seasonal or ephemeral, impacting local rates of extinction (Longman *et al.* 2023; Schindler 1974).

Adaptive responses to variation in the biotic and abiotic environmental elements can occur over extremely short intervals (Bitter *et al.* 2024; Grainger *et al.* 2021; Rudman *et al.* 2022), reinforcing the notion that evolutionary and ecological processes can occur on the same timescale and potentially interact (Carroll *et al.* 2007; Hairston *et al.* 2005). One of the mechanisms by which populations change in response to environmental variation is adaptive tracking (Bitter *et al.* 2024; Rudman *et al.* 2022). In environments where resources are variable, and this variability creates distinct selection pressures, then we would predict populations could have the ability to adaptively track this variation, modifying evolutionary outcomes. What remains unknown is whether populations can adaptively track the distinct selective regimes associated with the fine-scale variation in environmental resources that shift over short timescales [e.g., weeks/months] and, if so, the extent to which such resource variation then simultaneously impacts both ecological and evolutionary dynamics in natural populations. Alternatively, variation in the resource environment may promote populations to respond via other means, such as trait plasticity (Charmantier *et al.* 2008; Ghalambor *et al.* 2007; Schmidt & Conde 2006) or bet-hedging (Gillespie 1977; Simons 2011; Stearns 1976).

Natural populations of *Drosophila melanogaster* experience pronounced seasonal variation in the resource environment: in the ancestral range, the putative ancestral substrate marula (Mansourian *et al.* 2018) is seasonally ephemeral, as are the predominant fruit substrates in temperate regions (Markow 2015; Reaume & Sokolowski 2006). Seasonal environmental change, including variation in resource availability, affects population size (Dempster *et al.* 1995; Lawson *et al.* 2015), relative abundance

of competing Drosophila species (Grainger *et al.* 2021), and patterns of rapid adaptation at both the phenotypic (Behrman *et al.* 2015; Behrman & Schmidt 2022; Schmidt & Conde 2006) and genomic (Bergland *et al.* 2014; Machado *et al.* 2021) levels. Similarly, experiments conducted in field mesocosms over the spring-to-fall seasonal progression have shown that *D. melanogaster* populations adaptively track shifts in the biotic and abiotic environment over weekly to monthly timescales (Bitter *et al.* 2024; Grainger *et al.* 2021; Rudman *et al.* 2019, 2022). However, the role of variation in the resource environment in contributing to the ecological and evolutionary dynamics of populations remains unresolved.

Dietary manipulations in the laboratory have revealed the direct influence of caloric and macronutrient variation on *D. melanogaster* life history traits, including viability, fecundity, body size, lifespan, and appendage size (Bakker 1959, 1962; Chiang & Hodson 1950; Rodrigues *et al.* 2015; Shingleton *et al.* 2017; Skorupa *et al.* 2008; Wang & Clark 1995). Diet variation also affects patterns of genomic evolution (Kawecki *et al.* 2021) and multiple phenotypes, including mate choice (Schultzhaus *et al.* 2017) and ethanol tolerance (Cavener & Clegg 1981). Natural populations are also characterized by standing genetic variation for macronutrient tolerance (Havula *et al.* 2022), suggesting populations could evolve rapidly in response to shifts in the resource landscape.

Here, we conducted a manipulative field experiment to examine the effects of the nutritional environment on population dynamics and patterns of seasonal adaptation. Throughout a five-month experiment, we tracked census size, fitness-associated phenotypes, and allele frequencies genome-wide across replicate populations to determine how direct alterations of the nutritional environment impact ecological dynamics, phenotypic trajectories, and patterns of genomic evolution. We hypothesized that the dietary treatment would affect population demographic patterns and that nutritional variation might cause divergence in selective pressures, generating distinct patterns of adaptation across resource environments.

METHODS

Field Mesocosm Experimental Setup

Experimental populations were created using the same methodology as described in (Rudman *et al.* 2022). Briefly, a panel of eighty inbred lines originally collected in June 2012 (Linvilla Orchards, Media, PA, USA) were outcrossed and expanded for four generations of recombination. In the next generation, experimental flies were collected as a single 12h cohort from density-controlled cultures; collections were combined in groups of 2500 flies (equal sex ratios) and randomly assigned to an experimental treatment and replicate field cage (N = 6 cages per treatment).

Two distinct food substrates were developed to simulate variation in the resource environment. Our low-quality nutritional environment (LQ) is largely pureed apples and mimics a natural diet regularly experienced in temperate *D. melanogaster* populations. Alternatively, the high-quality nutritional environment (HQ) is a standard cornmeal molasses-based medium used in laboratory culture and prior experiments in this system (Bitter *et al.* 2024; Rajpurohit *et al.* 2016; Rudman *et al.* 2019). The principal differences between the nutritional treatments are caloric content and the ratio of protein to carbohydrates (recipes and nutritional information are given in Table S1).

185 Each population was maintained outdoors in a $2m^3$ mesh enclosure containing a
186 fruitless, dwarf peach tree. The only food source and egg-laying substrate was 400ml of
187 the respective dietary treatment contained in 900ml aluminum loaf pans, which were
188 replaced every other day for the duration of the experiment (July 11th - November 23rd,
189 2020). After 2d of oviposition, each loaf pan was covered with a screen mesh lid and
190 embryos were allowed to develop separately in a small cage until eclosion ceased. Loaf
191 pans of media within experimental cages were protected from rain and direct sun on
192 shelving units oriented away from direct sunlight.
193
194 *Measurement of population size and fitness-associated phenotypes*
195 To observe how the nutritional treatments affected population dynamics,
196 population size was estimated bi-weekly. At a standardized photoperiodic timepoint, 4
197 permanent ceiling quadrats in each cage were photographed. The number of adult *D.*
198 *melanogaster* in each sample photograph was counted (using a modified cell counting
199 protocol with ImageJ (O'Brien *et al.* 2016)) and corrected for total mesocosm surface
200 area to estimate population size (Rudman *et al.* 2019, 2022).
201 In addition to population demographics, we phenotyped each population for a
202 variety of fitness-associated traits, which have been shown to respond to variability in
203 the seasonal and resource environments (Behrman *et al.* 2015; Behrman & Schmidt
204 2022; Rudman *et al.* 2022). Our primary goal was to examine changes in the genetic
205 composition of experimental populations over seasonal time and whether patterns of
206 evolutionary change were altered by dietary treatment. Thus, all phenotypes at all
207 timepoints were assayed in a common garden, laboratory environment. Eggs were
208 collected overnight from each population on fresh loaf pans and returned to the lab for
209 two generations of density-controlled, common-garden rearing (25˚C, 12L:12D) on their
210 respective diets. Fitness-associated phenotypes of the F2 generation were measured
211 for each field cage on density and age-controlled replicates (N=3 per cage per
212 timepoint). Fecundity was measured as the total number of eggs laid by a group of five
213 females over three days (Paaby *et al.* 2014). The development time for males and
214 females was estimated as time from oviposition to eclosion for groups of 50 eggs by
215 recording eclosion events 3x per day at 09:00, 13:00, and 17:00 and scoring by sex
216 (Behrman *et al.* 2015); viability was estimated as the percentage of eggs laid that
217 emerged as adults. Starvation resistance was measured separately for males and
218 females as the time to death for replicate groups of 10 individuals kept in vials on 1%
219 agar. Thorax length was measured as the longest length across the dorsal shield in
220 lateral view for 15 ethanol-preserved females. Measurements were recorded using a
221 Leica MZ9.5 microscope, with an Olympus DP73 camera and CellSens standard
222 measuring software (Betancourt *et al.* 2021). We assayed each of these phenotypes in
223 the founding population five times during the experiment (Fig. 1). All project data and
224 analyses are available at https://github.com/jkbeltz/Nutritional_Quality_Analysis.git.
225 Temperature data were measured using a shielded, non-aspirated
226 temperature/relative-humidity sensor (CS-215L) mounted at 2m height, located centrally
227 among populations, and logged with a CR-1000 datalogger (all Campbell Scientific,
228 Logan, UT). Temperature was logged every 15 minutes as an average of the previous 5
229 minutes. These data were used to construct degree day models (Kamiyama *et al.* 2020)
230 to estimate generation times between each sampling interval throughout the

experiment. 180-degree days were used to represent a single generation. Rates of phenotypic evolution were then calculated in haldanes (Gingerich 2001) for all traits across the entire experiment as well as between each sampling interval.

In addition to the standard, common garden phenotyping of all cage populations at all timepoints on their diet of origin, an additional protocol was conducted at two timepoints (t2 on 18 September, representing the end of the summer period and expansion, and t4 on 25 October, before the winter collapse). Individuals from each population were reared on both dietary treatments to examine 1) diet-associated plasticity for the five assayed phenotypes and 2) whether such patterns of plasticity changed throughout the experiment. Eggs were collected overnight from each population on fresh loaf pans on their respective diets and returned to the lab where individual eggs were manually removed and transferred to both diets. Fitness-associated phenotypes were measured on density and age-controlled replicates in the F2 generation following the same protocols described above.

Phenotypic data was analyzed in R (v4.3.2) using mixed-effect linear models, where time, treatment, and/ or phenotypic treatment were fixed values (Tables S2-S4), and individual cage/ sampling date was a random effect (lme4, fit by REML). T-tests, ANOVAs using the Satterthwaite method (Satterthwaite 1946), and MANOVA were utilized to test the significance of phenotypic variation across treatments in multidimensional space (Table S6).

Microbial Sampling and Sequencing

Since the Drosophila-associated microbiota shifts over space and time (Chandler *et al.* 2011; Wong *et al.* 2013), affects host phenotypes (Chandler *et al.* 2011; Keebaugh *et al.* 2018; Wong *et al.* 2014), and varies with dietary substrate (Massey *et al.* 2024; Yakovleva *et al.* 2024), we examined the host and environmental microbiota in all populations at two timepoints. On September 18 and October 25, approximately 100 adults were directly sampled by aspiration from each cage for microbial sequencing. Additionally, a sample of older food (which has been in the environment for at least 10 days, and still had flies actively eclosing) was collected from each cage to assess the microbial community associated with each dietary substrate. These samples, along with common-garden F1s from phenotyping samples, were stored at -80˚C; upon completion of the experiment, DNA was extracted from three replicate pools of five male and five female flies, and amplicon libraries were generated using 16s rRNA V1V2 primers (Forward: 5'-AGAGTTTGATCCTGGCTCAG-3', Reverse: 5'-TGCTGCCTCCCGTAGGAGT-3') and Q5 polymerase before being paired-end sequenced with Illumina MiSeq to 15x coverage at the University of Pennsylvania / Children's Hospital of Philadelphia microbiome core, following previously described protocols (López-Aladid *et al.* 2023). All reads identified as Wolbachia sp. were excluded from the analysis. All sequence analysis was performed using QIIME2 (following code provided at https://github.com/jbisanz/16Spipelines) and visualizations generated with R (version 4.3.2). Raw sequence data are available at https://github.com/jkbeltz/Nutritional_Quality_Analysis.git.

Whole Genome Sequencing and Allele Frequency Estimation

Next, we examined how the experimental manipulation affected patterns of allele frequency change over seasonal time. Specifically, during each of the five phenotyping collection timepoints (Fig 1), 100 F1 adult female flies were randomly selected from each cohort of field-collected eggs, preserved in 80% ethanol, and stored at -80° C. An additional pair of replicate samples was collected from the founding population used to initiate the experiment. Genomic DNA was later extracted from all pools using the New England Biolabs Monarch Genomic DNA Purification Kit. Whole-genome sequencing libraries were derived from each pooled sample with Illumina DNA Prep Tagmentation kit, followed by sequencing on the Illumina NovaSeq 6000 flow cell using 150-bp, paired-end sequencing reads. Raw sequencing reads were trimmed of adapter sequences and bases with quality score < 20 and subsequently aligned to the v5.39 *D. melanogaster* genome using bwa and default parameters (Li & Durbin 2009). Deduplication of aligned reads was then conducted using Picard tools (http://broadinstitute.github.io/picard/). We downsampled aligned reads for each sample to obtain an equivalent, genome-wide coverage of 7x across samples. Haplotype-informed allele frequencies were then computed for each sample using the genome sequences of the founding inbred strains and a local inference method (Kessner *et al.* 2013) and pipeline (Tilk *et al.* 2019). This method produces an accuracy of allele frequency estimates at a genome-wide depth of 5x comparable to standard pooled sequencing approaches and 100x coverage (Rudman *et al.* 2022; Tilk *et al.* 2019). The final set of allele frequencies was then filtered to obtain only those single nucleotide polymorphisms (SNPs) with an average minor allele frequency > 0.02 in founder populations and >0.01 in at least one of the evolved samples, resulting in 1.9 M SNPs for our final assessment of genomic changes throughout the experiment.

Analysis of Genome-Wide Allele frequencies

Analysis of genome-wide allele frequency data was conducted in R v. 3.5.6. We first quantified and visualized patterns of genomic variation between treatments and through time using Principal Component Analysis (*prcomp* package). Allele frequencies were centered and scaled prior to PCA, and samples were projected onto the first, second, and third principal components. Through this, we inferred the extent to which variance in genome-wide allele frequencies throughout the experiment was predominantly driven by collection timepoint, treatment, or a combination thereof.

Next, we identified individual SNPs with systematic frequency movement across replicates throughout the experiment using a generalized linear model (GLM) independently for each treatment. As drift is unlikely to produce coordinated allele frequency across independent populations, we infer that SNPs identified via this approach are subject to direct selection associated with causative variants or indirect selection dominated by patterns of linkage disequilibrium. Prior to GLM regression, allele frequencies were weighted by effective coverage (Tilk et al. 2019), and the total number of chromosomes sequenced (N = 200). The normalized frequencies were fit to a GLM of the form: allele frequency ~ timepoint, using a quasi-binomial error variance structure (Wiberg *et al.* 2017). GLM's were run separately for samples collected from each treatment, and p-values were adjusted using a Benjamini-Hochberg false

discovery rate correction. The distribution of SNPs was then visualized using Manhattan plots.

We then used this regression-based approach to identify SNPs that evolved differentially between treatments and throughout the experiment. Specifically, we once again used coverage and population-size normalized allele frequency data across all timepoints and treatments and ran a GLM of the form: allele frequency ~ timepoint + treatment + treatment*timepoint. We identified those SNPs with a significant treatment by time interaction as those with an FDR-corrected p-value < 0.1.

RESULTS

The ecological impact of variation in resource quality

Census estimates reveal that variation in the nutritional environment did not produce significant differences in population size across the two treatment groups, with both exhibiting similar patterns of seasonal growth throughout the summer, achievement of maximum size in early September, and subsequent autumnal decline (Fig. 1). There was no overall effect of treatment on population size across the experiment (Table S2a). Furthermore, no individual pairwise comparison between treatment groups was significant at any individual timepoint after multiple testing corrections (Table S2b). These results demonstrate that, contrary to our prediction, resource quality alone does not drive population dynamics in this system.

The impact of nutritional variation on trait evolution

Our longitudinal phenotypic analysis revealed patterns of rapid evolutionary change across all surveyed traits (Fig. 2A-G, Table S3). The two dietary treatments had a pronounced and direct effect on phenotype, as expected; this is evident in the phenotypes exhibited by the founding population cultured on the two diets, as well as in the separation between treatments in the multivariate analysis (Fig. 2G). Our primary interest, however, was to examine whether patterns of evolution over seasonal time were parallel or divergent between the two dietary treatments, as demonstrated by a significant interaction between time and treatment. We find that two traits show distinct patterns of evolutionary change between treatments over seasonal time: viability and body size (Table S3). Viability remained roughly consistent over time for the LQ treatment; for the HQ treatment, viability remained constant until the last sampling interval, over which it declined by approximately 30%. Similarly, body size was generally constant over time for both treatments from t1 to t4. Over the last sampling interval, however, body size evolved to be larger in the LQ treatment while continuing to decrease in the HQ treatment. These differential patterns of phenotypic evolution over seasonal time were concentrated at the last timepoint, where rates of evolution were also observed to be noticeably higher as temperatures and population sizes declined.

Rates of phenotypic evolution in haldanes (H) were determined for all traits between each sampling timepoint for both treatment groups (Fig. 2I). Rates increased throughout the experiment, with the fastest rates observed in the final time interval for all phenotypes. We found no effect of the nutritional environment on the pace of evolutionary change (Table S4); thus, variation in resource availability affected evolutionary trajectories but not rates of change over generational time. This response is consistent with the largely parallel trait response of both treatments over time.

364 At two timepoints (t2 & t4), all population trait values were assayed on both diets
365 in a common garden laboratory environment. All traits exhibited pronounced plasticity in
366 response to the dietary environment (Fig. S1). However, we found no evidence that the
367 treatment manipulation affected how nutritional plasticity changed over time (Table S5),
368 as no three-way interaction (time x experimental treatment x assay diet) was significant
369 for any of the measured phenotypes. For multiple traits, however, we did observe shifts
370 in nutritional plasticity over time, suggesting that the evolution of plasticity may be an
371 essential component of rapid adaptation to environmental change in this system.
372 When conducting an experimental manipulation of dietary sources, we would be
373 remiss not to address the potential effects of the microbial community (Figs. S3-S).
374 Regular sampling and 16s sequencing of the mesocosm food supply, as well as wild-
375 caught and F1 common garden-reared adults, revealed that LQ samples tended to hold
376 more lactic acid bacteria (LAB) in lab culturing and greater acetic acid bacteria (AAB) in
377 the field, relative to HQ populations that were more consistent across environments
378 (Fig. S6). Overall, the total bacterial abundance of the food substrate (Fig. S5) and
379 diversity (Fig. S3) / community composition (Fig. S4, Table S7) of the *D. melanogaster*
380 host populations were not significantly affected by treatment. While this level of
381 sequencing resolution cannot exclude the impact of subtle variation of individual taxa,
382 these results indicate that the observed population dynamics and patterns of
383 evolutionary change reflect a host response to the dietary treatments and not direct or
384 indirect effects associated with distinct microbial communities.
385
386 *Impact of nutritional variation on genomic evolution*
387 PCA suggested that the variance in allele frequencies across all replicate
388 populations and treatments was primarily driven by seasonal time and secondarily by
389 the resource environment (i.e., the sample segregation along PCs 1 and 2
390 corresponded with collection timepoint and resource environment, respectively (Fig.
391 3A-B). Throughout the experiment (t1-t5), both treatment groups exhibited strong,
392 parallel genome-wide signals of allele frequency change (Fig. 3C&D), with 47,706 SNPs
393 responding significantly to time across all samples, consistent with patterns observed in
394 prior experiments in this system (Bitter *et al.* 2024; Rudman *et al.* 2022). The absolute
395 number of SNPs exhibiting seasonal frequency change was less than those responding
396 to season and was distinct between the two dietary treatments: specifically, 2,203 SNPs
397 in the LQ treatment and 29,303 SNPs in the HQ treatment exhibited an FDR corrected
398 P value < 0.05 and an effect size greater than 3%.
399 Given the observed divergent patterns of phenotypic evolution across treatments
400 (Fig. 2), and the suggestion that genome-wide allele frequencies between treatments
401 were different throughout the experiment (Fig. 3A-B), we next sought to identify SNPs
402 with treatment-specific trajectories. Specifically, we used a generalized linear model to
403 identify SNPs whose frequency was significantly impacted by the interaction between
404 treatment and time. We identified 14 SNPs, 10 of which are on chromosome 3R
405 (positions 12959498 – 12998860) and four on 3L (one at position 14360958 and three
406 others between positions 16911479 – 16911529; Fig. 3E, Table S6). Visualizing the
407 trajectory of these SNPs throughout the experiment exemplifies their treatment-specific
408 dynamics. Specifically, each SNP exhibited a magnitude of total allele frequency
409 change of greater than ~3%, but in all cases, the direction of allele frequency movement

410 was the opposite between treatments. These patterns thus indicate a set of loci under
411 strong selection in both nutrient environments but selected in the opposite direction (Fig.
412 3F). In concert, the genomic data revealed pervasive seasonal evolution within each
413 treatment and evidence of localized divergent responses to selection. Thus, while the
414 patterns of evolution are primarily driven by parallel adaptive responses to a changing
415 seasonal environment, we find evidence that variation in the resource environment
416 contributes to differential patterns of evolution at both the genomic and phenotypic
417 levels.

418 **DISCUSSION**

419 We report the observations from a large-scale, replicated field experiment in
420 which the resource environment for half of the populations was significantly reduced
421 (20% fewer calories, 60% reduction in protein). Over the 5-month experiment, we
422 observed no measurable distinction in population demographics, but a significant
423 difference in the evolutionary response to dietary treatment. We show that the trajectory
424 of phenotypic evolution is altered by dietary treatment, as are patterns of allele
425 frequency change at multiple regions of the genome. Given the parallelism among
426 replicates in patterns of evolutionary change, we interpret these patterns as adaptive:
427 diet impacts how experimental populations adapt to the changing seasonal
428 environment, despite having no pronounced effect on patterns of population size over
429 time.
430 We emphasize that our dietary treatments do not represent extremes. The HQ
431 diet is a typical rich diet for laboratory culture and the diet used in prior mesocosm field
432 experiments in this system (Bergland *et al.* 2014; Bitter *et al.* 2024; Grainger *et al.* 2021;
433 Rudman *et al.* 2019, 2022). The LQ diet was designed to mimic a natural, late-season
434 diet in temperate environments. Natural populations of *D. melanogaster* in temperate
435 North America persist on a wide range of substrates that vary in nutritional/caloric
436 content and are seasonally ephemeral as well as spatially variable (e.g., strawberries,
437 citrus, cherries, grapes, persimmons, paw paws, peaches, pears, apples, etc.). The
438 results we present here suggest that variation in the dietary environment can result in
439 distinct selection pressures that may contribute to spatially and temporally variable
440 selection and, in turn, to the maintenance of genetic variation in natural populations.
441 The effects of dietary treatment on evolutionary dynamics were not mirrored in
442 population demographics, highlighting the disconnect between responses in this
443 system.
444 Ecologically, we hypothesized that resource quality would impact population
445 dynamics, whereby the LQ replicates would exhibit a limited rate of growth and reduced
446 carrying capacity relative to the HQ treatment. This was rejected, as no distinction
447 between treatments was observed in population growth patterns or maximum
448 population size. Several potential explanations exist for these results. First, our
449 experimental populations may not have been limited by the resource environment, and
450 therefore, the effect of diet (e.g., calories, protein: carbohydrates) was lessened. This is
451 unlikely, considering all available food (400 ml) was consumed by the populations within
452 14d, and the reproductive output of mesocosm populations during the summer is
453 ~10,000 – 30,000 eggs per cage every 2d (Rudman *et al.* 2022). Second, the dietary
454 treatment could have altered rates of senescence and average lifespan, such that total

455 population size could be equivalent between treatment populations with different
456 underlying age structures. While diet has pronounced effects on lifespan in *D.*
457 *melanogaster* (Skorupa *et al.* 2008; Zanco *et al.* 2021), a model developed to estimate
458 lifespan from macronutrient content (Lee *et al.* 2008) predicts that both our treatment
459 groups would have similar lifespans. Third, treatment differences in the level of
460 intraspecific competition may also explain this result: the higher fecundity and viability of
461 the HQ populations should more rapidly increase density, potentially limiting population
462 size to match that of the LQ populations that are more resource-limited. However, in
463 that instance we would expect HQ to surpass LQ populations initially until becoming
464 competition-limited (Connell 1983); instead, we see LQ census size exceeding HQ
465 throughout the first two months of the experiment. Differences in substrate crowding
466 caused by treatment effects on body size (flies were ~10% larger on HQ) may also be a
467 factor limiting the expansion of the HQ populations due to greater intra-specific
468 competition. Finally, our dietary manipulation may have indirectly altered the associated
469 microbial community, thereby causing indirect host effects. We observed no significant
470 difference in total bacterial abundance and only subtle taxonomic distinctions between
471 treatments. This suggests that any additional microbial-derived protein or mutualistic
472 interactive effects should be equivalent between treatments. Variation in the microbial
473 community is likely not sufficient or distinct enough to alter season-long population
474 demographics. Regardless, our results demonstrate that a 20% increase in the energy
475 input does not influence the rate of population growth, maximum size, or persistence.
476 We speculate that a combination of factors may contribute to this, but overall, the
477 ecological dynamics in our system are likely primarily driven by intra-specific
478 competition, which may be modulated in multiple ways by the nutritional environment.
479 Evolutionarily, all populations primarily responded in parallel to seasonal change.
480 Variation in the nutritional environment did not restrict the capacity, rate, or magnitude
481 of the adaptive response of populations; it did cause divergence in evolutionary
482 trajectories for a subset of phenotypes (viability and body size) and had a quantifiable
483 impact on patterns of allele frequency shifts over the course of the experiment. The
484 treatment divergence, both genomically and phenotypically, demonstrates that these
485 populations can adaptively track and respond to shifts in the resource environment, as
486 well as the seasonal environment, concurrently. We identified 14 SNPs that
487 demonstrated opposite patterns of allele frequency change between treatments. Over
488 half of the SNPs with treatment-by-time interactions fell within a ~40kb window on
489 chromosomal arm 3R, while the remaining were found on 3L in two regions separated
490 by 2.5 Mbp. These 14 SNPs do not represent 14 independent loci of selection; given the
491 recombinational landscape in *D. melanogaster* (Comeron *et al.* 2012; Hunter *et al.*
492 2016) and the composition of the founding population, we speculate that this is likely
493 driven by selection on three genomic regions with strong linkage disequilibrium in each.
494 Overall, the divergence in the selection landscape between treatment groups suggests
495 that the genetic architecture underlying this treatment effect is relatively simple, which
496 stands in stark contrast to the highly polygenic seasonal adaptive response.
497 The highest rates of phenotypic change (Fig. 2I) and the greatest divergence in
498 trait evolution between treatment groups (Fig. 2D, E, F & 3C) were observed between
499 the final sampling timepoints when temperatures dipped below freezing. This suggests
500 that selection pressures may be most pronounced during this pre-winter phase of the

season, magnifying the effect of our dietary treatments. Similarly, increased rates of evolutionary change during this period (post-frost) have been previously observed (Behrman & Schmidt 2022; Bergland *et al.* 2014; Bitter *et al.* 2024; Grainger *et al.* 2021), suggesting that this relatively punctuated selective landscape may be critical in establishing seasonal cycling of both phenotypic means and allele frequencies (Schmidt and Conde 2006; Bergland et al. 2014; Behrman and Schmidt 2022). The dynamics of overwintering and seasonal adaptation in *D. melanogaster* populations may also be strongly impacted by intense selection in the pre-winter phase, and variation in the resource environment may be especially consequential during this period (Huang *et al.* 2021; Kimura *et al.* 1992; Kimura & Yoshida 1995; Saunders *et al.* 1990).

D. melanogaster populations can adaptively track seasonal heterogeneity (Bitter *et al.* 2024; Rudman *et al.* 2022), and we now report a simultaneous adaptive response to an additional axis of environmental variation. Critically, we demonstrate that environmental variation can alter adaptive outcomes with no effect on demographic trajectories. The adaptive and divergent responses to subtle variations in food supply may also affect patterns of mate choice (Schultzhaus *et al.* 2017) and other behaviors associated with food supply (Rosenberg *et al.* 2018). This ability to track and respond to fine-scale variation may drive the maintenance of genetic and phenotypic diversity globally, as natural populations respond to multiple axes of variation concurrently.

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FIGURES

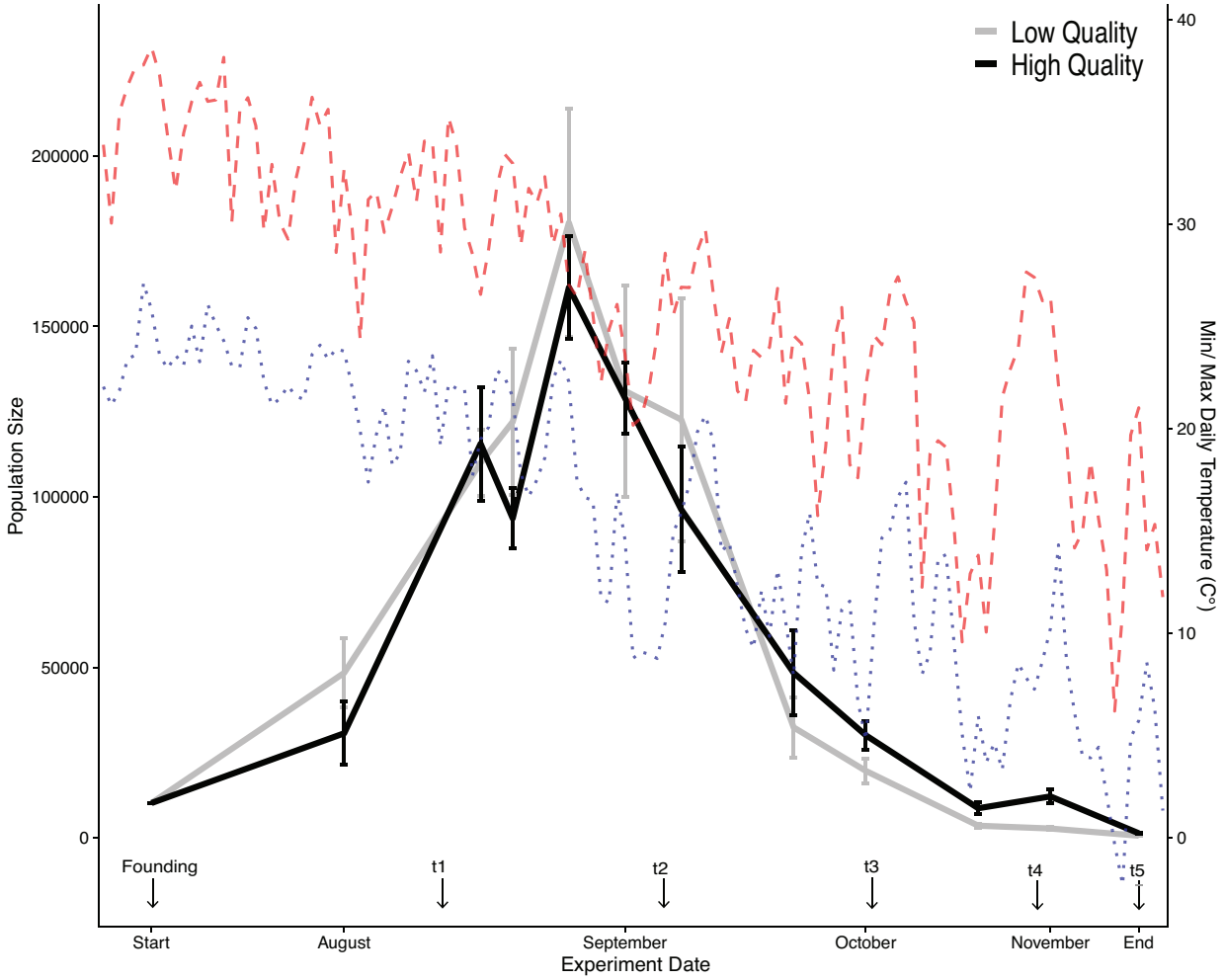

Figure 1. Mean (s.e.) estimated census size across replicated, seasonally evolving populations of *D. melanogaster* reared in either a low- or high-quality nutritional environment, along with the daily minimum (blue) and maximum (red) temperatures throughout the experiment. Census estimates were obtained on 7/30, 8/12, 8/21, 8/30, 9/2, 9/18, 9/26, 10/1, 10/20, 11/2, and 11/14. The founding of the experimental populations and all phenotyping sampling timepoints are labeled on the x-axis.

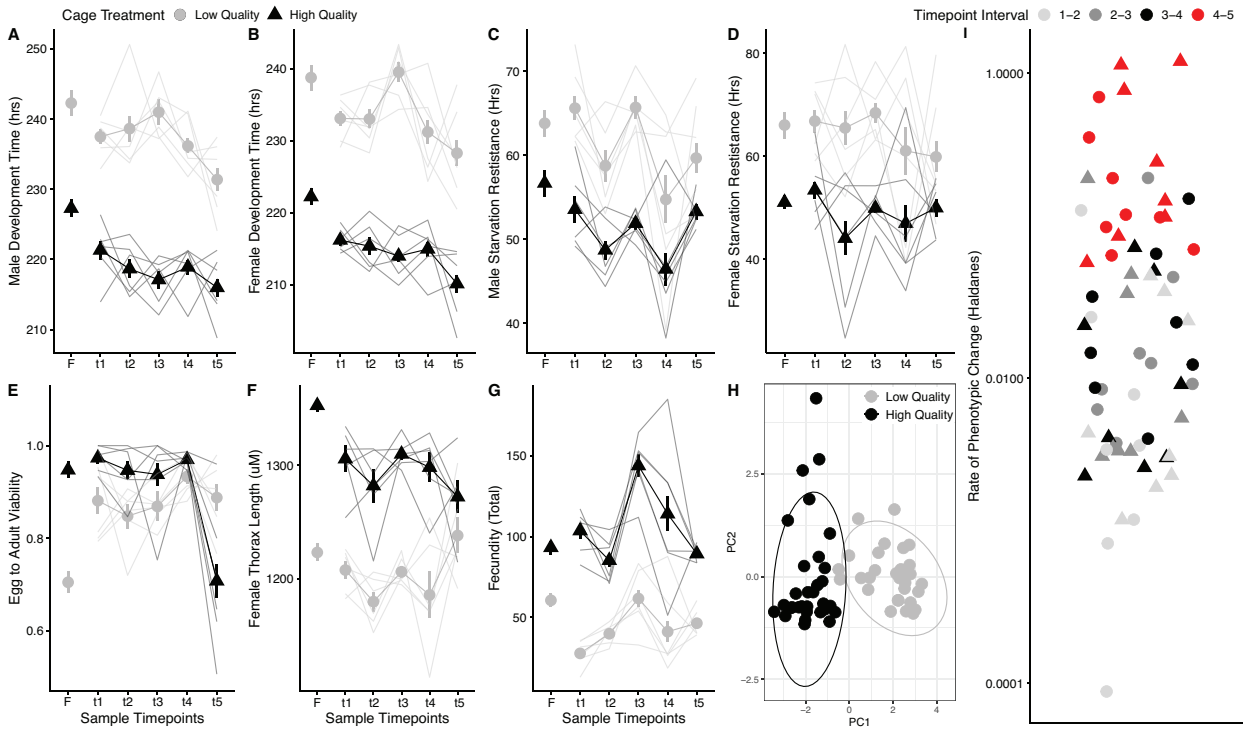

Figure 2. Patterns and rates of phenotypic evolution for replicate populations of *D. melanogaster* over seasonal time in two distinct resource environments. Mean trait values (± s.e.) are depicted by bolded lines with symbols (LQ in grey, HQ in black), and individual replicate cages are given as non-bolded lines (A-G). Founder populations are included for presentation purposes only. Variation in the nutritional environment impacts all assayed traits and shifts patterns of seasonal adaptation for two traits (E,F). When all trait means across all timepoints are used to construct a principal component analysis (H), a strong effect of nutritional environment is observed with a significant effect in a MANOVA ($F_{2,58}$=1.28E+02, p=2.2E-16). Rates of phenotypic evolution in Haldanes were calculated for all traits between each sampling interval (I) and showed no significant impact of nutritional treatment (Table S4).

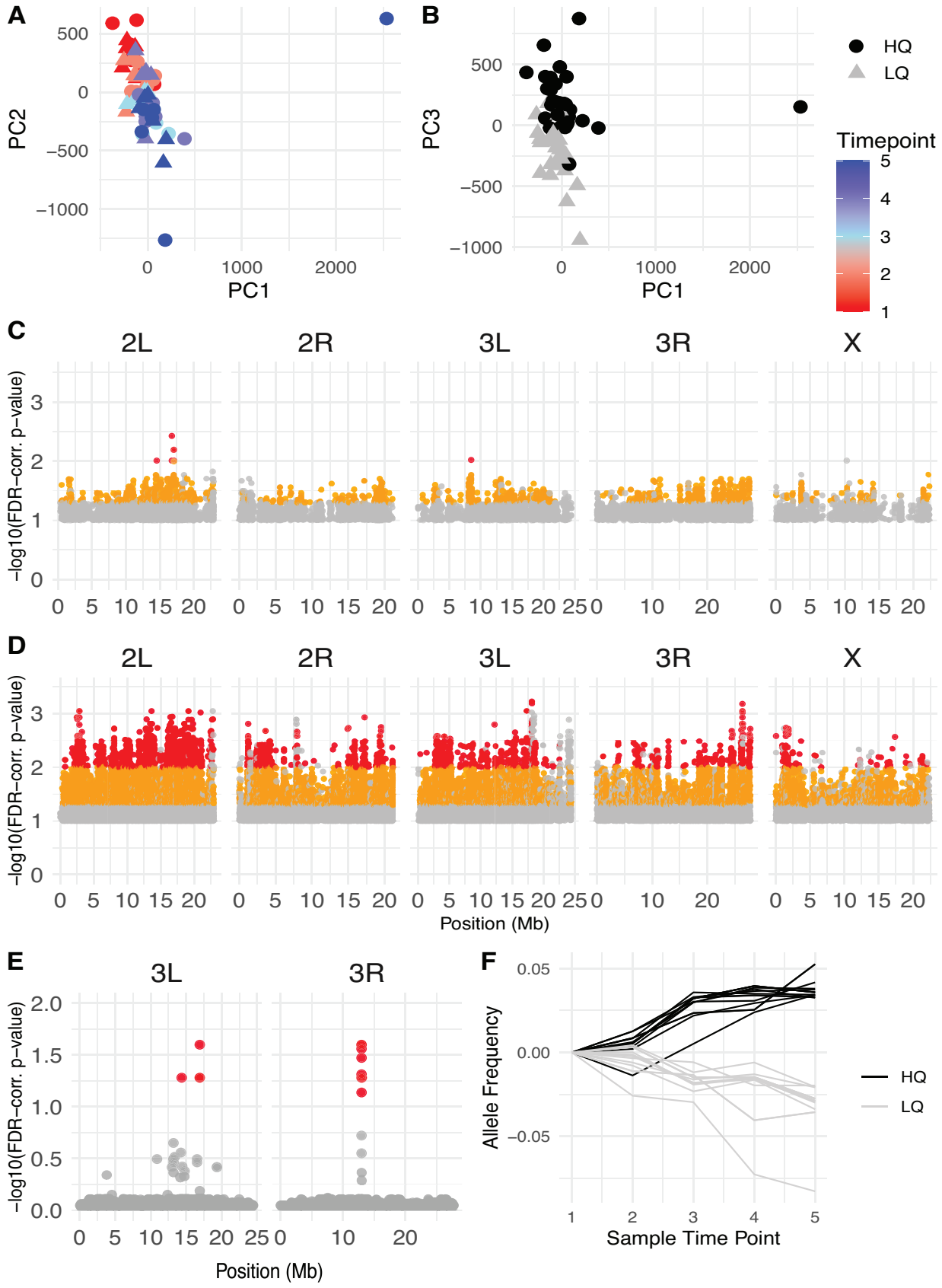
A
PC2
PC1
B
PC3
PC1
HQ
LQ
Timepoint
C
2L
2R
3L
3R
X
−log10(FDR−corr. p−value)
D
2L
2R
3L
3R
X
−log10(FDR−corr. p−value)
Position (Mb)
E
3L
3R
−log10(FDR−corr. p−value)
Position (Mb)
F
Allele Frequency
Sample Time Point
HQ
LQ

Figure 3. Genomic analysis based on the change in SNP allele frequencies for the LQ and HQ populations over experimental time (t1 → t5). (A/B) Principal component analysis across all samples using allele frequency data from 1.9 million SNPs. In (A), samples are projected onto the first two principal components and colored by collection time point. MANOVA revealed a significant effect of time overall ($F=32.65$, $p=5.07\times10^{-10}$), as well as in the LQ ($F=17.31$, $p=2.11\times10^{-5}$) and HQ ($F=3.61$, $p=3.16\times10^{-2}$) populations alone, but no effect of treatment or the interaction. Principal components one and two explain 6.62% and 5.21% of the variation, respectively. In (B), samples are projected onto the first and third principal components and colored by treatment. Here, the MANOVA reveals a significant effect of treatment ($F=25.88$, $p=1.31\times10^{-8}$), as well as time ($F=5.21$, $p=8.49\times10^{-3}$). Seasonal time is the strongest driver of SNP allele frequency shifts, but the quality of the nutritional environment produces clear divergence across PC3. Principal components one and three explain 6.62% and 4.76% of the variation, respectively. C/D: Manhattan plots depicting SNPs shifting in parallel across replicates through time in LQ (C) and HQ (D). Point color corresponds to FDR-corrected p-value and effect size (grey: FDR < 0.1 and effect size > 0.5%; orange: FDR < 0.05 and effect size > 1%; red: FDR < 0.01 and effect size > 2%). E/F: Identification of alleles that shift differentially over time between treatments (FDR < 0.1). Fourteen SNPs were identified, 9 of which mapped to a ~40kb window on 3R, and the remaining five are localized to two regions of 3L separated by roughly 2.5 million bp. (E) Manhattan plot depicting SNPs that in a GLM show a significant effect of the interaction of treatment and time. (F) The mean frequency of each of these alleles across all cage replicates in both treatment groups across sample timepoints.

SUPPLEMENTAL MATERIALS

Table S1:

Nutritional composition for both dietary treatments. The lab standard diet (HQ) consists of 81.0% distilled water, 8.0% molasses (Golden Barrel Sulfur-Free Black Strap Molasses), 5.3% cornmeal (Flystuff 62-100), 3.0% ethanol, 1.5% inactive dry yeast (Flystuff 62-108), 0.73% soy flour (Flystuff 62-115), 0.5% agar (Flystuff 66-103), and 0.17% methylparaben (Apex Bioresearch Products 20-258) by weight. The semi-natural apple-based diet (LQ) consists of 55.3% unsweetened applesauce (Regal #10 Can Unsweetened Applesauce), 39.0% distilled water, 3.0% ethanol, 1.5% inactive dry yeast (Flystuff 62-108), 0.65% agar (Flystuff 66-103), and 0.17% methylparaben (Apex Bioresearch Products 20-258) by weight. Preparation of both the LQ and HQ diets followed the same cooking protocol. Macronutrient calculations were done using the Drosophila Dietary Composition Calculator (https://brodericklab.com/DDCC.php).

Nutritional Facts / 100 ml	**Cornmeal Molasses Diet (High Quality)**	**Apple Diet (Low Quality)**
Calories	64.52	47.18
Fiber (g)	1.43	2.409
Sugars (g)	5.91	9.016
Protein (g)	1.73	0.43
Fat (g)	0.45	0.1
Carbohydrates (g)	14.99	7.95
Protein: Carbohydrates	1:8	1:14

Table S2a & b

A linear mixed-effects model was constructed to determine the effect of nutritional quality on census size across replicate mesocosms. The model was tested across all 11 timepoints, and pairwise comparisons were made across the treatment group at each timepoint. ANOVA was used to assess the impact of sampling time and treatment on census size across all phases of the experiment. The model was constructed in R using lme4, and ANOVA was used for post hoc analysis.

Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']

a) $yij = \beta 0 + \beta 1 X1ij + \beta 2 X2ij + \beta 4 X1ijX2ij + ui + vj + \varepsilon ij$

Response Variable (y = Pop Size)
Fixed effects ($X1$ = Timepoint (1:11) , $X2$ = Cage Treatment (LQ vs. HQ))
Random Effects (u = Cage Replicate, v = Timepoint)

Parameters (df)	**Time (46)**		**Cage Treatment (10)**		**Time : Cage Treatment (46)**	
Test	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*
All Timepoints	3.84×10^{1}	**1.00×10^{-4}**	1.27×10^{-1}	7.29×10^{-1}	6.29×10^{-1}	8.01×10^{-1}

b) Pairwise comparison of treatment groups at each timepoint from the model above. No significant effect treatment at any individual timepoint across the seasonal experiment.

	HQ Vs. LQ (10)	
Test	*t-ratio*	*(p-value)*
7/21/20	-4.23×10^{-15}	1.00×10^{0}
8/14/20	8.36×10^{-1}	4.23×10^{-1}
8/31/20	-2.64×10^{-1}	7.97×10^{-1}
9/4/20	1.34×10^{0}	2.11×10^{-1}
9/11/20	8.95×10^{-1}	3.92×10^{-1}
9/18/20	9.97×10^{-2}	9.23×10^{-1}
9/25/20	1.24×10^{0}	2.42×10^{-1}
10/9/20	-7.55×10^{-1}	4.67×10^{-1}
10/18/20	-4.92×10^{-1}	6.33×10^{-1}
11/1/20	-2.42×10^{-1}	8.14×10^{-1}
11/10/20	-4.44×10^{-1}	6.66×10^{-1}
11/21/20	-3.40×10^{-2}	9.74×10^{-1}

Table S3:

A linear mixed-effects model was constructed to determine if treatment (LQ & HQ), season (t1-5), or the interaction of both has effects on life history traits. Trait value is the response; treatment, timepoint, and the interaction term are predictors (individual cage treated as a random effect). Models were constructed for each trait individually in R using lme4, and anova() was used for post hoc analysis.

Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']

$$yij=\beta 0+\beta 1X1ij+\beta 2X2ij+\beta 4X1ijX2ij+ui+vj+\varepsilon ij$$

Response Variable (y = Phenotype)

Fixed effects ($X1$ = Timepoint (1:5) , $X2$ = Cage Treatment (LQ vs. HQ))

Random Effects (u = Cage Replicate, v = Timepoint)

Parameter (df)	**Time (3)**		**Cage Treatment (53)**		**Time : Cage Treatment (53)**	
Test	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*
Larval Development Time (Male)	7.87×10^{0}	**6.75×10^{-2}**	3.50×10^{1}	**2.50×10^{-7}**	2.83×10^{-1}	5.97×10^{-1}
Larval Development Time (Female)	2.60×10^{0}	2.05×10^{-1}	2.83×10^{1}	**2.14×10^{-6}**	1.80×10^{-2}	8.94×10^{-1}
Viability	1.31×10^{0}	3.36×10^{-1}	1.78×10^{1}	**9.49×10^{-5}**	1.67×10^{1}	**1.49×10^{-4}**
Starvation Resistance (Male)	5.66×10^{-1}	5.07×10^{-1}	1.34×10^{1}	**5.78×10^{-4}**	1.76×10^{0}	1.91×10^{-1}
Starvation Resistance (Female)	1.42×10^{0}	2.39×10^{-1}	7.09×10^{0}	**1.01×10^{-2}**	5.82×10^{-1}	4.49×10^{-1}
Fecundity	1.09×10^{-1}	7.63×10^{-1}	2.29×10^{1}	**1.41×10^{-5}**	9.69×10^{-1}	3.29×10^{-1}
Thorax Length (Female)	3.50×10^{-2}	8.64×10^{-1}	3.02×10^{1}	**1.14×10^{-6}**	4.00×10^{0}	**5.07×10^{-2}**

Table S4a & b

Linear model constructed to determine the effect of resource quality treatments on rates of evolution in haldane's, for 8 life history phenotypes across 3 timepoint sampling intervals (2-4, 2-5, 4-5).

a) Overall, ANOVA on the linear model finds a significant effect of sampling interval, cage treatment, and the interaction term on rates of evolution.

Parameter (df)	***Time Interval (2)***		***Cage Treatment (2)***		***Time Interval : Cage Treatment (4)***	
Test	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*
All Intervals	59.75×10^{0}	5.89×10^{-15}	3.919×10^{0}	3.54×10^{-1}	1.34×10^{0}	2.65 x 10-1

b) Pairwise comparison of rates of evolution for each pair of timepoint intervals, across all cage treatment levels

	Interval 2-4 VS. Interval 2-5 (60)		***Interval 2-4 VS. Interval 4-5 (60)***		***Interval 2-5 VS. Interval 4-5 (60)***	
Test	*t-ratio*	*(p-value)*	*t-ratio*	*(p-value)*	*t-ratio*	*(p-value)*
Interval	-2.25×10^{0}	7.06×10^{-2}	-10.6×10^{0}	$\mathbf{1.0 \times 10^{-4}}$	-7.874	$\mathbf{1.0 \times 10^{-4}}$

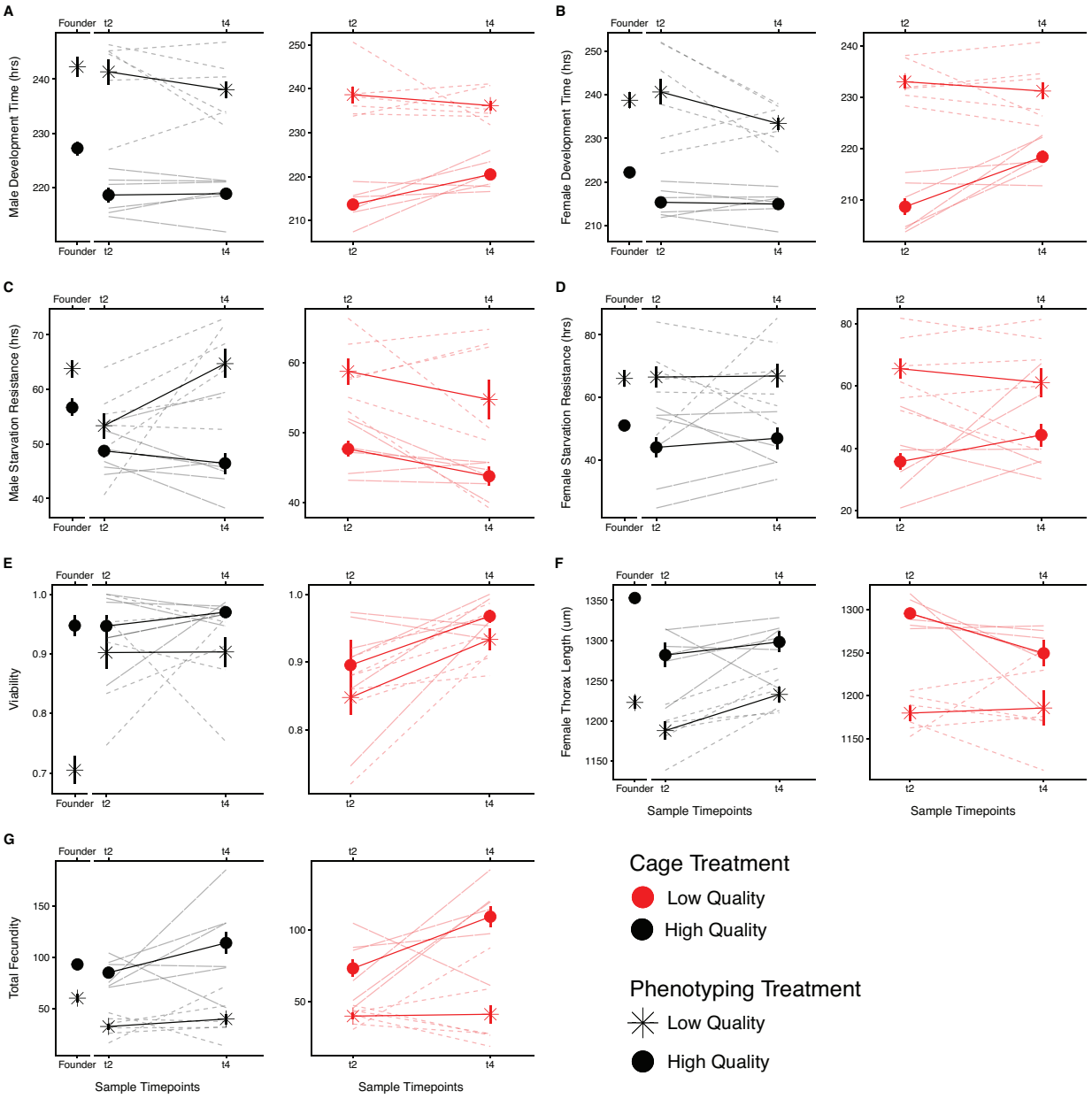

Figure S1. Mean (± one s.e.) phenotypes for replicate populations (N=6) reared on the low-quality (LQ, red) and high-quality (HQ, black) nutritional environments and assayed on LQ (asterisk/dash) and HQ (solid) environments at two experimental time points (mid-September & early November). Thinner dashed and solid lines represent individual replicate populations. Assaying each population in both treatment environments allowed us to determine if the plastic response to diet is evolving differentially across treatments and/or over seasonal time. The founder population, which was reared on the HQ diet, was also assayed in both environments, and is included purely for presentation purposes. We find that across treatments and time, assay diet primarily determines trait value. The mixed-effect linear model (Table S5) found that the phenotyping treatment (assay diet) significantly impacted all mean trait values, and some traits also showed an interaction between assay diet and time. We find no indication that the plastic response is evolving differentially across treatment groups and over time.

Table S5 :

A three-way linear mixed-effects model was constructed to determine whether treatment (LQ vs. HQ), seasonal time (t2 vs. t4), assay diet (LQ vs. HQ), as well as any interaction terms, explain the patterns of variation across all life history traits. The model treated individual cage values and sampling timepoint as random effects. This model was constructed for each trait variable, and an ANOVA post hoc was conducted on each trained model. F-values and P-values are reported, and significant P-values are bolded. The model was constructed in R using lme4, with post hoc testing using anova(). As described in Fig S1, trait values were strongly driven by assay diet and the interaction of time and assay diet. Very little consistent effect of treatment was detected, and there is very little explanation offered by the interaction terms, showing that the plastic response is not evolving differentially across treatment and timepoint.

Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']

$yij=\beta 0+\beta 1X1ij+\beta 2X2ij+\beta 4X1ijX2ij+ui+vj+\varepsilon ij$

Response Variable (y = Phenotype)

Fixed effects ($X1$ = Timepoint , X2= Assay Diet $X3$ = Cage Treatment)

Random Effects (u = Cage Replicate, v = Timepoint)

Parameter (Df)	Test	Larval Development Time (Male)	Larval Development Time (Female)	Viability	Starvation Resistance (Male)	Starvation Resistance (Female)	Fecundity	Thorax Length (Female)
Timepoint (10)	*F-Value*	7.0×10^{-2}	2.0×10^{0}	6.0×10^{0}	3.1×10^{-2}	2.4×10^{-1}	4.6×10^{0}	2.6×10^{-1}
	(p-value)	8.0×10^{-1}	9.6×10^{-1}	**3.4×10^{-2}**	8.6×10^{-1}	6.3×10^{-1}	5.8×10^{-2}	6.2×10^{-1}
Assay Diet (30)	*F-Value*	2.7×10^{2}	1.8×10^{2}	6.8×10^{0}	4.6×10^{1}	4.2 x 101	1.1×10^{2}	1.0×10^{2}
	(p-value)	**1.0×10^{-4}**	**1.0×10^{-4}**	**1.7×10^{-2}**	**1.0×10^{-4}**	**1.0×10^{-4}**	**1.0×10^{-4}**	**1.0×10^{-4}**
Treatment (33)	*F-Value*	1.1×10^{0}	2.7×10^{0}	1.1×10^{0}	7.6×10^{-1}	8.9×10^{-1}	6.8×10^{-2}	5.1×10^{0}
	(p-value)	3.1×10^{-1}	1.3×10^{-1}	3.2×10^{-1}	4.0×10^{-1}	3.7×10^{-1}	8.0×10^{-1}	**4.7×10^{-2}**
Timepoint : Assay Diet (30)	*F-Value*	6.5×10^{0}	9.3×10^{0}	1.4×10^{-2}	4.1×10^{0}	1.3×10^{0}	6.9×10^{0}	6.0×10^{0}
	(p-value)	**1.9×10^{-2}**	**6.4×10^{-3}**	9.1×10^{-1}	5.5×10^{-2}	2.7×10^{-1}	**1.6×10^{-2}**	**2.4×10^{-2}**
Timepoint : Treatment (30)	*F-Value*	2.2×10^{0}	6.6×10^{0}	3.2×10^{0}	6.6×10^{1}	3.5×10^{-3}	1.8×10^{-3}	6.4×10^{0}
	(p-value)	1.7×10^{-1}	**2.8×10^{2}**	1.0×10^{-1}	**2.8×10^{-2}**	9.5×10^{-1}	9.7×10^{-1}	**3.0×10^{-2}**
Assay Diet : Treatment (30)	*F-Value*	5.0×10^{-2}	1.2×10^{0}	1.5×10^{-1}	1.3×10^{-2}	9.7×10^{-2}	1.2×10^{0}	4.0×10^{-1}
	(p-value)	8.3×10^{-1}	2.9×10^{-1}	7.0×10^{-1}	9.1×10^{-1}	7.6×10^{-1}	2.8×10^{-1}	5.4×10^{-1}
Time : Assay Diet: Treatment (30)	*F-Value*	1.3×10^{0}	6.1×10^{-1}	2.3×10^{-1}	4.3×10^{0}	5.7×10^{-1}	3.9×10^{-1}	5.0×10^{-1}
	(p-value)	2.7×10^{-1}	4.5×10^{-1}	6.4×10^{-1}	5.2×10^{-2}	4.6×10^{-1}	5.4×10^{-1}	4.9×10^{-1}

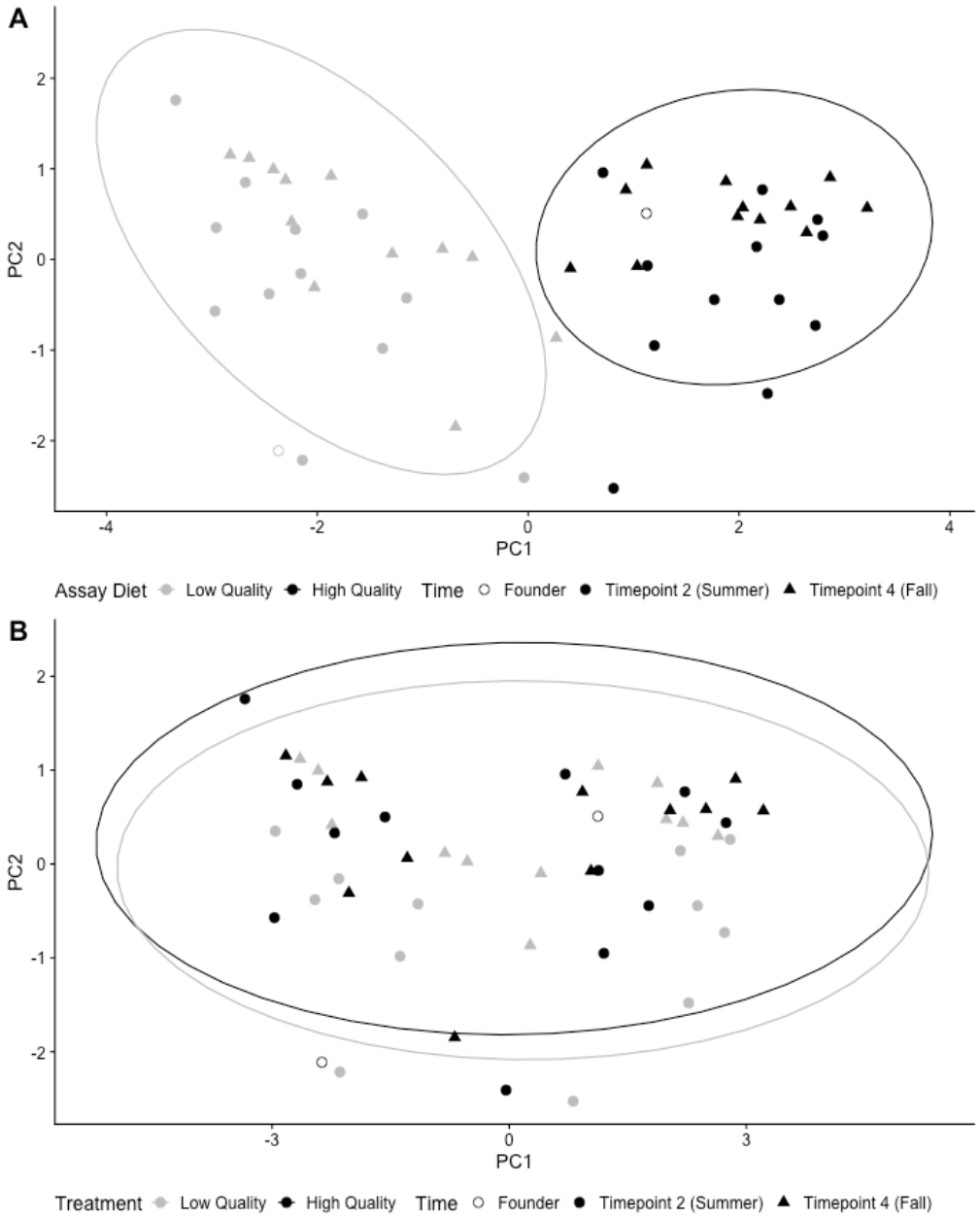

Figure S2. Principal component analysis was constructed using mean trait values across seven assayed traits (Fig. S1) of replicate populations (N=6) of *D. melanogaster*, reared in two distinct resource environments, collected at two timepoints, and assayed in both dietary environments. When points are shaded by the assay diet (A), we see a clear separation and a significant effect of the assay diet ($F_{2,47}$=1.23E+02, p=2.2E-16). When the same points are shaded not by assay diet but by mesocosm treatment (B), we see complete overlap in the groupings ($F_{2,47}$=6.36E-01, p=5.34E-01). This further demonstrates that when populations are assayed in both respective environments, the assay environment drives the trait values and that the plastic response to resource variation is not evolving differently across treatments.

Table S6 :

SNPs and genes associated with divergent selection between the HQ and LQ treatment groups. These SNPs showed a significant interaction between time and treatment in the GLM. Locations are listed in *D. melanogaster* genome version r5 dm3.

SNP position	Gene Identity
3R:12959498	None
3R:12960332	CG31267
3R:12960369	CG31267
3R:12961464	None
3R:12961492	None
3R:12961501	None
3R:12962391	CG5246
3R:12963490	None
3R:12964265	CG5255
3R:12998860	Mur89F
3L:14360958	fz
3L:16911479	Lmpt
3L:16911491	Lmpt
3L:16911529	Lmpt

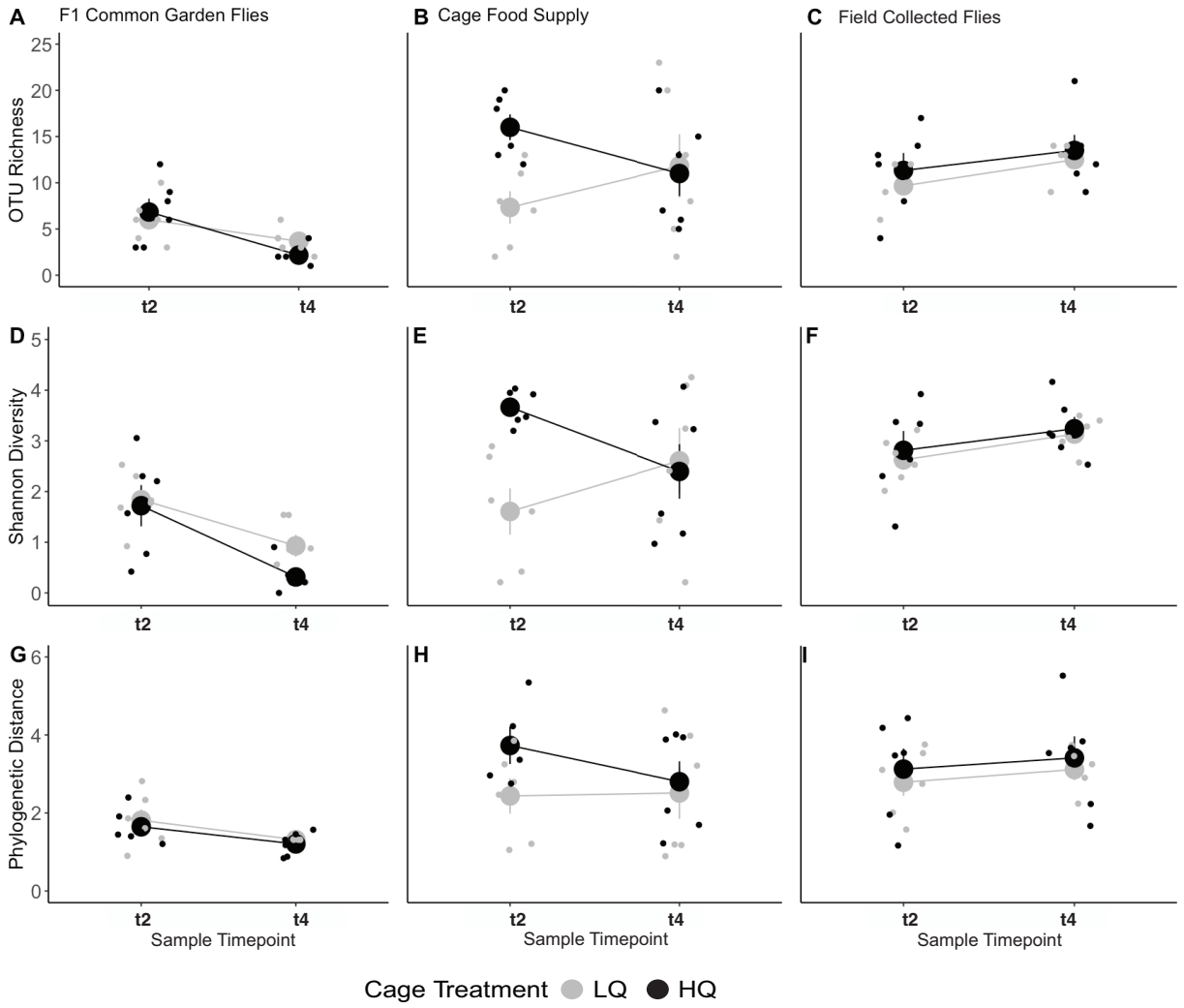

Figure S3. Alpha diversity metrics for bacterial communities in all populations and timepoints. Bacterial richness and diversity (Shannon & Faith_PD) for field-collected adults, food supplies, and lab-reared F1s at t2 (summer-evolved) and t4 (fall-evolved). Seasonality drives all alpha diversity metrics in field samples and F1-reared adult flies, with no significant separation in treatment means at either timepoint. For the cage food supply, we see greater richness and diversity in the HQ treatment in t2 and a convergence in t4, relative to diversity/richness in the LQ treatment. The nutritional manipulation had no effect on the microbial diversity of the host in either the field or the lab. This suggests that observed patterns of phenotypic and genomic evolutionary change are likely driven directly by nutritional manipulation and not an indirect effect of microbial diversity. Furthermore, the fact that the manipulation altered the food supply with no effect on bacterial associations suggests that the host largely controls the composition of its microbiome (top-down) as opposed to being dictated by the environment (bottom-up).

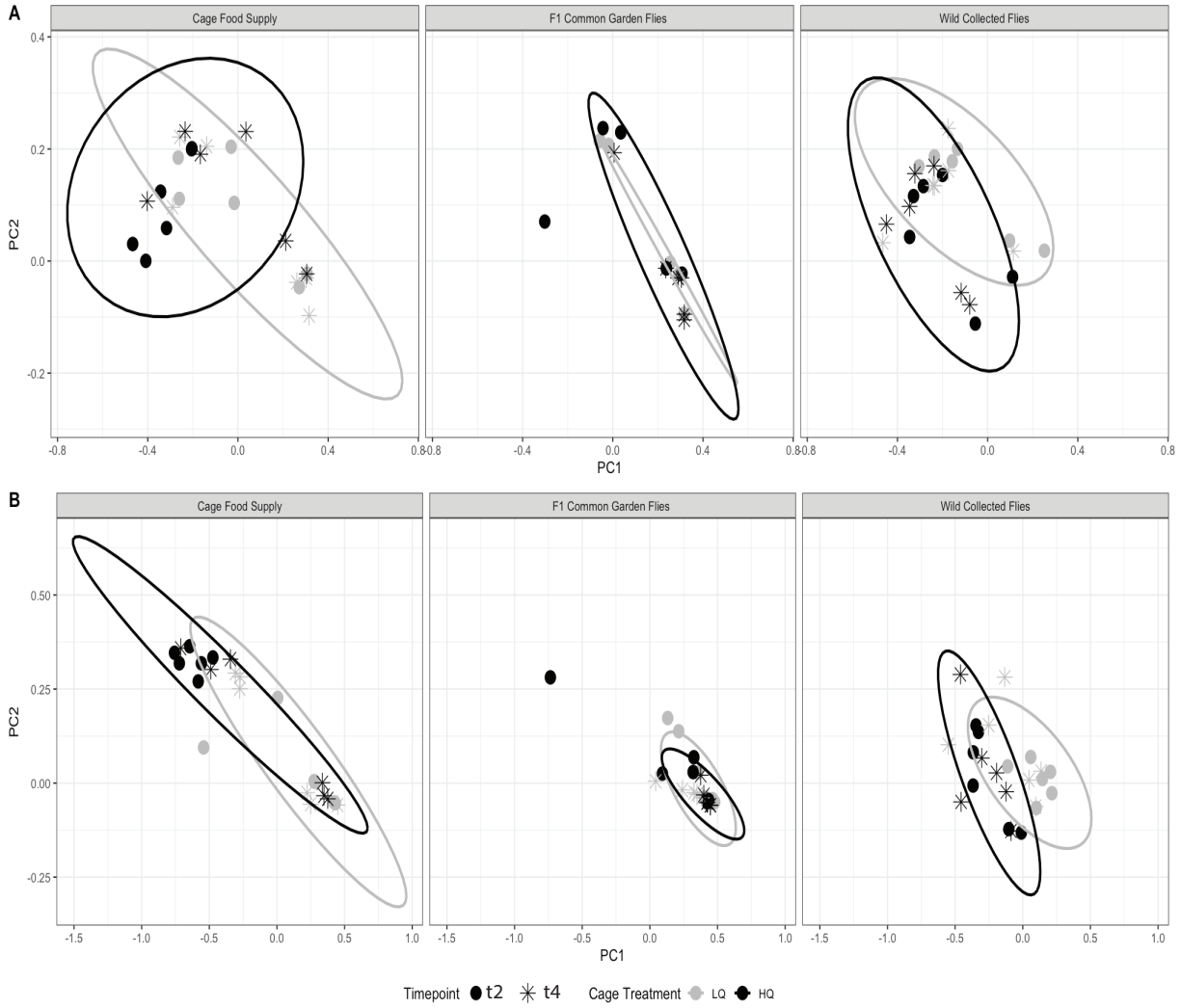

Figure S4. Principal component analysis was constructed using the relative abundance of all nonsingleton bacterial taxa in unweighted (a) and weighted (b) unifrac distance. Sequence variants were identified in field collected flies, cage food, and F1 flies reared in a single generation in a lab common garden, at experimental t2 (Summer Evolved, solid dots) and t4 (Fall Evolved, asterisks) for both LQ (grey) and HQ (black) populations. The MANOVA for significance in centroid position found no effect of nutritional quality on bacterial community composition and no effect of treatment, or the interaction term, for either unifrac distances in all samples.

Table S7 :

To assess whether the variance in the bacterial communities is significant between treatment groups for all sample types, we conducted MANOVA tests on the first two axes of variation for the weighted and unweighted Unifrac distance matrices constructed using the relative abundance of all non-singleton sequence variance. Like the alpha diversity metrics, the only clear effect on the microbial communities was observed in the cage food supply samples, which were significantly shaped by the treatment group. The bacterial communities of field-collected and F1 common garden flies showed no effect from cage treatment, time, or the interaction term. These findings again suggest that the ecological and evolutionary effects observed due to the treatment are not driven by variation in the microbial communities between treatments.

Response Variable - Weighted and Unweighted Unifrac Matrices, PC1 & PC2
Fixed effects - Sample Timepoint (t2 Vs. t4), Cage Treatment (LQ Vs. HQ)

Sample Type	**MANOVA Parameters**	**Time (2,19)**		**Cage Treatment (2,19)**		**Time : Cage Treatment (2,19)**	
	Test	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*	*F-Value*	*(p-value)*
Cage Food Supply	*Unweighted Unifrac Distance*	1.91×10^{0}	1.76×10^{-1}	2.05×10^{0}	1.57×10^{-1}	2.34×10^{0}	1.24×10^{-1}
	Weighted Unifrac Distance	1.30×10^{0}	2.95×10^{-1}	4.29×10^{0}	**2.91×10^{-2}**	2.63×10^{0}	9.85×10^{-2}
F1 Common Garden Flies	*Unweighted Unifrac Distance*	3.34×10^{0}	5.76×10^{-2}	3.91×10^{-1}	6.82×10^{-1}	6.46×10^{-2}	9.38×10^{-1}
	Weighted Unifrac Distance	2.96×10^{0}	3.64×10^{-1}	1.93×10^{-1}	8.26×10^{-1}	3.07×10^{0}	6.99×10^{-2}
Field Collected Flies	*Unweighted Unifrac Distance*	1.23×10^{0}	3.14×10^{-1}	2.97×10^{0}	6.84×10^{-2}	1.63×10^{-1}	8.51×10^{-1}
	Weighted Unifrac Distance	1.12×10^{0}	3.48×10^{-1}	2.15×10^{0}	1.01×10^{-1}	8.13×10^{-1}	4.58×10^{-1}

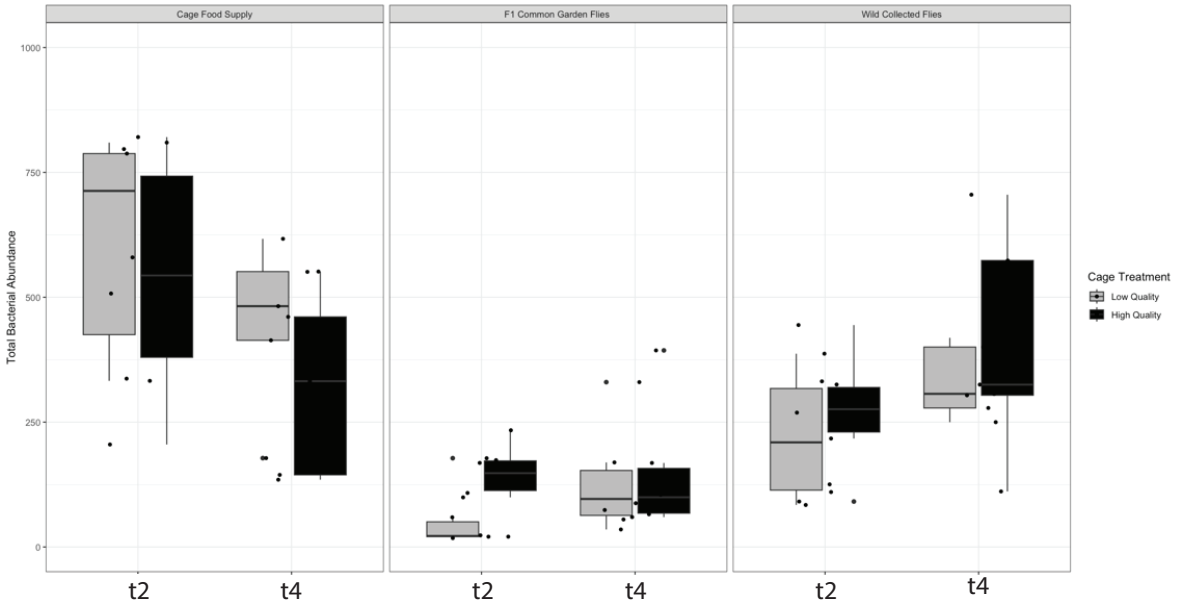

Figure S5. Total abundance of bacteria per sample across treatment, season, and sample type. Sequence variants were identified in wild-collected flies, cage food, and F1 flies reared for a single generation in a lab common garden, at experimental t2 (Summer Evolved, solid dots) and t4 (Fall Evolved, asterisks), for both Low Quality (LQ, Grey) and High Quality (HQ, Black) populations. The sequencing procedure and analysis protocol can be found in the methods. Raw counts of bacterial reads per sample were adjusted for the DNA concentration determined immediately after extraction to account for differences in DNA volume per sample. Seasonal change from summer to fall causes a decline in bacterial abundance in the food supply and an increase within wild-collected adults. T-tests revealed no significant effect of treatment on total bacterial abundance for wild adults and food at either time point. However, the HQ F1 common garden flies consistently have a greater abundance of bacteria than the LQ populations ($p=4.21E-02$). This result suggests that field treatment may impact how fly hosts take up bacterial taxa when brought back to a lab setting. Critically, the lack of effect of treatment on total bacterial abundance reveals that the volume of bacterial cells present on the food substrate is consistent across the two dietary treatments.

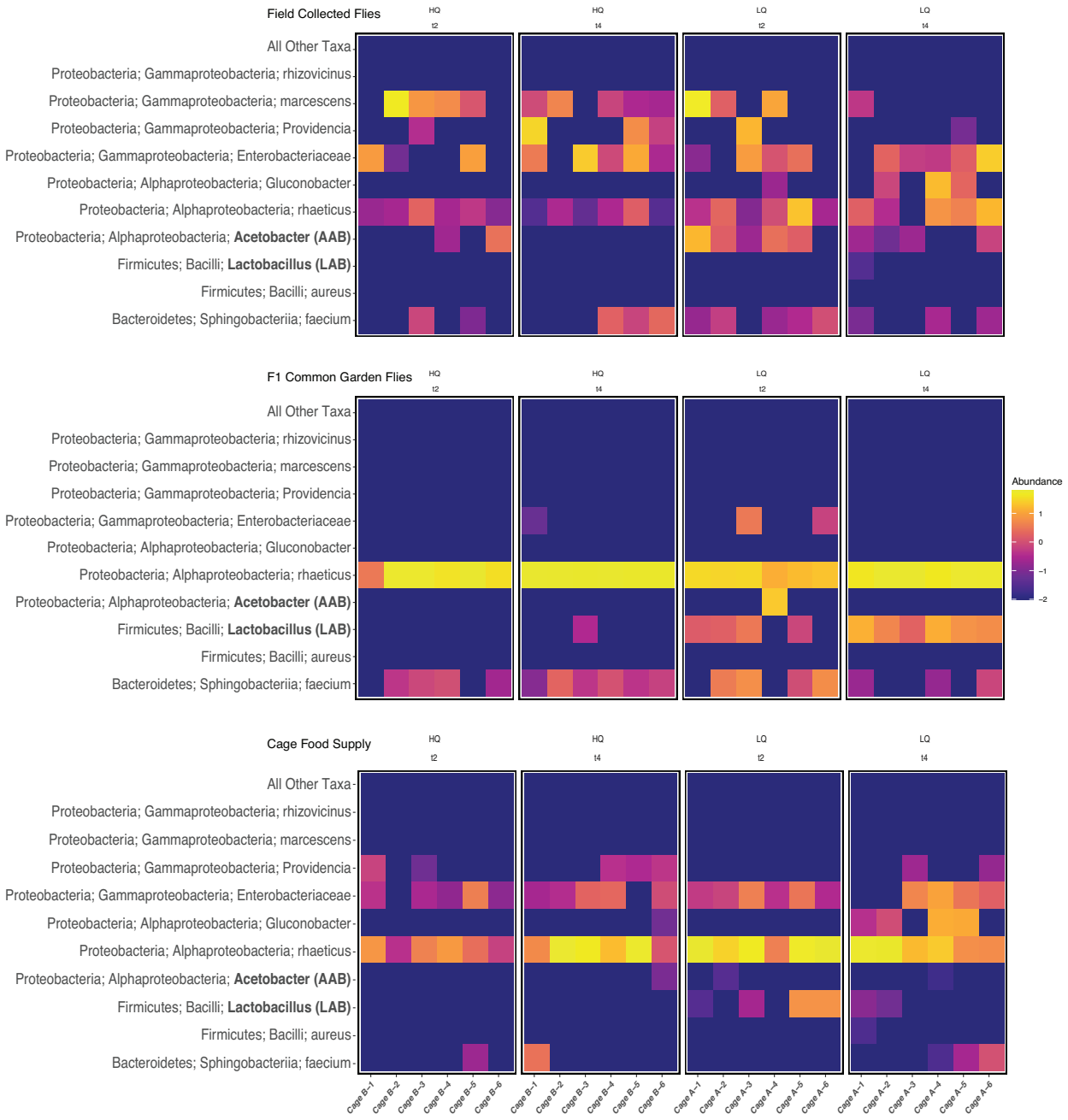

Figure S6. This heatmap illustrates the relative abundance of the ten most abundant bacterial taxa identified within and around the 12 experimental populations of D. melanogaster raised in one of two distinct nutritional environments. Major microbial groups are represented as rows, with each replicate cage as a column. HQ treatment samples are displayed on the left, while LQ samples are on the right, organized by timepoint (t2 and t4). Bacterial sequence variants were identified from samples of field-collected flies (top panels), cage food (middle), and flies raised for a single generation in a common garden laboratory environment (bottom). Bacterial sequence variants are classified by Phylum, Class, and then the lowest taxonomic groups

identified with that variant (primarily Genus and species). The sequencing process and analysis protocol can be found in the methods.

We observe a generally high level of consistency in the most dominant microbial taxa across treatment groups and time, with two notable exceptions. First, Lactobacillus are virtually absent in the field-collected flies from both treatments and timepoints, as well as in the HQ treatment samples from the food supply and F1 laboratory flies. In contrast, Lactobacillus are more abundant in the LQ treatment samples from the cage food and F1 laboratory flies at both timepoints. Second, field-collected flies from the LQ treatment show a higher prevalence of Acetobacter taxa than those collected from the HQ treatment, a pattern that is consistent across the two sampling timepoints. Interestingly, Acetobacter is found at low abundance and prevalence in the F1 and cage food samples at both timepoints. We also observe a greater prevalence of Gluconobacter taxa in the LQ populations, especially in the later sampling timepoint. As expected, we see clear differences between field and lab host samples (Ludington & Ja 2020). When LQ and HQ samples are brought into a standardized lab environment, flies from 10 out of 12 LQ cages acquire Lactobacillus, while only 1 of 12 from HQ do the same. This suggests that the fly populations are evolving differently between dietary treatments and are “filtering” microbes in distinct ways. Overall, the total bacterial communities are not significantly different between treatments when flies are sampled directly from the field. However, given the level of sequencing resolution employed here, it is impossible to exclude the potential effects of treatment and seasonal evolution on individual bacterial taxa and strains.