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Rapid divergence and convergence of life-history in experimentally evolved *Drosophila melanogaster*

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Laboratory selection experiments are alluring in their simplicity, power, and ability to inform us about how evolution works. A longstanding challenge facing evolution experiments with metazoans is that significant generational turnover takes a long time. In this work, we present data from a unique system of experimentally evolved laboratory populations of *Drosophila melanogaster* that have experienced three distinct life-history selection regimes. The goal of our study was to determine how quickly populations of a certain selection regime diverge phenotypically from their ancestors, and how quickly they converge with independently derived populations that share a selection regime. Our results indicate that phenotypic divergence from an ancestral population occurs rapidly, within dozens of generations, regardless of that population's evolutionary history. Similarly, populations sharing a selection treatment converge on common phenotypes in this same time frame, regardless of selection pressures those populations may have experienced in the past. These patterns of convergence and divergence emerged much faster than expected, suggesting that intermediate evolutionary history has transient effects in this system. The results we draw from this system are applicable to other experimental evolution projects, and suggest that many relevant questions can be sufficiently tested on shorter timescales than previously thought.

KEY WORDS: **Adaptation, selection-experimental, population biology.**

Evolutionary biologists have long used laboratory selection to explore hypotheses about adaptation. Such experimental evolution can quickly, dramatically, and reproducibly shape phenotypes in model species (Garland and Rose 2009). When adequately replicated, experimentally evolved populations can be used to test general theories about evolution in well-defined settings, albeit ones that may be significantly different from any that actually exist in the wild (cf. Garland and Rose 2009; Rose et al. 2011; Barrick and Lenski 2013).

But there is an important bifurcation in the experimental evolution literature, that between (i) studies of rarely recombining microbes, like *Escherichia coli* (e.g., Lenski et al. 1991), and (ii) studies of outbreeding species that recombine sexually and maintain standing genetic variation, such as *Drosophila melanogaster* (e.g., Luckinbill et al. 1984). Recombination and standing genetic variation are either absent or rare in many paradigms for microbial experimental evolution (e.g., Tenaillon et al. 2012), though genetic variation can arise from mutator substitutions in some cases after many generations of clonal evolution (e.g., Barrick et al. 2009). The lack of recombination in such clonal evolution experiments gives rise to very different patterns of adaptation from those observed in experiments with outbreeding sexual species. Clonal evolution features selective sweeps, clonal interference, and whole-genome hitchhiking, all of which both purge genetic

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variation and slow the genome-wide response to selection (reviewed by Burke 2012; Kawecki et al. 2012). By contrast, it has been found that experimental evolution in outbred *Drosophila* populations involves abundant standing genetic variation, many genomic sites that respond to selection, and rapid immediate responses to selection (Burke et al. 2010; Burke 2012; OrozcoterWengel et al. 2012; Rose et al. 2015). While the differences between these two types of experimental evolution are considerable, ideas from one type of system can serve as useful sources of hypotheses for the other.

For example, an issue of great interest in the microbial evolution literature has been the extent to which multiple replicated lines converge on similar phenotypic and genetic outcomes, the so-called "chance versus necessity" or "history versus selection" issue (e.g., Woods et al. 2006; Tenaillon et al. 2012). Here, we study experimental evolution in 30 populations of *D. melanogaster*, with a view to addressing the repeatability of phenotypic evolution in outbreeding, sexually reproducing populations, which for clarity we hereafter term "Mendelian" populations.

Research on the Mendelian experimental evolution paradigm has already produced some useful initial findings concerning divergence, convergence, and the repeatability of evolution. First, it is clear that phenotypic divergence occurs rapidly in Mendelian populations subject to new types of selection. Phenotypes of newly selected populations differentiate from their ancestors in tens of generations; this has been shown in multiple *Drosophila* experiments (e.g., Luckinbill et al. 1984; Rose et al. 1992; Chippindale et al. 1997; Zhou et al. 2007; Turner et al. 2011; Turner and Miller 2012), as well as in other insects (e.g., Roff et al. 1999; Beldade et al. 2002; Zera 2005; Michalczyk et al. 2011), mice (Swallow et al. 1999; Chan et al. 2012), domesticated birds (Johansson et al. 2010; Stringham et al. 2012), and foxes (Trut et al. 2004). Second, independent replicate populations experiencing identical selection pressures quickly converge on common phenotypes (Teotonio and Rose 2000; Simões et al. 2008; Fox et al. 2011; Fragata et al. 2014). However, studies that assess the rapidity of convergence and divergence simultaneously, to assess the importance of evolutionary history, are lacking. Here, we carry out simultaneous comparisons of convergence and divergence to determine the degree to which selection might erase or preserve the signature of history for specific fitness traits.

We present life-history data from two sets of populations: 15 long-standing populations and 15 recently derived populations. Five populations from each set are subject to one of three regimes of experimental evolution: (1) selection for accelerated larval development, (2) the ancestral laboratory selection regime of two-week life cycles, and (3) selection for postponed reproduction. Put another way, we present the results from three tests of evolutionary convergence and divergence involving ten populations each, with large-scale parallel assays of life-history characters. Our results reveal in significant detail how Mendelian experimental evolution produces both phenotypic divergence and convergence, on time-scales vastly compressed compared to those of clonal evolution.

Methods

EXPERIMENTAL EVOLUTION REGIMES

This study uses large, deliberately outbred, lab populations of *D. melanogaster* selected for different patterns of age-specific reproduction. All the lines used in the current study originate from an ancestral "IV" population first collected from South Amherst, MA in 1975 by Phillip Ives (vid. Rose 1984), and then cultured in the lab using two-week discrete generations. These ancestral IV flies were subsequently used in February 1980 to create five "O" (old) replicate lines, using females of increasing ages over successive generations until these flies were maintained on a 10-week generation cycle (Rose 1984). The IV flies were also used to found five additional "B" lines in February 1980, lines that have since been cultured using the same protocol as the IV populations from which they were derived. Detailed descriptions of the subsequent history and culture methods for these lines can be found in Rose et al. (2004).

The populations of the O selection treatment are the ancestors of five additional experimental treatments that along with the B selection treatment make up the six focal treatments of this study. These treatments belong to one of two temporal designations ("longstanding" or "recent") and one of three selection types ("A," "B," or "C," described below). Thus, these six treatments provide opportunities to study the differences between populations that have the same selection regime but were established long ago versus recently and also to study the differences between populations that diverged from the same ancestor recently but experience selection for different life histories. See Figure 1 for an overview of the experimental evolution design of the present study.

We call these six selection treatments ACO, AO, B, BO, CO, and nCO, with each letter referencing a selection regime and evolutionary history. The CO populations were derived from the original O treatment after 57 generations of O-type selection in 1989. The "C" in CO indicates a 28-day selection regime while the O represents the CO treatment's most recent common ancestor. The ACO lines are the only populations that did not directly originate from the O treatment, but were instead derived from the CO populations after 27 generations of C-type selection in 1992. The AO, BO, and nCO lines ("n" stands for *new* CO treatment) were derived from the O treatment around 2007 after 153, 150, and 159 generations of O selection, respectively. Thus, these three

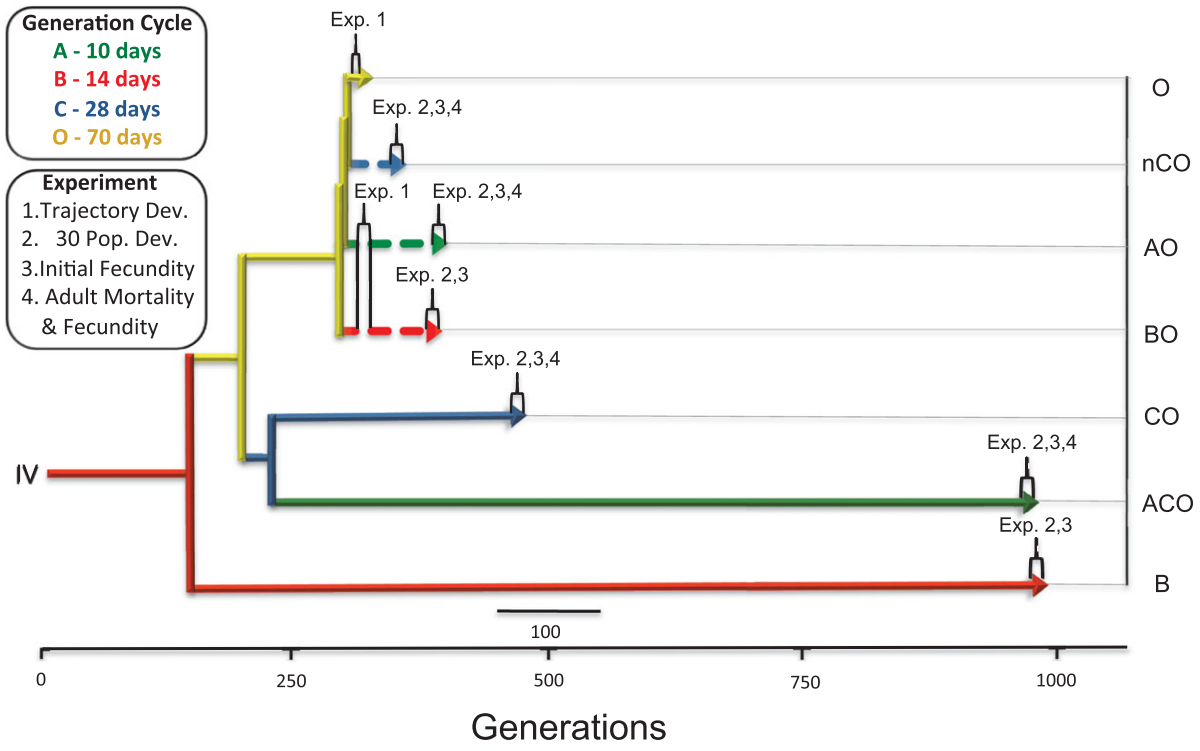

Figure 1. **Schematic of population selection history. Abbreviated illustration of the evolutionary relationships between the focal selection treatments of this study. All treatments share ancestry, some more recently than others. The first capitalized letter of a treatment name indicates the type of life-history regime for which it has sustained selection: A (10-day cycle), B (14-day cycle), or C (28-day cycle). The four experiments of this study are labeled above the lines indicating the treatments that they involve.**

treatments were derived relatively recently, while the CO, ACO, and B treatments are longstanding.

This system of 30 populations is now maintained using three distinct selection regimes: A, B, and C. *A selection regime*: the ten ACO and AO populations spend the first 9 days of life in 8-dram glass vials, and at day 10 adults are transferred to a Plexiglass "cage" in which they are given fresh food and allowed to oviposit for 24 hours. *B selection regime*: the ten B and BO populations spend 14 days in 8-dram vials, and are then allowed 1–2 hours in fresh vials to oviposit before adults are discarded. *C selection regime:* the ten CO and nCO populations develop in vials for 14 days prior to being transferred to Plexiglass cages. C flies are then given 48 hours to oviposit before eggs are collected on day 28. All populations are supplied with food made from cooked bananas, barley malt, yeast (3.6% w/v), corn syrup, and agar. The populations that spend time in cages are also supplied with a 5% live yeast paste on the food surface to promote oviposition 24–48 hours prior to egg collection. Lastly, all populations are kept at 23°C and left in a 24-hour light cycle room. See Figure S1.

EXPERIMENTAL COMPARISONS

Four experimental comparisons were performed: (1) The *evolutionary trajectories of development time* (from egg to adult eclosion from pupae) among AO, BO, and O populations at three time points after the derivation of the AO and BO stocks from the O stocks; (2) *Common-garden comparison of development times* from (a) hatching to the start of pupariation and (b) hatching to adult eclosion from pupae assayed in parallel among flies from the AO, ACO, B, BO, CO, and nCO treatments; (3) *Common-garden comparison of initial fecundity* of flies from the AO, ACO, B, BO, CO, and nCO treatments; and (4) *Parallel simultaneous cohort comparisons of adult fecundity and survivorship* of flies from the AO, ACO, CO, and nCO treatments. Table 1 outlines the numbers of generations elapsed in each selection treatment at the time it was sampled for each experiment. The goal of each of these four comparisons was to determine the degree to which divergence has occurred among the three different selection treatments, as well as the degree to which convergence has occurred between the longstanding and recently derived treatments. For each of the four experiments described, all replicate populations assayed were reared in parallel on a B-type culture schedule for two generations before each experiment to minimize maternal effects.

Experiment 1: Trajectories of selection for accelerated development

After two standard rearing generations, 14-day-old adult flies were put in cages and given food supplemented with yeast paste.

Table 1. Number of generations elapsed in each experimental selection treatment at the time of each experiment.

Selection treatment	Experiment 1 *Assay 1*	*Assay 2*	*Assay 3*	Experiments 2, 3	Experiment 4
ACO				761	752
AO	15	22	28	169	160
B				852	
BO	13	19	24	135	
CO				298	295
nCO				45	42

These numbers do not include "standardizing" generation cycles prior to each assay.

The food plates were changed twice hourly to ensure that females did not retain eggs, and create a cohort of flies of closely synchronized ages. Females were given 1–2 hours to oviposit on a third plate, and those eggs were used for the assays. We measured average development time of the AO, BO, and O treatments by collecting ~60 eggs from each population using fine paintbrushes and placed them into vials containing 5 mL of food. Ten vials were used for each replicate population. These vials were kept at 25°C and checked for eclosed flies every 8 hours following eclosion of the first fly. At each check, eclosed flies were sexed and development time (hours to eclosion) was recorded.

Experiment 2: Parallel assays of two developmental stages in 30 populations

For this experiment, two phases of metamorphosis were measured, from hatching to the onset of pupariation, and from hatching to adult eclosion. After the two generations of standardized rearing, each population from the ACO, AO, B, BO, CO, and nCO treatments laid eggs on a food-free agar plate. From each such plate, 30 first-instar larvae were individually transferred to a food vial, three vials per population. For the subsequent 14 days, vials were monitored every four hours for presence of newly formed pupal casings, as well as newly eclosed adult flies. Newly formed pupae were noted and the time recorded, while newly eclosed flies were collected, sexed, and counted.

Experiment 3: Initial fecundity of newly eclosed adults

Early-life fecundity measures were collected from the newly eclosed adults from Experiment 2, in all replicate populations of the ACO, AO, B, BO, CO, and nCO treatments. Newly eclosed flies were collected every 12 hours, sorted into 40 mating pairs, and then placed into vials to mate and lay eggs. Every 12 hours until day 14 of age from egg, the mating pair was given a vial cap containing fresh food to lay eggs. The eggs laid on the old vial caps were placed on a flatbed scanner to create digital image for egg counting purposes.

Experiment 4: Adult mortality and fecundity from day 14 onward

For this experiment, adult mortality was measured in all replicate populations of the ACO, AO, CO, and nCO selection treatments. After two generations of standardized rearing, ~1200 adult flies were emptied into Plexiglass cages. Cages were supplied with fresh food daily. Dead flies were counted and eggs collected from cages at the same time every day until all flies in the experiment died.

Mortality data were obtained from the 30 experimental populations over all adult ages. Each assayed cohort began as four cages containing ~1200 flies each, where the volume of a cage was 13.2H × 18.5W × 22.4L cm^3. We redistributed and combined flies periodically to maintain this 1200 flies/cage density as the number individuals in the cohort declined. When a cohort fell to 600 individuals (50% cage density), flies were transferred to a half-cage, at 300 individuals, the cohort was transferred to a quarter-cage, and at 100 individuals, the cohort was transferred to a single 8 dram vial. Flies were briefly anesthetized using carbon dioxide during these consolidations. The number of dead flies in each cage or vial was recorded over all daily intervals.

A fecundity measure was made in concert with the mortality assay of experiment 4. After the daily mortality count, flies were given a new plate of food. Eggs that had been laid on the surface of the old food plate were collected through a filtration process onto a membrane using a modified Buchner funnel. A digital image of the membrane was then taken and the number of eggs laid were counted using ImageJ software. A video demonstrating this procedure is presented in Supporting Information. Carbon dioxide can make females eject eggs in greater than normal numbers. To compensate for this problem, the number of eggs laid during the 24 hours after exposure to carbon dioxide was removed from the fecundity analysis.

STATISTICAL METHODS

Experiment 1

In experiment 1, we measured development times for three selection treatments: O, AO, and BO. These measurements were made

with three different assays corresponding to three discrete time-points over the course of the derivation of these treatments. The AO populations were measured 15, 22, and 28 generations after their derivation from the O populations, and the BO populations were measured 13, 19, and 24 generations after their derivation from the O populations. Since our primary interest is in the differentiation of these populations from their O ancestor, in each assay development times were scaled for each sex by the development time in the appropriate O ancestral population. Thus, the O1 male development time was subtracted from the AO1 male development time at each sample time and so on.

We let each measured development time difference at generation t be $y_{ijkm}(t)$, where the subscripts indicate different levels of the experiment. These levels include selection treatment ($i = 1$ (AO) and 2 (BO)), sex ($j = 1$ (female), 2 (male)), population ($k = 1, \ldots,10$), and vial ($m = 1,\ldots,10$). Then a mixed effects linear model for development time differences is,

$$y_{ijkm}(t) = \mu + \alpha\delta_i + \beta\delta_j + \pi\delta_i\delta_j + \left(\gamma + \theta\delta_i + \varphi\delta_j + \omega\delta_i\delta_j\right)t + b_k + c_{km} + \varepsilon_{ijkmt}, \quad (1)$$

where $\delta_s = 0$ if $s = 1$ and 1 otherwise. The random components, b_k, c_{km}, and ε_{ijkmt} correspond to the random variation from populations, vials nested within populations, and residual variation, respectively. These are all assumed to be independent, normally distributed with zero mean and variances σ_b^2, σ_c^2, and σ_ε^2, respectively. Parameters of equation (1) were estimated by the restricted maximum likelihood techniques implemented by the *lme* function in R (R Core team 2014).

To determine if the development time of, say, the AO populations was significantly different from the O populations at any of the three measured generations reduces to determining if any of the predicted development time differences were significantly different from 0. As an example the development time difference for the AO females at generation t would be from equation (1), $\mu+\gamma t$. The variance of this difference is $\mathrm{Var}(\mu) + t^2\mathrm{Var}(\gamma) + 2t\mathrm{Cov}(\mu,\gamma)$. A consequence of the assumption of normally distributed random effects is that the errors on the parameter estimates are assumed to have a t-distribution which for the large sample sizes here can safely be assumed to be approximately normally distributed. Since there are three different generations, the confidence intervals on these predictions need Bonferroni correction (Miller 1966) so that all three intervals have a 5% chance of the true difference being outside these intervals. We used the same approach with the BO populations. We also ran a second test to determine if the male and female development time differences differed within a selection treatment. This was also done by taking the difference of the male and female values at each generation and testing for a difference of zero.

Experiment 2

The same basic analysis as that of Experiment 1 was used for pupal and adult development time. However, this experiment did not test samples at different times. Sex cannot be determined for pupae, and since the sample sizes were much smaller than experiment 1, we decided to increase our power to detect differences in selection regimes by pooling sexes. Results from experiment 1 suggest these differences tend to be small in any case. To test for convergence, we tested paired treatment groups that share the same recent selection (i.e., CO vs. nCO, AO vs. ACO, and B vs. BO) for effects of selection on mean development time. The observations consisted of the development time of individuals (y_{ikm}) from selection treatment ($i = 1$ (ACO or CO or B), 2 (AO or nCO or BO)), population-k ($k = 1$,..10), and vial m ($m = 1, \ldots 3$), and are assumed to be described by,

$$y_{ikm} = \mu + \alpha\delta_i + b_k + c_{km} + \varepsilon_{ikm}, \quad (2)$$

where $\delta_s = 0$, if $s = 1$ and 1 otherwise, and b_k, c_{km}, and ε_{ikm} are independent standard normal random variables with zero means and variance σ_b^2, σ_c^2 , and σ_ε^2, respectively. Statistically testing for a significant effect of selection regime on development time corresponds to determining if α is significantly different from 0.

To test for divergence, the six selection regimes were reclassified to three different categories: AO and ACO to A; CO and nCO to C; B and BO to B. The effects of selection regime were then evaluated with equation (2). Parameters of equation (2) were estimated by the restricted maximum likelihood techniques implemented by the *lme* function in R (R Core team 2014).

Experiment 3

We again tested for convergence between paired selection treatments (i.e., CO vs. nCO, AO vs. ACO, and B vs. BO) for effects of selection on fecundity over 3–4 consecutive ages. The observations consisted of fecundity at a particular age (t) but within a small age interval ($k = 1, 2,..,m$). These age intervals were chosen to span the ages, such that all comparison populations still had live flies. Within each interval, fecundity rates were modeled by a straight line and allowing selection regime ($j = 1$ (ACO or CO or B), $j = 2$ (AO or nCO or BO)) to affect the intercept of that line but not the slope. However, slopes were allowed to vary between intervals. As with the other analyses, populations ($i = 1,\ldots,10$) were assumed to contribute random variation to these measures. With this notation, the fecundity at age-t, interval-k, selection regime-j, and population-i, is y_{ijkt} and is described by,

$$y_{ijkt} = \alpha + \beta_k + \delta_j\gamma_j + (\omega + \pi_k\delta_k)t + \delta_k\delta_j\mu_{jk} + c_i + \varepsilon_{ijkt}, \quad (3)$$

where $\delta_s = 0$ if $s = 1$ and 1 otherwise, and c_i and ε_{ijkt} are independent standard normal random variables with variance σ_c^2 and σ_ε^2, respectively. The effects of selection on the intercept are

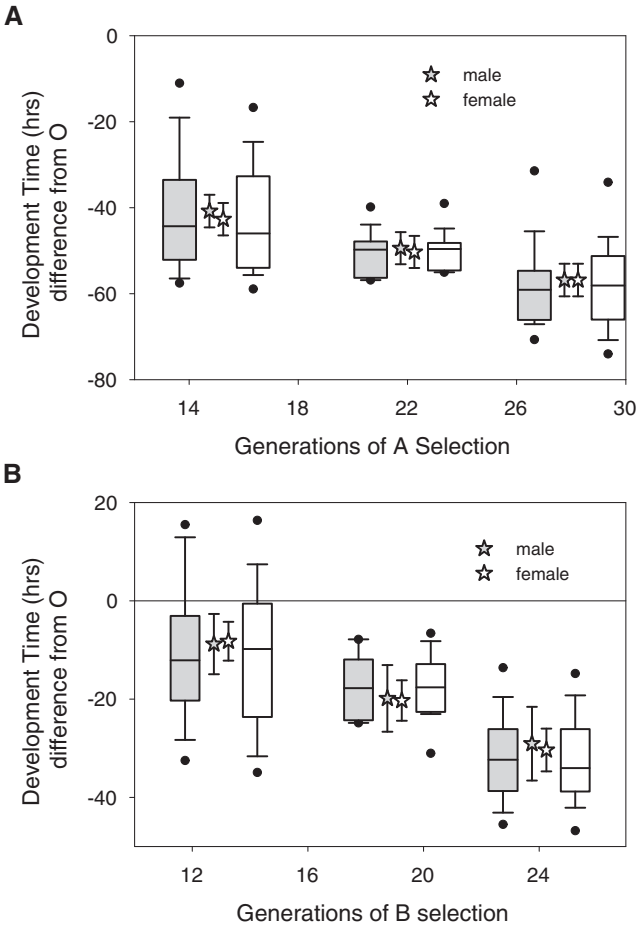

Figure 2. Evolutionary trajectory of mean egg-to-adult development time in the newly derived AO and BO treatments. (A) The development time differences from the O populations for AO males and females. The stars show the predicted development time differences (eq. 1) for each sex and the bars are simultaneous 95% confidence intervals. Each of the three intervals does not include 0 suggesting the AO populations are developing significantly faster than their O ancestors. To the left and right of the stars are box plots for the entire sample at that generation. The top and bottom of the box are 75th and 25th percentiles respectively. The line in the box is the median. The upper and lower bars are 90th and 10 percentiles respectively and the top and bottom points are 95th and 5th percentiles. (B) The development time differences (from the O populations) for BO males and females. Symbols follow the conventions of Figure 2A.

assessed by considering the magnitude and variance of both γ_j and μ_{jk}.

Divergence was tested by making the same reclassification as described in the methods for experiment 2. Equation (3) was then used to assess the effects of the three selection treatments. For both the convergence and divergence analysis, we used the Bonferroni correction to adjust the significance level for each pair-wise comparison made by dividing the significance level by the number of age intervals used in the analysis (0.05/n, where n is he number of age interval used).

Experiment 4

The same basic analysis as that of Experiment 3 was used to test for convergence and divergence of life-time fecundity. However, this experiment did not contain all six selection treatments, instead it only contained four (ACO, AO, CO, and nCO). For convergence the same pairing was used (i.e., ACO vs. AO and CO vs. nCO) and for divergence we used same classification used in Experiment 2.

Empirical examination of mortality in these populations showed low and relatively constant mortality at young ages, especially in the "C" type populations, then an exponential rate of increase, followed by a slowing of mortality at advanced ages. These patterns have relatively straightforward evolutionary explanations that we incorporate into a simple demographic model. In "C" type populations, there is a prolonged adult, prereproductive period during which evolution would be expected to reduce mortality. At late life, mortality rates are also expected to level off, albeit at very high rates, for reasons we have discussed previously (Mueller et al. 2012). These considerations lead to a model of mortality rates at age-t,

$$u(t) = \begin{cases} Aexp\ (\alpha bd_1) & if\ \ t \le bd_1 \\ \exp(\alpha t) & if\ bd_1 < t < bd_2 \\ Aexp\ (\alpha bd_2) & if\ \ t \ge bd_2 \end{cases}. \qquad (4)$$

In this model, there is a constant mortality plateau up to age-bd_1, followed by a Gompertz pattern of exponential increase in mortality and finally ending with a second plateau in mortality after age-bd_2. To estimate the parameters of equation (3), we collected mortality rates over discrete intervals of time, typically one day in length. At each interval, the number of survivors at the start of the interval N_i and the number of deaths over the ensuing interval, d_i, were observed. The observed number of survivors and deaths over each discrete time interval can then be used to calculate maximum likelihood estimates of the four parameters in equation (4) using the discrete interval mortality rates predicted from equation (4), which are equal to $1 - p_{t+1}p_t^{-1}$, where $p_t = exp\{-\int_0^t u(t)\}$ (Mueller et al. 1995). With this estimation procedure, early and late plateaus are not forced on the data, since the maximum likelihood estimates can be undefined with either bd_1 can be less than 1 or that bd_2 can be greater than the oldest fly in an experiment. Indeed these parameters were undefined several times with these data.

The model parameters from equation (4) were estimated for each of the 10 "C" type populations (five nCO and five CO), 10 "A" type populations (five AO and five ACO) and for each sex, giving rise to 40 estimates of each parameter. We then used a simple linear-mixed effects model to determine if either sex

or selection regime had a significant impact on these parameters. For comparisons within A and C groups, there were only 20 total samples. For comparisons between different selection types, there were a total of 40 samples, except when bd_1 and bd_2 were undefined.

Results

OVERALL DEVELOPMENT TIME RESULTS FROM EXPERIMENT 1

Figure 2 shows the average egg to eclosion development time values for the AO and BO populations during the course of their derivation. These data show a pattern of rapid divergence for the five newly derived AO populations (Fig. 2A) and the five newly derived BO populations (Fig. 2B) from their O ancestors, and this pattern emerges in fewer than 15 generations. In addition to diverging from the ancestral treatment, the newly derived treatments rapidly diverged from one another.

Comparing males and females within a selection treatment revealed that males were generally further differentiated from their ancestral O population than were females. However, the difference between males and females was small, ranging from 0.7 to 1.1 hours in the AO populations and from –0.6 to 1.3 hours in the BO populations. Due to the large sample sizes in this experiment, these small differences were significantly different from 0 in generations 15 and 22 in the AO populations and in generation 24 in the BO populations. Since we utilized a simultaneous inference scheme, we take these results as evidence that sex is important to our model predictions, which supports the use of equation (1) with effects of sex on the intercept and slope of our linear model as our model.

Both the AO (Fig. 2A) and BO (Fig. 2B) populations show highly significant deviations from the O population development times. At the last sample time, the AO populations are developing about 60 hours faster than the Os, while the BO populations are developing about 35 hours faster. Over the time course of this experiment, these development time differences decrease significantly. For both BO and AO males and females, a test of whether the slopes are equal to 0 show significant differences with $P < 0.0001$ in each case.

Variation in development times that is not accounted for by the linear model, which could arise due to drift generating differences among populations, accounts for only about 7% of the total variation. Variation due to uncontrolled vial differences accounts for only about 2% of the variation.

DEVELOPMENT TIME RESULTS FROM EXPERIMENT 2

Figure 3 illustrates patterns of convergence and divergence observed for the developmental characters measured in Experiment 2. First, measures of pupariation and eclosion taken within matched A, B, and C-type treatments, both long-standing and recently derived, are very similar. Thus, both of these metrics of development are highly convergent, regardless of a population's specific evolutionary history. Second, populations that share the same selection regime recently (e.g., ACO and AO) are markedly different from those that do not; that is to say, A-type development is more rapid than B-type development, which is more rapid than C-type development. Thus, both the time from hatching to pupariation and the time from hatching to eclosion are developmental characters that have unambiguously diverged in the A, B, and C treatment groups of contemporary populations.

Adult and pupal development times show no significant differences when populations subjected to the same selection regimes are compared, despite the differences in duration of the shared selection regime (Table 2A). For pupal development time, these tests could have detected differences at 7% of the mean and for adult development times about 4% of the mean (Table 2A), thus these are not insensitive tests. However, when populations subjected to different selection regimes are compared we see significant development time differences, with A type selection resulting in the fastest development time followed by B type selection and then C type selection (Table 2B).

EARLY FECUNDITY RESULTS FROM EXPERIMENT 3

We observe patterns from Experiment 3 that largely parallel those of Experiment 2 (Fig. 4). We observe similar early-life fecundity trajectories in the matched long-standing and recently derived A, B, and C-type selection treatments. Again populations that share the same selection regime recently (e.g., ACO and AO) are markedly different from those that do not. Qualitatively in keeping with the results of Experiment 2, the A, B, and C treatment groups of populations are clearly divergent from each other, although this is most obvious in the earliest ages assayed. Statistically, comparisons made between treatments of the same type return with no difference, with the exception of ACO versus AO at ages 12, 13, and 14 (bold values in Table 3; hours 276–336, $P < 0.0125$). In contrast, comparisons made between selection types return with statistically significant differences, with the exception of B-type versus C-type after 11 days of age (bold values in Table 4, hours 276–336, $P > 0.0166$). Our significance thresholds vary between experiments and sometimes between treatment comparisons within an experiment due to the number of tests involved in each comparison.

ADULT LIFE-HISTORY RESULTS FROM EXPERIMENT 4

Figures 5 and 6 reiterate the convergence and divergence patterns observed in the other experiments. Within A and C-type treatments, long-standing and newly derived populations are not significantly different from one another (Table 5). On the other hand, populations that do not share the same selection regime are

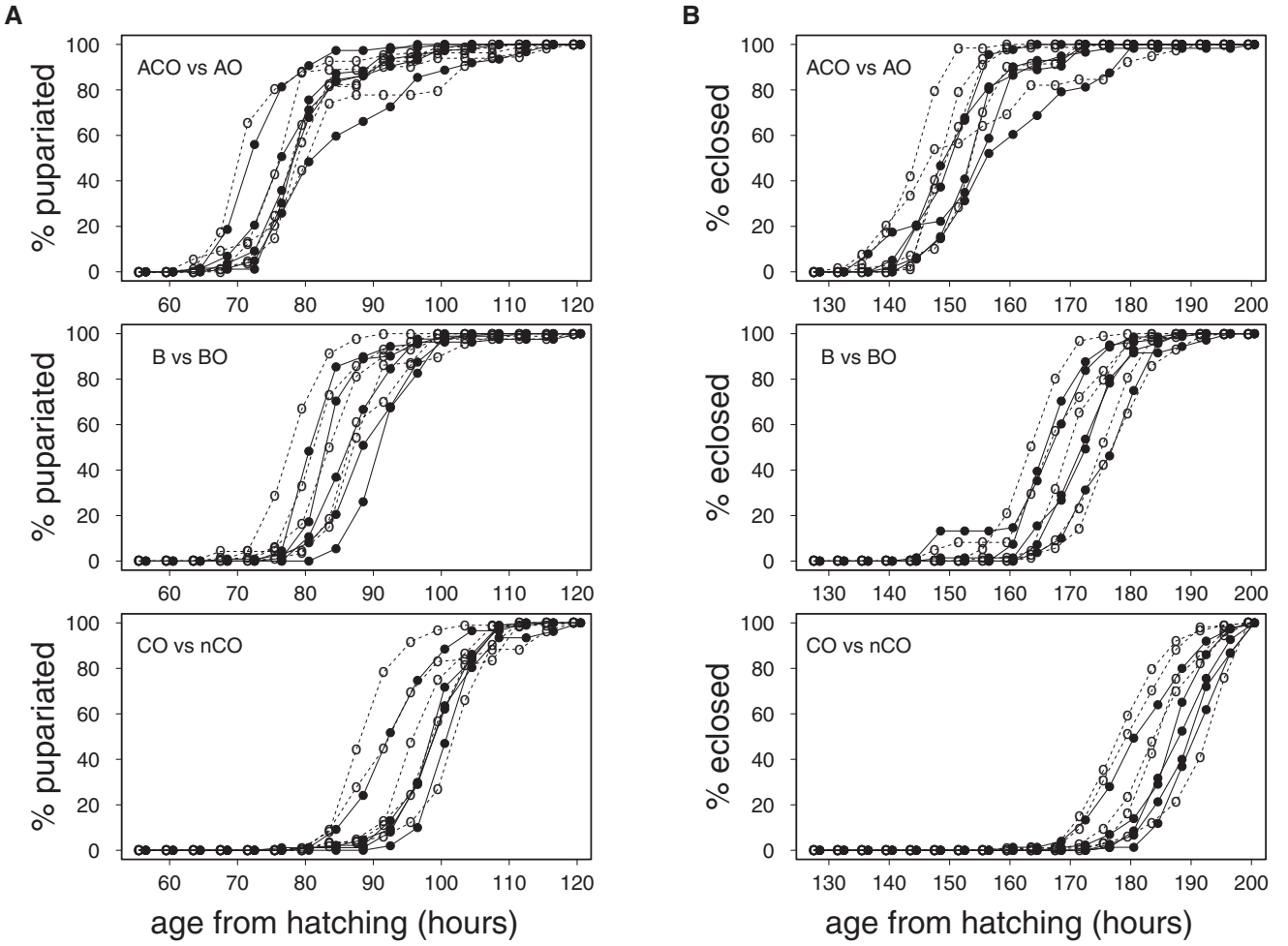

Figure 3. Comparison of development time in all six contemporary selection treatments. (A) Time to pupariation from thirty cohorts of our six laboratory populations. Open circles and dashed lines represent the percentage of cohort pupariated by each hour assayed in the five replicated longstanding populations (ACO in the top panel, B in the middle panel, CO in the bottom panel), and solid circles and solid lines represent percentage of cohort pupariated per hour assayed in the five replicated newer derived populations. (B) Time to eclosion from thirty cohorts of our six laboratory populations. The percentage of each cohort that reached eclosion by each hour assayed is expressed following the same conventions as A.

Table 2. Convergence and divergence in development time among contemporary populations.

	Comparison	Pupae	Diff	95% CI	Adult	Diff	95% CI
A	AO	80.5			155.0		
	ACO	81.3	(ACO–AO) 0.8	±5.8	152.3	(AO–ACO) 2.7	±5.4
	BO	88.1			172.1		
	B	86.4	(BO–B) 1.7	±5.9	173.1	(B–BO) 1.0	±7.4
	nCO	99.4			188.4		
	CO	98.2	(nCO-CO) 1.2	±6.1	186.1	(nCO–CO) 2.3	±6.6
B	A	80.9	(B-A) 6.4	±3.6	153.6	(B–A) 19.0	±4.0
	B	87.3	(C-B) 11.5	±3.6	172.6	(C–B) 14.6	±4.0
	C	98.8	(C-A) 17.9	±3.6	187.2	(C–A) 33.6	±4.0

Egg-to-pupa and egg-to-adult development times (hours) in Experiment 2. The 95% confidence intervals are computed for the development time differences using equation (2). Newly derived selection treatments of similar types (A) show no significant differences from long-established selection treatments of the same type. Conversely, selection regimes of the different types (B) are all significantly diverged from one another.

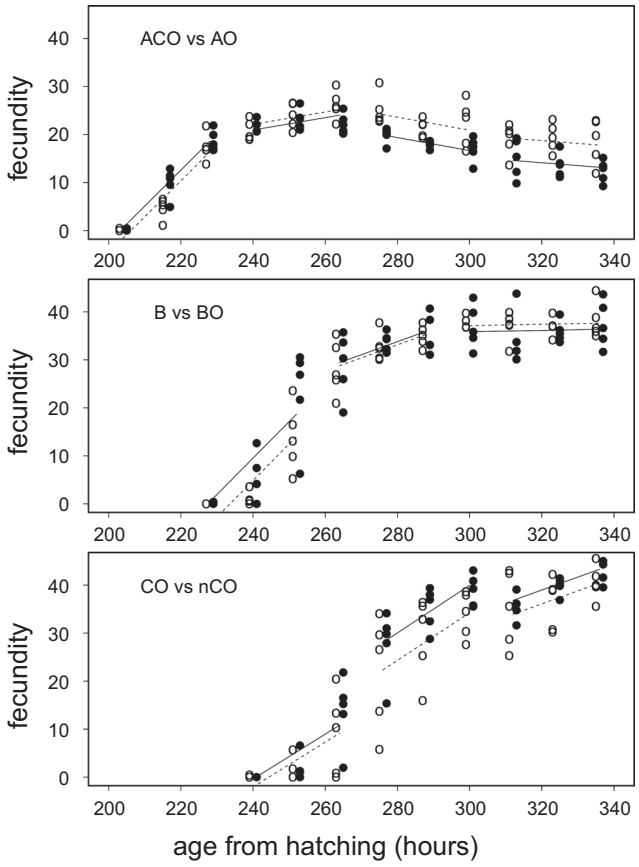

Figure 4. Early-life fecundity comparison in all six contemporary selection treatments. Initial age-specific fecundity prior to day 14 from 30 cohorts of our six laboratory selection treatments. Open circles and dashed lines represent average eggs laid per female per day as a function of age in the five replicated longstanding populations (ACO in the top panel, B in the middle panel, CO in the bottom panel), and solid circles and solid lines represent the five replicated newer derived populations (AO in the top panel, BO in the middle panel, nCO in the bottom panel).

highly divergent from one another. A notable exception from this trend is evident in middle-period fecundity, between ages 17 and 25 days from egg, when there is no detectable difference in fecundity among those flies that are still alive in A and C cohorts. But in keeping with the results from Experiment 3, fecundity prior to age 16 is significantly different among selection treatments (Table 6, $P < 0.005$), and furthermore, fecundity after age 25 is significantly different (Table 6, $P < 0.005$), as A flies die in large numbers after this age (Fig. 5).

The C-type populations show smaller values of the parameters A and α compared to A-type populations, and these differences are significant for A ($p = 0.0007$) but not for α ($p = 0.0403$; Fig. 7A; Table S10). The C-type populations show greater values of bd_1 ($P = 0.0014$) and bd_2 ($P < 0.0001$; Fig. 7B; Table S10). So with respect to at least three parameters that determine the trajectory of age-specific mortality, there is evidence of significant differentiation between the C-type and A-type populations. Differentiation of bd_2 between A and C types has been previously documented (Rose et al. 2002), and is found again here. No significant differentiation is observed within the selection types (Tables S11–S12).

Discussion

OVERVIEW OF RESULTS: RAPID DIVERGENCE AND CONVERGENCE

First, when comparing the degree of phenotypic *divergence* among populations of the three recently derived treatments (AO, BO, and nCO) with those of the long-standing treatments (ACO, B, and CO), it is apparent that life-history differentiation is remarkably similar when the comparison is made between these two sets of treatments. In effect, hundreds of additional generations of A, B, and C-type selection seem to have yielded at most minor increases in life-history differentiation.

Second, and conversely, there is a high degree of phenotypic convergence within each of the three sets of A, B, and C-type populations. There are some exceptions to this general pattern. For example, (i) early fecundity over age 12, 13, and 14 days was significantly different between the ACO and AO populations, and (ii) early fecundity over ages 12 and 13 days was significantly different between the CO and nCO populations.

We find that the phenotypes of our newly derived populations usually converged with those of longstanding populations sharing the same selection regime within 200 generations. We also find that these newly derived populations, all initiated from a common ancestor, significantly diverge from one another within this time frame. The rapidity of convergence and divergence suggests that in this particular set of 30 populations, recent evolutionary history is highly predictive of phenotype. We do not presume that phenotypic divergence and convergence in these experiments necessarily involves the same underlying genetic mechanisms. In Mendelian evolution experiments, selection treatments sometimes produce common phenotypes in independent replicates that are the result of different genetic "solutions" (e.g., Garland et al. 2002; Kawecki and Mery 2006). We will explore the degree of convergence and divergence in our populations at the genotypic level in future work.

Given that Mendelian populations maintain a considerable amount of standing genetic variation that is reshuffled every generation by recombination, it is certainly reasonable to expect selection histories to be erased quickly even in moderately sized populations. Whether or not this occurs appears to depend on the details of the experiment in question. Populations of a bean weevil collected from different geographic origins continued to differ in host preference (Kawecki and Mery 2003), and in a

Table 3. Convergence of early-life fecundity.

Age range (hours)	*ACO vs. AO*	*Age range (hours)*	*B vs.BO*	*Age range (hours)*	*CO vs. nCO*
204–228	0.025				
240–264	0.381	228–252	0.04	240–264	0.568
276–300	**0.011**	264–288	0.764	276–300	0.090
312–336	**0.008**	300–336	0.561	312–336	0.362

Calculated *P*-values for the linear-mixed effects model of convergence on initial fecundity between populations (Experiment 3). Fecundity estimates are compared between selection treatments within the same age intervals, although these intervals vary slightly by comparison. Bold values indicate significant nonconvergence. More information provided in Tables S1–S3.

Table 4. Divergence of early-life fecundity.

Age range (hours)	*A-type vs. B-type*	*A-type vs. C-type*	*B-type vs. C-type*
240–264	3.26×10^{-4}	2.57×10^{-12}	1.73×10^{-8}
276–300	4.74×10^{-9}	1.90×10^{-6}	**0.013**
312–336	5.29×10^{-12}	6.46×10^{-13}	**0.216**

Calculated *P*-values for the linear-mixed effects model of divergence on initial fecundity between selection regimes for Experiment 3. Bold values indicate nonsignificant divergence. More information provided in Tables S4–S6.

number of life-history traits (Bieri and Kawecki 2003), despite 120 generations of adaptation to a common laboratory environment. Populations of *Drosophila* collected from different locations and reared in a uniform laboratory environment have previously been shown to converge for some phenotypes but not others (Cohan and Hoffman 1989; Griffiths et al. 2005; Simoes et al. 2007; Simões et al. 2008; Santos et al. 2010). This could be attributable to stochastic effects during the initial founding phase, insufficient sampling of natural variation, or both.

It is also conceivable that the speed of convergence among populations with different lab evolutionary histories is trait specific. One version of this hypothesis is that history should play a greater role in the convergence of traits less directly related to fitness; that is to say, fitness traits should converge faster and more consistently than traits less obviously associated with fitness as determined by a particular selection regime. This is a classic result of evolution experiments with asexual populations (e.g., Lenski and Travisano 1994; Travisano et al. 1995), but remains ambiguous in experiments with Mendelian populations (e.g., Joshi et al. 2003). Our finding that early fecundity was the same between the long-established ACO and newly derived AO populations prior to age 13, but diverged after age 13, is potentially consistent with this idea. Early fecundity is the primary fitness trait in the A-type selection regime, as eggs are collected within 10 days from hatching in a single generation (Fig. S1). Thus fecundity after this age should be effectively decoupled from fitness.

Another aspect of the hypothesis that the effect of history on convergence is trait-specific is that past selection might continue to affect the adaptation of populations to new selection pressures if the past selection gave rise to particular patterns of genotype by environment (G × E) interactions. We have invoked this explanation before in a study of reverse evolution with some of the same populations as in the current study (Teotonio and Rose 2000). Notably, that study showed somewhat less phenotypic convergence for some characters and populations than we have generally found here. Recent work by Fragata et al. (2014) reports that strong initial differentiation among populations of *D. subobscura* is diminished within 22 generations of a common selection environment, both for traits expected to be correlated with fitness and those that were not. Overall, our results are consistent with this result and support the view that past evolutionary history generally has transient effects in the face of ongoing selection and recombination.

NOVEL AGING RESULT: EARLY-ADULT MORTALITY PLATEAUS

The second major finding that emerged from this study was the virtual absence of aging between ages 14 and 28 days, from egg, in the age-specific mortalities and fecundities of the ten C cohorts assayed in Experiment 4. These cohorts were derived from populations that had been cultured for about 350 (CO) and 200 (nCO) generations without reproduction during, or of course before, this period of adult life. Thus, there has been full-intensity selection for continued survival to least up to the age of 28 throughout these hundreds of generations. From this standpoint, then, it is perhaps unsurprising that we find little statistically detectable aging during this period of adult life, despite the reproductive maturity of these fruit flies (Rose et al. 2007).

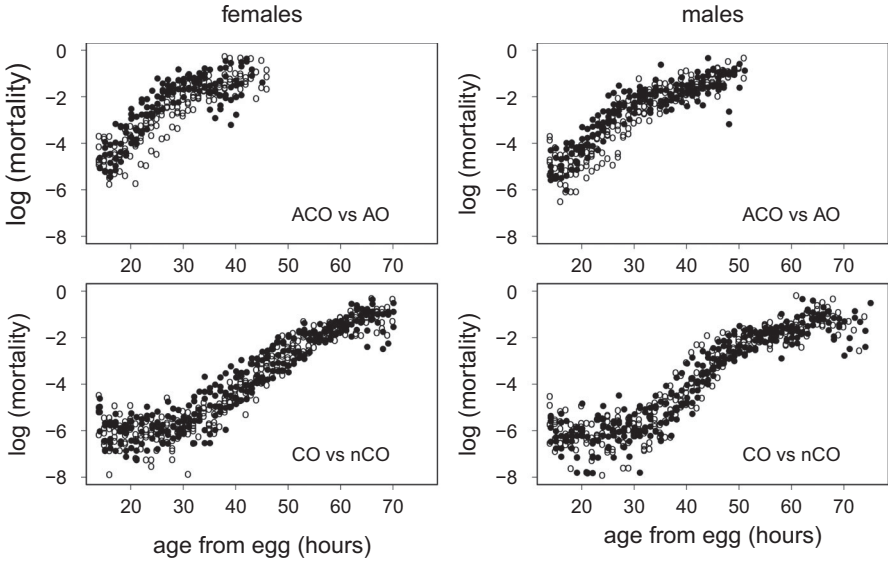

Figure 5. Adult age-specific mortality among males and females from A-type and C-type selection treatments. Points represent log-transformed mortality per population in each selection treatment, and are presented separately for females (left panels) and males (right panels). Open circles represent longstanding populations (ACO in the top panels and CO in the bottom panels), and solid circles represent newer derived populations (AO in the top panels and nCO in the bottom panels).

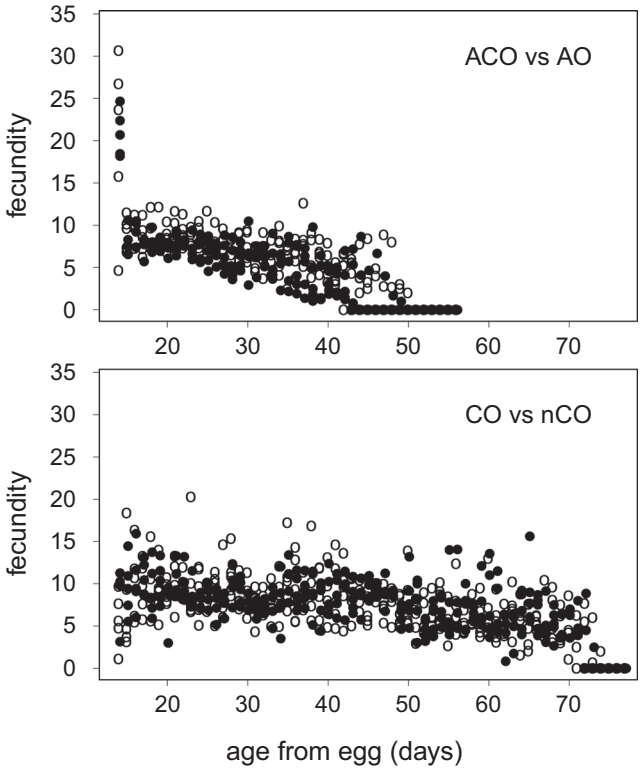

Figure 6. Adult age-specific fecundity from A-type and C-type selection treatments. Points represent average number of eggs laid per female per day as a function of age. Open circles represent longstanding populations (ACO in the top panel, CO in the bottom panel), where closed circles represent newer derived populations (AO in the top panel, nCO in the bottom panel).

But prior studies of ours did not reveal this pattern; for example, previous experiments with the CO lines do not reveal an absence of aging during the same period (Rose et al. 2002). Rather, aging appears to start soon after the age of 16 or 18 days from egg in the cohorts assayed at that time, when the CO populations were well over 150 generations of selection for survival until at least 28 days, bearing in mind their history of O selection prior to their derivation as CO stocks in 1989 (vid. Rose et al. 1992; Fig. 6). This disparity relative to the present data is particularly obvious for the female cohort data from the 2002 study of Rose et al., which shows a pattern of increasing mortality between ages 14 and 28 days from egg, unlike the data found in our present comparison of A and C type mortality rates.

At present, our interpretation is that this disparity was due to our earlier use of vial assays of age-specific adult life-history. The A, O, and C type populations are cultured using adults laying eggs in cages, with C populations living in cages for the two weeks between ages 14 and 28 from egg. Thus, we suggest, the present assay was performed under conditions more representative of the conditions that selection was actually focused on over the last 200 or 350 generations of the culture of the nCO and CO populations, respectively. Conversely, our earlier assays were conducted under conditions that did not closely reflect the circumstances of C-type selection. Our conclusion is that, if we had used the same type of cage assay as that employed in the present study before, we would have previously detected the virtual absence of aging during the 14–28 day life-history period.

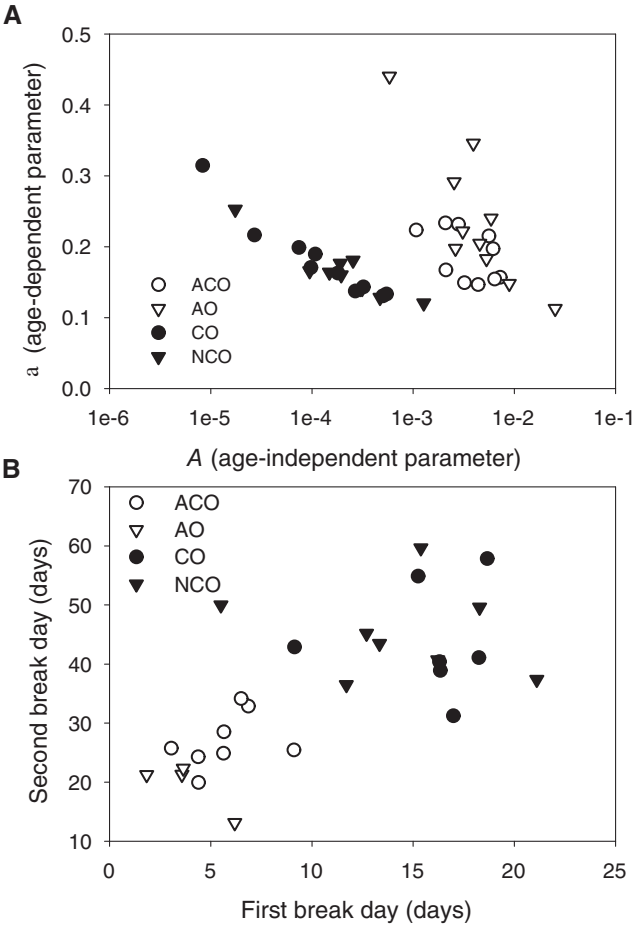

Figure 7. Parameter estimates for the mortality model of equation (4). (A) The parameters A and α for the A-type (open symbols) and C-type populations (solid symbols). (B) The parameters bd_1 and bd_2 for the A-type and C-type populations. The only parameter that does not show significant differentiation is α ($P = 0.0403$).

OVERALL SYSTEM OF 30 DIFFERENTIATED POPULATIONS

Our observation of rapid convergence and divergence in these experiments supports the idea that experimental evolution research with Mendelian populations does not require the kind of very long sustained selection experiments that we have done, at least for some of our most pressing questions. While long-term experiments may not be necessary to sufficiently address questions about the role of convergence and divergence in adaptation, we feel that the long-established lines of our *Drosophila* system provide an invaluable resource for the dissection of particular fitness traits. Our populations provide a spectrum of phenotypes that may be of interest to evolutionary and developmental biologists alike. Our A, B, and C-type selection regimes have produced populations with different and finely tuned life-histories, at both the juvenile and adult stage. These differences provide opportunities for multiple types of investigation, including comparative physiology, detailed developmental biology, mechanistic research on aging, speciation, the evolution of mating strategies and social behavior.

We are not aware of a longer standing Mendelian resource as highly replicated as ours. Further, our work here suggests that, with the creation of our newly derived A, B, and C-type

Table 5. Convergence of A-type and C-type female fecundity.

Age range (days)	*ACO vs. AO*	*CO vs. nCO*
14–16	0.889	0.137
17–19	0.637	0.801
20–22	0.528	0.91
23–25	0.45	0.713
26–28	0.573	0.667
29–31	0.758	0.942
32–34	0.639	0.899
35–37	0.357	0.982
38–40	0.272	0.89
41–43	0.502	0.97
44–46	0.574	0.654
47–49	0.384	0.732
50–52	0.989	0.95
53–55	0.717	0.865
56–58		0.742
59–61		0.519
62–64		0.759
65–67		0.467
68–70		0.895
71–73		0.907
74–76		0.762

Calculated *P*-values for the linear-mixed effects model of convergence on adult age-specific fecundity in Experiment 4 between AO versus ACO and CO versus nCO. More information provided in Tables S7–S8.

Table 6. Divergence of A-type and C-type female fecundity.

Age range (days)	*Female fecundity*
14–16	2.03×10^{-5}
17–19	**0.193**
20–22	**0.195**
23–25	**0.089**
26–28	0.004
29–31	0.002
32–34	4.45×10^{-4}
35–37	1.11×10^{-5}
38–40	2.71×10^{-5}
41–43	2.48×10^{-5}
44–46	8.94×10^{-5}

Calculated *P*-values for the linear-mixed effects model of divergence on female age-specific fecundity in Experiment 4 between A-type selection and C-type selection. Bold values indicate nonsignificant divergence. More information provided in Table S9.

selection regimes, we have effectively doubled this replication from fivefold to tenfold. And with respect to characters that are highly divergent among the three selection times, we have generated a 30-population system that is ripe for interrogation from multiple perspectives.

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DATA ARCHIVING

Data files have been deposited in Dryad: http://dx.doi.org/10.5061/dryad.133n7

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Detailed schematic for A, B, and C culture protocols.

Supplementary Video. This short Youtubevideo outlines our methods for counting eggs during fecundity assays (title, Fecundity: Live Demonstration). Link created 2/17/15.

Table S1. Extended summary statistics for Experiment 3: ACO vs. AO early fecundity. Here, as in Table 3, bold values indicate significant non-convergence.

Table S2. Extended summary statistics for Experiment 3: B vs. BO early fecundity (to go along with Table 3 in main text).

Table S3. Extended summary statistics for Experiment 3: CO vs. nCO early fecundity (to go along with Table 3 in main text).

Table S4. Extended summary statistics for Experiment 3: A-type vs. B-type early fecundity (to go along with Table 4 in main text).

Table S5. Extended summary statistics for Experiment 3: A-type vs. C-type early fecundity (to go along with Table 4 in main text).

Table S6. Extended summary statistics for Experiment 3: B-type vs. C-type early fecundity. Here, as in Table 4, bold values indicate non-significant divergence.

Table S7. Extended summary statistics for Experiment 4: ACO vs. AO adult age-specific fecundity (to go along with Table 5 in main text)

Table S8. Extended summary statistics for Experiment 4: CO vs. nCO adult age-specific fecundity (to go along with Table 5 in main text)

Table S9. Extended summary statistics for Experiment 4: A-type vs. C-type adult age-specific fecundity. Here, as in Table 5, bold values indicate non-significant divergence.

Table S10. Extended summary statistics for Experiment 4: A-type vs. C-type adult age-specific mortality using the two-stage Gompertz model.

Table S11. Extended summary statistics for Experiment 4: ACO vs. AO adult age-specific mortality using the two-stage Gompertz model.

Table S12. Extended summary statistics for Experiment 4: CO vs. nCO adult age-specific mortality using the two-stage Gompertz model.