The devil in the details of life-history evolution: instability and reversal of genetic correlations during selection on *Drosophila* development

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Abstract

The evolutionary relationships between three major components of Darwinian fitness, development rate, growth rate and preadult survival, were estimated using a comparison of 55 distinct populations of *Drosophila melanogaster* variously selected for age-specific fertility, environmental-stress tolerance and accelerated development. Development rate displayed a strong net negative evolutionary correlation with weight at eclosion across all selection treatments, consistent with the existence of a size-versus-time tradeoff between these characters. However, within the data set, the magnitude of the evolutionary correlation depended upon the particular selection treatments contrasted. A previously proposed tradeoff between preadult viability and growth rate was apparent only under weak selection for juvenile fitness components. Direct selection for rapid development led to sharp reductions in both growth rates and viability. These data add to the mounting results from experimental evolution that illustrate the sensitivity of evolutionary correlations to (i) genotype-by-environment (G × E) interaction, (ii) complex functional-trait interactions, and (iii) character definition. Instability, disappearance and reversal of patterns of genetic covariation often occur over short evolutionary time frames and as the direct product of selection, rather than some stochastic process. We suggest that the functional architecture of fitness is a rapidly evolving matrix with reticulate properties, a matrix that we understand only poorly.

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Introduction

The assumption that organisms evolve within a definable, stable genetic architecture has been the motivation for numerous investigations into the genetic relationships among functional traits. In particular, the concept of tradeoffs—negative genetic covariances between fitness-enhancing characters—that constrain the evolution of fitness is central to life-history theory (e.g. Lande 1982; Cheverud 1984; Maynard Smith *et al*. 1985; Clark 1987). For example, Stearns (1992, p. 72) states that 'at least 45 tradeoffs are readily defined between life-history traits'. He goes on to provide an extensive list of cautionary statements and illustrative problems showing 'why we might observe the "wrong" tradeoff'. The problems highlighted by Stearns include fixation of alleles governing a tradeoff, variation in both acquisition and allocation of resources, genotype-by-environment interaction (henceforth G × E), and problems associated with measurement of life-history traits. Other authors have catalogued their own lists of potential confounds (e.g. Houle 1991; Reznick 1992; Roff 1992; Rose *et al*. 1996).

In general, when an empirical study generates the 'wrong' tradeoff there are many ways to explain the result without calling into question the existence of an underlying tradeoff. Here we argue that the very notion of a tractable genetic architecture underlying life history is a sanguine intellectual mistake. We question the existence of fundamental evolutionary tradeoffs in life history on the basis

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of a review of evidence collected from laboratory evolution experiments. Finally we present new results from an analysis of 55 long-term laboratory-evolved populations in which we can demonstrate the reversal of an otherwise robust genetic correlation in a subset of selected lines. The devil, we submit, is in the details of the adaptive process.

At the outset it is important to distinguish between the various kinds of tradeoffs that have been hypothesized. We will categorize these as acquisition and allocation tradeoffs, although in reality these grade together. First, acquisition tradeoffs are the result of behavioural or physiological 'decisions' made by the organism concerning the mode of resource acquisition and the kind and efficiency of uptake from the forage. An organism may suffer increased predation as a result of foraging activity (an ecological tradeoff), or it may have to decide how efficiently and selectively it extracts nutrition from what it acquires (a physiological tradeoff). Second, an organism allocates energy to competing functions, such as growth or reproduction, which therefore trade off with each other. Both varieties of tradeoff, acquisitive and allocative, posit a linkage between traits in which net fitness is constrained. But these constraints also depend on the environment in which functional traits are expressed. Because environments change, this generates a potential ecological tradeoff between specialist and generalist organismal niches; a form of variation captured in the reaction norm. While theoretically sound at any single instant in time, do these principles actually constrain evolution in a meaningful way?

Few practitioners of life-history theory would argue for the immutability of tradeoffs over geological time, because this would require fitness to be an absolute and universal measure, and diverged taxa are expected to have different functional traits. The genetic architecture of fitness implicit in life-history theory is plainly supposed to be microevolutionary in impact. As clearly articulated by Reznick (1992), the first problem with tradeoffs is the identification of the effects of alleles against a particular genetic background, making the population the appropriate unit of study. Thus, while phenotypic manipulations performed on individuals may sometimes illuminate physiological or behavioural tradeoffs, in the absence of parallel additive genetic variation for the characters involved in such a tradeoff, the evolutionary response may not follow the prediction from the manipulative experiment. In fact, there is no apodictic argument that shows that standing genetic variation will trace the pathways involved in phenotypic tradeoffs. In some of our earlier research on dietary restriction, for example, phenotypic tradeoffs between fertility and longevity bore both striking analogies and disanalogies to evolutionary responses in the same *Drosophila melanogaster* populations (Chippindale *et al*. 1993, 1997). We will return to these experimental results a little later in our discussion of G × E interactions.

The second problem with measuring tradeoffs is the degree to which the standing genetic variation predicts the evolutionary response. That is to ask: given that we can measure a genetic correlation between two traits, to what degree is the correlation stable over evolutionary time? Can a genetic architecture between life-history components be defined over tens, hundreds or thousands of generations? Selection has a winnowing effect on allelic diversity and therefore the additive genetic variation may be depleted at some or all loci during an evolutionary response. Genetic drift is a related issue, first because of loss of variation and second because functional traits may vary in the degree to which they are sensitive to inbreeding, modifying the genetic correlation between characters when populations are finite and small. Finally, G × E interaction can in theory lead to reversal in the sign of a genetic correlation between traits when their expression depends upon a variable environmental factor. Populations evolved in different environments may therefore vary widely in their intertrait correlations, even when tested in a 'common garden'. All of these problems, as well as several not listed, present particular difficulties for the measurement of evolutionary genetic correlations in wild populations, either by direct observation over time or by comparative analysis. By allowing control over the environment of selection and the genetic background of populations under study, laboratory evolution offers our best opportunity to resolve the question of the stability of genetic correlations over short periods of evolutionary time. Below we review some recent results that bear upon this issue.

Chippindale *et al*. (1993) and Leroi *et al*. (1994a,b) documented the apparent disappearance of the well-known life-history tradeoff between early fertility and late fertility / survival described by Rose (1984). These papers demonstrated that, under the standard assay conditions used by Rose, populations selected for high late-life fertility (O populations) had also evolved substantially higher early-life fertility than early-reproduced (B) populations, contrary to theory and earlier results. Upon detailed dissection of the experimental protocols, Leroi *et al*. (1994a,b) concluded that the 'right tradeoff' could be observed under conditions matching the culture protocol followed for the short-lived lines. These authors further documented the steady reversal of the genetic correlation under the 'standard' protocol over the course of the preceding decade. Rose *et al*. (1996) dubbed the appearance and disappearance of the tradeoff the 'Cheshire cat syndrome' after the feline phantasm created by Lewis Carroll. While trivial in their details to all but a hardcore experimentalist, these results have profound implications for our present topic. Ten years of evolution in populations selected for late-life fertility corresponded to just 55 generations, with the early-fecundity tradeoff disappearing after approximately 20 generations. It is therefore apparent that (i) these populations adapted to subtle differences in cul-

ture protocols with great rapidity and (ii) the resulting G × E interaction completely obscured the negative genetic correlation observed early in selection.

With such a degree of evolutionary sensitivity, it is hardly surprising that a recent survey of life-history correlations in *Drosophila* reciprocally tested in different laboratories revealed substantial incongruence between test environments (Ackerman *et al*. 2001). These authors found that female fecundity was particularly sensitive to the test environment, even when considerable effort was made to replicate conditions occurring in the lab of origin. In the antagonistic pleiotropy example given above, the authors were fortunate enough to have a checklist of known environmental differences to apply to the problem. When populations are subjected to truly novel conditions, as during the initial domestication of wild-caught animals, the genetic correlations between life-history traits are likely to shift radically (see Matos *et al*. 2000, 2002; Sgrò and Partridge 2000, 2001, and comment on the latter (Matos and Avelar 2001)). These kinds of results have prompted some authors (e.g. Rose *et al*. 1996) to suggest that the ideal experimental material for laboratory evolution experiments will be populations that have largely ceased to adapt to the specific conditions of lab culture and selection. Plainly this dictum presents a major challenge to the survey of naturally occurring variation and has, therefore, prompted some strong criticism of the relevance of laboratory evolution to natural adaptation (Gibbs 1999; Hoffmann and Harshman 2000). However, for testing the more abstract question of the stability of genetic correlations between life-history characters, there are clear advantages to using laboratory-adapted populations and stable, controlled environmental conditions.

The G × E interaction alluded to is one manifestation of a more general problem for the concept of simple overarching tradeoffs in functional evolution: allocation tradeoffs may be obscured by the evolution of acquisitive traits (Houle 1991). It is simple to envision a negative correlation between two traits sharing a common (limited) resource pool. But if there are loci affecting the size of the shared resource pool, say via acquisition of energy, then the value of the two traits may shrink or grow simultaneously with selection on the acquisition loci. Thus a positive evolutionary correlation may be observed between two characters that remain negatively genetically correlated. Houle (1991) also points out that acquisition traits, being at the 'front end' of an organism's interaction with its world, may be more likely to evolve than allocation functions within the functioning organism.

Recently, Phelan *et al*. (2003) and Archer *et al*. (2003) demonstrated that genetic correlations among fitness components in *Drosophila* can break down and even reverse over relatively short periods of evolutionary time. These authors looked at the comparative and dynamic aspects of the positive genetic correlation between stress resistance and longevity in a large collection of populations with common ancestry but varied selection treatments. Both analyses show the positive genetic correlation between stress and longevity with selection for greater longevity, and early in selection for stress tolerance, or with mild-stress selection. But the correlation reversed with strong sustained selection for stress resistance. Thus, while small increases in either desiccation or starvation resistance always lead to increased longevity, as the populations evolved very high stress resistance the longevity ceased to increase. These studies were able to rule out confounding factors such as inbreeding depression or G × E interaction and isolate the effect as a product of natural selection.

Several earlier studies have also documented shifting character correlations under selection in morphological characters (Clayton *et al*. 1957; Bell and Burris 1973; Wilkinson *et al*. 1990; see also review in Bell 1997, pp. 269–271). Wilkinson *et al*. (1990), for example, showed that the correlation between several measures of body size and sternopleural bristle number switched from weakly negative to strongly positive after 23 generations of selection for greater or lesser thorax size in *Drosophila*. Genetic correlations between traits can disappear, reverse, and otherwise change within short periods of evolutionary time.

In the present study we examine the genetic correlations between juvenile fitness components under selection in *D. melanogaster*. Specifically, we used a large artificially constructed phylogeny (55 populations) selected in several fundamentally different ways to examine the stability of the previously inferred tradeoffs between development rate and body size (Partridge and Fowler 1992; Chippindale *et al*. 1994, 1996, 1997; Zwaan *et al*. 1995; Nunney 1996; Prasad *et al*. 2000), and between growth rate and viability (Chippindale *et al*. 1994, 1996, 1997). We document another instance in which an apparent tradeoff reverses under selection and discuss the significance of this observation from the perspective of the complexity of the matrix of functional-trait relationships.

Materials and methods

Fiftyfive populations from a broad spectrum of selection treatments were used in these experiments. All ultimately descend from a single population of *D. melanogaster* designated IV. The origin and early maintenance of the IV population were described by Rose (1984). Briefly, the IV population was mass-sampled from the wild in Massachusetts in 1975 and subsequently maintained as an outbred population on two-week discrete generations under standard laboratory conditions. These conditions were: constant light, 25°C temperature, high relative humidity, and low to moderate densities (50–150 per 25 mm × 95 mm shell vial) with abundant banana / molasses food.

All of the IV-derived selection treatments have been described elsewhere (see table 1). These populations can

be broadly grouped as purely demographically selected (selection on the timing of reproduction; 24 populations), environmental-stress selected (selection on adult survival of adverse environmental conditions; 20 populations), and accelerated-development selected (selection on fast development and early reproduction; 10 populations). To clarify the phylogenetic affiliations of these groups of populations, a simplified phylogeny of the selection treatments is given in figure 1. A summary of selection protocols and references to published descriptions of stocks is given in table 1. In all treatments except RU (no #5 population) and IV (a single population), the numbering of derived populations has been retained in the phylogeny. For example, SB_5 and CB_5 were both derived from B_5, and therefore founded from an independent population that had been isolated from all other B populations by several hundred generations.

For the first two weeks from egg laying, the standard conditions applied to the IV population (see above) have been retained in all of the derived selection treatments except ACB and ACO (see below). At the larval densities employed during selection, this two-week rearing period

Table 1. A summary of selection treatments used in the study, with references to published descriptions.

Name	Rep.	Year founded	Ancestor treatment	Selection treatment	Gen. time	Gens.	Described by
IV	1	1975	Wild (MA, USA)	Early-life fertility; ancestral population	14	490	Ives (1970); Rose (1984)
B	5	1980	IV	Early-life fertility, as IV; baseline populations	14	380	Rose (1984)
O	5	1980	IV	Longevity and late-life fertility	70	82	Rose (1984)
RU	4	1985	O	Early-life fertility, as IV; reverse selected	14	250	Service *et al*. (1988)
C	5	1988	O	Weak adult starvation stress; control to D	20–22	130	Rose *et al*. (1990)
D	5	1988	O	Adult desiccation stress	20–22	130	Rose *et al*. (1990)
CB	5	1989	B	Mid-life fertility; control to SB, ACB	28–35	77	Rose *et al*. (1992)
CO	5	1989	O	Mid-life fertility; control to SO, ACO	28–35	77	Rose *et al*. (1992)
SB	5	1989	B	Adult starvation stress	28–35	77	Rose *et al*. (1992)
SO	5	1989	O	Adult starvation stress	28–35	77	Rose *et al*. (1992)
ACB	5	1991	CB	Accelerated development, early-life fertility	8	100	Chippindale *et al*. (1997)
ACO	5	1991	CO	Accelerated development, early-life fertility	8	100	Chippindale *et al*. (1997)

Rep., Number of populations within selection treatment; gen. time, generation time in days; gens., number of generations at time of assay (approximated for IV, B and RU).

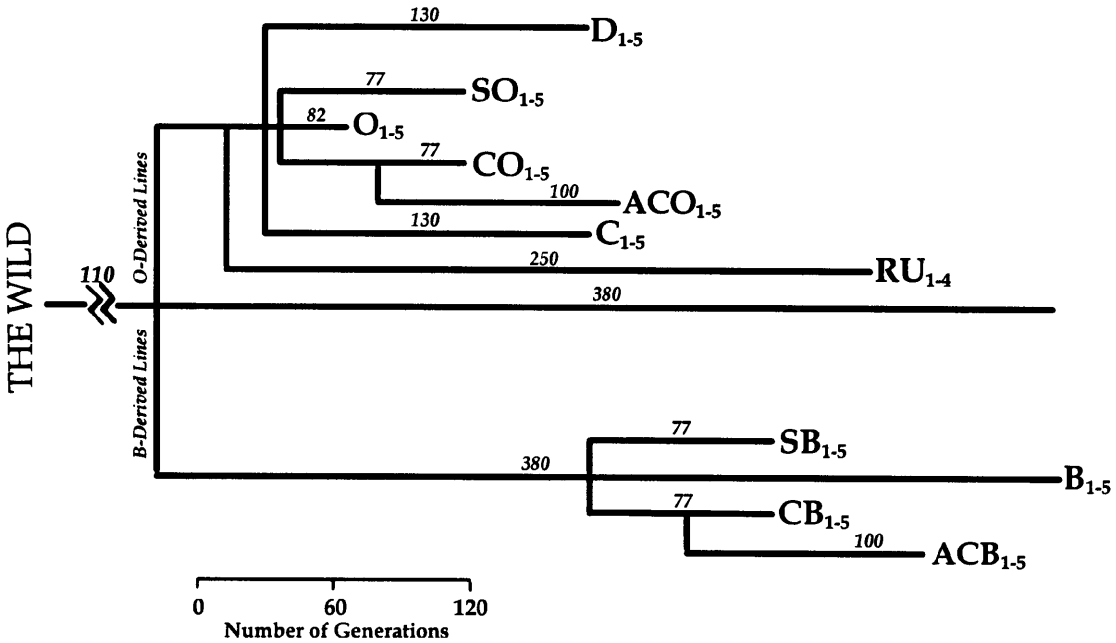

Figure 1. Phylogeny of selection treatments used in the study. All selection treatments are replicated five-fold except for RU (four replicates) and the base population IV. After the establishment of the B and O selection treatments from IV, all subsequent treatments were founded from either B-derived or O-derived populations, and retain the same numbered replicate structure (e.g. CB_1 and SB_1 were derived from B_1). A summary of the treatments is given in table 1.

is sufficient to allow the emergence of all normally developing adults. Occasionally, errors in the estimation of egg numbers at the beginning of a generation led to crowding for some or all vials within a population. In crowded vials developmental time is increased and it is therefore likely that populations have experienced some weak and sporadic direct selection owing to this factor (Chippindale *et al*. 1994). However, occasional crowding is unlikely to have afflicted one selection treatment more than any other, and is therefore not a viable explanation for evolved differences in development time. Following the two-week rearing period, all selection treatments with a lengthened adult phase have made use of plexiglas population cages to facilitate feeding and collection of eggs. The B and RU selection treatments have been maintained in a fashion identical to IV in all respects. The ACB and ACO (accelerated development) selection treatments had been selected for rapid development and extremely early fertility by transferring only the first 15–20% of emerging adults to population cages and then collecting the first available eggs. The entire life cycle of ACB and ACO typically took eight days at the time these experiments were conducted (100 generations of selection).

***Preparation of selection treatments for experiments and general handling*:** The various selected lines were assembled for the experiment by collecting 1000–2000 eggs from each population following routine selection. These samples were reared and held as adults in population cages to synchronize egg laying among the different selection treatments for subsequent generations. Two full generations were then maintained without strong selection as follows: The flies were reared on medium from the same food batch using the standard two-week rearing period, then transferred to population cages for oviposition. The total generation time of these pre-experimental generations was 2.5–3 weeks. Population sizes were 1200–2000 adults. Thus, a large unstressed sample of each population was established prior to experimentation, eliminating trans-generation effects that may result from selection (e.g. Crill *et al*. 1996), and ensuring abundant eggs for the experiment.

Since several people participated in the setting of experiments and collection of data, efforts were made to distribute handling effects. For example, in the counting of eggs, each population sample was split between two counters, and counters were rotated across selection treatments. Similarly, selection treatments were physically and temporally interspersed in all parts of the experiments to reduce the potential for environmental noise to have directional effects (e.g. local temperature or light variation in an incubator affecting whole selection treatments differentially). Interspersion was carried out by grouping the populations by replicate number and handling these as blocks. Since most selection treatments consisted of five replicate populations, there were five blocks, each made up from the same-numbered representatives of each selection treatment. Blocks were randomly positioned and rotated at six-hour intervals to homogenize any differences resulting from position effects.

***Development time and viability*:** One-week-old adults from the second unselected generation were provided with fresh yeast paste *ad libitum* for three days. The females were therefore at or close to their maximum daily fecundity, and were unlikely to be holding back any advanced-stage embryos (Chippindale *et al*. 1993). Eggs were collected by inserting Petri plates, partially filled with standard medium, into the population cages for 4 h. At the end of this interval, the plates were removed and labelled. Using fine brushes, exactly 60 eggs were counted into each of 10 vials filled with 5 ml (± 0.5 ml) of standard medium. The vials were then incubated at 25°C until adults started emerging from the pupae. Checks on emerging adults were conducted at six-hour intervals, at 1:00, 7:00, 13:00 and 23:00. Flies were transferred at these hours to holding vials provisioned with food before counting.

Viability was estimated from three sources: (i) egg to larva, (ii) egg to pupa, and (iii) pupa to adult. To measure egg-to-larva viability, each person who counted eggs into the experimental vials for a given population also counted 100 eggs onto a Petri dish filled with food-coloured agar. The 110 Petri dishes (two per population) prepared in this way were then incubated for 48 h before being scored for unhatched eggs. The dishes were rechecked two days later. The unhatched egg total was used to compute hatchability for each population. To measure viability from egg to pupa, pupae were counted by dotting them with a marker at the end of the experiment. Pupae that had formed on the stopper had been carefully removed and transplanted to the base of the vials between pupation and adult emergence, so these were not lost. Measurements of survival to adulthood were drawn from the totals of adults counted from the development time estimates. After eight consecutive checks without emergence, the six-hour development time checks were terminated. However, for an additional week the vials were checked for extremely late adults on a daily basis. The vials were then left for another week before the tallying of pupae totals.

***Dry weight*:** Dry weight was measured on recently eclosed flies from the development time experiment. Because of the scale of this experiment, it was impossible to count and freeze every fly from every population within a suitably short time from emergence. Instead, we chose one of the six-hour eclosion intervals for each population for the sampling of flies for weight estimates. At the densities employed, the distribution of emergence times is stereotyped: The first six-hour interval consists of a few females per vial, the second is almost exclusively female,

the third consists of about half female and half male, and from there male eclosion predominates in mirror image to the beginning of eclosion. It is the third interval (from 12–18 h after the first female emerged in any particular population) that was targeted for the collection of samples for weight measurement. Samples from this peak interval were counted and flash frozen on dry ice within 3 h. Approximately 200 adults were frozen from each population, of which 60 of each sex were dried and weighed. These measures were taken on groups of 10 flies of the same sex using a Cahn electronic microbalance (resolution to 1 μg). Measurements were blocked so that the selection treatments and sexes were interspersed (see general procedures described above). Because of the number of flies that were weighed (6600 in total), there were 30 separate rounds of weighing, each featuring one-sixth of the measurements for a particular replicate population number. The six separate weights generated for a given sex of each population were used to calculate the population mean.

Results

Development time

Development time ranged from 191.8 (female) / 195.8 (male) hours for the fastest population (ACB_4) to 251.0 (f) / 253.3 (m) hours for the slowest (D_3). Over the complete data set, the effect of selection was highly significant (ANOVA; table 2a). Across accelerated development and purely demographically selected treatments, development time scaled upward with total generation time (figure 2 abscissa), from the ACB and ACO treatments (8–9 days generation time) to Bs (14 days) to CBs and COs (28–35 days) and Os (70 days). ACB and ACO treatments were significantly faster than all other treatments (Tukey's HSD, $Q = 3.30$, $P < 0.05$). The CB and CO selection treatments are handled in exact parallel under selection, but were derived from opposite ends of the development time spectrum (B and O, respectively). As might be expected, development time has converged upon an intermediate position, though the two selection treatments remain distinct. The RU selection treatment presents an interesting case, as more than 200 generations of reverse selection from a 10-week cycle to a 2-week cycle has failed to return these lines to the speed of the Bs.

Selection for adult stress resistance led to significantly extended development times (D > all others; D, SO, SB, and C > all others except O; Tukey's HSD, $Q = 3.30$, $P < 0.05$). These populations may be compared to their matched controls and their differences attributed to the effects of selection for stress, rather than demographic features of the selection regime. A fully crossed three-factor analysis of variance (effects: selection treatment (S or C), ancestry (B or O), and sex) was performed on the SB and SO populations and their respective controls. This ANOVA revealed a highly significant effect of selection treatment (S > C; $P < 0.01$) and ancestry (B or O; $P < 0.05$). No other factors or interactions were significant in this ANOVA. Desiccation-resistance selection had an even more drastic slowing effect upon development than did starvation selection (D > all others; Tukey's HSD, $Q = 3.30$, $P < 0.05$), with the D treatment averaging over 10 days from egg to adult (245.09 h and 245.68 h, for females and males respectively). Because the Ds have matched controls (C) selected for starvation for the same interval in

Table 2. Summary of analyses from main data set.

Character	Source	Sum of squares	d.f.	*F* ratio	*P*
a. Development time	Selection	22481.12	10	190.89	< 0.0001
	Sex	157.62	1	13.38	< 0.001
	Selection × Sex	42.60	10	0.36	0.96
b. Dry weight	Selection	335578.17	10	108.60	< 0.0001
	Sex	140603.74	1	455.03	< 0.0001
	Selection × Sex	7425.11	10	2.40	0.01
c. Growth rate	Selection	3.53	10	70.02	< 0.0001
	Sex	2.95	1	585.18	< 0.0001
	Selection × Sex	0.09	10	1.81	0.07
d. Egg hatchability	Selection	10.77×10^{-3}	10	3.30	< 0.01
e. Larval viability	Selection	32.19×10^{-3}	10	2.60	0.01
f. Pupal viability	Selection	16.54×10^{-3}	10	3.90	< 0.001
g. Egg-to-adult viability	Selection	90.15×10^{-3}	10	9.73	< 0.0001

All analysis of variance based upon population means (55 populations total; typically five replicate populations per selection treatment). Sexes were pooled for viability analysis.

which Ds are desiccated, we may isolate the effect of desiccation over and above the part of the selection response that is due to starvation selection. Desiccation-selected populations were 10.0 h slower developing than their controls, on average. Although the D selection treatment does not have a matched, unstressed control, the Ds were significantly slower than even the Os (Tukey's test as noted above) despite having a much shorter generation time.

Development time was sexually dimorphic, with males being significantly slower developing than females in all selection treatments (figure 2; table 2a). The analysis reported in table 2 also suggests no significant selection-by-sex interaction term. However, an ANOVA on the difference in development time (i.e. M–F) between the sexes revealed a strong effect of selection ($F_{11,34} = 3.89$, $P = 0.0006$). The pattern was for the slowest-developing, i.e. stress-selected and postponed-ageing, populations (D, SB, SO, C, O, ascending in order) to have the smallest levels of dimorphism (all less than 2-h difference between sexes; D and SB significantly different from all others by Tukey's HSD ($Q = 3.45$, $P < 0.05$)). At the other end of the spectrum, fast-development-selected populations had the highest levels of dimorphism (over 4 h average difference; significantly different from all others by Tukey's HSD ($Q = 3.45$, $P < 0.05$)). These differences are small but consistent, and because the greatest differences are found in the fastest-developing lines (and vice versa), the relative differences are even greater than the raw differences.

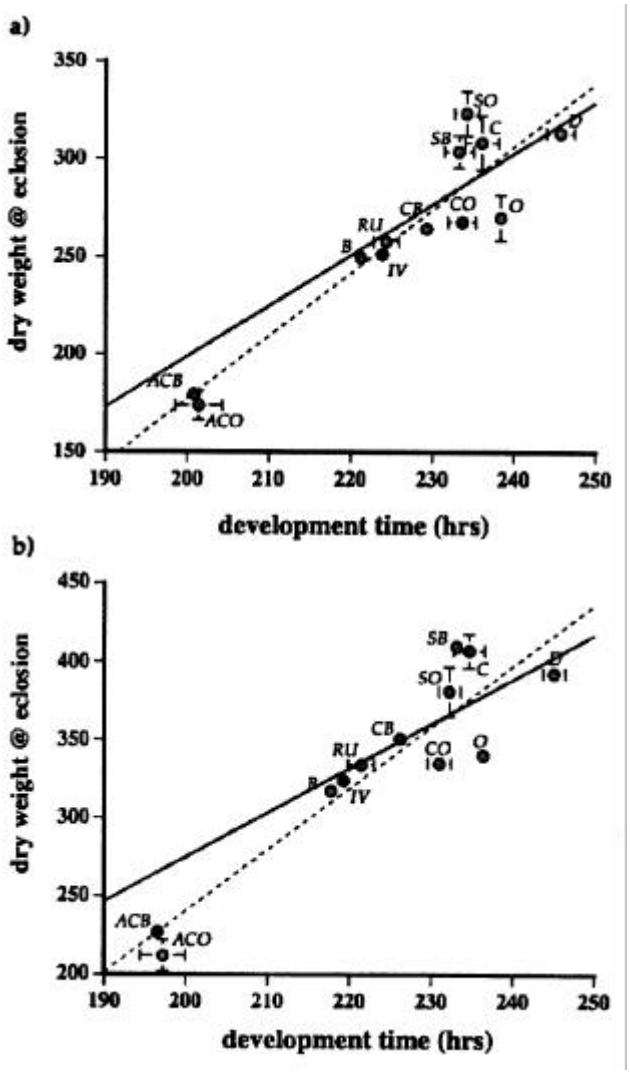

Figure 2. Relationship between development time and dry body weight at eclosion. The solid line shows the least squares linear regression with the ACB and ACO selection treatments excluded and the dotted line shows it with these treatments included; (a) data for males, (b) data for females. Details of the statistics and their rationale are given in the text.

Body size

Dry weight at emergence from pupa is a measure of net growth. There was a substantial effect of selection on this character (figure 2 ordinate). At the extremes, stress-selected treatments (SO, C, D and SB, in that order) were most massive while accelerated-selection treatments (ACO, ACB) were minuscule by comparison. For example, the starvation-selected populations were approximately 80% heavier than the accelerated populations, on average. The range across individual populations for males was 161.6 μg (ACB_4) to 374.7 μg (SO_1), and for females 200.9 μg (ACO_3) to 437.4 μg (SB_1). Demographically selected treatments exhibited relatively slight differences in weight. The Bs were smallest for both sexes at 249.8 μg (male) and 317.3 μg (female). The heaviest males of the demographic-selection treatments were the Os, 8.2% heavier than Bs at 270.2 μg. O females were 7.2% heavier than B females at 340.1 μg.

Analysis of variance (factors: selection treatment and sex) detected highly significant variation among selection treatments and between sexes (table 2b). A *post hoc* test (Tukey's HSD on LS means, sexes pooled; $Q = 1.98$, $P < 0.05$) grouped stress-selected treatments (SO, C, D, SB, in descending order) together as significantly heavier than all demographically selected (CB, O, CO, RU, B, in descending order), which in turn were all significantly heavier than the accelerated-selection treatments (ACO and ACB). There was a significant interaction between selection treatment and sex for dry weight (table 2b) that was difficult to ascribe to any feature of selection except perhaps development time, with the trend towards greater weight differential in slower-developing populations. Specifically, the absolute difference in size between females and males in the accelerated-development treatments was smaller (48 μg for ACB and 39 μg for ACO) than in all other selection treatments (ranging between 67 μg (CO) and 99 μg (C)) but a *post hoc* test (Tukey's HSD, $Q = 3.45$, $P < 0.05$) registered significant differences only between the ACB / ACO selection treatments and the C / SB / CB

selection treatments. When sexual size dimorphism (SSD) was considered as a relative measure (i.e. M / F dry weight) there was no significant variation among selection treatments ($F_{11,54} = 0.63$, $P = 0.80$). Indeed, relative SSD was highly conserved despite the radical differences in development time and selection protocols. All selection treatments fell between 0.75 (CB) and 0.80 (ACO) for the ratio of male to female dry weight.

Viability

Survivorship was measured for all major preadult stages: egg to larva, larva to pupa, and pupa to adult. These data are reported in table 3 and figure 3. Differences were slight within each specific stage yet selection treatment had a significant bearing upon survival in all stages (table 2, d,e,f) and overall (table 2, g). First, egg hatchabilities ranged between 90.6% (ACO) and 96.2% (O). Accelerated-development selection treatments had the lowest embryonic survival while O-derived demographic treatments (O, CO, RU) had the highest survival rates (table 3). As larvae, again the O selection treatment had the highest survival rate (97.6%) and an accelerated population (ACB) had the lowest survival rate (93.2%). And as pupae, O-derived selection treatments (CO, C, SO, RU) showed the highest survival while accelerated-selection treatments again had the lowest survival rates.

Total (egg to adult) viability was estimated independently of the data collected for each stage by summing the adult counts from the development time results. These results (given in figure 3) matched closely those obtained by multiplying survival estimates from each preadult stage. The range of values was 74.8% (ACB) to 90.8% (O). Accelerated-development selection had the strongest impact on overall viability. Relative to their controls, ACB viability had declined by 13.8% and ACO viability by 10.4%, both differences being significant even in the relatively conservative Tukey's *post hoc* test (table 3). Consistent with earlier findings (Chippindale *et al*. 1994, 1996), the B selection treatment had lower viability than the O

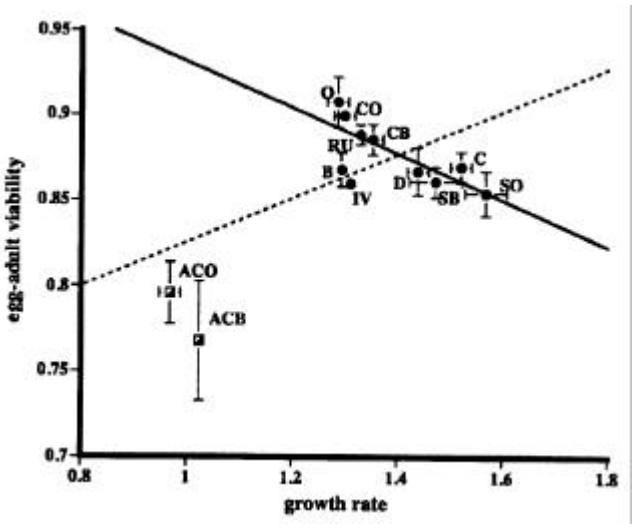

Figure 3. Relationship between net growth rate and egg-to-adult viability. The solid line shows the least squares linear regression with the ACB and ACO selection treatments excluded and the dotted line shows it with these treatments included. Details of the statistics and their rationale are given in the text.

Table 3. Stage-specific and overall viability estimates (as percentages) for the 11 replicated selection treatments.

	Stage				
Selection	Egg	Larva	Pupa	Egg to adult	
B	94.7 ± 0.4	95.77 ± 0.51	96.57 ± 0.61	86.81 ± 1.04	B, C
O	96.2 ± 0.5	97.60 ± 0.83	96.97 ± 0.88	90.80 ± 1.47	A
RU	95.0 ± 1.3	96.59 ± 0.93	97.29 ± 0.17	88.87 ± 0.55	A, B, C
CB	94.6 ± 1.0	97.27 ± 1.34	96.37 ± 0.64	88.59 ± 0.93	A, B, C
SB	94.6 ± 0.4	95.46 ± 1.39	96.03 ± 0.92	86.10 ± 0.92	C
ACB	93.2 ± 0.6	88.43 ± 2.51	93.17 ± 0.87	74.80 ± 3.54	D
CO	95.3 ± 0.3	96.39 ± 2.04	98.30 ± 0.69	89.99 ± 2.00	A, B
SO	93.7 ± 1.0	94.10 ± 1.42	97.63 ± 0.78	85.43 ± 1.26	C
ACO	90.6 ± 1.2	95.47 ± 1.71	93.50 ± 0.75	79.60 ± 1.85	D
C	93.3 ± 1.0	94.93 ± 0.83	98.73 ± 1.70	86.97 ± 0.88	A, B
D	94.7 ± 0.6	97.24 ± 1.18	94.77 ± 1.18	86.71 ± 1.39	C
Mean	94.17	95.39	96.30	85.88	
SE	0.44	0.77	0.55	1.42	

The letters in the rightmost column give the statistical relationships from Tukey's HSD test on least squares means ($Q = 3.40$, $P < 0.05$) for egg-to-adult viability; selection treatments sharing the same letter are not significantly different. Values are mean ± standard error.

selection treatment (O–B = 4.0%; significant by Tukey's HSD test) and demographically selected populations with later reproduction (CB, CO, O) generally had higher survival rates than early-reproducing populations.

Starvation selection was again (see Chippindale *et al*. 1996) found to reduce viability relative to controls kept on the same schedule (CB–SB = 2.5% and CO–SO = 4.6%). This difference was only significant for the CO-minus-SO contrast in the Tukey's HSD test (table 3); however, the replicated nature of the selection treatments, in addition to the replication of populations within each, allowed an additional analysis of variance to be conducted (factors: selection treatment (S or Control), ancestor (O or B)). This ANOVA confirmed a strong effect of stress selection ($F_{1,19} = 6.81$, $P = 0.019$) but no effect of ancestry ($F_{1,19} = 0.075$, $P = 0.79$). Desiccation-selected populations had overall viability similar to that of starvation-selected populations and were not different from their controls (table 3).

Net growth rate

We have seen that the general form of selection applied correlates with both body mass and development time, with stress-selected lines being big and slow-developing at one end of the spectrum and the accelerated-development treatments being small and fast-developing at the other. One may estimate the net growth rate from the ratio of body weight at eclosion to development time. Figure 3 (ordinate) shows these data for the average across the sexes, while the overall analysis (with sex as a factor) is given in table 2c. There was a strong effect of selection treatment apparent when the data were pooled across the sexes (one-factor ANOVA; $F_{10,54} = 55.7$, $P < 0.0001$). The data form three natural groupings, each significantly different in *post hoc* testing (Tukey's HSD, $Q = 3.45$, $P < 0.05$). Populations selected for adult stress resistance (SO, C, SB and D in descending order), were significantly faster growing than purely demographically selected treatments (CB, RU, CO, B and O in descending order), which in turn all had higher net growth rate than the accelerated-selected treatments (ACB and ACO).

Females had higher growth rates than males, as reflected by the sex factor in the ANOVA reported in table 2c. The nearly significant sex × selection interaction reported from the ANOVA ($P = 0.07$) reflects the smaller absolute difference between males and females in growth rate in the low-growth-rate populations, particularly the A-selected populations, contrasted with the stress-selected populations. We also examined the relative growth rates of males and females (i.e. M / F) and found no suggestion of an effect of selection treatment (one-factor ANOVA; $F_{10,54} = 0.61$, $P = 0.81$). With the tight range of values from 0.75 (CB) to 0.80 (ACO) there were no significant differences between any two selection treatments or grouping by general selection type.

Correlations among characters

We examined two potential associations between characters, based on predictions from theory or findings in previous work. We conservatively used the selection treatment means ($n = 9$ for purposes of testing the correlation coefficient) rather than the data for all 55 populations. First, we tested the correlation between development time and body size (figure 2). For both sexes, a strong and positive association between development time and body size was apparent, as estimated by the least squares regressions shown in figure 2 ($y = 3.24x - 469.9$, $r^2 = 0.865$, $P < 0.01$ for males; $y = 3.91x - 541.2$, $r^2 = 0.854$, $P < 0.01$ for females). The accelerated-selection treatments exert strong leverage on the regression. When the regression is calculated with the ACB and ACO points removed, we obtain a shallower slope and lower significance in the regression ($y = 2.61x - 323.7$, $r^2 = 0.482$, $P < 0.05$ for males; $y = 2.83x - 290.8$; $r^2 = 0.455$, $P < 0.05$ for females). The second pattern we looked for was a correlation between growth rate and viability, since earlier work had suggested a negative association. This prediction was not borne out when the accelerated populations were included in the analysis because they were so unusually low in both growth rate and viability. Figure 3 shows regressions calculated with and without the ACB and ACO populations. When the A-selected treatments are included in the regression, we obtain a positive slope ($y = 0.14x + 0.67$, $r^2 = 0.385$, $P < 0.05$). When the ACB and ACO populations are treated as outliers and excluded we obtain a strongly negative slope ($y = -0.14x + 1.01$, $r^2 = 0.618$, $P < 0.01$). The significance of these contrasts is discussed below.

Discussion

In this study we have taken a snapshot of a large evolving laboratory phylogeny to ask what, if any, relationships among juvenile fitness components are preserved through the course of adaptation to several very different selection regimes. From the data it is apparent that the magnitude and even the sign of the inferred evolutionary correlation between two characters depends strongly on the specific selection history of the populations included in the analysis. Most strikingly, we found that the evolutionary correlation between growth rate and preadult viability was negative among most selection treatments (i.e. consistent with a tradeoff) but strongly positive in populations selected directly for rapid growth and development. We first discuss this general problem and its specific manifestations, and then explore several other interesting patterns to emerge from this survey.

Reversal of the tradeoff between growth and viability

Prior to the present work, we had hypothesized a tradeoff between growth rate and preadult viability using data from demographically selected (Chippindale *et al*. 1994)

and stress-resistance-selected (Chippindale *et al*. 1996, 1998) populations. That relationship appears to hold up in the present data set for populations with such adult selection treatments. For example, adult-stress-selected populations (C, D, SB and SO) evolved higher growth rates than their controls or ancestors and suffered from reduced juvenile survivorship. This appears to reflect an innate risk to the higher growth rates favoured by selection for stress tolerance. The populations directly selected for rapid development (ACB and ACO), however, exhibit a very different pattern: Compared with their controls (CB and CO) these populations displayed both reduced viability and reduced net growth rates, with the control populations showing 33% higher net mean growth rates than the 'A' populations. This observation is consistent, both quantitatively and qualitatively, with the results reported by Prasad *et al*. (2000) for a similar selection experiment on accelerated development. In other words, the negative evolutionary correlation apparent in other populations has been supplanted by a positive evolutionary correlation in ACB and ACO populations.

Given the importance of body size in *Drosophila* (e.g. Robertson 1957; Partridge and Farquhar 1983; Mueller 1985; Partridge *et al*. 1987), and the strong directional selection for fast development in the ACB and ACO populations, decreased growth rate is expected to have multifarious negative fitness consequences. In fact, the accelerated-development populations show lower fecundity, lower adult stress resistance and lower longevity relative to their controls and their ancestors (Chippindale *et al*., in press). We know that viability has trended steadily downward with decreasing development time in these populations (Chippindale *et al*. 1997), so the key question is why body size has declined disproportionately rapidly under this selection protocol. One possibility is that the A lines became inbred, and so generally less fit than their controls and other populations in the laboratory. Another explanation might be that a G × E interaction arose owing to differences in culture protocols and test conditions. Both of these are unlikely reasons for the reversal in the evolutionary correlation in this specific instance. First, census population sizes were of the order of 10^3 in the A populations, comparable to their controls. And while it is true that the accelerated-selection protocol necessitated four times more generations in the same calendar time period (specifically 100 in the ACB and ACO lines compared with 26 in their controls), a full diallel cross among ACO populations failed to reveal evidence of inbreeding depression for viability or development time (Chippindale *et al*. 1997) or fecundity (A. K. Chippindale, unpublished data). The second hypothesis is also unlikely because all features of the rearing environment were common to the test populations throughout selection, and the experimental assays carefully reproduced those conditions. Finally, we have found extraordinary consistency within selection treatments among the 10 independently selected populations ($ACB_{1–5}$ and $ACO_{1–5}$), suggesting that drift or linkage disequilibrium are unlikely candidates to explain the evolutionary correlation. We therefore conclude that the reduction in net growth rate is most likely to be a product of selection.

We suggest that the apparent reduction in growth rate is an artifact of trait definition. Development is the coordination of growth with ontogeny; there are stages in which one or the other process may be more important. By estimating growth rate as the ratio of final size to total development time one ignores the intricacies of development. Thus, the duration of a nongrowing stage may have little or nothing to do with final body size, but much to do with survival. For example, if weakened selection on development time primarily slowed stages that improve viability without affecting active growing phases, then we would observe increased egg-to-adult development time, reduced net growth rate, and increased viability, with adults being equivalent sizes. This scenario appears to be played out in our demographically selected populations. Because populations with extended life cycles (O, CB, CO) do not need to be sexually mature at 14 days of age, as do our 'baseline' treatments (B, IV, RU), development stretches out with little change in body size (present data; Chippindale *et al*. 1994, 1996, 1998). The main benefit to slow development appears to be improved juvenile viability among these treatments. For stress-selected populations, increased body size and greater storage of nutrients by larvae is critical to fitness (Chippindale *et al*. 1996, 1998; Djawdan *et al*. 1998). For these populations we may envision selection for longer feeding stages and higher growth rate during these stages predominating. We therefore observe a big, slow-developing, low-viability phenotype. Finally, the accelerated populations are simultaneously selected on all components of juvenile fitness. Because there are likely to be hundreds or even thousands of loci affecting development, their selection trajectory may be dictated by (i) the relative cost / benefit ratio of selection on a locus affecting a particular developmental stage and (ii) the exhaustion of genetic variation at that locus and the recruitment of a new variable locus to the response. Unfortunately we lack data taken throughout the evolution of the accelerated populations that would cast light on the nature of the correlated responses to selection for faster development. It would be interesting, for example, to see if the A populations initially traced the pathway defined by the negative correlation seen between the other selection treatments, increasing growth rate, before reversing the correlation to create a hook-shaped selection response.

Is the genetic correlation between development time and body size 'fundamental'?

A glance at figure 3 suggests that the tradeoff between

development rate (inverse of development time) and body size is very robust ($r^2 \approx 0.85$ in each sex). Overall, each hour of development translates into 3.2 (males) to 3.9 (females) μg of adult dry tissue. It is intuitive that, all else being equal, longer development will result in greater size, and this correlation has been observed in other recent selection experiments with *Drosophila* (e.g. Partridge and Fowler 1992; Chippindale *et al*. 1994, 1996, 1997; Zwaan *et al*. 1995; Nunney 1996; Prasad *et al*. 2000). The common link among these studies is the use of relatively outbred stocks and replicated selection lines. In 'artificial' selection designs employing very small populations, any real genetic relationship may be obscured by inbreeding depression and a suite of other confounding factors. Furthermore, the environmental manipulation of larval density produces a positive correlation between development rate and size, as flies become both small and slow-developing (Lints and Gruwez 1972; Chippindale *et al*. 1994; Houle and Rowe 2003). In natural populations ecological factors such as increased risk of exposure to predation from foraging (e.g. Rowe and Ludwig 1991) may generate an inverse relationship between development time and adult body size. Therefore there are many circumstances in which 'all else' will not be equal where the evolutionary correlation between these traits is concerned. The present work comes very close to controlling these confounding factors, although we see exceptions even within this data set. As pointed out above, a number of specific comparisons of selection treatments within the present data deviate quantitatively from the overall pattern. The positive relationship is weaker or nonexistent, for example, within any of the clusters of selection types (demographic, stress, or development). Given that each main selection treatment is replicated five-fold (except IV and RU), comparing any two points on figure 3 would constitute a large experiment by the standards of present life-history literature. We therefore conclude that, although a tradeoff between development rate and body size is apparent in the case of the conditions and most of the populations described herein, many forms of selection and G × E interaction can undermine this evolutionary correlation.

Sexual dimorphism

One of the more interesting results to emerge from this survey is the extraordinary consistency of sexual dimorphism in the face of sustained and intense selection. Dimorphism in the speed of growth and resultant size of the imago in *D. melanogaster* is highly stereotyped, but poorly understood evolutionarily. Females are substantially larger than males, but emerge earlier under normal laboratory conditions. Although some of the overall difference in development time between the sexes may result from a lengthening of the pupal period in males (Nunney 1996), this could, at best, account for 1% of the size difference at eclosion. The simple fact is that females have dramatically higher rates of larval growth. In the present study, this growth rate advantage of females over males ranged from 25% to 33% more dry mass per hour of development. In the context of a tradeoff between growth rate and survival this presents a further problem, for the two sexes have equivalent survival probability despite large differences in growth rate.

So why do females grow so much more rapidly? Roper *et al*. (1993) suggested that males emerge later and attain smaller sizes because it takes them less time than for females to become reproductively mature. In other words, selection is more intense for early female eclosion. But such an argument about *relative* development rate would be difficult to defend for conditions where generations overlap (e.g. the wild). And furthermore there is no other evidence of weaker selection for male developmental rate, such as a higher phenotypic variance in emergence time. The sexes also scale together very neatly, irrespective of the selection treatment, both here and in other studies (Partridge and Fowler 1992; Chippindale *et al*. 1994, 1996, 1997; Nunney 1996). This relationship is preserved despite stronger selection for earlier male emergence in the ACB and ACO regimes that directly results from the dimorphism in development time (Chippindale *et al*. 1997, but see Prasad *et al*. 2000).

One explanation for this paradoxical situation is a lack of genetic variation for size dimorphism. To this we may add two hypotheses; the first is a 'lock and key' hypothesis and the second involves intrinsically greater expenses to male growth. The lock and key hypothesis is simple: owing to the mechanics of mating, sexual selection acts on the mechanical fit between male and female bodies, and this 'fit' is genetically correlated with body size. The second hypothesis is that there is something energetically 'expensive' about male development in fruit flies that limits equivalent accumulation of body mass. One suggestion has been that spermatogenesis is costly (Nunney 1996, reporting a personal communication from L. Partridge) and delays growth and eclosion. A novel variant of this hypothesis is that selection acts upon male ontogenetic fidelity. In this model, males are smaller because those males that are better formed are chosen preferentially by females, or exhibit better performance in traits relevant to mating success (e.g. courtship, flight). This hypothesis could be tested by measuring the fluctuating asymmetry (FA) of males and females (as an index of developmental stability) with clear predictions: males should be more symmetrical than females, and symmetry should be positively correlated with male mating success. Against this hypothesis, Shakarad *et al*. (2001) failed to observe differences in sternopleural bristle FA in *D. melanogaster* populations selected for rapid development and their controls, or between the sexes in these populations.

However, both their 'A-type' populations (Prasad *et al*. 2000) and ours exhibit lower growth rates than their controls, so it is possible that growth rate does still affect developmental fidelity.

Conclusions

After decades of research on the hundreds of selection lines derived from the IV *D. melanogaster* population, many of our earlier conceptions about the nature of life-history evolution have been broken down or even reversed. Here and in other papers (Leroi *et al*. 1994a,b; Archer *et al*. 2003; Phelan *et al*. 2003; Chippindale *et al*., in press) we have empirically demonstrated nonlinear and even reversing evolutionary correlations under long-term selection.

Far from being distraught by the lack of simple, predictable patterns in experimental evolution, we are instead impressed by the ingenuity of selection, the complexity of trait associations, and the sensitivity of animal populations to small changes in their environment. Some of the problems plainly stem from grouping as simple 'traits' or 'characters' portions of the phenotype governed by many loci and multiple discrete functional steps (e.g. development, longevity). Under these circumstances it is inevitable that allele frequencies will vary idiosyncratically in particular populations and change throughout selection, and that selection will exploit some loci but not others at any given point in a protracted selection response. The matrix of associations among components of a major character, such as any life-history character, is bound to be complex, with that complexity multiplied by the multiple interacting characters that define each summative life-history character.

These findings present a major challenge for the investigation of life-history evolution, even under the simplified conditions of laboratory culture. As biologists seeking to understand the patterns and consequences of natural selection, we need to acknowledge that our parsing of the text of evolution may be radically different from the actual meaning and substructure of that text. The true story, the devils at work, may be much richer in causally important details than we have imagined.

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