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Life-history evolution in the laboratory*

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Abstract

A common perception about evolutionary biology is that it largely pertains to the study of fossils or of biological diversity Similarly there is often an impression that the life history of a species is some kind of fixed characteristic of that species I use results from ongoing studies in my laboratory on direct and correlated responses to selection for faster development and early reproduction in the fruitfly *Drosophila melanogaster* to illustrate the feasibility of rigorously studying the evolution of life histories in the laboratory I show that (a) evolutionary biology can be a rigorous experimental science (b) very often traits that we think should evolve do not do so due to genetic constraints and (c) there are great advantages of working with laboratory systems if one is trying to understand the evolutionary process

Keywords Development time mortality life span growth rate *Drosophila melanogaster*

1 Introduction

Evolutionary biology, taken in the broadest sense, is today a vast field encompassing many different areas, and utilizing many different methodologies Unlike many areas in suborganismal biology, evolutionary biology rests upon a very well-developed and mathematically sophisticated substratum of theory, deduced from the axioms of the principles of Mendelian inheritance This feature makes it different from many other areas in biology in that it permits a kind of rigorous feedback between theory and experiment, reminiscent more of the 'hard science' picture of physics than of what most people think about when they consider biology Within evolutionary biology itself we can delineate four broad areas of research which differ considerably in the issues they address, and the methodology they use

Palaeontology and, today, molecular systematics, are primarily concerned with understanding *patterns* of biological diversity in time, the focus being on reconstructing past events Understanding spatial *patterns* in the diversity of extant life forms constitutes the domain of biogeography, nowadays often called biodiversity Many evolutionary biologists concern themselves with trying to understand why and how extant traits in species may have evolved The focus here is on extant populations or species as *products* of evolution, and possible fitness consequences of extant traits are the principal object of study For want of a better label, and in order to contrast it with the fourth area of evolutionary biology research, I will call this broad approach evolutionary ecology Finally, there is evolutionary genetics wherein the major inter-

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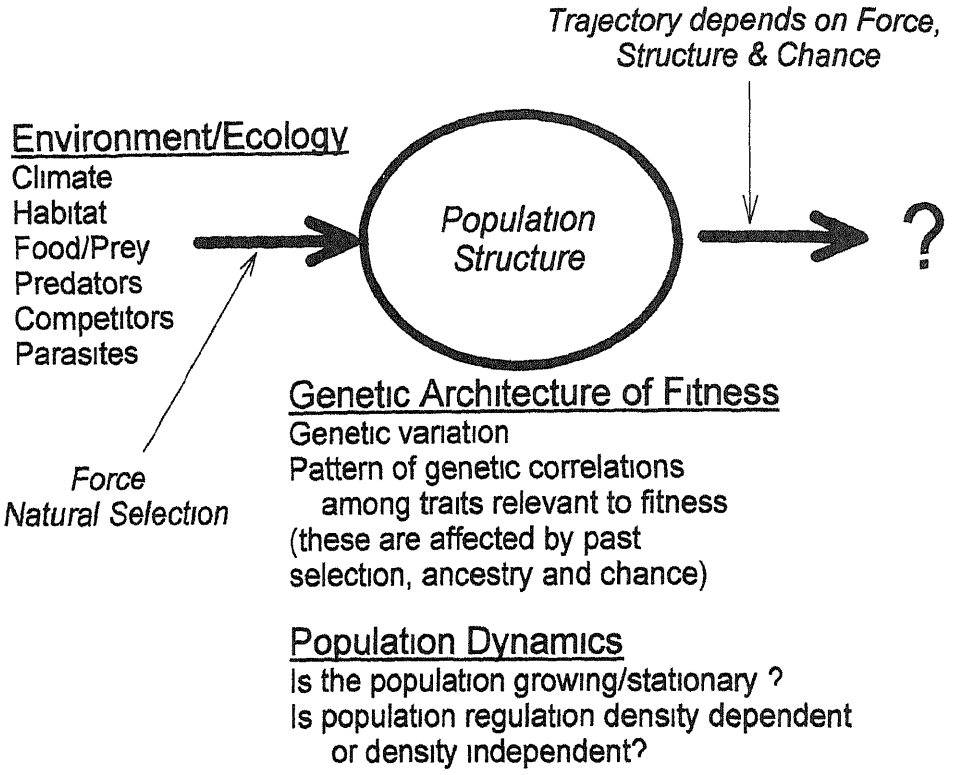

FIG 1 The evolutionary trajectory of a population in phenotypic space can be viewed as analogous to the trajectory of a body upon which a force acts The evolutionary trajectory is the result of an interaction between the force of natural selection (itself a composite force, being the resolution of myriad ecological factors) and the structure of the population (in terms of the genetic architecture of fitness components and the nature of population growth)

est is in the dynamics of the evolutionary *process* Here, one is not typically interested in a particular extant trait or species *per se*, but is rather trying to elucidate broad principles of how adaptive evolution occurs in response to certain clearly defined selection pressures

The evolutionary trajectory of a population is a resolution of the force of natural selection acting on it, the genetic structure of the population, its past selection history and ancestry, and chance in the form of genetic drift (Fig 1) [1 2] The approach used by many practitioners of evolutionary genetics is to work with well-characterized laboratory systems where one can simplify and control the selection pressures, allow for and quantify historical effects, and circumvent the problems of chance by working with replicated populations [3–6] In such studies, the logic of one's approach is to study the evolutionary trajectory of a well-characterized set of populations under a certain set of selection pressures and use this information to draw inferences about the genetic architecture of fitness in the population

One area in evolutionary genetics that is extensively studied is life-history evolution [7 8] From an evolutionary point of view, the life history of an organism primarily refers to the

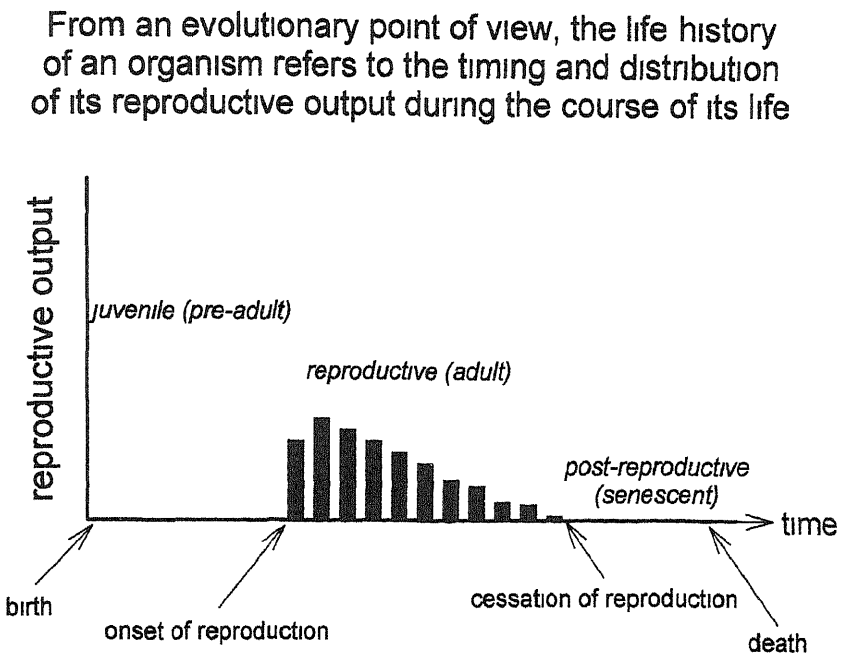

FIG 2 Illustration of what is meant by the life history of an organism The timing of the events depicted is evolutionarily significant because the force of natural selection acting on genes is maximum when genes are expressed prior to the onset of reproduction declines during the reproductive phase and is zero after the cessation of reproduction (at least in species lacking parental care)

timing and distribution of its reproductive output during the course of its life (Fig 2) The fruit-fly *Drosophila melanogaster* is a good model system for studying questions in life-history evolution It has a short life cycle and can turn over a generation from egg to egg in about 10 days at 25°C (Fig 3) In this paper, I will describe some results from ongoing studies in my laboratory aimed at understanding how populations of *D melanogaster* evolve when under selection to develop to adulthood fast and reproduce relatively early in adult life

2 Materials and methods

2 1 *Experimental populations*

This study was conducted on eight laboratory populations of *D melanogaster* four populations selected for fast development from egg to adult and early reproduction (FEJ-1 4, fast development, early reproduction, derived from JB populations), and the control populations from which the selected lines were derived (JB-1 4) The derivation and maintenance of the JB populations and their ancestors have been described in detail elsewhere[9 10] and I, therefore,

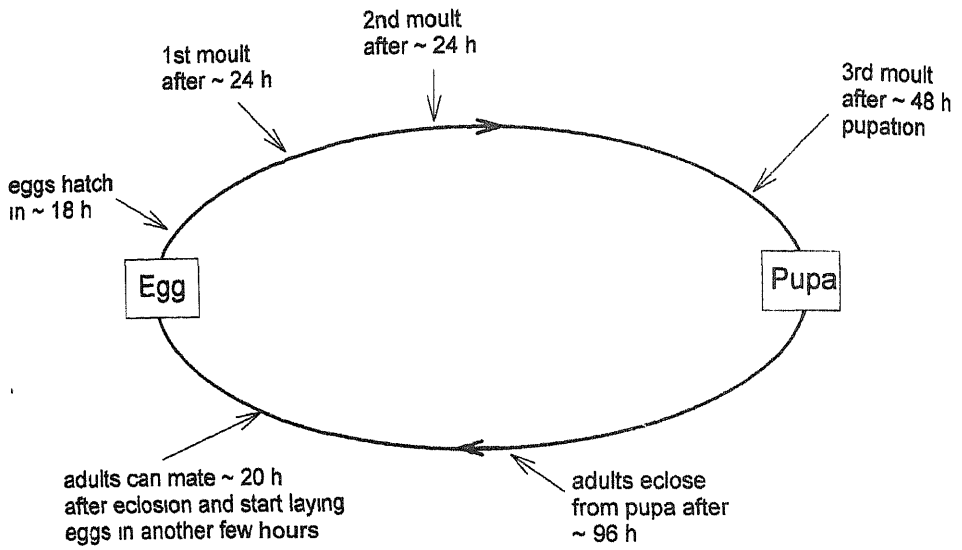

FIG 3 The life cycle of the control *Drosophila melanogaster* populations (JB) used in this study at 25 C and constant light

restrict myself to details pertinent here Briefly, the JB populations are maintained at 25°C on a 21-day discrete-generation cycle, under constant light, at moderate densities of ~ 60-80 larvae per 8-dram vial (9 0 cm h × 2 4 cm dia) containing approximately 6 ml of banana-jaggery food medium Every generation, adults of each population are allowed to oviposit for about 18 h on petri-plates of fresh banana food placed in a plexiglass cage ($25 \times 20 \times 15$ cm^3) From these petri-plates, ~ 60–80 eggs are collected into each of the 40 vials in which larvae then develop into adults Adults eclosing from these vials are transferred to fresh vials on day 12, 14 and 16 after egg-lay On the 18th day after egg-lay, adult flies are transferred into plexiglass cages and supplied with banana food supplemented with live yeast paste for two days, after which eggs are collected to initiate the next generation and the adults discarded The population typically consists of about 1,500 flies at this stage

The FEJ populations are maintained on a similar regime except that 80 vials of ~ 60–80 eggs are collected per population, and once the pupae darken, the vials are closely monitored and only the first 20% or so of eclosing flies per vial, regardless of sex are dumped into cages to constitute the pool of breeding adults The flies in the cages are supplied with yeasted food medium for two days and then allowed to oviposit for ~ 1 h on a fresh food plate The number of breeding adults in the FEJ populations is 800–1,000 Thus, the major differences between the two types of population are (a) FEJ eggs are collected around day 11, while those of JB are collected on day 21 after egg-lay, (b) the egg-laying window is ~ 1 h for FEJ and ~ 18 h for JB, and (c) only the first 20% or so of eclosing flies contribute to the next generation in FEJ, whereas in JB populations all flies eclosing on or before day 12 contribute to the next genera-

tion (this is sufficient time for practically all the surviving individuals to eclose at the moderate densities used to maintain these populations)

2 2 *Collection of adults for assays*

Prior to assaying, all populations were passed through a full generation of common rearing to obviate any parental effects due to differences in maintenance regime From the running cultures of each of the FEJ and JB populations, 20 vials of ~ 60–80 eggs were collected Adults eclosing in these vials were collected into cages 14 days after egg-lay The progeny of these adults (henceforth referred to as standardised flies) were used for the various assays

2 3 *Development time and survivorship assays*

These assays were conducted every 10 generations Standardised flies of each JB and FEJ population were supplied with yeasted agar plates in the cages for 2 h Eggs were collected off these plates with the help of a moistened brush and placed in vials containing 5 ml banana food at a density of 30 eggs per vial eight such vials were set up per population Once the pupae darkened, the vials were checked every 4 h and any eclosed adults were removed sexed and the time of their eclosion recorded These 4-hourly checks were continued until three consecutive days passed with no eclosion recorded from any vial From these records, data on egg-to-eclosion development time and survivorship were obtained At generation 56 of the selection the durations of each larval instar and the pupal stage were also assayed on all the FEJ and JB populations

2 4 *Dry weight assays*

These assays were conducted every 10 generations after the 20th generation of selection Freshly eclosed flies were killed by freezing, dried for 18 h at ~ 70°C and weighed in batches of 5 males or 5 females Six batches each of males and females were weighed for each FEJ and JB population The weight data were also used to estimate larval growth rates for each FEJ and JB population by dividing population mean dry weight by the mean development time

2 5 *Life-span assays*

Life-span assays were conducted after 10, 20 and 30 generations of selection, respectively The assays after 10 and 30 generations of selection were conducted on reproducing flies, while the generation 20 assay was conducted on virgin females Standardised flies of each JB and FEJ population were supplied with yeasted banana food plates in the cages for 2 h Eggs were collected off these plates and placed in vials containing 5 ml banana food at a density of ~ 60–80 eggs per vial Three such vials were set up per population Flies eclosing in these vials were used to set up the life-span assay following the method of Joshi *et al* [11] One-day-old flies were placed in vials (4 males and 4 females per vial in the generation 10 and 30 assays, 8 females per vial in the generation 20 assay) containing ~ 3 ml of banana food Ten such vials were set up for each JB and FEJ population The flies were transferred to fresh food vials every third day until all flies had died All vials were checked for deaths daily, dead flies in a vial were not replaced over the course of the assay Adult life span (henceforth life span) was measured as

the time, in days, from eclosion to death In analysing life-span data, we focussed on females only, as female longevity is more directly relevant to fitness It is females who need to survive in order to lay eggs at a given age, males can, in principle, inseminate females and then die, but still have the eggs resulting from that insemination being laid up to several weeks later

2 6 *Behavioural assays on larvae*

At generation 65 of selection, four larval behaviours, all of which are known to be energy costly, were assayed Larval feeding rate was measured as the number of cephalopharyngeal sclerite retractions per minute on 48-h old JB larvae and 42-h old FEJ larvae (matched to the same physiological age as the JB larvae) following the technique of Joshi and Mueller [10] Pupation height was measured at densities of 30 larvae per vial (20 0 cm h × 2 5 cm dia) as the height above the medium that the larvae pupated [12] Larval digging behaviour was measured using a method modified from that of Godoy–Herrera [13] Vials (9 0 cm h × 2 5 cm dia) were prepared with 3 0 ml of charcoal-impregnated food overlaid with a 5 0 mm thick layer of regular banana food medium Larvae that dug into the charcoal food were detected after finishing feeding by the presence of charcoal particles in the gut, and were classified as diggers The proportion of diggers among larvae of each JB and FEJ population was thus estimated Larval foraging path length was measured as the distance traversed by a 48-h old larva (42-h old for FEJ populations) in a five-minute period while feeding in a petridish containing agar overlaid with a 50% yeast suspension, following Sokolowski [14]

3 Results and discussion

3 1 *Development time and survivorship*

A strong and consistent direct response to selection on egg-to-eclosion development time was seen, with the mean difference between FEJ and JB populations increasing from ~ 6 h at generation 10 to ~ 30 h at generation 60 of selection (Fig 4) Separate analyses of variance (ANOVA) done on the data from generations 10, 20, 30, 40, 50 and 60 revealed significant fixed effects of selection regime and sex ($P < 0\ 01$ in all cases), and no significant sex × selection interaction ($P > 0\ 1$ in all cases), suggesting that the sexes were not responding differentially to selection for faster development Until the 40th generation of selection, egg-to-eclosion survivorship in individual JB and FEJ populations varied considerably, but did not differ significantly between the selection regimes (Fig 4) However, survivorship of FEJ populations was significantly lower than the JB controls at generation 50 and 60 of selection (Fig 4) Data from stage-specific development time assays at generation 56 of selection revealed significant reductions of ~ 4, 10, and 8 h, respectively, in the duration of the first and third instars and pupal stage of the FEJ populations, relative to the JB controls There was no significant change in the duration of the second instar

Reduction in the duration of pre-adult development in *Drosophila* is not, in itself, new, with three recent studies having reported successful selection for faster development [15–17] The magnitude of the response to selection for shorter development time observed by us is consistent with that seen in these previous studies [15–17] Our observation of a survivorship cost to faster development becoming apparent only after 40 generations of selection is also consistent

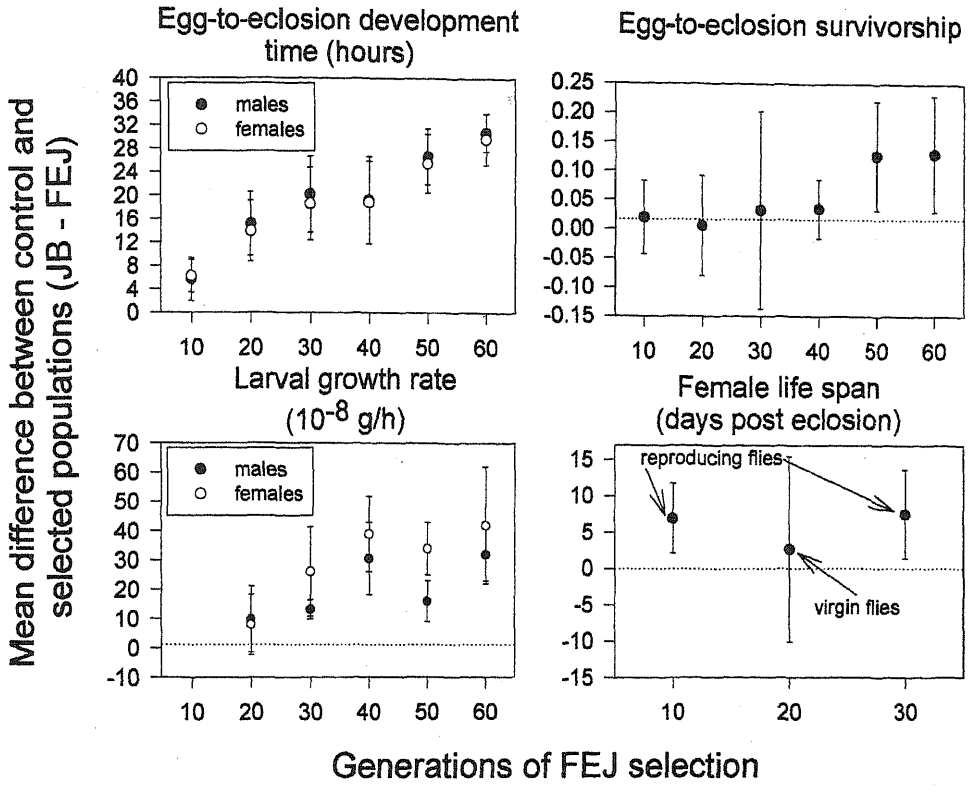

FIG. 4. This composite depicts the main direct and correlated responses to selection for faster development and early reproduction. In all four panels, the mean difference between the trait values of the control (JB) and selected (FEJ) populations is plotted as a function of generations of FEJ selection. Error bars are 95% confidence intervals about the mean difference, based upon variation among the pair-wise differences between each matched JB and FEJ population; any mean difference for which the error bar does not overlap zero is, therefore, statistically significant at the 0.05 level.

with the one previous study of comparable duration in terms of the number of generations of selection.[17] Evidently, it is possible to reduce development time to a degree without incurring a cost in terms of reduced pre-adult survivorship; thereafter, a fairly steep cost (a survivorship reduction of ~ 15%) accompanies further reductions in development time.

One difference between our results and those of Chippindale *et al.*[17] who used flies that share ancestry with our populations is that we observed a significant reduction in the duration of the pupal stage, whereas populations selected for faster development by Chippindale *et al.*[17] showed only a marginal reduction in pupal duration. In fact, it has been a general belief amongst *Drosophila* workers that it is not possible to markedly reduce the pupal duration by selection, presumably because of the large-scale developmental changes that need to occur during this important stage in metamorphosis. Our selection regime, however, differed from that of Chippindale *et al.*[17] in one important respect: our FEJ populations had two full days after eclosion before eggs were collected for initiating the next generation, whereas Chippin-

dale *et al* [17] collected eggs as soon as enough were laid, which was often within a day or less after eclosion We speculate that the additional two-day period prior to egg collection in our FEJ populations has allowed a reduction in the pupal duration to evolve because the flies can use the additional two days to, perhaps, mature the eggs in their ovaries, which otherwise may need to be done during the pupal phase itself We do not, at this time, have an explanation of why the duration of the second larval instar has not responded to selection

There has now accumulated a considerable body of work on life-history evolution in *Drosophila* species that primarily deals with the elucidation of trade-offs between components of fitness, especially those generated by antagonistic pleiotropy [4 5 7] In these studies, trade-offs between larval and adult fitness components have received relatively less attention, even though selection on juvenile stages in holometabolous insects can have profound effects on traits directly relevant to adult fitness [17 18] Most work on trade-offs linking larval and adult fitness components in *Drosophila* has centred around the relationship between development time, adult size and adult life span,[19] and I shall discuss our results on correlated responses to selection for faster development against the backdrop of prior work on the interrelationship between these three traits

3 2 *Dry weight at eclosion and larval growth rate*

In the course of 60 generations of evolution in the FEJ populations dry weight at eclosion of both males and females underwent a decrease of ~ 36% More interestingly the larval growth rate (dry weight at eclosion/development time) in FEJ males and females also decreased (Fig 4), with the difference in larval growth rate at generation 60 being 0 32 and 0 43 × µg/h for males and females, respectively

Large body size in *Drosophila* tends to be positively correlated with both male mating success[20] and female fecundity [21] Consequently it has been thought that there is a trade-off between faster development and adult size, and that this trade-off in part, has shaped the evolution of larval growth rates in nature [22 23] In different studies on *Drosophila*, direct selection for fast development has been seen to yield correlated decreases in adult weight,[15 17] and this notion of a trade-off between fast development and adult size is also supported by quantitative genetic studies of fitness effects of chromosome inversions in *D buzzatii* [24] Our present results are clearly consistent with previous studies with respect to the trade-off between development time and weight at eclosion It should be noted, however, that selection for faster development under extremely crowded conditions does not result in the evolution of smaller body size in *Drosophila*[18], or alter the larval growth rate[15], suggesting that even this fairly consistently seen trade-off may be susceptible to environmental effects, especially density

Our observation that the FEJ populations have evolved a reduced larval growth rate is somewhat counter-intuitive because individuals in the FEJ populations are under selection to develop fast and also be reasonably large, and therefore, more fecund, at eclosion One may, therefore, naively expect larval growth rates to have increased during the course of FEJ selection We speculate that pre-adult development in *Drosophila* consists of distinct phases during which either weight gain or developmental processes take precedence, respectively If so, it may be that the fitness cost of reduction in periods of weight gain is less than that of reduction

in periods when developmental processes are occurring, thereby explaining why larval growth rate in the FEJ populations has slowed down over time Possibly, if the selection regime was such that both shorter development and larger adult size (perhaps through longer adult life span) were at a premium, larval growth rates would actually increase during selection There is some evidence from the lepidopteran *Epiritta autumnata*, that short development time and larger adult size can evolve simultaneously [25] Although previous studies in which faster development was selected for[15 17] did not explicitly address the issue of larval growth rate, as opposed to development time, some data from other studies are consistent with our result that shorter development time is accompanied by a slower larval growth rate [16 26 27]

3 3 *Life span*

Compared to our understanding of the relationship between development time and survivorship or weight at eclosion, the picture regarding the relationship between life span and development time in *Drosophila* is rather more unclear In one study, longevity of reproducing females did not change as a correlated response to selection for faster development alone, without concommitant selection for early reproduction [16] However, this may be due to the fact that the selected flies were small and less fecund, and their lowered fecundity may have offset any decreased life span that evolved as a correlated response to selection for fast development In another study, selection for faster development and reproduction at day 14 from egg-lay also did not yield a correlated change in longevity, although in this case longevity was assayed on virgins [15] In two other studies, selection for increased late age fecundity (and, therefore, also indirect selection for increased life span) was observed to yield a correlated increase in development time, although in one case the flies taking longer to develop were heavier than controls and had lower egg-to-adult viability,[28] whereas in the other case slower developing flies did not significantly differ in weight at eclosion from controls, but had higher egg-to-adult viability [19] However, in another study in which selection was directly for increased life span, rather than late life fecundity[29] the evolution of higher life span was not accompanied by a correlated change in development time Moreover, a study of several sets of populations with different mean longevity ranging from ~30 days to over 90 days revealed that there was no significant among-population correlation between development time and life span[19], suggesting that there may not be a fundamental physiological link between development time and adult life span contrary to the developmental theory of ageing [30]

One of the problems in unravelling the genetic cross-connections between development time and life span is that the relationship between these two life-history traits is likely to be mediated through reproductive output Since selection for faster development often also involves selection for relatively early reproduction[15 17], as well as indirect selection for smaller size (which can itself have effects on fecundity), it is not clear a priori exactly how we might expect longevity to respond to selection on development time and vice versa Moreover, different laboratories tend to use different strains of flies from geographically disparate sources as well as protocols differing in potentially important respects, such as population sizes and the degree to which density is controlled, it is, therefore, not clear whether differences among results from different laboratories are due to differences in experimental protocols, or flies, or both

We observed a clear correlated decrease in female life span after 10 generations of selection (Fig 4) When life span was assayed on reproducing females, the FEJ females lived about 7 days less, on average, than their JB counterparts, a life-span reduction of about 20% This difference in life span of reproducing females was similar when assayed at generations 10 and 30 of FEJ selection, suggesting that there is a lower limit to which life span can decrease, perhaps due to a 'pleiotropic echo' of traits conferring high fitness very early in life [31 32] We also observed that the life span of virgin females from FEJ and JB populations did not differ significantly, when assayed at generation 20 of FEJ selection (Fig 4) Thus, the reduction of life span, when assayed on reproducing flies, in the FEJ populations is likely to be causally related to some aspect(s) of reproduction, rather than being a reflection of some direct link between development time and life span It is possible that FEJ females are expending a proportionately greater fraction of their body mass on early life egg production compared to the JB controls

3 4 *Larval behaviour*

For all four larval behaviours studied individuals from the FEJ populations differed significantly from those of the control populations (Fig 5), in a direction suggesting the evolution of

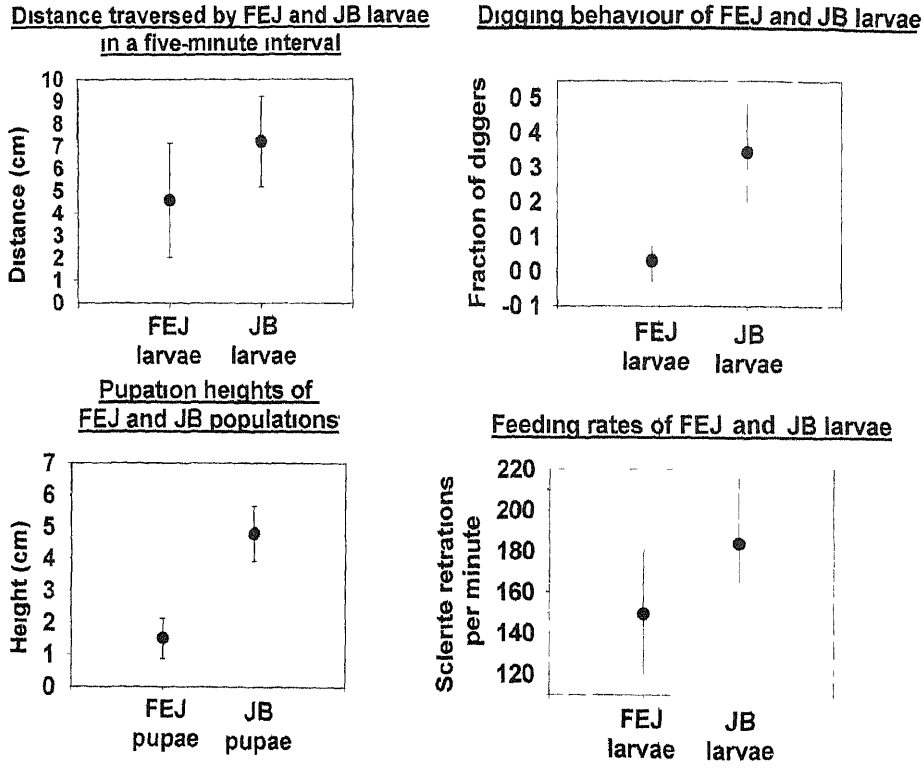

FIG 5 This composite depicts the difference between the trait values of the control (JB) and selected FEJ populations for four larval behaviours that are known to be energy costly All differences between FEJ and JB populations are statistically significant at the 0 05 level

a general syndrome of decreased energy expenditure in the FEJ individuals Mean larval feeding rates in the FEJ populations were lower than those in the JB populations by about one standard deviation, a difference similar in magnitude to that observed in populations subjected to very high densities and their controls [10] Foraging path lengths, pupation heights and fraction of diggers in the population were also less in the FEJ populations, as compared to the JB controls

4 Conclusions

Overall, our results clearly suggest that selection on development time has multifarious effects on the whole life history in *Drosophila* Flies from the faster developing populations are smaller at eclosion, and through adult life, live less long, are less fecund at early ages, and show reduced levels of energy-expensive larval behaviours Somewhat counter-intuitively, they also exhibit reduced larval growth rates, which is not what one would predict from simple optimization arguments This highlights a very general but often ignored problem in evolutionary studies although evolution does optimize, typically we do not know a priori what the genetic constraints on such evolutionary change are and, consequently, predictions from simple optimization arguments based on primarily ecological constraints are often likely to be wrong I also hope that the work discussed here serves to highlight the fact that evolutionary genetics is a rigorous experimental science and that, indeed, laboratory systems offer opportunities for well replicated and controlled studies of the evolutionary process that, in many ways, far exceed those presented by natural populations

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