

Evolution of reduced pre-adult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time

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Summary

Four large ($n > 1000$) populations of *Drosophila melanogaster*, derived from control populations maintained on a 3 week discrete generation cycle, were subjected to selection for fast development and early reproduction. Egg to eclosion survivorship and development time and dry weight at eclosion were monitored every 10 generations. Over 70 generations of selection, development time in the selected populations decreased by approximately 36 h relative to controls, a 20% decline. The difference in male and female development time was also reduced in the selected populations. Flies from the selected populations were increasingly lighter at eclosion than controls, with the reduction in dry weight at eclosion over 70 generations of selection being approximately 45% in males and 39% in females. Larval growth rate (dry weight at eclosion/development time) was also reduced in the selected lines over 70 generations, relative to controls, by approximately 32% in males and 24% in females. However, part of this relative reduction was due to an increase in growth rate of the controls populations, presumably an expression of adaptation to conditions in our laboratory. After 50 generations of selection had elapsed, a considerable and increasing pre-adult viability cost to faster development became apparent, with viability in the selected populations being about 22% less than that of controls at generation 70 of selection.

1. Introduction

In recent years, much empirical work on life-history evolution has focused upon the elucidation of trade-offs between components of fitness, especially those generated by antagonistic pleiotropy, and the bulk of this work has been done on *Drosophila* species (Rose *et al.*, 1987, 1996; Joshi, 1997). There is now clear evidence for multiple trade-offs between components of adult fitness in *Drosophila*, for example negative effects of early reproduction upon later reproduction and adult survival/longevity (Rose, 1984; Service *et al.*, 1985, 1988; Roper *et al.*, 1993; Zwaan, 1993; Leroi *et al.*, 1994; Joshi *et al.*, 1996), as well as between larval components of fitness, such as rate of food acquisition and the efficiency of its utilization

(Mueller, 1990; Joshi & Mueller, 1996; Santos *et al.*, 1997), or the rate of food acquisition and survival to eclosion, especially in the presence of nitrogenous metabolic wastes (Borash *et al.*, 1998). Trade-offs between larval and adult fitness components, however, have not received as much attention, even though selection on juvenile stages in organisms with a complex life-cycle, such as holometabolous insects, can have profound effects on traits directly relevant to adult fitness (Chippindale *et al.*, 1997; Santos *et al.*, 1997).

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 lifespan, and, unfortunately, studies in different laboratories have tended to yield somewhat discordant results (e.g. see discussion in Chippindale *et al.*, 1994). In this paper, we focus on the relationship between development time and adult size at eclosion. Adult size at eclosion is an important fitness trait in

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holometabolous insects such as *Drosophila*, being at the junction of the pre-adult and adult life-stages. Adult size at eclosion is, thus, a good example of a trait that is determined largely by resource acquisition and utilization during the larval stage but exerts its effects on fitness through adult life-history components such as fecundity and lifespan. Large body size in *Drosophila* tends to be positively correlated with both male mating success (Partridge *et al.*, 1987) and female fecundity (Mueller, 1985). 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 (Santos *et al.*, 1988; Partridge & Fowler, 1993). In different studies on *Drosophila*, direct selection for fast development has been seen to yield correlated decreases in adult weight (Zwaan *et al.*, 1995; Nunney, 1996; Chippindale *et al.*, 1997). Selection for larger flies has also been seen to result in correlated increases in development time, but selection for smaller flies did not result in the evolution of decreased development time (Partridge & Fowler, 1993). The 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* (Betran *et al.*, 1998). However, subjecting *Drosophila* populations to extreme larval crowding, a scenario in which faster development is also under indirect selection because food runs out well before most larvae have finished development, does not result in the evolution of smaller body size (Santos *et al.*, 1997). It is likely, therefore, that even this fairly consistently seen trade-off between fast development and adult size may be susceptible to environmental effects, especially density.

There is also some evidence of a trade-off between faster development and pre-adult survivorship. In a survey of laboratory populations subjected to varying demographic maintenance regimes, Chippindale *et al.* (1994) observed a negative correlation between development time and pre-adult survivorship. Similarly, selection for faster development was seen to result in decreased pre-adult survivorship (Chippindale *et al.*, 1997). However, in a study using flies from a different ancestry than those used by Chippindale *et al.* (1994, 1997), no significant differences were observed in pre-adult survivorship between control lines and those selected for faster development (Zwaan *et al.*, 1995).

Given the importance of large body size due to the correlation of size and fecundity, and the trade-off between faster development and adult body size, it is reasonable to expect that, all else being equal, selection for faster pre-adult development will result in the evolution of a greater larval growth rate (i.e. the rate at which weight is put on during development). If such

selection does not result in the evolution of a higher larval growth rate, it would indicate the existence of some hitherto unexplored constraints on the optimization of growth rate and body size. Due to the impact of body size on adult components of fitness, such constraints, if they exist, would be of considerable significance in shaping the evolution of life-histories under scenarios where faster development is selectively favoured.

In this paper, we report results from the first 70 generations of a continuing experiment in which we subjected four large, outbred populations of *Drosophila melanogaster* to selection for fast development and early reproduction, relative to their ancestral populations that served as controls, and compared the selected populations and their controls for egg to eclosion development time and survivorship, dry weight at eclosion, and larval growth rate. Our populations share common ancestors (the B populations of Rose, 1984) with all populations used in studies in the Rose laboratory (Service *et al.*, 1985, 1988; Leroi *et al.*, 1994; Chippindale *et al.*, 1994, 1997). Our study, therefore, also acts as a useful repetition, with some differences in protocol, of a similar study carried out by Chippindale *et al.* (1997); such repetition is helpful in assessing how robust results from laboratory studies in life-history evolution are (Joshi & Mueller, 1996; Reznick & Ghalambor, 1999; Harshman & Hoffmann, 2000).

2. Materials and methods

(i) Experimental populations

This study was conducted on eight laboratory populations of *D. melanogaster*: four populations selected for fast development from egg to eclosion and early reproduction (FEJ-1...4; Fast development, Early reproduction, derived from JB populations), and the four control populations from which the selected lines were derived (JB-1...4; first described by Sheeba *et al.*, 1998). The JB populations are maintained at 25 °C on a 21 day discrete-generation cycle, under constant light, at moderate densities of approximately 60–80 larvae per 8 dram vial (9.0 cm high × 2.4 cm diameter) 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-jaggery food placed in a Plexiglas cage (25 × 20 × 15 cm³). From these Petri plates, approximately 60–80 eggs are collected into each of 40 vials in which larvae then develop into adults. Adults eclosing from these vials are transferred to fresh food vials on day 12, 14 and 16 after egg-lay. On the 18th day after egg-lay, adult flies are transferred into Plexiglas cages and supplied with banana-jaggery food supplemented with live yeast and acetic acid paste for 2 days, after which eggs are collected to

initiate the next generation and the adults discarded. The population typically consists of about 1600–1800 flies at this stage.

The FEJ populations are maintained on a similar regime except that 80 vials of approximately 60–80 eggs are collected per population, and once the pupae darken, the vials are closely monitored and only the first 25% or so of eclosing flies per vial, regardless of sex, are transferred into cages to constitute the pool of breeding adults. The flies in the cages are supplied with yeasted food medium for 2 days and then allowed to oviposit for about 1 h on a fresh food plate. The number of breeding adults in the FEJ populations is typically 1000–1200. Thus, the differences between the two types of population are: (a) FEJ eggs are collected around day 11 (day 10 in later generations of selection) while those of JB are collected on day 21 after egg-lay, (b) the egg-laying window is approximately 1 h for FEJ and 18 h for JB, (c) only the first 25% 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 generation (this is sufficient time for practically all the surviving individuals to eclose at the moderate densities used to maintain these populations), and (d) the number of breeding adults in the JB populations is somewhat greater than in the FEJ populations.

Each FEJ population was derived from one JB population. Therefore, FEJ and JB populations bearing the same numerical subscript are more closely related than different FEJ or JB populations are amongst themselves (FEJ-*i*, JB-*i* are more closely related than FEJ-*i* and FEJ-*j* or JB-*i* and JB-*j*; *i*, *j* = 1, ..., 4). Populations bearing the same numerical subscript were, consequently, treated as blocks in the statistical analyses. The four JB populations were derived from four of the five UU populations described in Joshi & Mueller (1996), and had been independent evolutionary entities in the laboratory for over 450 generations since their derivation from a common ancestral population established from wild-caught flies in South Amherst, Massachusetts (Ives, 1970). For about 110 generations preceding the derivation of the JB populations, the UU populations had been maintained on a 21 day discrete-generation cycle in the laboratory, prior to which the ancestors of the UU populations, the B populations of Rose (1984), had been maintained on a 14 day discrete-generation cycle for approximately 350 generations. Both the B populations and the UU populations were maintained on banana-molasses food at 25 °C under constant light. The maintenance regime of the JB populations is essentially the same as that of their UU ancestors, except for the fact that the banana-jaggery food, though very similar, is not exactly identical to banana-molasses food.

(ii) *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 approximately 60–80 eggs were collected. Adults eclosing in these vials were collected into cages 12 days after egg-lay, by which time all flies would have eclosed. The progeny of these adults (henceforth referred to as standardized flies) were used for the various assays.

(iii) *Development time and survivorship assays*

Development time and survivorship assays were conducted every 10 generations during the course of selection. Standardized flies of each JB and FEJ population were supplied with yeasted agar plates in the cages for 1 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. Sixteen such vials were set up per population (8 for the generation 10 and 20 assays). 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 3 consecutive days passed with no eclosion recorded from any vial.

(iv) *Dry weight assays*

Dry weight assays were conducted every 10 generations, from generation 20 onward. Freshly eclosed adults (< 2 h post-eclosion) originating from eggs laid by standardized flies were collected, killed by freezing, dried for 18 h at approximately 70 °C and weighed in batches of 5 males or 5 females. The flies collected for the assays were reared at a larval density of 30 eggs per vial, and all flies eclosing during the 10–14 h period of bulk eclosion in each vial were collected at 2 h intervals and frozen. From these frozen flies, six batches each of males and females were chosen haphazardly and weighed for each FEJ and JB population. Data on dry weight at eclosion and egg to eclosion development time were also used to estimate larval growth rates for each FEJ and JB population by dividing population mean dry weight at eclosion by the mean development time.

(v) *Statistical analyses*

In selection experiments, the unit of replication is the population, implying that the error variation for

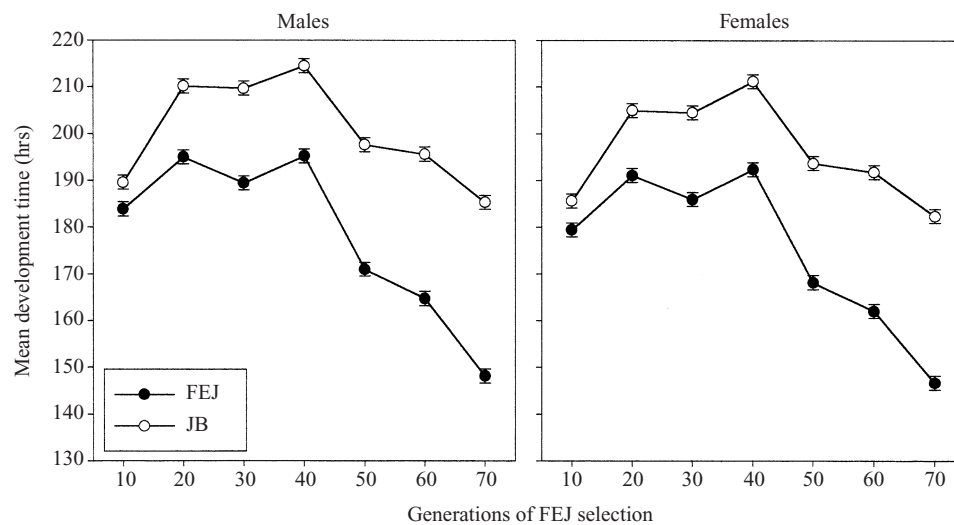


Fig. 1. Mean egg to eclosion development time of males and females from the selected (FEJ) and control (JB) populations over the course of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomized block ANOVA, and can, therefore, be used for visual hypothesis testing.

testing hypotheses regarding selection regime is the variation among population means (Chippindale *et al.*, 1994; Rose *et al.*, 1996). Consequently, for each of the four traits considered (development time, pre-adult survivorship, dry weight at eclosion, and larval growth rate), the primary units of analyses were the population mean trait values for a given combination of block \times generation of assay \times sex (except for survivorship) \times selection regime. For each trait, two analyses were done. Analyses of variance (ANOVAs) were conducted according to a mixed model, completely randomized block design wherein time (generation of assay), sex (except in the case of survivorship) and selection regime were treated as fixed factors, crossed amongst themselves, and with random blocks based on ancestry (see last paragraph of Section 2.i, above). In such a design, it is not possible to test for significance of block or any interactions involving block; these effects are anyway not of great interest in laboratory selection studies. All ANOVAs were done on untransformed data, since the units of analysis were mean values, which can be assumed to follow a normal distribution to a reasonable degree. In addition to the ANOVAs, the mean trait values for each combination of block \times generation \times sex (except for survivorship) \times selection regime were linearly regressed upon time in generations, to detect any long-term directional changes in mean trait values in either the control or selected populations in the course of the 70 generations of selection. In the case of development time, the difference between males and females in each population was also regressed over time in generations to determine whether selection for faster development ameliorated the development time

advantage of females typically seen in *Drosophila* cultures. All statistical analyses were implemented using STATISTICA for Windows, release 5.0 B (StatSoft, 1995).

3. Results

(i) Egg to eclosion development time

We observed a strong and consistent direct response to selection on egg to eclosion development time, with the mean difference between FEJ and JB populations increasing from approximately 6 h at generation 10 to approximately 36 h (a 20% reduction, relative to controls) at generation 70 of selection (Fig. 1). Although the absolute values of development time changed considerably from assay to assay, the difference in development time between the selected and control lines underwent an almost linear increase over time. The ANOVA revealed significant main effects of time, sex (males took longer to develop than females) and selection regime, as well as significant time \times sex and time \times selection regime interactions (Table 1). The regression of development time on time in generations had a significantly negative slope in the FEJ populations, whereas the slope of the JB populations did not differ significantly from zero (Table 2), and this difference in long-term trends in the selected and control populations is reflected in the strong time \times selection regime interaction in the ANOVA.

As selection proceeded, the difference between male and female mean development time was consistently reduced in the FEJ populations, declining from 4.5 h

Table 1. Results of analysis of variance (ANOVA) on mean egg to eclosion development time in the FEJ and JB populations

Source	df	MS	F	P
Time	6	3200.36	23.34	< 0.001
Sex	1	385.97	1331.72	< 0.001
Selection regime (Sel)	1	14314.01	1487.28	< 0.001
Time × Sex	6	2.92	5.82	0.002
Time × Sel	6	434.66	44.75	< 0.001
Sex × Sel	1	6.72	6.86	0.079
Time × Sex × Sel	6	0.61	0.59	0.733

The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table.

Table 2. Summary of the results of linear regressions of mean trait value over time in generations for development time (in h), dry weight at eclosion (in mg) and larval growth rate (in µg/h)

Trait	Selection regime			
	FEJ		JB	
	Males	Females	Males	Females
Development time	-0.6654 (1.6×10^{-6} ; 0.59)	-0.6216 (4.0×10^{-6} ; 0.57)	-0.1920 (0.0784; 0.11)	-0.1680 (0.1179; 0.09)
Dry weight at eclosion	-0.0017 (4.4×10^{-11} ; 0.87)	-0.0019 (6.6×10^{-9} ; 0.79)	-0.0003 (0.1357; 0.10)	-0.0003 (0.2388; 0.06)
Larval growth rate	-0.0039 (8.7×10^{-5} ; 0.51)	-0.0034 (0.0126; 0.25)	+0.0019 (0.0418; 0.18)	+0.0027 (0.0216; 0.22)

Entries are the slopes of the regressions, followed by *P* values and coefficients of determination (*R*²), respectively, in parentheses.

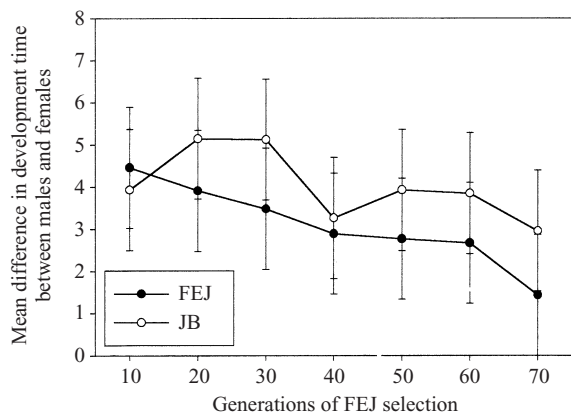


Fig. 2. Mean difference between males and females (in hours) in egg to eclosion development time in the selected (FEJ) and control (JB) populations over the course of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomized block ANOVA, and can, therefore, be used for visual hypothesis testing.

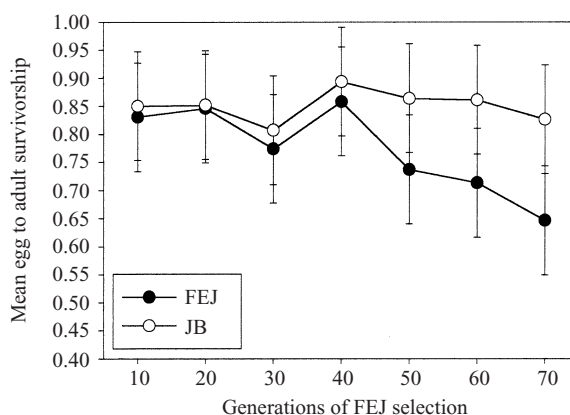


Fig. 3. Mean egg to eclosion survivorship in the selected (FEJ) and control (JB) populations over the course of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomized block ANOVA, and can, therefore, be used for visual hypothesis testing.

Table 3. Results of analysis of variance (ANOVA) on mean egg to eclosion survivorship in the FEJ and JB populations

Source	df	MS	F	P
Time	6	0.0176	5.211	0.0029
Selection regime (Sel)	1	0.0859	24.108	0.0162
Time × Sel	6	0.0100	2.354	0.0744

The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table.

at generation 10 to 1.4 h at generation 70 (Fig. 2). Regressing the difference between males and females over time in generations revealed a significantly negative slope in the FEJ populations (slope = -0.0438 ; $P < 10^{-5}$; $R^2 = 0.60$), whereas the slope in the JB populations did not differ significantly from zero (slope = -0.0240 ; $P = 0.0921$; $R^2 = 0.11$). The mean difference between males and females, averaged across selection regimes, also declined with time as a consequence of the decline in the FEJ difference, giving rise to the significant time × sex interaction in the ANOVA (Table 1), although the time × sex × selection regime interaction was not significant. Overall, the difference between male and female development times was less in the FEJ populations, compared with the JB controls at every generation except the tenth, although only the differences observed at generations 30 and 70 were statistically significant (Fig. 2).

(ii) Pre-adult survivorship

Egg to adult survivorship did not differ significantly between the FEJ and JB populations for the first 40

generations of selection, even though survivorship in the FEJ populations was consistently lower than in the JB populations by approximately 0.03 (Fig. 3). After the fortieth generation of selection, an increasing survivorship cost to faster development became apparent, with the FEJ populations showing significantly reduced survivorship compared with the JB controls. Between generations 40 and 70, the mean difference in survivorship of the FEJ and JB populations increased from 0.035 to 0.179 (Fig. 3), yielding a 22% reduction in FEJ survivorship at generation 70. Pre-adult survivorship in the JB populations remained within the range 0.8–0.9 throughout the 70 generations of selection, which is the typical range of pre-adult survivorship in these populations and their ancestors. The ANOVA revealed significant main effects of time and selection regime, whereas the time × sel interaction was not significant (Table 3). Regressions of pre-adult survivorship on time in generations revealed a significantly negative slope in the FEJ populations (slope = -0.0031 ; $P = 0.0005$; $R^2 = 0.38$), whereas the slope in the JB populations did not differ significantly from zero (Slope = $+0.0001$; $P = 0.98$; $R^2 < 10^{-4}$).

(iii) Dry weight at eclosion

Dry weight at eclosion of both males and females in the FEJ populations was significantly lower than in their JB counterparts from the twentieth generation of selection onward, and continued to decrease as selection proceeded (Fig. 4). Over the 70 generations of selection, dry weight at eclosion of FEJ flies underwent a reduction of approximately 45% in males and 39% in females, relative to the JB controls. The slope of the regression of dry weight at eclosion on time in generations was significantly negative for

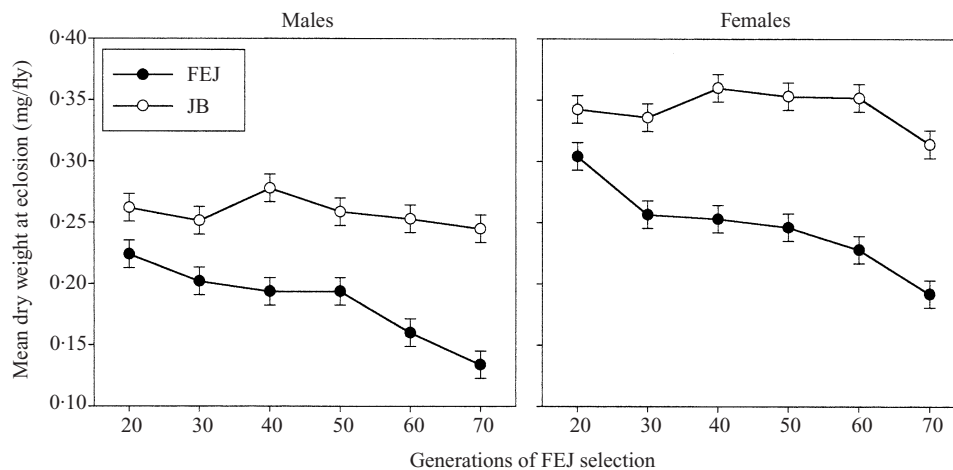


Fig. 4. Mean dry weight at eclosion of males and females from the selected (FEJ) and control (JB) populations over the course of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomized block ANOVA, and can, therefore, be used for visual hypothesis testing.

Table 4. Results of analysis of variance (ANOVA) on mean dry weight at eclosion in the FEJ and JB populations

Source	df	MS	F	P
Time	5	0.007331	18.52	< 0.001
Sex	1	0.129607	1925.96	< 0.001
Selection regime (Sel)	1	0.172808	626.96	< 0.001
Time × Sex	5	0.000213	2.42	0.085
Time × Sel	5	0.003432	26.57	< 0.001
Sex × Sel	1	0.003115	35.11	0.010
Time × Sex × Sel	5	0.000223	4.01	0.017

The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table.

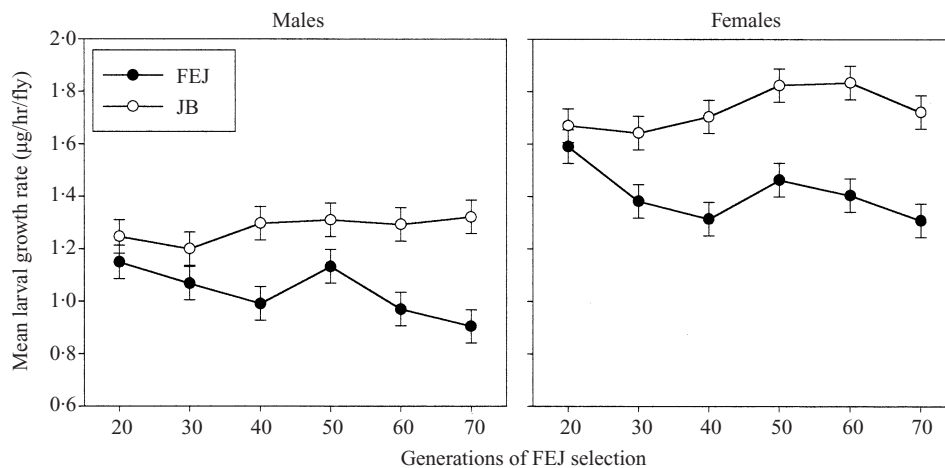


Fig. 5. Mean larval growth rate of males and females from the selected (FEJ) and control (JB) populations over the course of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomized block ANOVA, and can, therefore, be used for visual hypothesis testing.

Table 5. Results of analysis of variance (ANOVA) on mean larval growth rate in the FEJ and JB populations

Source	df	MS	F	P
Time	5	0.041143	3.73	0.022
Sex	1	4.200594	1429.065	< 0.001
Selection regime (Sel)	1	1.941244	229.54	< 0.001
Time × Sex	5	0.008189	4.29	0.013
Time × Sel	5	0.061501	11.90	< 0.001
Sex × Sel	1	0.039050	13.87	0.034
Time × Sex × Sel	5	0.006129	3.41	0.030

The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table.

FEJ males and females, but not significantly different from zero for JB males and females (Table 2). Consistent with these observations, the ANOVA revealed significant main effects of time, sex (females

heavier than males) and selection regime (JB heavier than FEJ), as well as significant time × selection regime, sex × selection regime, and time × sex × selection regime interactions (Table 4).

(iv) *Larval growth rate*

Interestingly, the mean larval growth rate (dry weight at eclosion/development time) in the FEJ populations decreased relative to the JB controls as selection proceeded, with FEJ individuals putting on less weight per unit time during pre-adult development than their JB counterparts (Fig. 5). FEJ males had significantly lower larval growth rates than JB males from generation 40 onward, whereas in the case of females, the FEJ–JB difference was significant from generation 30 onward (Fig. 5). Regressions of larval growth rate on time in generations revealed that the increased divergence between selected and control populations was due to declining growth rate in the FEJ populations, coupled with an increase in growth rate of the JB populations over the 70 generations of selection (Table 2). As in the case of dry weight at eclosion, the overall relative reduction of larval growth rate in the FEJ populations was greater for males (~32%) than females (~24%). All fixed main effects and interactions in the ANOVA were significant (Table 5).

4. Discussion

Successful selection for faster development has been achieved in *Drosophila* in several studies in recent years, and a correlated decrease in adult size/weight has been consistently observed (Zwaan *et al.*, 1995; Nunney, 1996; Chippindale *et al.*, 1997). Other lines of work have also supported the notion of a trade-off between development time and adult size in *Drosophila* (Partridge & Fowler, 1993; Betran *et al.*, 1998), and our results are consistent with this notion (Figs. 1, 4). Indeed, this trade-off is often seen in studies on other insects as well (Miyatake, 1995, 1998; Tucic *et al.*, 1997).

The magnitude of the direct response to selection that we observed in this study is greater than even that seen by Chippindale *et al.* (1997), who recorded a 17% decrease in development time after 125 generations of selection for fast development and early reproduction. The study by Chippindale *et al.* (1997) is the only previous study on *Drosophila* in which selection for faster development was continued for a large number of generations; other studies have typically been of about 15 generations in duration (Zwaan *et al.*, 1995; Nunney, 1996). Moreover, the populations we used share common ancestry with the populations used by Chippindale *et al.* (1997), making a detailed comparison of our results and theirs all the more meaningful.

Our observation of a correlated decrease in pre-adult survivorship in the FEJ populations is consistent with the negative correlation between development time and pre-adult survivorship seen in a survey of

populations maintained under varying demographic regimes (Chippindale *et al.*, 1994), as well as with the results of a study in which faster development was directly selected for (Chippindale *et al.*, 1997). As also noticed by Chippindale *et al.* (1997), the survivorship cost of faster development became apparent in our study only after 50 generations of selection had elapsed (Fig. 3). Yet, by this time, differences in development time (Fig. 1) and dry weight at eclosion (Fig. 4) between selected and control lines were already considerable, indicating that it is possible to reduce development considerably, at the expense of putting on weight, without seriously compromising pre-adult survivorship. This is also a possible explanation for why no trade-off between development time and pre-adult survivorship was observed by Zwaan *et al.* (1995) in a selection study lasting only 16 generations, although the possibility of a different genetic architecture of traits related to development and survival in their populations cannot be altogether discounted (Chippindale *et al.*, 1997; Harshman & Hoffmann, 2000).

The amelioration of the difference in male and female development times in our study (Fig. 2) is counter to observations made by Zwaan *et al.* (1995) and Chippindale *et al.* (1997). Even though females in *Drosophila* cultures typically eclose earlier than males and, therefore, males should experience stronger selection in a regime selecting for faster development, male and female development times have not previously been seen to respond differentially to selection. Possible explanations for this apparent paradox have been that the difference in male and female development times can be ameliorated by either high (Zwaan *et al.*, 1995) or variable (Joshi *et al.*, 1999) larval densities in the culture vials, or the suggestions that sex-specific expression of heritable variation for development time is lacking in these populations (Chippindale *et al.*, 1997), or that the sexual dimorphism in development time is subject to strong canalizing influences (Chippindale *et al.*, 1997). While one or more of these explanations may, in fact, be operating to ameliorate the selection differential between males and females in a culture subjected to truncation selection for fast development, our results clearly indicate that the sexual dimorphism in development time in *Drosophila* can, nevertheless, respond to selection in the manner expected, with males gradually narrowing the development time gap with females.

As in the case of the trade-off between development time and pre-adult survivorship, it is possible to argue that a reduction of the male–female difference in development time was not observed by Zwaan *et al.* (1995) because of the short duration of their study, or perhaps because of differences in the genetic composition of the base populations used. Indeed, in our

study too, the reduction of the male–female difference in development time as selection proceeds is observed only when data from 70 generations are considered. It is not clear, however, why such a reduction was not seen in the study by Chippindale *et al.* (1997), given the similarities between our study and theirs: they continued selection for 125 generations, and their flies and ours share common ancestry. There are, however, two major differences between our selection protocol and that used by Chippindale *et al.* (1997). In the FEJ populations, flies had 48 h after eclosion before eggs were collected for initiating the next generation, whereas in the selected lines of Chippindale *et al.* (1997) eggs were collected as soon as enough were available, typically within 24 h of eclosion. It is possible that FEJ males, not being under as strong selection for early sexual maturity as males in the populations of Chippindale *et al.* (1997), were able to undergo a reduction in the duration of some phase of development that could be compensated for after eclosion. Our selection intensity was also somewhat greater than that of Chippindale *et al.* (1997), as evidenced by the more rapid response to selection we observed, and population numbers in the FEJ populations were also greater (80 vials of 60–80 eggs versus 50 vials). It is, therefore, also possible that such a reduction in the male–female difference in development time may have been seen by Chippindale *et al.* had they continued selection for longer.

Perhaps the most interesting result in the present study is that larval growth rate actually decreased in the FEJ populations that were selected for shorter development time. All else being equal, it is not unreasonable to expect that the FEJ populations would have evolved a higher larval growth rate, being under selection for both a shorter development time, and fecundity at 2 days of adult age. Yet, larval growth rate, the average rate of dry weight gain over the course of development from egg to eclosion, clearly decreased in the FEJ populations as selection proceeded (Fig. 5). In the case of both dry weight at eclosion and larval growth rate, the fractional reduction, relative to controls, in FEJ males was greater than that seen in FEJ females. This is not altogether surprising, given that weight at eclosion is clearly important to females due to its relationship to early life fecundity (Mueller, 1985), whereas male size is not strongly related to reproductive success in laboratory cultures maintained at low larval densities (Joshi *et al.*, 1999). Part of the decrease in growth rate of the FEJ populations relative to the JB controls was actually due to an increase in JB growth rates as selection proceeded (Fig. 5, Table 2). We suspect that this may be an expression of the JB populations adapting to some novel aspect of rearing in our laboratory, perhaps the banana-jaggery food.

Although previous studies in which faster devel-

opment was selected for (Zwaan *et al.*, 1995; Nunney, 1996; Chippindale *et al.*, 1997) 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. Nunney (1996) selected for shorter egg hatch to pupation time, and if we divide the mean dry weight of eclosing adults in his control and selected populations by the larval development time, the average larval growth rates obtained are 2.44 and 3.13 $\mu\text{g}/\text{h}$ for control males and females, respectively, and 2.28 and 2.81 $\mu\text{g}/\text{h}$ for males and females from the selected lines. In another study, larval growth rates of female *D. melanogaster* from two geographically distinct populations were found to vary with development time (Azevedo *et al.*, 1997). A population from Ecuador had a mean development time of 208.5 h and mean larval growth rate of 1.394 $\mu\text{g}/\text{h}$, whereas a North Carolina population had a mean development time and larval growth rate of 215.6 h and 1.449 $\mu\text{g}/\text{h}$, respectively. Similarly, the average larval growth rates obtained by Tucic *et al.* (1997) in a study of density-dependent selection on the bean weevil *Acanthoscelides obtectus* were 0.156 and 0.186 mg/day for males and females from slow-developing lines, and 0.155 and 0.183 mg/day for males and females from faster-developing lines, respectively. Although none of these data from the literature permit testing for statistical significance, the trend observed is consistent with our findings of a slower larval growth rate in the FEJ populations.

Overall, our results clearly suggest that there is more to the evolution of faster development than merely a reduction in development time. Perhaps 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, and the duration of periods of weight gain is the first to be reduced in response to selection for faster development. Both the slowing down of larval growth rate in the FEJ populations, and the relatively late observation of reduced pre-adult survivorship, are consistent with this scenario. Possibly, if the selection regime was such that both shorter development and larger adult size (perhaps through longer adult lifespan) were at a premium, larval growth rates would actually increase during selection. There is some evidence from the lepidopteran *Epirrita autumnata* that short development time and larger adult size can evolve simultaneously (Kause *et al.*, 1999), and studies on the melon fly *Bactrocera cucurbitae* suggest that short development and higher early life fecundity can also be successfully selected for

simultaneously (Miyatake, 1998). The possibility that males, and perhaps females, in the FEJ populations are achieving some part of their reduction in development time by postponing some aspect(s) of reproductive development until after eclosion is a tantalizing one; it may also be a partial explanation of why we obtained a stronger response to selection than Chippindale *et al.* (1997). Further studies on the time course of larval weight gain, and on the timing of the attainment of sexual maturity in the FEJ and JB populations, may help to clarify these issues.

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