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RESEARCH ARTICLE

Correlated changes in life history traits in response to selection for faster pre-adult development in the fruit fly *Drosophila melanogaster*

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ABSTRACT

Insects including the fruit fly *Drosophila melanogaster* are under intense pressure to develop rapidly because they inhabit ephemeral habitats. We have previously shown that when selection for faster development was artificially imposed on *D. melanogaster* in the laboratory, reduction of pre-adult development time and shortening of the clock period occurs, suggesting a role for circadian clocks in the regulation of life history traits. Circadian clocks in *D. melanogaster* have also been implicated in the control of metabolic pathways, ageing processes, oxidative stress and defense responses to exogenous stressors. In order to rigorously examine correlations between pre-adult development time and other life history traits, we assayed pre-adult survivorship, starvation and desiccation resistance, body size and body weight, fecundity and adult lifespan in faster developing populations of *D. melanogaster*. The results revealed that selection for faster pre-adult development significantly reduced several adult fitness traits in the faster developing flies without affecting pre-adult survivorship. Although overall fecundity of faster developing flies was reduced, their egg output per unit body weight was significantly higher than that of controls, indicating that reduction in adult lifespan might be due to disproportionate investment in reproduction. Thus our results suggest that selection for faster pre-adult development in *D. melanogaster* yields flies with higher reproductive fitness. Because these flies also have shorter clock periods, our results can be taken to suggest that pre-adult development time and circadian clock period are correlated with various adult life history traits in *D. melanogaster*, implying that circadian clocks may have adaptive significance.

KEY WORDS: Fitness, Fecundity, Lifespan, Starvation, Desiccation, Circadian

INTRODUCTION

In nature, insect populations including those of the fruit fly *Drosophila melanogaster* are subjected to directional selection for faster development because they inhabit ephemeral habitats such as rotting fruits (Prasad and Joshi, 2003). Several studies on life history traits in different model organisms, including *Drosophila*, led to the development of life history theory, which posits that natural selection enhances organismal fitness, and that the trait combinations under selection are constrained by trade-offs (see Prasad and Joshi, 2003). Trade-offs are observed at phenotypic as well as genetic levels; hence assessment of trade-offs is necessary at both levels. Previous studies on flies selected for faster pre-adult development and early reproduction showed shortening of development time (Chippindale et al., 1994; Chippindale et al., 1997; Prasad et al., 2000) and trade-offs between development time and pre-adult viability (Chippindale et al., 1997; Prasad et al., 2000), adult weight (Chippindale et al., 2004), fecundity (Prasad et al., 2000; Chippindale et al., 2004) and adult lifespan (Chippindale et al., 1994). Populations selected for slower development and delayed reproduction showed increased pre-adult development time (Chippindale et al., 1994) and extended adult lifespan (Partridge and Fowler, 1992; Djawdan et al., 1996). While a study on the butterfly *Bicyclus anynana* reported a negative correlation between pre-adult development time and adult lifespan (Pijpe et al., 2006), others have reported positive [melon flies, *Bactrocera cucurbitae* (Miyatake, 1997a); *D. melanogaster* (Chippindale et al., 2004)] or no correlation [*D. melanogaster* (Zwaan et al., 1991)]. This suggests that correlation between pre-adult development and adult fitness traits depends on the selection protocol and/or the genetic architecture of the founder populations.

In insects, circadian clocks, which rhythmically regulate most behavioural and metabolic processes, have been implicated in the regulation of life history traits such as pre-adult development time (Kyriacou et al., 1990; Paranjpe et al., 2005) and adult lifespan (Miyatake, 1997a; Klarsfeld and Rouyer, 1998). Correlation between development time and circadian clocks was also reported in laboratory selection studies on melon flies (Miyatake, 1997b; Shimizu et al., 1997) and fruit flies (Kumar et al., 2006; Takahashi et al., 2013; Yadav and Sharma, 2013a). Disruption of circadian timing systems results in reduced reproductive output in the gypsy moth, *Lymantria dispar* (Giebultowicz et al., 1990), and *D. melanogaster* (Beaver et al., 2002; Beaver et al., 2003), shortening of adult lifespan in *D. melanogaster* (Hendricks et al., 2003; Kumar et al., 2005), reduction in vegetative growth and survivorship in *Arabidopsis thaliana* (Dodd et al., 2005), and increased predation in free-living ground squirrels *Spermophilus lateralis* (DeCoursey et al., 1997) and chipmunks *Tamias striatus* (DeCoursey et al., 2000). Furthermore, circadian clocks in resonance with environmental light/dark (LD) cycles have been shown to enhance adult lifespan of *D. melanogaster* (Pittendrigh and Minis, 1972; Klarsfeld and Rouyer, 1998) and blow flies *Phormia terraenovae* (von Saint Paul and Aschoff, 1978), and competitive ability in cyanobacteria *Synechococcus* sp. (Ouyang et al., 1998). Taken together, these studies suggest that circadian clocks play an important role in the regulation of organismal fitness.

Circadian clocks in *Drosophila* have also been implicated in the regulation of the response to exogenous stressors (Gorbacheva et al., 2005; Gachon et al., 2006; Lee and Edery, 2008; Krishnan et al., 2008). Resistance to starvation and desiccation is often used as a measure to assess fitness in *Drosophila* (Service et al., 1985; Service et al., 1988; Zwaan et al., 1991; Chippindale et al., 1996), and has

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led to the widespread acceptance of the stress theory of ageing, which posits that pre-adult and adult fitness traits are correlated with the extent of stress resistance (Parsons, 2003). In *D. melanogaster*, studies on selection for resistance to starvation and desiccation reported a correlated increase in pre-adult development time (Chippindale et al., 1996; Harshman et al., 1999), while selection for extended lifespan resulted in increased starvation and desiccation resistance (Service et al., 1988; Graves et al., 1992). Studies examining the correlation between circadian clocks and pre-adult and adult fitness traits have always been carried out separately, often in different model organisms, under varying selection or maintenance protocols, yielding contradictory and inconclusive outcomes. For instance, studies on selection for faster pre-adult development (Chippindale et al., 1994; Chippindale et al., 1997; Prasad et al., 2000) were carried out in *D. melanogaster* under rhythm-abolishing laboratory conditions of constant light (LL) by imposing additional selection pressure on the age of reproduction [early life (Prasad et al., 2000) or very early (Chippindale et al., 1994; Chippindale et al., 1997)]. Such studies were also carried out under different LD cycles in the laboratory (Miyatake, 1997a; Miyatake, 1997b; Shimizu et al., 1997) or under natural conditions (Pijpe et al., 2006) on several insect species, such as melon flies and the butterfly *B. anynana*. Therefore, a comprehensive study is required that critically examines circadian clocks and a variety of fitness components in large outbred populations, for a better understanding of the role of circadian clocks in the regulation of life history traits.

To examine the consequence of rapid pre-adult development and faster circadian clocks on pre-adult and adult fitness traits, we used four large outbred faster developing (FD) populations of *D. melanogaster*. After 55 generations of selection, these populations started developing as pre-adults significantly faster (~29 h; ~12%) and had periods (τ) of circadian activity/rest rhythm ~0.5 h shorter than the controls [baseline developing (BD) flies] (Yadav and Sharma, 2013a). The FD flies were raised under constant dark (DD) conditions to avoid any physiological or behavioural consequences of LL, and to ensure that their circadian clocks were free-running. These flies, which develop considerably faster and have shorter clock periods than controls, served as an appropriate system to study the correlation between circadian clocks and life history traits. We analyzed the pre-adult survivorship, resistance to starvation and desiccation, body weight and body length, fecundity and adult lifespan in these flies to assess some of their key pre-adult and adult fitness traits. The results revealed that FD flies with shorter clock periods have a smaller body size and body weight, reduced resistance to starvation and desiccation, reduced adult lifespan and lower fecundity than controls. However, their mid- and late-life fecundity per unit body weight was significantly higher compared with that of controls, suggesting that selection for faster pre-adult development yields flies with faster circadian clocks and higher reproductive fitness.

RESULTS

Pre-adult survivorship of faster developing flies remains unchanged

The pre-adult survivorship of FD and BD flies did not differ except in the 10th and 20th generation assays (Fig. 1A). ANOVA revealed a statistically significant effect of generation; however, the effect of stock and the generation × stock interaction was not statistically

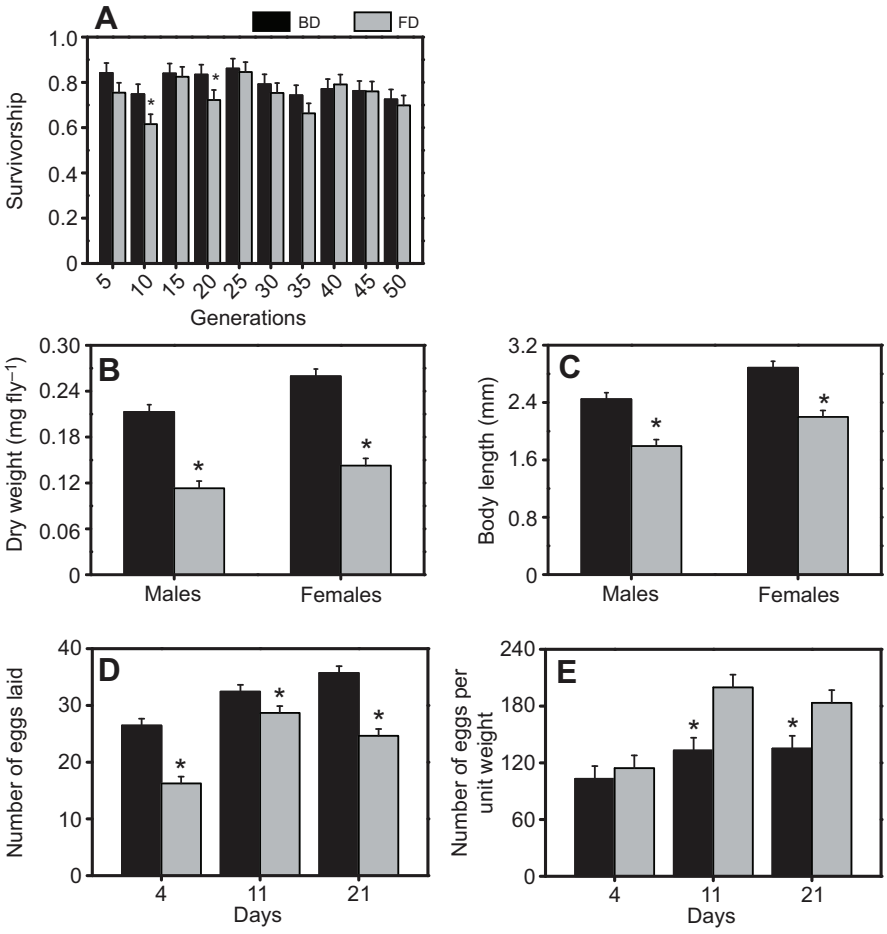

Fig. 1. Pre-adult and adult fitness of faster developing (FD) flies. (A) Mean pre-adult survivorship of selected (FD) and control (BD) populations under constant darkness (DD) across generations. A total of 10 vials (30 eggs per vial) were used for four replicate populations at every generation. (B) Mean dry weight and (C) body length at emergence of virgin males and females from FD and BD stocks show a significant effect of selection for faster development. (D) Mean fecundity (number of eggs layed) and (E) mean fecundity per unit body weight of females from FD and BD stocks at early (4 days post-emergence), mid (11 days) and late life stages (21 days). Error bars represent 95% confidence interval around the mean for visual hypothesis testing. Asterisks indicate statistically significant differences between selected and control populations (*P<0.05).

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Table 1. ANOVA details of the following assays: pre-adult survivorship under constant dark (DD), dry weight at emergence, body size/length at emergence, fecundity under DD and fecundity per unit body weight

Assay	Effect	d.f.	MS effect	d.f. error	MS error	*F*	*P*
Pre-adult survivorship	Generation (G)	9	0.024	27	0.003	7.47	0.0001
	Stock (P)	1	0.048	3	0.009	4.94	0.11
	Block (B)	3	0.007	0	0	–	–
	G × P	9	0.005	27	0.004	1.25	0.31
	G × B	27	0.003	0	0	–	–
	P × B	3	0.009	0	0	–	–
	G × P × B	27	0.004	0	0	–	–
Dry weight	Sex (S)	1	0.58	3	0.01	389.41	0.0003
	Stock (P)	1	4.71	3	0.09	54.59	0.005
	Block (B)	3	0.04	0	0	–	–
	S × P	1	0.03	3	0.01	16.29	0.027
	S × B	3	0.01	0	0	–	–
	P × B	3	0.09	0	0	–	–
	S × P × B	3	0.01	0	0	–	–
Body size/length	Sex (S)	1	0.72	3	0.01	519.29	0.0002
	Stock (P)	1	1.79	3	0.01	541.39	0.0002
	Block (B)	3	0.01	0	0	–	–
	S × P	1	0.01	3	0.01	0.55	0.51
	S × B	3	0.01	0	0	–	–
	P × B	3	0.01	0	0	–	–
	S × P × B	3	0.01	0	0	–	–
Fecundity	Age (A)	2	240.81	6	8.15	29.55	0.0008
	Stock (P)	1	407.37	3	2.96	137.72	0.001
	Block (B)	3	35.87	0	0	–	–
	A × P	2	9.54	6	2.63	3.63	0.09
	A × B	6	8.15	0	0	–	–
	P × B	3	2.96	0	0	–	–
	A × P × B	6	2.63	0	0	–	–
Fecundity per unit body weight	Age (A)	2	7907.71	6	289.09	27.35	0.0009
	Stock (P)	1	10,668.33	3	1023.72	10.42	0.048
	Block (B)	3	1860.07	0	0	–	–
	A × P	2	1588.06	6	91.85	17.29	0.003
	A × B	6	289.09	0	0	–	–
	P × B	3	1023.72	0	0	–	–
	A × P × B	6	91.85	0	0	–	–

significant (Table 1). Thus, selection for faster development does not affect the pre-adult survivorship.

Reduced dry weight and body size in faster developing flies

Dry weight at emergence of FD male and female flies was significantly lower compared with that of BD controls (Fig. 1B). Dry weight of FD flies was reduced by ~47% in males and ~45% in females compared with BD controls. ANOVA revealed a statistically significant effect of sex, stock and their interaction (Table 1). *Post hoc* multiple comparisons using Tukey's honestly significant difference (HSD) test revealed that dry weight at emergence of males was significantly lower than that of females.

Body size (length) at emergence of both male and female FD flies was significantly smaller compared with that of BD controls (Fig. 1C). ANOVA revealed a statistically significant effect of sex and stock, but not of their interaction (Table 1). *Post hoc* multiple comparisons using Tukey's HSD test revealed that body size at emergence of males was significantly smaller than that of females. These results suggest that selection for faster development yields flies with reduced body weight and body size.

Higher fecundity per unit body weight in faster developing flies

After 50 generations of selection, the average egg output (i.e. fecundity) of FD females at three life stages (early-life, mid-life and late-life) was significantly lower compared with BD controls (*post hoc* multiple comparisons using Tukey's HSD test; Fig. 1D). ANOVA revealed a statistically significant effect of life stage and stock; however, the effect of the life stage × stock interaction was not statistically significant (Table 1). These results suggest that selection for faster pre-adult development yields females with reduced reproductive output.

ANOVA on fecundity per unit body weight data revealed a statistically significant effect of life stage, stock and their interaction (Table 1). *Post hoc* multiple comparisons using Tukey's HSD test showed that except during the early-life stage, fecundity per unit body weight of FD flies was significantly higher than that of BD controls (Fig. 1E). These results suggest that although FD flies have lower egg output, their fecundity per unit body weight was significantly greater than that of BD controls.

Lower starvation resistance in faster developing flies

Based on the survivorship curve for starvation resistance, it is clear that FD flies have a lower starvation resistance compared with BD controls (Fig. 2A–D). ANOVA revealed a statistically significant effect of light regime, stock, sex and the light regime × stock interaction; however, the effects of the stock × sex and light regime × stock × sex interactions were not statistically significant (Table 2). *Post hoc* multiple comparisons using Tukey's test revealed that flies

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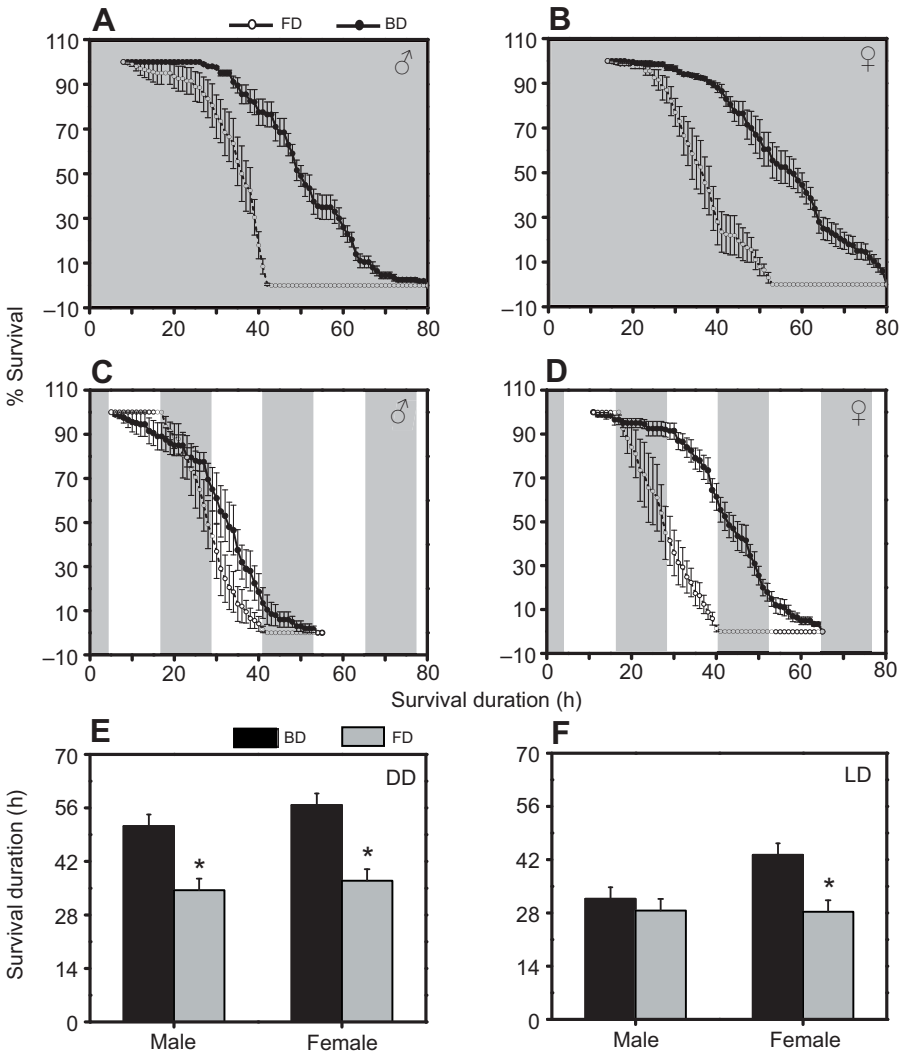

Fig. 2. Starvation resistance of faster developing (FD) flies. Survivorship curves of virgin males and females from selected (FD) and control (BD) stocks during starved condition under (A,B) constant darkness (DD) and (C,D) light/dark (LD) cycles. Mean survivorship of virgin males and females under starved condition in (E) DD and (F) LD. All other details are the same as in Fig. 1.

from both the stocks had lower starvation resistance under LD compared with DD. Females had significantly higher starvation resistance compared with males (Fig. 2E,F). Under both regimes, starvation resistance of FD flies was lower than that of BD controls, suggesting that selection for faster pre-adult development results in reduced resistance to starvation.

Lower starvation and desiccation resistance in faster developing flies

FD flies had lower starvation and desiccation resistance compared with BD controls (Fig. 3A–D). ANOVA revealed a statistically significant effect of light regime, stock, sex and the light regime × stock and stock × sex interactions; however, the effects of the light × stock and light regime × stock × sex interactions were not statistically significant (Table 2). *Post hoc* multiple comparisons using Tukey's test revealed that flies from both the stocks had higher resistance to starvation and desiccation under DD compared with LD (Fig. 3E,F). Under both conditions, starvation and desiccation resistance of FD females was significantly lower compared with BD controls; however, that of males did not differ. Moreover, as expected, starvation and desiccation resistance of females was significantly higher than that of males (Fig. 3E,F). This suggests that exposure to LD adversely affects the starvation and desiccation resistance of dark-reared flies, and selection for faster pre-adult development reduces resistance to starvation and desiccation.

Shorter lifespan in faster developing flies

Adult lifespan of FD flies was shorter by ~10 days under DD and by ~9 days under LD than that of BD controls (Fig. 4A–C). ANOVA revealed a statistically significant effect of light regime and stock; however, the effect of the light regime × stock interaction was not statistically significant (Table 3). *Post hoc* multiple comparisons using Tukey's test revealed that the mean lifespan of flies from both the stocks was shorter under LD compared with DD, and in a given light regime, lifespan of FD flies was significantly shorter compared with BD controls (Fig. 4A–C). This suggests that exposure to LD adversely affects the lifespan of dark-reared flies, and selection for faster pre-adult development yields flies with reduced adult lifespan.

To re-confirm our results on lifespan, we repeated this assay once again after 10 generations; however, this time lifespan was assayed only under DD. ANOVA revealed that the adult lifespan of FD flies was significantly shorter than that of BD controls (Fig. 4D,E, Table 3). These results thus suggest that selection for faster pre-adult development results in flies with reduced mean adult lifespan.

DISCUSSION

The results of our study revealed that selection for faster pre-adult development in *D. melanogaster* yields flies with compromised adult fitness, albeit at no cost to their pre-adult survivorship (Fig. 1A). Although FD flies had lower starvation and desiccation

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Table 2. ANOVA details of starvation resistance and starvation and desiccation resistance assays under DD and LD

Assay	Effect	d.f.	MS effect	d.f. error	MS error	*F*	*P*
Starvation resistance	Light regime (L)	1	1133.59	3	12.08	93.81	0.002
	Stock (P)	1	1497.96	3	23.95	62.54	0.004
	Sex (S)	1	185.76	3	3.75	49.55	0.006
	Block (B)	3	39.94	0	0	–	–
	L × P	1	170.66	3	0.58	294.85	0.0004
	L × S	1	5.53	3	9.36	0.59	0.50
	P × S	1	110.63	3	29.56	3.74	0.15
	L × B	3	12.08	0	0	–	–
	P × B	3	23.95	0	0	–	–
	S × B	3	3.75	0	0	–	–
	L × P × S	1	38.41	3	5.54	6.93	0.08
	L × P × B	3	0.58	0	0	–	–
	L × S × B	3	9.36	0	0	–	–
	P × S × B	3	29.56	0	0	–	–
	L × P × S × B	3	5.54	0	0	–	–
Starvation and desiccation resistance	Light regime (L)	1	761.64	3	3.84	198.51	0.0008
	Stock (P)	1	246.21	3	19.41	12.68	0.038
	Sex (S)	1	381.97	3	1.17	327.29	0.0004
	Block (B)	3	37.49	0	0	–	–
	L × P	1	33.63	3	2.47	13.61	0.03
	L × S	1	0.59	3	4.69	0.13	0.75
	P × S	1	199.42	3	1.65	121.01	0.002
	L × B	3	3.84	0	0	–	–
	P × B	3	19.41	0	0	–	–
	S × B	3	1.17	0	0	–	–
	L × P × S	1	2.56	3	0.59	4.36	0.13
	L × P × B	3	2.47	0	0	–	–
	L × S × B	3	4.69	0	0	–	–
	P × S × B	3	1.65	0	0	–	–
	L × P × S × B	3	0.59	0	0	–	–

resistance, reduced body weight/size, lower fecundity and adult lifespan, their fecundity per unit body weight was significantly higher, suggesting that reproductive fitness of FD flies is higher than that of the controls.

Pre-adult survivorship

In previous studies carried out on the same set of *D. melanogaster* populations, selection for faster pre-adult development was reported to cause a significant reduction in pre-adult survivorship (Chippindale et al., 1997; Prasad et al., 2000). However, our study revealed that pre-adult survivorship of FD flies remains unaffected, suggesting that selection for faster pre-adult development is not always accompanied by a cost to pre-adult survivorship. Our results are in line with those of Zwaan et al. (Zwaan et al., 1995), who also reported no difference in pre-adult survivorship between FD and control flies. Such differences in the outcome of apparently similar studies could be due to the additional selection pressure imposed in terms of early or very early reproduction (Chippindale et al., 1997; Prasad et al., 2000) and/or the fact that previous studies were carried out under arrhythmicity-inducing LL conditions, which is known to speed-up pre-adult development in insects (Paranjpe et al., 2005; Lone and Sharma, 2008).

Body weight and body size

In *D. melanogaster*, greater body weight/size is associated with higher adult fitness (Chippindale et al., 1996; Harshman et al., 1999). Studies have shown that body size is correlated with desiccation resistance (Rose, 1984), reproductive success (Zwaan et al., 1995) and fecundity (Robertson, 1957). Previous studies on selection for faster pre-adult development revealed a correlated reduction in adult body size and/or body weight (Chippindale et al., 2004). Consistently, in the present study, body weight/size at emergence of the FD flies was smaller compared with that of controls, suggesting a trade-off between pre-adult development time and adult body size/weight (Fig. 1B,C).

Starvation and desiccation resistance

Starvation resistance is often used as a marker of adult fitness in insects, and is known to co-vary with adult lifespan in laboratory populations of *D. melanogaster* (Service et al., 1988; Zwaan et al., 1991; Graves et al., 1992) and in natural populations of the butterfly *B. anynana* (Pijpe et al., 2008). In *D. melanogaster*, laboratory selection for extended lifespan is reported to result in increased resistance to starvation and desiccation (Service et al., 1985; Service et al., 1988; Graves et al., 1992; Chippindale et al., 1994), while flies subjected to selection for increased resistance to starvation and desiccation evolve higher level of metabolic reserves, such as lipids (Chippindale et al., 1996; Djawdan et al., 1997; Djawdan et al., 1998), carbohydrates (Graves et al., 1992; Chippindale et al., 1998), water content, dry weight and increased pre-adult development time (Chippindale et al., 1996; Chippindale et al., 1998). Previous studies have also reported an association between starvation resistance and energy reserves, particularly carbohydrate metabolic reserves (Djawdan et al., 1997; Djawdan et al., 1998). The results of the present study revealed that FD flies have reduced starvation and/or desiccation resistance compared with controls (Figs 2, 3). Additionally, we observed that starvation resistance of flies was reduced under LD compared with DD, suggesting a deleterious effect of the LD condition (Fig. 2). Such a light regime mediated differences in resistance to desiccation, and starvation is likely to be due to the fact that our flies were reared under DD, and exposure to non-native light regime might have caused adverse effect on stress

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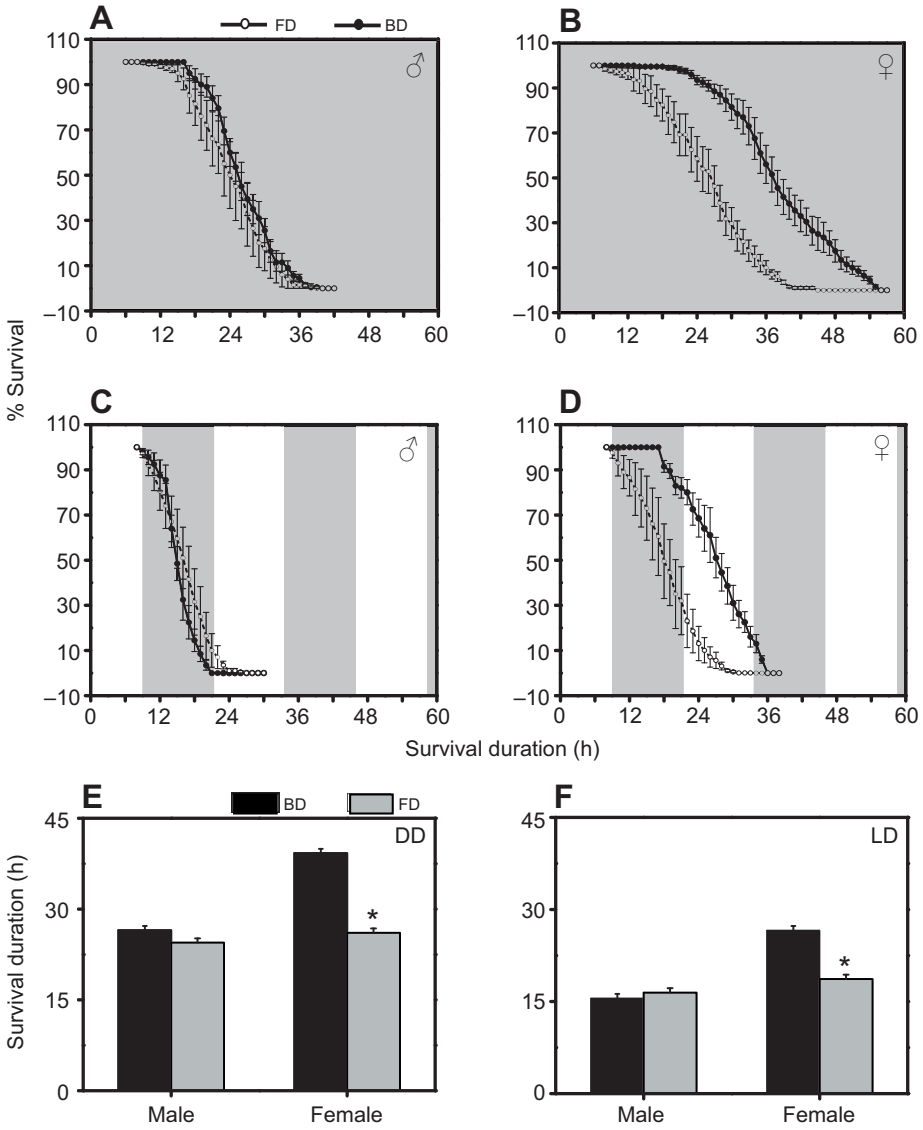

Fig. 3. Starvation and desiccation resistance of faster developing (FD) flies. Survivorship curves of virgin males and females from selected (FD) and control (BD) stocks during starvation and desiccation conditions under (A,B) constant darkness (DD) and (C,D) light/dark (LD) cycles. Mean survivorship of virgin males and females under starvation and desiccation conditions in (E) DD and (F) LD. All other details are the same as in Fig. 1.

resistance. Interestingly, starvation and desiccation resistance of males did not show such a light regime-mediated effect (Fig. 3).

Adult lifespan

In insects, adult lifespan is significantly reduced because of deleterious effects of light, including that of LL or non-24 h LD cycles (Pittendrigh and Minis, 1972; von Saint-Paul and Aschoff, 1978), while DD is known to stimulate the defense system and enhance adult lifespan (Shostal and Moskalev, 2013). Similarly, a few other studies have reported higher adult lifespan in flies maintained under DD compared with those kept in LD or LL (Allemand et al., 1973; Sheeba et al., 2000). Thus, we expected enhanced adult lifespan in flies maintained under DD compared to those kept in LD. Indeed, we did observe a small but significantly higher adult lifespan in flies maintained under DD compared with those kept in LD (Fig. 4A–C). Shortened lifespan in FD flies is largely selection-mediated and clock-independent because their clocks would run with the same pace under LD, and therefore clock-mediated differences will disappear under this condition. However, adult lifespan of the FD flies was found to reduce considerably by the 75th generation without a concurrent shortening of clock period (Yadav and Sharma, 2013b).

Reproductive output

In *D. melanogaster*, egg output increases during the first 2–4 days post emergence and remains high for the next 20 days, and thereafter declines gradually (Rose, 1984; Novoseltsev et al., 2002). Our populations, which were maintained on a discrete generation cycle, where egg collection occurred on day 11, showed higher fecundity on day 11 compared with day 4 (Fig. 1D). In addition, we observed that fecundity of the FD females was lower compared with that of controls (Fig. 1D), implying a trade-off between development time and reproductive output, quite unlike the increased fecundity in FD populations of melon fly, *B. cucurbitae* (Miyatake, 1997b). There is also a reasonable body of evidence from studies in *D. melanogaster* to suggest that an increase in reproductive output comes at the cost of reduced adult lifespan (Partridge et al., 1987; Chippindale et al., 1993; Djawdan et al., 1996; Partridge et al., 1999; Sgrò and Partridge, 1999). However, in our study we found that both adult lifespan and fecundity were reduced in the FD flies. This prompted us to examine egg output per unit body weight in our flies. Interestingly, the analyses revealed that FD flies have higher fecundity per unit body weight, particularly during the mid and late-life stages, which suggests that reduction in their adult lifespan was due to disproportionate investment in reproduction (Fig. 1D).

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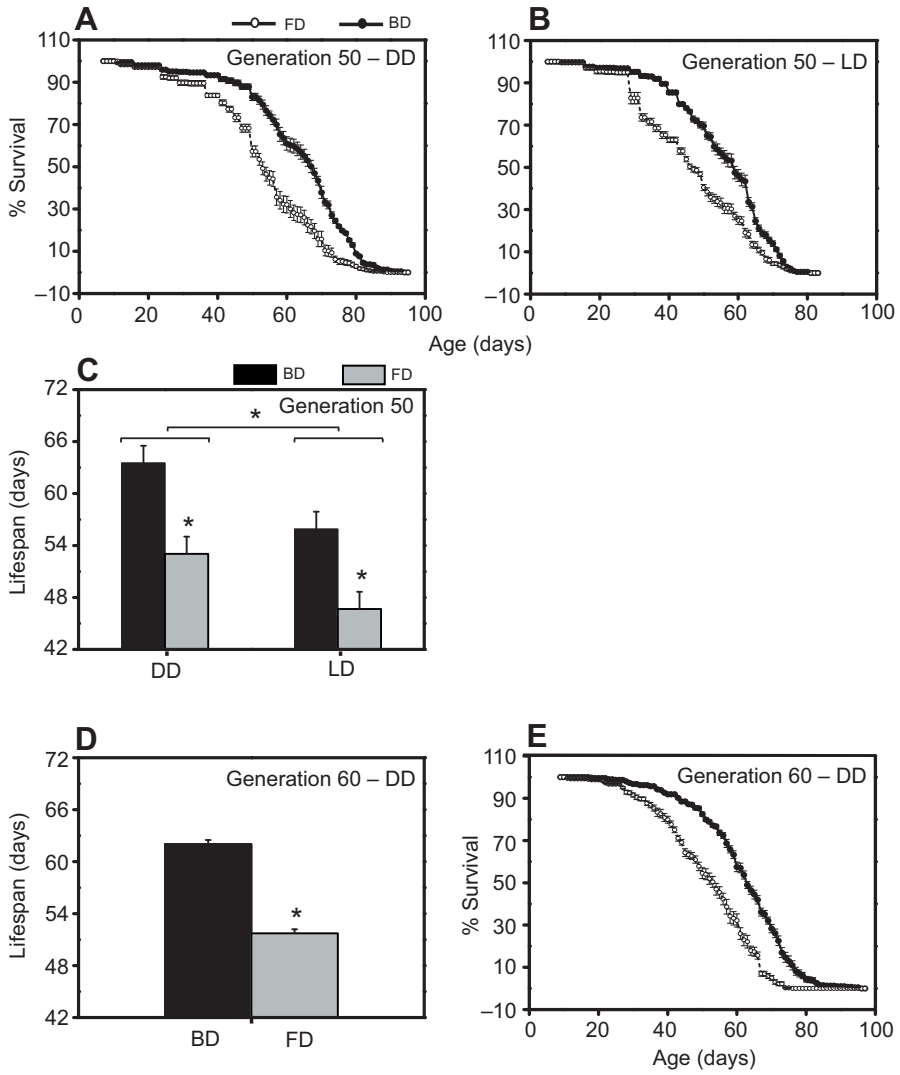

Fig. 4. Adult lifespan of faster developing (FD) flies. Survivorship curves under (A) constant darkness (DD) and (B) light/dark (LD) cycles of selected (FD) and control (BD) flies after 50 generations of selection, and (C) their mean adult lifespan. (D) Mean adult lifespan and (E) survivorship curves of flies after 60 generations of selection. All other details are the same as in Fig. 1.

Thus, our study reveals correlations of pre-adult development time with several key life history traits in wild-type populations of *D. melanogaster*, of which that with reproductive output is the most crucial. Additionally, we previously showed that the clock period of FD flies is significantly shorter than that of controls (Yadav and Sharma, 2013a). Taken together, these results suggest a possible role of circadian clocks in the regulation of life history traits in *D. melanogaster*. However, traits such as pre-adult development time and survivorship, adult lifespan and fecundity, and clock period are polygenic, and the underlying genes are likely to have pleiotropic effects. Therefore, it is quite possible that mutations altering circadian phenotypes might have also altered life history traits, resulting in correlation between circadian clocks and life history traits.

Conclusions

Although the results of our study appear to suggest that FD flies have lower adult fitness, their egg output per unit body weight is significantly higher than, and their pre-adult survivorship is comparable with, those of the controls. These results, along with

Table 3. ANOVA details of lifespan assays under different light regimes

Assay	Effect	d.f.	MS effect	d.f. error	MS error	*F*	*P*
DD and LD, generation 50	Light regime (L)	1	195.66	3	3.12	62.82	0.004
	Stock (S)	1	389.08	3	12.43	31.31	0.01
	Block (B)	3	6.08	0	0	–	–
	L × S	1	1.57	3	12.79	0.123	0.75
	L × B	3	3.12	0	0	–	–
	S × B	3	12.43	0	0	–	–
	L × S × B	3	12.79	0	0	–	–
DD, generation 60	Stock (S)	1	212.42	3	11.95	17.78	0.02
	Block (B)	3	2.45	0	0	–	–
	S × B	3	11.95	0	0	–	

those reported previously on circadian clocks, can be taken to suggest that selection for faster pre-adult development results in a correlated increase in reproductive output and shortening of circadian clock period, implying a connection between pre-adult development time, circadian timing systems and adult life history traits in *D. melanogaster*.

MATERIALS AND METHODS

Experimental populations

This study was performed on four large outbred populations (N≈1200 breeding adults, roughly equal number of males and females) each of the baseline developing (BD) and faster developing (FD) stocks of *D. melanogaster*, maintained under DD at constant temperature (25±0.5°C; mean ± s.d.) and humidity (75±5%). While origin and maintenance of these populations are described in detail elsewhere (Yadav and Sharma, 2013a), briefly, four baseline populations ($BD_{1–4}$) were maintained under DD for approximately 100 generations prior to the initiation of four FD populations ($FD_{1–4}$). For example, the FD_1 population was derived from the BD_1 population. Thus, each FD population was derived from its corresponding BD population, and therefore, FD and BD populations bearing identical numerical subscripts are more closely related to each other than to the populations with which they share a selection regime. Temperature and relative humidity were monitored continuously using a Quartz Precision Thermo-Hygrograph (Isuzu Seisakusho, Tokyo, Japan), and were found to be constant throughout the study.

All replicate populations were maintained in plexiglass cages (25×20×15 cm^3) and supplemented with banana-jaggery food medium (henceforth referred to as banana medium) at moderate larval and adult densities, on a 21 day discrete generation cycle. To start a new generation, adult flies were provided with banana medium supplemented with live yeast paste for 2 days prior to the egg collection. Twelve hours before egg collection, the yeasted food plate was replaced by a fresh food plate. From this plate, 60–80 eggs were dispensed into glass vials (9 cm height×2.4 cm diameter). All adults emerging from 24 such vials were transferred on the 12th day after egg collection into a plexiglass cage. The stock maintenance protocol for the FD populations was similar to that of BD, except that the number of vials used for each population was 80 (because only the first 25%, i.e. 12–15 flies, were collected from each vial) and the FD flies were directly transferred to breeding cages on the day of emergence, whereas the control flies were transferred into breeding cages only after all the flies had emerged. To start the next generation, eggs were collected after 21 days from the previous egg collection date.

Light treatments and light source

All our assays were performed under conditions of constant temperature (25±0.5°C; mean ± s.d.) and humidity (75±5%) inside controlled cubicles either in 12 h:12 h LD or DD regimes. The light phase of the LD cycles (lights on at 08:00 h and lights off at 20:00 h) was created using fluorescent white light (12 W Liliput Spiral B-22, SAMSON Lighting Pvt., Chennai, India) of intensity ~100 lx (1.1 W m^{-2}), while red light of λ>650 nm was used during the dark phase of 12 h:12 h LD and DD. The white light source largely consisted of three wavelengths (436, 546 and 612 nm). Light intensity was measured using a LI-COR lightmeter L250 (Lincoln, NE, USA).

Stock standardization

To eliminate possible non-genetic effects of parental rearing conditions, before starting every assay, all populations were subjected to a common rearing condition for one generation (standardization). For this, from the running cultures of $FD_{1–4}$ and $BD_{1–4}$ populations, eggs laid on banana medium over a 12 h window were collected into glass vials. From each population, approximately 50–60 eggs were collected and transferred into each of the 24 glass vials. These vials were kept under DD until all adult flies emerged. After 12 days of egg collection, adults were transferred into plexiglass cages. Such caged populations will be referred as ‘standardized populations’ from which eggs laid on banana medium over 2 or 12 h window were used for various fitness assays.

Fitness assays

Pre-adult survivorship assay

Pre-adult survivorship was assayed at regular intervals of five generations under DD. Flies from the standardized populations of $FD_{1–4}$ and $BD_{1–4}$ stocks were allowed to lay eggs for 2 h (08:00 to 10:00 h) on banana medium, of which exactly 30 eggs were dispensed into each glass vial containing ~10 ml banana medium. Eggs were collected under DD illuminated by red light (λ>650 nm), and 10 glass vials for each replicate population were used, which remained in DD for the duration of the experiment. Thus, a total of 80 vials were used in this assay (10 vials×4 replicates×2 stocks). Pre-adult survivorship (proportion) was estimated by dividing the total number of adults emerging from a vial by the total number of eggs dispensed into that vial.

Adult lifespan assay

Adult lifespan assays were performed twice – at the 50th and 60th generations. From the running culture of each standardized population, 60–80 eggs were collected and transferred into vials with 6 ml banana medium. After 7–8 days, freshly emerged virgin males were collected every 5–6 h throughout the day, anesthetized with CO_2 and separated under red light before being transferred into DD or 12 h:12 h LD. Ten virgin males were placed in each vial containing ~6 ml of corn food, and 10 such vials were used for each population. Thus 40 vials (4 blocks×10 vials) of FD and 40 of BD stocks were used for this assay under each of the light regimes (LD and DD). The vials were checked every day for deaths, and flies were transferred into fresh food vials every third day.

Fecundity assay

The experimental setup was similar to that used in the adult lifespan assay, except that fecundity was assayed only in the DD condition. However, instead of 10 virgin males, one male–female pair was placed into a glass vial with 1 ml unyeasted banana medium, which was replaced every 12 h by a fresh vial containing banana medium. The number of eggs laid during early-life (3, 4 and 5 days), mid-life (10, 11 and 12 days) and late-life (20, 21 and 22 days) stages was counted every 12 h. The average number of eggs laid by a female at ages 3, 4 and 5 was considered as the reproductive output of the fly at age 4 days. Similarly, the average number of eggs laid at ages 10, 11, 12 and 20, 21 and 22 days was considered as the reproductive output of the fly at ages 11 and 21 days, respectively.

Dry weight assay

Eggs were collected from standardized populations at a density of 30 eggs per vial and transferred into DD. Flies emerging during the emergence peak, which spanned ~6 h, were collected at 1 h intervals. Virgin males and females were separated using CO_2, killed by freezing, dried for 36 h at 70°C and finally weighed in groups of 10 males or 10 females. Five groups of dried flies were chosen randomly from each of the FD and BD populations and weighed.

Body size/length assay

Collection of eggs, adult fly separation and experimental setup were similar to those in the dry weight assay except that flies were not frozen or dried. Briefly, head to abdomen length was measured for 30 males and 30 females per population. Body length of anesthetized flies was measured using a microscope with a measuring scale (least count 0.1 mm) from Yucon Instrument Co. (Guangxi, China).

Starvation resistance assay

The experimental setup was similar to that previously described for the adult lifespan assay. The only difference here was that 10 virgin males or 10 virgin females were placed in a vial without any food, but were provided with 3 ml of agar (1.2%) to prevent desiccation. Forty vials (4 blocks×10 vials) of FD and 40 of BD were used under each of the light regimes (LD and DD). These vials were checked every 1 h for deaths.

Starvation and desiccation resistance assay

The experimental setup was similar to that described previously for the starvation resistance assay. The only difference was that flies were placed in completely dried vials, without any food medium, agar or desiccating agent.

Statistical analyses

For the adult lifespan assay, mean lifespan (in days) was used as data for a mixed model ANOVA in which replicate populations (block) were treated as a random factor, and stock (population) and light regime were treated as fixed factors crossed with blocks. In all cases, the block average of replicate populations was used as the unit of analysis and hence only the fixed factors could be tested for significance. *Post hoc* multiple comparisons were carried out using Tukey's honestly significant difference (HSD) test. Data from starvation and starvation and desiccation resistance assays were analyzed similarly. For the analysis of reproductive output, mean egg output data were used in a two-way mixed model ANOVA with stock and age as fixed factors and block as a random factor. All statistical analyses were implemented in STATISTICA for Windows Release 5.0 B (StatSoft, Tulsa, OK, USA).

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Competing interests

The authors declare no competing financial interests.

Author contributions

P.Y. and V.K.S. conceived and designed the experiments. P.Y. performed the experiments. P.Y. and V.K.S. performed the analyses, wrote the manuscript and approved the final version.

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