Evolution of Life-history Traits, Canalization and Reproductive Isolation in Laboratory Populations of *Drosophila melanogaster* Selected for Faster Pre-adult Development and Early Reproduction

A Thesis

Submitted for the Degree of

Doctor of Philosophy

By

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Dedicated To

My Family, Friends

& Rahul

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DECLARATION

I hereby declare that the work embodied in my thesis entitled "**EVOLUTION OF LIFE-HISTORY TRAITS, CANALIZATION AND REPRODUCTIVE ISOLATION IN LABORATORY POPULATIONS OF *DROSOPHILA MELANOGASTER* SELECTED FOR FASTER PRE-ADULT DEVELOPMENT AND EARLY REPRODUCTION**" has been carried out by me at Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India, under the supervision of Prof. Amitabh Joshi, and that this work has not been submitted elsewhere for any degree or diploma.

In keeping with the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described has been based on the findings of other investigators. Any omission, which might have occurred by oversight or error of misjudgment, is regretted.

S Ghosh

Shampa Ghosh Modak

Place: Bangalore

Date: February 16, 2009

Evolutionary & Organismal Biology Unit

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16 February, 2009

CERTIFICATE

This is to certify that the work described in the thesis entitled "**EVOLUTION OF LIFE-HISTORY TRAITS, CANALIZATION AND REPRODUCTIVE ISOLATION IN LABORATORY POPULATIONS OF *DROSOPHILA MELANOGASTER* SELECTED FOR FASTER PRE-ADULT DEVELOPMENT AND EARLY REPRODUCTION**" is the result of investigations carried out by Ms. Shampa Ghosh Modak in the Evolutionary and Organismal Biology Unit of the Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560 064, under my supervision, and that the results presented in the thesis have not previously formed the basis for the award of any other diploma, degree or fellowship.

A Joshi

Amitabh Joshi, Ph.D.

Professor

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Ghosh Modak, S., Satish, K. M., Mohan, J. and Joshi, A. Evolution of canalized development time in laboratory populations of *Drosophila melanogaster* selected for rapid development.

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Summary

The life-history of an organism refers to the probability of survival and reproductive output at different life-stages and is, thus, intimately related to the realization of an individual's Darwinian fitness. Any phenotype must necessarily impinge upon the life-history if it is to have an adaptive significance. Hence, an understanding of the genetic architecture of fitness-related traits — how different phenotypes are genetically correlated with one another, and with components of the life-history — is fundamental to the understanding of adaptive evolution. Experimental evolution approaches using long-term studies of the evolutionary responses of *Drosophila melanogaster* populations to specific selection pressures in the laboratory have been an important means of studying the genetic architecture of fitness-related traits and life-history evolution over the past two decades or so.

In holometabolous insects like *Drosophila*, adult fitness is greatly dependent on the extent of resource acquisition and assimilation during the larval stage. Consequently, both pre-adult survival and the duration of larval development are important to overall fitness. In this thesis, I present results from an ongoing study of the evolutionary consequences of long-term selection in our laboratory for reduced pre-adult duration in *D. melanogaster.* Four populations (FEJs) were selected for faster pre-adult development and early reproduction (day 3 post-eclosion) for close to 400 generations, leading to a 25% reduction in pre-adult development time compared to the four ancestral control populations (JBs). A previous thesis from this laboratory detailed the evolution of the life-history of the FEJs over the first 200 generations of selection, and also the correlated evolution of various larval and adult traits related to resource acquisition and utilization. In an extension of this line of investigation, I studied various further changes in the life-history of the

FEJs between 200 and 300 generations of selection for faster development and also conducted crosses to examine the genetic control of development time and associated traits. In addition, I also obtained preliminary evidence of a tradeoff between developmental rate and pathogen resistance in these populations. In a major expansion of the scope of evolutionary questions addressed with these populations, I found evidence that long-term directional selection for rapid development has led to the evolution of (a) greater canalization of development time along two environmental axes, and (b) incipient reproductive isolation between selected and control populations. I also initiated a similar selection study with populations of *D. ananassae*, to ask whether the pattern of genetic correlations between development time and traits relevant to larval competitive ability in this species is similar to that seen in *D. melanogaster*. The results indicate that the genetic architecture of these traits is quite different in these two phylogenetically and ecologically similar species, thus recommending caution when extrapolating results from selection studies on *D. melanogaster* to other species with a similar ecology.

Studies of the FEJs had earlier revealed a peculiar pattern of evolution first toward but then later away from the optimal life-history of rapid development coupled with relatively high fecundity around day 3 post-eclosion, even if the latter meant reduced subsequent fecundity and lifespan. At generation 30, FEJs seemed to be evolving towards the predicted optimal life-history: they developed faster and had a shorter lifespan than JBs, but produced more eggs per unit dry weight in early life. However, at generation 70, FEJ and JB lifespan was the same, and FEJ fecundity per unit dry weight early in life was significantly lower than the JBs. It was hypothesized that this counter-intuitive evolution away from the optimal life-history was due to the FEJs having undergone a reduction in size and lipid content large enough to trigger a physiological switch inducing greater resource allocation for somatic maintenance as opposed to reproduction. *D. melanogaster* is known to undergo nutritional-status-dependent allocation switching, thought to be adaptive under fluctuating food availability in the wild. The FEJs also showed a greater starvation resistance per unit lipid than the JBs, an observation consistent with the 'physiological switch' hypothesis since lipid mobilized to the ovaries for reproduction is unavailable for resisting starvation in *D. melanogaster*. The conclusion, thus, was that this past adaptation had become a constraint that the FEJs had not been able to overcome over the first 70 generations of selection. After 200 further generations, I found that ongoing selection had led to the FEJs circumventing the maladaptive constraint of the 'physiological switch'. FEJ lifespan at generation 270 was lower than the JBs and, despite undergoing further reduction in both total and fractional lipid levels, FEJs produced a significantly higher number of eggs per unit lipid than the JBs. In addition to producing more eggs per unit lipid, FEJ populations also showed a 70% higher starvation resistance per unit lipid compared to JBs. Thus, continued FEJ selection seems to have ultimately resulted in the evolution of greater efficiency of lipid usage in these populations. Alternatively, the FEJs may have evolved a reduced level of egg provisioning than the JBs as a means of reconciling the need for high early life fecundity and very low lipid levels at eclosion.

Another aspect of somatic maintenance, the pathogen resistance of adult flies was measured by assaying the time to death of FEJs and JBs in presence/absence of *E. coli* cultures on LB agar medium. *E. coli* induced a significant reduction in lifespan of both FEJs and JBs, but the percent reduction in lifespan due to presence of *E. coli* was significantly higher in FEJs, suggesting a possible tradeoff between developmental rate and pathogen resistance in *D. melanogaster*. It is very likely that this tradeoff is mediated by reduced energy reserves in the FEJs.

Canalization helps to maintain phenotypic constancy of a trait, buffering it against the effects of genetic and environmental noise. It is believed that this helps express phenotypes favoured in the most commonly experienced environment and suppresses deviations from the phenotypic optima. Thus, traits closely related to fitness have been predicted to get canalized in course of selection. In FEJs, the nature of the selection has imposed an extraordinary fitness premium on rapid development, which is not the case for the JBs. After 300 generations of selection, I studied the development time of the FEJs and JBs at nine different combinations of temperature and density (18°C, 25°C and 28°C crossed with 30, 70 and 300 eggs per vial). The objective of this experiment was to investigate whether or not development time has evolved to become more canalized across environments in FEJs compared to the JBs. The FEJs developed faster than JBs in all nine environments and had a smaller coefficient of variation (CV) of development time compared to the JBs. The changes in mean and CV of the trait across density were significantly less in FEJs compared to JBs. Along the temperature axis, mean trait value changed in a similar manner for both types of populations, but the FEJ CV remained more consistent across temperature than that of the JBs. This suggested a reduced sensitivity of FEJ development time to changes in both rearing temperature and density, reflecting environmental canalization and providing empirical support for the notion that long-term directional selection for a life-history trait can lead to its canalization.

The role of divergent ecological selection in speciation is well known, with the principal mode of action thought to be adaptation to different environments. The effect of divergent life-history evolution on reproductive isolation has not been explored thus far. I found that two complementary, asymmetric isolating mechanisms have mediated the evolution of incipient reproductive isolation between the FEJs and JBs. The large size difference between the FEJ and JB flies evolved in response to about 400 generations of selection for faster development appears to play a crucial role in the isolation. Small FEJ males obtain few matings with large JB females, irrespective of the presence or absence of JB males, giving rise to unidirectional, pre-mating isolation mediated by sexual selection. Conversely, small FEJ females suffer increased mortality following mating with large JB males, resulting in viability-selection-driven, post-mating isolation in the opposite direction. The isolation is likely due to reduced size and activity levels in the selected populations, showing that early stages of ecological speciation can be a by-product of differential life-history evolution, even in the absence of major differences in habitat or resource use between populations. The results also show a complex interaction of sexual and natural selection, underscoring the need for detailed studies of the functional links between traits responding to divergent selection and the actual mechanisms of reproductive isolation.

Identifying genetic correlations among fitness-related traits by examining correlated responses to selection has been an important component in our understanding of life-history evolution in *Drosophila*. However, most such studies have been done on a single species, *D. melanogaster*, and there is little information about how conserved these correlations are across congeners. In view of this, I subjected four laboratory populations of *D. ananassae* to selection for faster development and early reproduction and studied the selection responses for 25 generations. Similar to *D. melanogaster*, developmental rate traded off with adult body size in *D. ananassae*. Faster development also resulted in a pre-adult survivorship cost in both the species, but the tradeoff seemed to be much stronger in case of *D. ananassae*. However, the tradeoffs between developmental rate and larval feeding rate, and between developmental rate and larval competitive ability observed in *D. melanogaster* were not found in *D. ananassae*. These results indicate that the genetic architecture of fitness-related traits is not conserved in these two species despite their being quite similar ecologically and phylogenetically. Hence, one should be cautious when extrapolating results obtained from *D. melanogaster* even to fairly similar congeners.