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Genetic analysis of body color polymorphism in *Drosophila melanogaster* through selection experiment.

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Abstract

Drosophila melanogaster is a widespread species that exhibits enormous variation in abdominal melanisation throughout its range. To gain insight into this variation, present work involves selection for abdominal melanisation. In 38 generations of selection for melanisation, an increase of ~2.5 fold in dark selected strain and a decrease of ~6-7 folds in light strain was observed in both the sexes as compared to control populations of *D. melanogaster*. Genetic crosses between dark and light strains obtained through selection produced intermediate offspring, but a clear maternal effect differentiated the reciprocal F_1's. F_1 flies showed higher plastic effect as compared to selected dark and light strains across various growth temperatures. Our results are novel in the occurrence of five body color phenotypes in ratio of 1:4:6:4:1 (two-gene) in both sexes of *D. melanogaster*.

Keywords: Abdominal melanisation, dark and light selected strains, *Drosophila melanogaster,* selection experiment.

Introduction

Body melanisation exhibits a large amount of variability in Drosophilids, resulting either from genetic polymorphism or phenotypic plasticity (Gibert *et al.,* 1998; Rajpurohit *et al.,* 2008; Parkash *et al.,* 2011). The color polymorphism in abdominal tergites was reported by da Cunha (1949) for *D. polymorpha.* In this species, both males and females show any one of the three types of abdominal tergite coloration: dark, intermediate or light. In four species of montium subgroup, discrete polymorphism for 6^{th} and 7^{th} abdominal segments occurs only in female individuals, but the dominance level of darker and lighter phenotypes vary between species (Ohnishi and Watanabe, 1985). Recent studies in montium species *D. jambulina* and *D. punjabiensis* have shown genetic polymorphism for body color morphs (Parkash *et al.,* 2009; Singh, 2011). *D. melanogaster* is known for its sexual dimorphism. Males possess a black abdomen (tergites 5 and 6) while females exhibit yellow tergites with black stripe at their posterior margin.

Several studies on melanisation include analyses of phenotypic plasticity (David *et al.,* 1990; Gibert *et al.,* 1998, 2000; Parkash *et al.,* 2011), evolutionary developmental basis of intra- and interspecific differences (Kopp *et al.,* 2000; Wittkopp *et al.,* 2002a, 2002b), a phylogenetic and

speciation context of trait evolution (Hollocher *et al.*, 2000a,b), and traditional quantitative trait loci approaches (Llopart *et al.*, 2002; Kopp *et al.*, 2003; Wittkopp *et al.*, 2003).

Abdominal melanisation has been extensively studied in *D. melanogaster* (Wittkopp *et al.*, 2003). Present work involves selection for melanisation in cosmopolitan *D. melanogaster* for the first time. The selected dark and light strains help in carrying out genetic crosses and thereby, report five body color phenotypes in ratio 1:4:6:4:1 for both sexes in *D. melanogaster*. Crosses between dark and light strains show clear maternal effect between reciprocal F_1's.

Materials and Methods

Selection for melanisation: Preparation of homozygous dark and light strains

The aim of selection experiment was to determine the extent to which the phenotype for melanisation might change as a result of selection. The flies used to start the selection experiment were collected in September-October months of 2007 from a highland locality Shimla. Thirty isofemale lines were initiated from flies collected with net sweeping and bait traps from fruit markets and godowns. The cultures were maintained at a constant growth temperature of 21°C in a biological oxygen demand incubator. The density was controlled by limited egg laying period (6-8 hours) on cornmeal-yeast-agar medium. An equal number of offspring from each isofemale line were pooled to produce a population of at least 3000 individuals in order to generate maximum variability. A mass population of this size was maintained on laboratory medium for five generations and then subdivided into 9 lines, each with ~300 individuals. Three of the lines were maintained on laboratory medium serving as controls, and the remaining six lines were subjected to selection for melanisation, *i.e.*, extreme dark and light female flies were assorted. From the assorted flies, 60 dark (D) and light (L) flies were used for egg laying separately in 5 replicates each. They were labeled as Dark (D_1, D_2, D_3, D_4, and D_5) and Light (L_1, L_2, L_3, L_4, and L_5) lines. Thereafter, every generation extreme dark and light flies were assorted out and other phenotypes obtained were discarded. For about 38 generations, this procedure was followed; thereafter, melanisation did not increase but selection was continued to stabilize the phenotype. Thus, very dark and very light flies obtained were used as parents to establish crosses (Figure 1).

Figure 1. Dark and light body color phenotypes obtained as a result of selection of melanisation for 38 generations in both sexes of *D. melanogaster*. These flies were further used as parents for carrying out genetic crosses.

Drosophila *strains and crosses*

Furthermore, in order to ascertain the genetic basis as well as allelic dominance, we carried out Mendelian crosses (F_1 and F_2 crosses) with these true breeding dark and light strains of *D. melanogaster.* We made 15 single pair matings using virgin 1 ♂ and 1 ♀ each of very dark and very light morph for obtaining F_1 progeny. For

investigating plastic effects of body melanisation, two replicates each of the above pair matings were then transferred to different growth temperatures (14, 17, 21, 25, and 28ºC). Six day-old flies from these different developmental temperatures were scored for melanisation. We randomly scored 100 ♀ as well as 100 ♂ flies (F_1); results differed greatly according to sex. Further, out of pooled F_1 progeny, 50 ♂ and 50 ♀ were randomly selected, and five replicates with 10 pairs each were used for F_2 progeny. F_2 progeny showed segregation for 5 types of body color phenotypes, *i.e*., very dark, dark, intermediate, light, and very light morphs.

Table 1. Data on female progeny scored for F_1 & F_2 genetic crosses between true breeding dark and light strains of *D. melanogaster*. Similar results were obtained for males (data not shown)

Genetic Crosses	Type/ Replicate	♀ flies scored x (n)	V. Light	Light	Intermediate	Dark	V. Dark
(A) Light ♀ * Dark ♂	F_1	200	0 (0 %)	0 (0 %)	200 (100%)	0 (0 %)	0 (0 %)
	F_2: 1.	300	20	69	119	70	22
	2.	270	19	57	124	53	17
$F_{1\,A}$ ♀ * $F_{1\,A}$ ♂	3.	340	22	78	130	85	25
	4.	315	25	80	110	77	23
	5.	294	27	66	103	73	25
(B) Dark ♀ * Light ♂	F_1	200	0 (0 %)	0 (0 %)	200 (100%)	0 (0 %)	0 (0 %)
	F_2: 1.	286	25	62	102	70	27
	2.	308	27	71	110	75	25
$F_{1\,B}$ ♀ * $F_{1\,B}$ ♂	3.	320	30	74	115	78	23
	4.	260	19	58	92	66	25
	5.	255	17	53	100	64	21

$F_{1\,A}$ = Light ♀ x Dark ♂; $F_{1\,B}$ = Dark ♀ x Light ♂

Results and Discussion

Melanisation is one of the most variable traits in the genus *Drosophila*: differences in body color are common among individuals within a population, among populations of the same species, and among closely related species (True, 2003; Wittkopp *et al.,* 2010). Present work involves selection for melanisation (38 generations) in *D. melanogaster* from a highland locality. The dark flies were ~96% and light flies ~4-5% melanic after 38 generations of selection (Figure 1). Table 1 shows results of genetic crosses between true breeding dark and light strains obtained after selection for melanisation in females of *D. melanogaster*. Similar results were obtained for males (data not shown). Crosses helped to analyze genetic basis as well as allelic dominance (if any) for body color polymorphism in this species (Table 1).

Figure 2 summarizes the five body color phenotypes obtained in F_2 generation, close to 2-gene ratio of 1:4:6:4:1 in both sexes of *D. melanogaster.* Mean ± SD for % body melanisation of F_1 flies obtained through genetic crosses (at 21°C) in *D. melanogaster* across five growth temperatures are given in Table 2. We observed significant differences across growth temperatures for F_1 reciprocal crosses. F_1 progeny obtained from genetic crosses with light female parents are less pigmented as compared to F_1 flies from dark female parent (Table 2). F_1 individuals from light ♀ × dark ♂ cross show higher plasticity than F_1 flies from dark ♀ × light ♂ cross. The selected strains were investigated over a range of growth temperatures. Flies of dark selected stains (although quite

dark) show slight decrease in melanisation at higher temperatures, whereas flies of light selected strain (very light) show some increase in melanisation at lower temperatures.

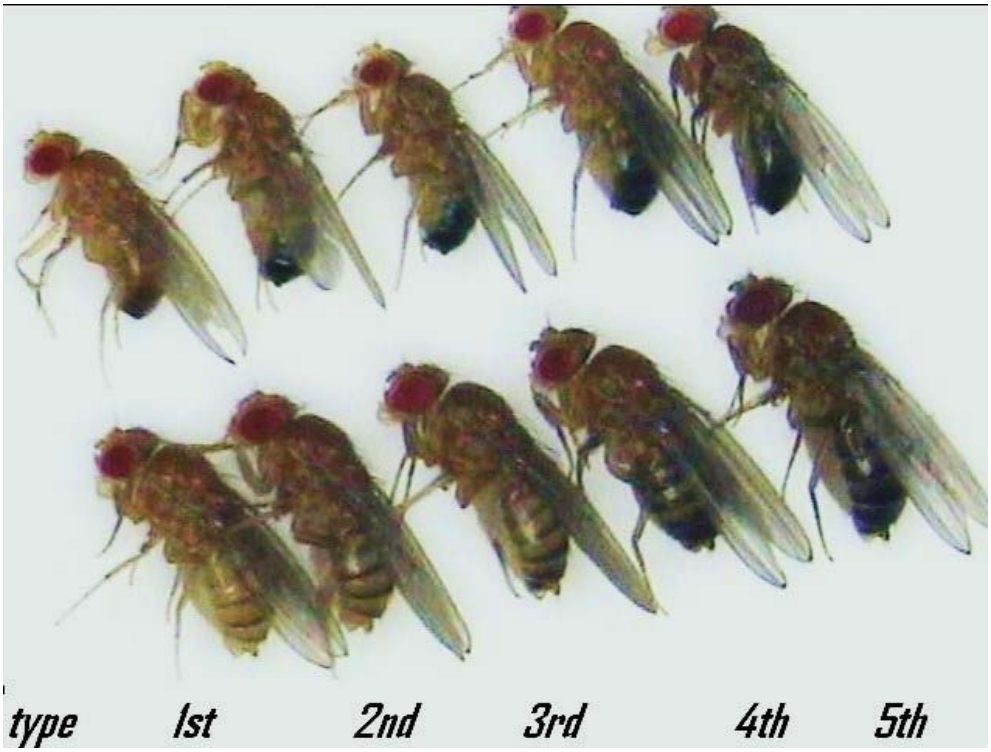

Figure 2. Five body color phenotypes (1st – very light; 2nd – light; 3rd – intermediate; 4th – dark and 5th – very dark) for F_2 crosses in both the sexes of *D. melanogaster*.

Table 2. Mean ± SD for % body melanisation of F_1 progeny obtained through *genetic crosses between true breeding dark and light strains of *D. melanogaster* (Dark ♀ x Light ♂; Light ♀ x Dark ♂) across five growth temperatures.

Temperature	Dark ♀ x Light ♂		Light ♀ x Dark ♂	
(°C)	♂	♀	♂	♀
14 (°C)	70.93 ± 5.22	84.50 ± 4.61	52.72 ± 2.68	68.10 ± 3.21
17 (°C)	56.70 ± 4.30	72.34 ± 3.82	49.20 ± 2.93	60.00 ± 2.56
21 (°C)	48.00 ± 4.71	59.60 ± 4.80	27.50 ± 4.22	41.32 ± 5.10
25 (°C)	36.20 ± 5.03	50.92 ± 5.65	19.00 ± 5.97	33.04 ± 4.27
28 (°C)	24.15 ± 3.17	31.00 ± 4.39	12.78 ± 3.96	14.00 ± 3.42
% Change	2.94	2.72	4.12	4.86

*Crosses were performed at 21 °C and thereafter eggs were transferred to different growth temperatures for obtaining F_1 flies.

The goal of the present study was to examine the striking variation in abdominal pigmentation displayed by cosmopolitan *Drosophila melanogaster*, to develop an understanding of why this trait is so variable in this species. Further investigation of present work will help *Drosophila* workers to

understand many more unknown queries about melanisation in cosmopolitan *Drosophila melanogaster.*

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Comparing selection schemes in BAC-recombineering method of tagging a novel *Drosophila* gene, *DmCSAS.*

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Abstract

Sialylation plays an important role in the *Drosophila* nervous system. However, the regulation of sialylation pathway remains poorly understood. We focused our analysis on a novel gene, CMP-Sialic acid synthetase (CSAS), that is predicted to be a key player of the pathway. In order to investigate its expression, we decided to introduce a tag sequence into the CSAS genomic locus within a BAC clone using recombineering strategy. We wanted to introduce the tag without any additional extraneous sequences in order to minimize the influence of the insert on the gene's function. We sought to modify existing recombineering protocols and test different selection and screening methods during recombineering. Our results confirmed the general utility of positive/negative selection approach using *rpsL*$^{+}$*-kana* marker. However, they also revealed the limitation of this strategy, as it did not allow unambiguously to identify recombinant clones, while resulting in enrichment rather than selection for desired recombineering events.