Effect of genetic variation on locomotion in laboratory stocks of *Drosophila melanogaster*.

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Abstract

Locomotory behavior was studied in five different stocks of *Drosophila melanogaster*. Out of these stocks, one was wild type and the remaining were mutants for different phenotypic traits. Individual flies of either sex were introduced in the locomotor chamber to observe its behavior and locomotory speed in one minute period of time. The observations of each group of flies with respect to their sex were also recorded. The results obtained indicate that there is significant variation in the locomotory speed of the flies belonging to different stocks (tested through one way ANOVA). Out of all these stocks, flies having curled wings showed maximum speed than other types, which may be due to their specific genetic quality. Further it was also observed that the two sexes show insignificant difference in their locomotory behavior, in all the stocks studied (as analyzed through student t- Test). The varying speed of locomotion observed in the stocks could be due to genetic variations.

Introduction

Drosophila is one of the useful animal models to study behavioral aspects. This insect can be cultured in laboratory conditions and can be employed in a number of behavior studies, both sexual and non sexual. Sexual behavior comprises courtship and mating behavior, and non sexual behavior includes feeding behavior, interactions with other members of the group as well as members of other groups, larval feeding, locomotion of adults and their larvae, pupation site preference, movements based on light source, effect of geotaxis, and so forth. Behavior genetic analysis is an approach to the study of organisms and their behavior that combines the concepts and methods of behavioral analysis from psychology and ethology (Sisodia and Singh, 2004).

The relationship between genes and behavior, however, is less straight-forward. Although genes can influence behavioral function at all these levels, they do not specify which behavior occurs where, when, and why. Behavioral phenotypes in *Drosophila* are associated with a large number of mutants belonging to more than 100 different genes. Many of them play a role during nervous system development and/or affect aspects of functioning of the nervous system, such as membrane excitability, synaptic transmission, or muscle contraction.

Several mutations have been isolated on the basis of aberrant locomotor activity. The "inactive" mutation was described by Kaplan (1977). O'Dell and Burnet (1988) reported that the locomotor activity is reduced in adult flies by the mutant genes inactive, inactive^2, hypoactive-C, and hypoactive E. The frequency of jumping is greatly reduced in all four mutants, and the threshold for the jumping response appears to be related to speed of locomotion. Differences in the expression of reactivity in lines selected for changes in locomotor activity have indicated that spontaneous activity and reactivity are at least partially under the control of different genes (Connolly, 1967; Van Dijken, 1982). Seven non-allelic hypoactive mutations, described by Honyk and Sheppard (1977) and Honyk

et al. (1980) were isolated using a screen for mutants of reduced flight abilities. Burnet *et al*. (1988) reported that the amounts and speed of locomotion are largely under independent genetic control. Diagana *et al*. (2002) created a mutation called *homer* and showed that flies homozygous for this mutation are viable and show coordinated locomotion, suggesting that *Homer* is not essential for basic neurotransmission. However, they also found that the *homer* mutant displays defects in behavioral plasticity and the control of locomotor activity. Mutations which have been found to cause abnormalities of the jumping response are *bendless* (Thomas, 1980; Thomas and Wyman, 1982), *jumpless* (Hall, 1982), and *non-jumper* (Thomas, 1980), which are associated with abnormalities affecting the giant nerve fiber. Vaj and Jayakar (1976) investigated the importance of autosomal genes in the determination of locomotor activity in *D. melanogaster* and found that chromosome 4 is most influential in controlling the locomotor activity. The pyokori behavior is genetically controlled by major genes(s) on the second chromosome. However, some minor genes affect the manifestation of the pyokori behavior (Nakashima-Tanaka and Matsukara, 1980).

In the present work, males and females of five different stocks have been utilized to study locomotory behavior and speed of locomotion. One wild type stock and four mutant stocks have been selected for this study. The purpose of this study is to see whether there is variation at the level of speed of locomotion in different mutant stocks of this species. We know that mutation at a single gene may lead to its effect on a number of other activities of the fly. Such pleiotropic effects of a gene can be envisaged in the present experiment as mutant types may show variations in their locomotor activity owing to differences in the loci carrying the mutations.

Materials and Methods

Locomotory behavior was observed in five different stocks of *Drosophila melanogaster*. These stocks have spent several generations in the lab. The details of these stocks are as follows.

Wild Type: Flies of this stock are normal for all the visible phenotypic traits, *i.e*., the flies have normal wings, normal eye color, normal body color, and they have spent several generations in the laboratory condition.

White Eye: These flies possess white eye color and the gene determining this trait is located on the X chromosome.

Sepia Eye: Flies of this trait possess brownish black eye color and the gene determining this trait is located on III chromosome.

Curled Wing: These flies are mutant for wing shape. Their wings are turned upward at the terminal region. They are unable to fly because of the abnormal shape of the wings. The gene determining this character is also located on III chromosome.

Black Body Color: These flies have darkly pigmented body color. The gene determining this character is located on II chromosome.

A square box with a lid was used for observing locomotion. For introducing and taking out flies, an aspirator was used. The box had 100 squares drawn on its roof, each with an area of 1 cm^2. A single fly was observed at a time. A fly was allowed to adapt in the new environment for 90 seconds. Locomotion was observed for the next 60 seconds. The number of squares (l cm^2 area each) treaded by a fly in that 60 seconds was counted and recorded. Speeds of locomotion of individual flies of both the sexes were also recorded from all the stocks.

Student t- Test was done to compare the speed of locomotion between the two sexes, in all the five stocks. One way ANOVA was applied to compare the mean speeds of locomotion among the flies (males and females taken together) of the five stocks.

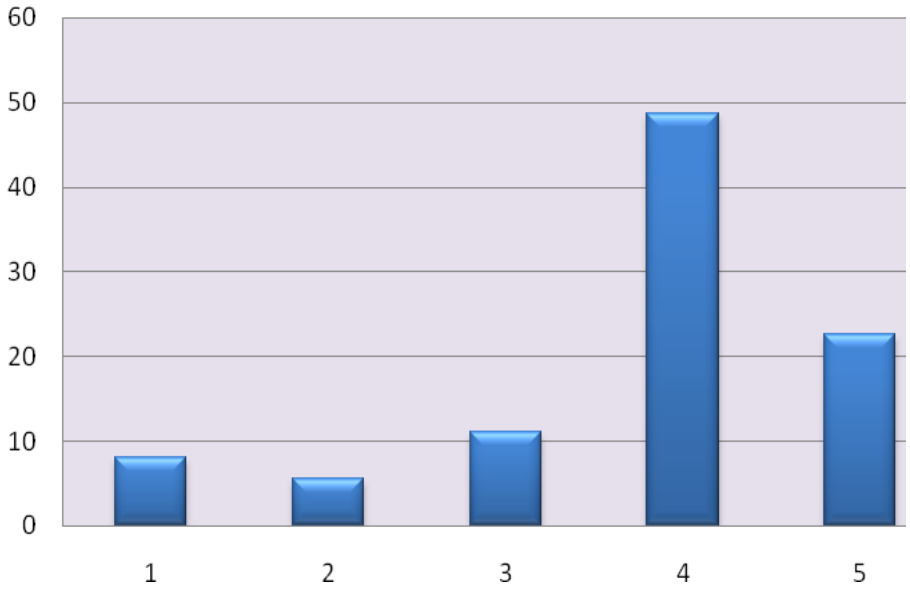

Figure 1. Mean rate of locomotion (in cm) in five different stocks of *D. melanogaster*.

1, wild type; 2, white eye; 3, black body; 4, curl wing; and 5, sepia eye.

Table 1. Student t- Test analysis to show differences in the mean speed of locomotion in the two sexes of five different stocks of *D. melanogaster*.

	Wild type	White eye	Black body	Curl wing	Sepia eye
t values	0.230	0.428	0.994	0.0586	0.440
P	0.820	0.671	0.327	0.954	0.662

df = 38 (for all five paired comparisons)

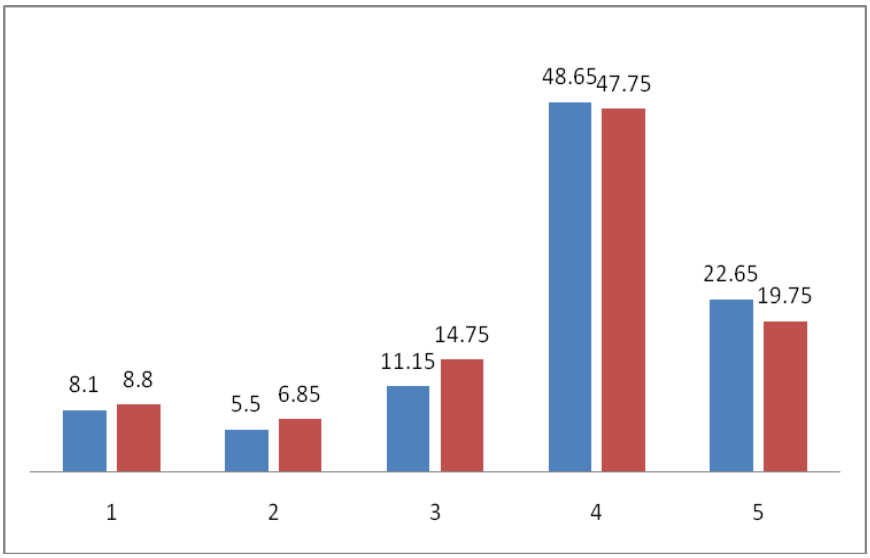

Figure 2. Mean rate of locomotion (in cm) in males and females of five different stocks of *D. melanogaster*.

1, wild type; 2, white eye; 3, black body; 4, curl wing; and 5, sepia eye.

Table 2. Result of one way ANOVA, to compare the mean speeds of locomotion across the five stocks.

Source of variation	*df*	ss	ms	F	*p*
Between	9	46285	5142.8	25.4	
Within	190	38459	202.4		< 0.001
Total	199	84744			

Results and Discussion

Comparison of mean rates of locomotion shows that white eyed flies moved less than other types (Figure 1). This was observed in both the sexes of the white eyed flies. Flies with curl wing were seen moving with much speed and almost non-stop throughout the period they were allowed to remain in the box. Both the sexes of this stock showed this trend. The wild type flies did not show a greater speed of locomotion than others except the white eyed flies. The second runner in this study was sepia eyed flies whose males moved faster than their females. When sex-wise comparisons were made, it was observed that males moved faster than females in three stocks (wild type, white eyed, and black bodied). However the difference was not significant in any of the cases (Table 1). Table 2 shows the result of one way ANOVA, comparing the mean speeds of locomotion of flies in five different stocks. It is clear that curl winged flies moved maximum distance in the stipulated time period compared to other flies. White eyed flies moved minimum distance. Figure 2 shows mean rate of locomotion per minute in males and females of five different stocks of *D. melanogaster*.

Wild type males were seen to move that side of the chamber where light intensity was comparatively more. They were moving faster but they were suddenly seen to stop at a place and started rubbing their forelegs. Female flies did not show inclination towards light and they moved to all directions. Some flies were observed hopping frequently in the chamber. White eyed males and females were often seen beating their wings frequently and rubbing their forelimbs while sitting at one position. Both sexes were observed to be unaffected by the presence or absence of light conditions. Sepia eyed females preferred to move towards the source of light. Few flies were observed hopping in the chamber instead of moving in a direction. Some were seen moving in a circular fashion and frequently rubbing their forelimbs. Some were walking in a zig-zag manner and a few beating their wings. Curl winged males and females were showing neutral response to light conditions. Flies were mostly hopping to short distances. These flies were also often seen moving in a zig-zag manner. Flies with black body color preferred to move towards higher intensity of light. Most flies of both sexes preferred to move on the roof of the chamber. These flies either moved in a zig-zag manner or in straight line. Most of the male flies of this stock were seen beating their wings. Thus, distinct variation in the speed of locomotion, recorded among the different stocks of *Drosophila melanogaster*, indicates genic effect on this behavior.

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References: Burnet, B., L. Burnet, K. Connolly, and N. Williamson 1988, Heredity 61: 111-119; Connolly, K., 1967, Anim. Behav. 15: 149-152; Diagana, T.T., U. Thomas, S.N. Prokopenko, B. Xiao, P.F. Worley, and J.B. Thomas 2002, J. Neuro. 22: 428-436; Honyk, T., and D.E. Sheppard 1977, Genetics 87: 95-104; Honyk, T., J. Szidonya, and D.T. Suzuki 1980, Mole. Gen. Genet. 177: 553-565; Kaplan, W.D., 1977, iav: inactive. Dros. Inf. Serv. 52: 1; Hall, J.C., 1982, In: *Comparative Insect Physiology, Biochemistry and Pharmacology* (Kerkut, G.A., and L.I. Gilbert, eds.), Pergamon Press, Oxford, pp. 287-373; Nakashima-Tanaka, E., and K. Matsukara 1980, Jap. J. Genet. 55: 275-282; O'Dell K.M.C., and B. Burnet 1988, Heredity 61: 199-207; Sisodia, S., and B.N. Singh 2004, Genetica 121: 207-217; Thomas, J.B., 1980, Neurosci. Abstr. 6: 742; Thomas,

J.B., and R.J. Wyman 1982, Nature 298: 650-651; Van Dijken, F.R., 1982, Thesis (Ph. D.), Rijksuniversiteit to Utrecht; Vaj, E., and S.D. Jayakar 1976, Atti. Ass. Genet. 21: 208-210.

Inversion polymorphism in a few south Indian populations of *Drosophila ananassae*.

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Introduction

The inversions were first detected in *Drosophila melanogaster* through the suppression of crossing-over by inversion heterozygotes (Sturtevant, 1926). Since flies with different karyotypes produced by inversions are externally indistinguishable, many investigators including Dobzhansky believed that the inversion karyotypes are adaptively neutral (White, 1977). This assumption is proved to be wrong, since many investigations demonstrated that inversion polymorphism in *Drosophila* are subject to natural selection and is an adaptive trait (Ayala *et al.,* 1974). The degree of inversion polymorphism varies in different species and also in different populations of the same species. Species like *D. pseudoobscura* (Dobzhansky and Sturtevant, 1938), *D. persimilis* (Mohn and Spiess, 1963), *D. subobscura* (Sperlich, 1961), *D. willistoni* (Prevosti, 1964), *D. ananassae* (Kikkawa,1938), *D. melanogaster* (Barnes, 1983), *D. robusta* (Carson and Stalker, 1947), *D. pavani* (Brncic, 1957), *D. paramelanica* (Stalker, 1960), *D. euronotus* (Stalker, 1963), *D. nasuta* (Ranganath and Krishnamurthy, 1978), *D. immigrans* (Toyofuku,1957), D. *nebulosa* (Pavan, 1946) possess large stores of inversions. These species have been termed as "champion species" by White (1977). On the other hand species such as *D. simulans, D. virilis,* and *D. novamexicana* do not seem to possess inversions in their natural populations (Aulard *et al*., 2002; Singh, 2008; White, 1977).

The most convincing evidence for the selective control of inversion frequencies comes from observations on inversion frequencies in geographic populations of different *Drosophila* species which showed seasonal, geographic, altitudinal, and latitudinal variations. In certain species, north-south clines in inversion frequencies (increase towards equator) have been reported (Krimbas and Powell, 1992). Dobzhansky *et al.* (1950), and Da Cunha and Dobzhansky (1954) have found a good correspondence between the mean number of heterozygous inversions and an index expressing environmental heterogeneity in natural populations of *D. willistoni*. Superiority of inversion heterokaryotypes over homokaryotypes has been demonstrated by Dobzhansky (1970). This led Dobzhansky and coworkers (1950) to suggest that chromosomal polymorphism is a device to cope with the diversities of environments.

Drosophila ananassae is one such species which exhibits high level of inversion polymorphism. It is a cosmopolitan domestic species having a unique status among *Drosophila.* Due to certain peculiarities such as male crossing over, high mutability, and high level of chromosomal polymorphism, it has been used for many genetic studies. This species harbors large numbers of inversions. Further it carries three well knit coextensive inversions, namely, 2LA on the left arm of the 2nd chromosome, 3LA on the left arm of the 3rd chromosome, and 3RA of the right arm of the 3rd chromosome. The extent of genetic polymorphism in various populations of *Drosophila ananassae* carrying these inversions has been studied; the adaptive significance of them has also been studied (Futch, 1966; Rajeswari and Krishnamurthy, 1969; Reddy and Krishnamurthy, 1974,