America. They found a great deal of genetic variation, and on an average 58% of loci were found to be polymorphic for *D. willistoni* and 71% polymorphic for *D. equinoxialis*. Acid Phosphatase was also studied in adult *D. melanogaster* and *D. simulans*, which revealed three types of variants in both the species (MacIntyre, 1966). The present study differs from the studies of Hegde (1979) as they have reported a total of 7 bands in *D. ananassae*.

Nagaraj (1985) reported 3 and 2 patterns of acid phosphatase in *D. bipectinata* and *D. malerkotliana*, respectively, from Uttara Kannada district. Dharwad, which is an adjacent district, showed 8 and 7 different patterns. The present study showed more patterns and bands in each pattern compared to earlier studies of Hegde (1979) and Nagaraj (1985). This provides evidence for the polymorphic nature of the acid phosphatase enzyme in *D. ananassae*, *D. bipectinata*, and *D. malerkotliana.*

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Age and sex related change in the heritability of locomotor behavior in *Drosophila melanogaster.*

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Abstract

Locomotor behavior is a crucial and fitness-related trait which has a polygenic basis. Here in this study we estimated basic quantitative genetic parameters of locomotion using isofemale lines of *D. melanogaster*. Negative geotaxis and startle response were used as component traits defining locomotion. We have estimated narrow sense heritabilities and its components for three different age groups for both sexes. Our results show that these indices can change with age and sex, though differently for geotaxis and startle response. Change in heritability of negative geotaxis with age was more or less negligible, whereas the heritability for startle response decreased with age. We infer this difference between the traits in amount of change they had with increasing age could indicate that the putative genes influencing additively each trait phenotype are distinct, and, accordingly, act differently. **Keywords:** Locomotion, aging, genetic variance, heritability, *Drosophila*

Introduction

Locomotor behavior is one the most important evolutionary features of an organism affecting its feeding, mating success, dispersal ability under normal or stresfull conditions, and its ability of predator

avoidance (Alexander, 2006). Locomotion is a complex trait in the sense of quantitative genetics and has been the subject of several genetics and genomic studies focusing on its genetic architecture (Anholt and Mackay, 2010). Decline in locomotor activity of *Drosophila* with age is an indication of functional senescence in which negative geotaxis, a component of locomotion, has been shown to be severely reduced by increasing age (Grotewiel *et al*., 2005). Differences in the level and pattern of decline in locomotor activity with age among the different lines of *Drosophila melanogaster* suggest that the pattern of locomotion with age may be genetic (Fernandez *et al*., 1999). Here in the present study, we measured the change in locomotion of *D. melanogaster* inbred lines with age and calculated the narrow sense heritabilities for both sexes per age category in order to see if the pattern of change with age could have a genetic component.

Material and Methods

Lines

For the negative geotaxis and startle response essays 7 isofemales lines were used. These lines were picked up from a collection of females caught in the eastern part of Black Sea region of Turkey, in Firtina Valley, a couple of kilometers West to the Ardeşen county, in 2012. That collection of isofemale lines has been highly inbred with brother sister matings since then and the 7 lines of our study were picked up after checking the status of inbreeding (not shown). They were all found completely inbred.

Locomotion

Negative geotaxis and startle response were measured to phenotype locomotion. For both negative geotaxis and startle response, we measured 7 highly inbred lines in which 10 individual measurements were made per line per sex. In negative geotaxis, banging essay was performed: individual flies were taken into empty vials and, after banging, each fly was observed for climbing distance in a period of 10 seconds. In startle response essays, after banging the empty vials each containing an individual fly, each fly was observed for moving activity time through a fixed duration of 45 seconds. 10 flies per sex and line were assayed both for negative geotaxis and startle response, and mean line phenotypic scores for each trait for each sex were estimated. All measurements were performed for three ages, namely, at 0 (eclosion), 15, and 30 days after emergence.

Heritability

For each age category, we estimated narrow-sense heritability (h^2) and associated parameters for each sex. Narrow-sense heritability estimates were performed after computing the genotypic variance (V_G) from the single classification ANOVA performed per sex. Additive genetic variance, V_A, was calculated as $V_G = 2 \times F \times V_A$, where F is the inbreeding coefficient (taken as unity as we used an isofemale line design)(Falconer and Mackay, 1996). Heritability was estimated from the general formula of $h^2 = V_A/V_P$, where V_P is the phenotypic variance.

Results

I. Among line variation and sex differences through aging

We found considerabe variation in mean scores of negative geotaxis and startle response both within an age category and across ages (Figures 1 and 2). Sexes also varied: there was a marked (and significant) difference between female and male scores for both traits. Interestingly, magnitude and direction of change between sexes seemed to be enlarged and reversed with increasing age for most of the lines. At zero age (eclosion), the number of the lines that gave female mean negative geotaxis scores larger than those of the males was almost half the total lines, but at age 30 almost all the lines had larger male scores (Figure 1). Moreover, the magnitude of the difference between female and male was conspicuously increased in the male direction compared to age 0. The same was almost true for startle response, though with a less emphasized pattern (Figure 2).

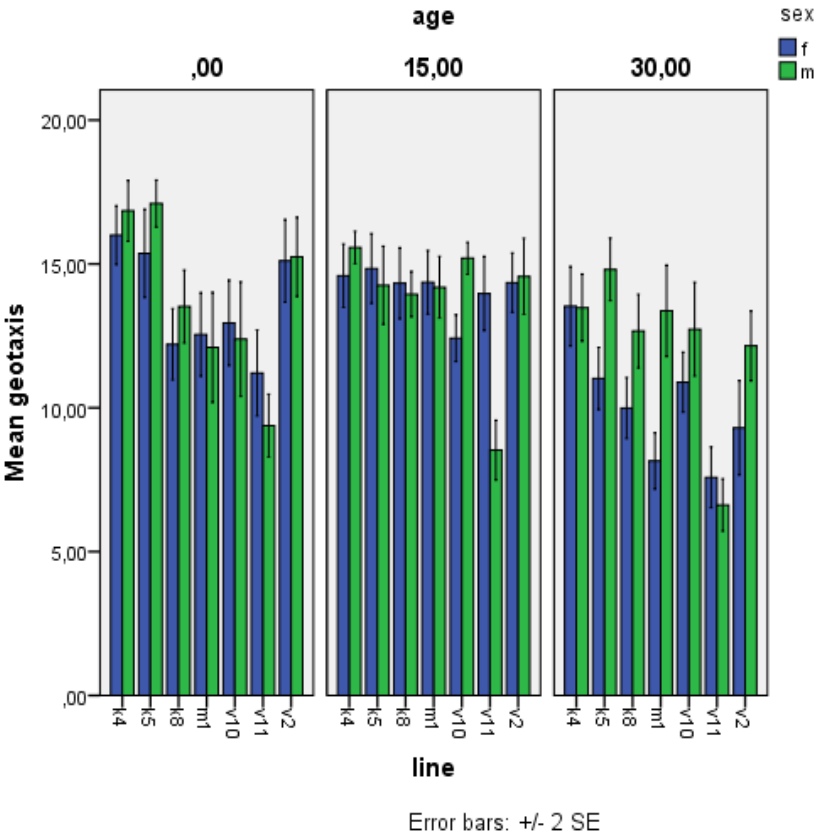


Figure 1. Among line variation in negative geotaxis and its response to aging.

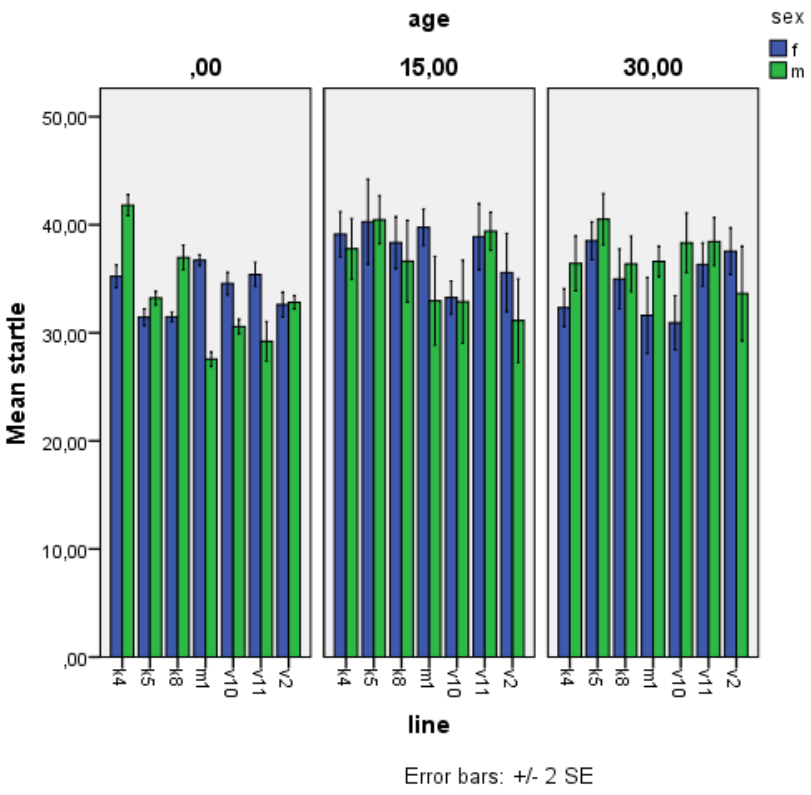


Figure 2. Among line variation in startle and its response to aging.

Table 1. Heritability and associated parameter estimations for negative geotaxis. V_P: phenotypic variance. V_G: genotypic variance. V_A: additive genetic variance. h^2: narrow sense-heritability.

Age (days) ♀♀	V_P	V_G	V_E	V_A	h^2
0 (eclosion)	7.4	2.9	**4.8**	1.5	**0.20**
15	3.4	0.3	**3.1**	0.2	**0.05**
30	6.8	3.6	**3.6**	1.8	**0.27**
Age (days) ♂♂					
0 (eclosion)	11.4	7.3	**5.0**	3.6	**0.32**
15	7.2	5.4	**2.5**	2.7	**0.37**
30	9.8	6.5	**4.2**	3.2	**0.33**

Table 2. Heritability and associated parameter estimations for startle-response. V_P: phenotypic variance. V_G: genotypic variance. V_A: additive genetic variance. h^2: narrow sense-heritability.

Age (days) ♀♀	V_P	V_G	V_E	V_A	h^2
0 (eclosion)	5.7	4.1	**2.1**	2.1	**0.36**
15	23.1	4.5	**19.1**	2.3	**0.10**
30	21.4	7.9	**14.7**	3.9	**0.19**
Age (days) ♂♂					
0 (eclosion)	23.1	23.6	**2.6**	11.8	**0.51**
15	36.3	10.2	**27.4**	5.1	**0.14**
30	21.2	2.8	**18.8**	1.4	**0.07**

II. Heritability and its change with age

The second part of our work entailed the genetic description of the age and sex dependent line variability in locomotion. For this purpose we estimated narrow sense heritability (h^2) and related parameters for each sex in each age category. Tables 1 and 2 show estimations in this respect.

Traits showed distinct patterns of change with age and sex for heritability. For negative geotaxis the amount of genetic variation as *h2* was mostly invariant between sexes across ages (Table 1), while the h^2 of startle response was consistenly decreased with age in both sexes (Table 2). This trait specifity in change of heritability with age may be highly likely to have resulted from the effect of differential amount of increase in environmental variance (V_E) through age. Although the amount of change in V_E was smaller and similar across ages for geotaxis, it seems that increasing age created increasing environmental variance for the startle (Table 1 and 2, V_E). The magnitude of the environment effect through age on heritability can be seen in Figure 3A and B.

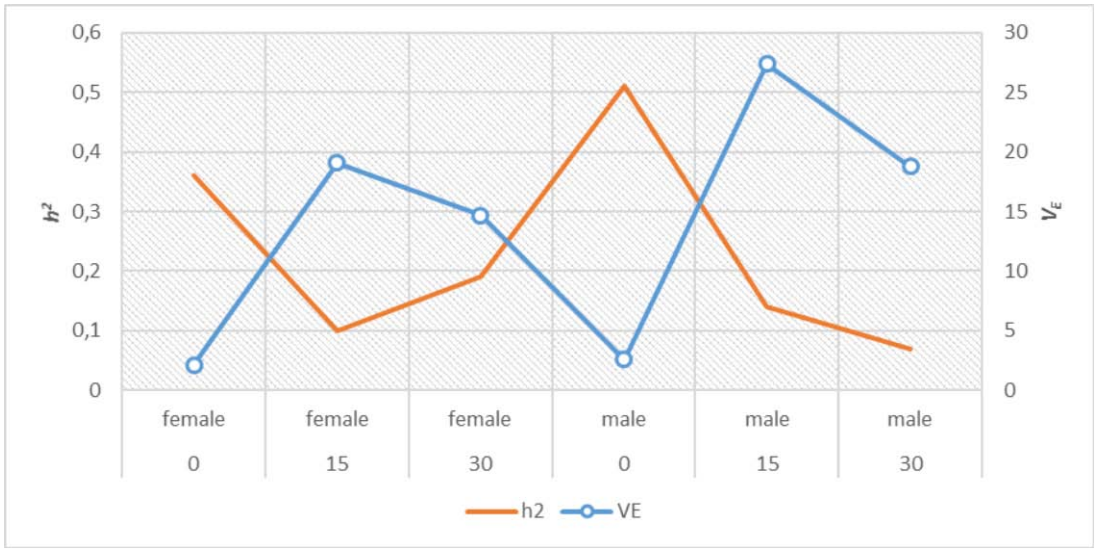
Discussion

In summary, both the high variability among lines and the conspicuous sex dependent change in that variability through aging suggest that the genes underlining locomotion may have been undergoing drastic change in expression through aging (Figures 1 and 2). This change should have contributions from many locations from the genome, as locomotion is a complex, polygenic trait. Consequently, this sex and trait based variability in locomotion with age among lines could be translated into variation in the direction and magnitude of narrow sense heritability with increasing age. However, we have found that in only one of the traits, startle response, the change in heritability seems to track aging (Table 2). Narrow sense heritability (h^2) is an indication of additive genetic variance that contributes to phenotypic variation in a trait (Falconer and Mackay, 1996). In this respect, our finding that this genetic variance for startle response can be different for different age categories suggests that the expression of the putative genes contributing to locomotion might be changing through age. Indeed it has been shown that the pattern of change in locomotion with age can be line specific, hence genetic (Fernandez *et al*., 1999). Our results for startle response could enforce this previous inference. On the other hand, we have found that the pattern of change (startle response) and stability (negative geotaxis) in the heritabilities are strongly affected by the amount of change in environmental variance (V_E) with age (Tables 1 and 2). The more uniform the environmental variance, the more constant is the heritability (negative geotaxis), and, *vice versa* (startle response) (Figure 3). Then, what could account for this differential effect of environmental variance on the pattern of change in heritability with age?

First, beside we used the same isofemale lines for each age category, experimental variance was also very low for each experimental setup. Aging, being a general deterioration in itself both for organismal maintenance and repair, provides highly variable cellular environment and can thus vary the operation of gene expression cascades. Thus, we suggest that the differential response in heritability with age may be due to distinct genotype-by-environment expression contributions from the genome for these traits. Both negative

Figure 3A (left). Change in the environmental variance and heritability of negative geotaxis in response to aging.

Figure 3B (below). Change in the environmental variance and heritability of startle in response to aging.



geotaxis and startle response are components of locomotion, and it is rather interesting that aging should have resulted in two distinct expression patterns in these two traits thought to be mirror images in their contribution to locomotion. This remarkable point should be accounted for, and we are planning experiments in which a mapping population of genomic lines will be used to discriminate the genes contributing to negative geotaxis and startle response at different ages. We hope this further experimentation will clarify the identities and association levels of the genes (hence their functional relatedness to locomotion) contributing to locomotion.

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