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Genetic analysis of adult locomotor activity in *Drosophila melanogaster*

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This study is an attempt to identify genes influencing spontaneous adult locomotor activity in *Drosophila melanogaster*. A wild type stock and 13 morphological marker stocks (six markers for chromosome X and seven for chromosome three have been used). Backcrosses have been set up to study linkage relationships between loci affecting the quantitative character and marker loci. The results suggest the presence of several genes influencing locomotor activity on both chromosomes analyzed.

INTRODUCTION

Both theoretical (Beckmann and Soller, 1983; Chotai, 1984; Weller, 1986) and experimental studies (Maroni *et al.*, 1982; Tanksley *et al.*, 1982; Vallejos and Tanksley, 1983; Schnee and Thomson Jr., 1984; Weller, 1987; Shrimpton and Robertson, 1988*a*; 1988*b*) concerning the detection and mapping of genes influencing quantitative characters seem to represent a renewed interest in the field of quantitative genetics. This is probably mainly due to the fact that in many economically important organisms a number of commercial traits are quantitative variables, as in the case of *Lycopersicon* (tomato), for which 22 loci affecting quantitative characters, for example *p*H, vitamin C concentration, reducible sugar concentration, etc., have been mapped (Weller 1987; Weller *et al.*, 1988). Thompson Jr. and Thoday (1979) reviewed the most important methods proposed for the location of genes influencing quantitative traits: almost all the approaches make use of the selection of lines that differ strongly for the character under study. Jayakar (1970) and Jayakar *et al.*, (1977), starting from Thoday's approach (1961; 1966) based on the detection of linkage between genetic markers and loci affecting quantitative traits, suggested an experimental and statistical methodology useful for any population of diploid organisms. A considerable advantage of Jayakar's approach is that it does not need to select lines for high or low values of the quantitative trait, but analyzes the distribution of the trait among the individuals of the segregating classes obtained from either backcrosses or intercrosses for a marker locus, situations which can be observed in nature besides being easy experiments to set up in the laboratory.

The quantitative character here studied is adult locomotor activity in *Drosophila melanogaster*, which has been the object of several investigations (Connolly, 1966; 1967; Angus, 1974; Burnet and Connolly, 1974; van Dijken *et al.*, 1977; 1979; van Dijken and Scharloo, 1979*a*, *b*; Kyriacou, 1981; Le Bourg *et al.*, 1984; Le Bourg and Lints, 1984; Lints *et al.*, 1984; 1985; Meehan and Wilson, 1987; Burnet *et al.*, 1988; O'Dell and Burnet, 1988). This is a complex behavioural trait for which significant levels of heritability have been generally demonstrated (Ewing, 1963; Connolly, 1966; Angus, 1974; van Dijken and Scharloo, 1979*a*), even if in one case no response to selection experiments was obtained (Lints *et al.*, 1985). The activity of *D. melanogaster* was first subdivided into two components, "spontaneous activity" and "reactivity" (Burnet and Connolly, 1974); more recently a third, distinct, category has been defined and named "stimulated activity" (Mehan and Wilson, 1987). Moreover the locomotor activity of single flies, as revealed by "open-field" observations, is characterized by two different behavioural components, the "amount" and "speed" of movement

* Deceased 21.1.1988.

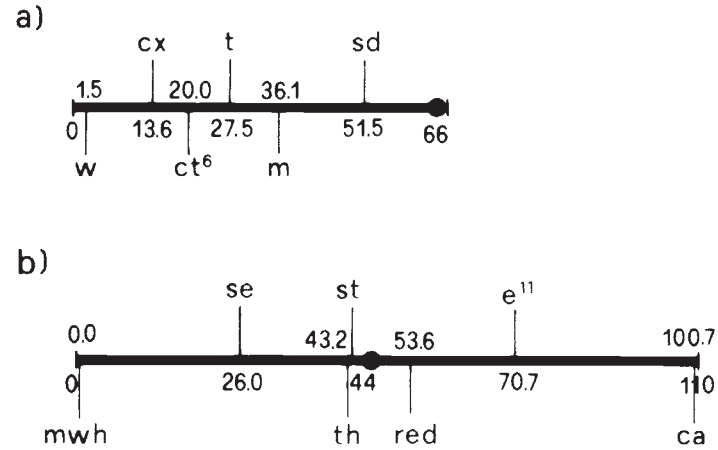

Figure 1 Genetic map of the morphological markers used in the experiments. a, chromosome X; b, chromosome 3.

(Burnet *et al.*, 1988). Sex differences in locomotor activity have been described, females being often more active than males, and also the decrease of activity with increasing age in both sexes (Le Bourg and Lints, 1984). Lack of correlation between spontaneous locomotor activity and fecundity, fertility and lifespan has also been reported for females from a wild laboratory strain (Le Bourg *et al.*, 1984).

A genetic analysis of locomotor activity in *D. melanogster* has been made by van Dijken *et al.* (1979) on three pairs of high and low locomotor activity lines obtained independently from two different base populations. The results of experiments using reciprocal crosses as well as chromosome substitutions suggested that a considerable part of activity differences was attributable to the effects of chromsome X. Minor effects were due to the second and third chromosomes; moreover significant interactions between chromosomes were also reported. Vaj and Jayakar (1976) in a study aimed to assess the importance of autosomal genes in the determination of locomotor activity in *D. melanogaster* have found that chromosome 4 is the most influential in controlling this character.

Burnet *et al.* (1988) commenting on the results obtained by Connolly (1966, 1967) argued that spontaneous activity and reactivity are probably under control of different genetic systems. The same authors also suggested that the amount and speed components of locomotion are affected by different genes, that those influencing the amount of locomotion are located on chromosome 3, and those responsible for speed on chromosome X and 2. Of course this does not exclude the existence of genes which could affect both the components. It is even likely that larval and adult locomotor activities be under control of different genes, because of lack of correlation between them (Sewell *et al.*, 1975; Thompson Jr. *et al.*, 1983; Burnet *et al.*, 1988). In this paper we analyze the genetical effects on adult locomotor activity of chromosomes X and 3 in *D. melanogaster* according to Jayakar's model (1970; 1977) extended to the case of sex-linked genes. To this purpose a total of 13 morphological markers have been utilized.

MATERIAL AND METHODS

Stocks and morphological markers

The wild-type stock (Cast-PD), used as a random source of individuals to study locomotor activity, was established in 1984 from a large sample collected in the wild in the neighbourhood of Padua (Italy). The morphological markers used in the experiments are shown in fig. 1. *w*, *cx*, ct^6, *m*, *se*, *th*, and e^{11} stocks were kept in our laboratory collections in homozygous condition. The other markers were obtained: *red* and *st* from Professor M. Gatti (Rome), *sd* from Professor S. Cavicchi (Bologna), *mwh* from Professor Wurgler (Zurich), *t* and *ca*, *K-pn* from the Umea Drosophila stock center. The stock *ca*, *K-pn* was used considering that no phenotypic effects are shown by *K-pn* either in homozygous or heterozygous conditions if *pn* (1 – 0·8) is not present. The stocks were carefully checked before use with respect to their phenotypic

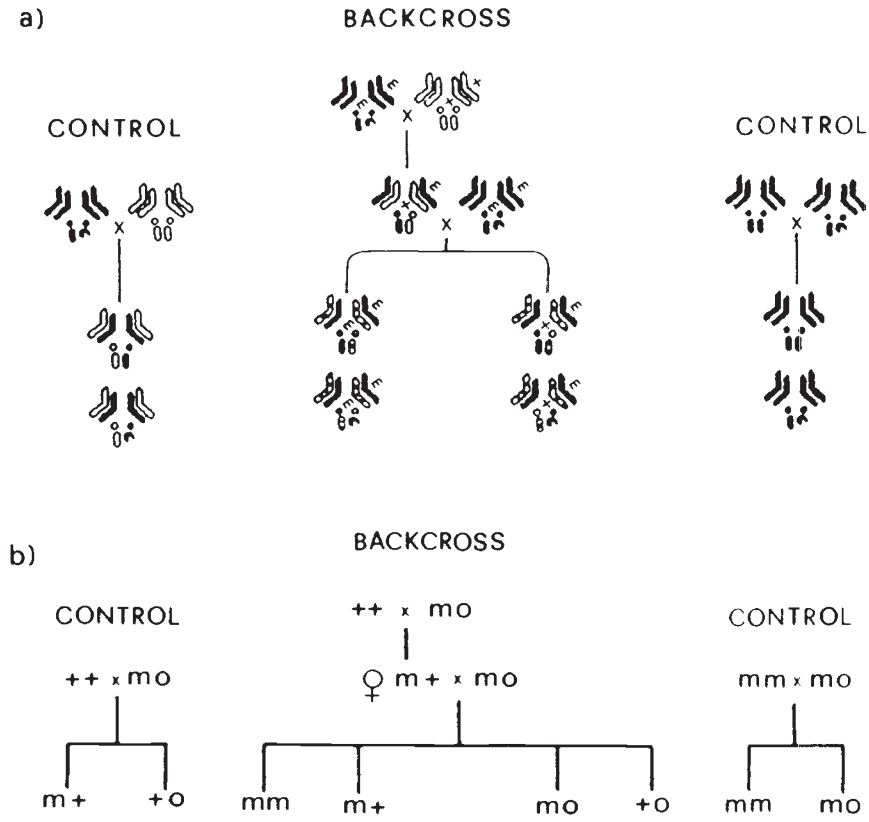

Figure 2 Scheme of the experimental design: backcrosses and control crosses with the wild type (white) and an autosomal marker (black) stocks (a). A simplified scheme for an X-linked marker is also shown (b).

characteristics according to Lindsley and Grell (1972).

Individual crosses were set up with 1–2 day old individuals, cultured at 24°C ± 1°C on a sucrose-yeast medium (Mittler and Bennet, 1962).

Experimental design

The experimental schemes adopted are shown in fig. 2. Only backcrosses with heterozygous females were used because of the lack of crossing-over in *Drosophila* male. For each marker, from a minimum of 9 to a maximum of 16 backcrosses were set up.

Individuals to be measured were randomly chosen from each control and backcross sibship, males and females approximately in equal number, unless the sibship size was small and therefore all the emerged flies were tested. As for *t* marker, only females were scored for the sake of accuracy in classifying the phenotypes.

Measurement of locomotor activity

Individuals were tested for locomotor activity 48–54 hrs after eclosion. No significant differences were observed within each control (and also within each backcross) in the mean scores of groups of flies examined on different days. Flies to be measured were transferred to fresh food vials, and scored always in the afternoon (between 1300 and 1800 h) under constant light (tungsten lamp—11·9 Watts $\times m^{-2}$) and temperature (24°C + 1°C) conditions. The locomotor activity score of a fly is its total walk—measured in degrees—during 90 seconds inside a horizontal glass tube (internal diameter = 3 mm) shaped as a 10 cm diameter ring. Similar apparatus had been used in previous studies by Connolly (1966) and by Vaj and Jayakar (1976). Flies were brought into the ring after weak anaesthesia with CO_2 and tested for locomotor activity after 15 minutes to reduce effects due to the "reactivity" component (Burnet *et al.*, 1988). In order to eliminate any possible influence on

locomotor behaviour due to chemical cues released by the flies, a battery of glass rings was used and the rings were systematically cleaned. In the experiments carried out with *th*, *w*, ct^6, *sd* morphological markers, control and backcross progeny was scored, in addition to locomotor activity, also for thorax-abdomen length by using a microscope equipped with a micrometer ocular. The same survey was done in a sample of the wild stock "Cast-PD".

Statistical analyses

Statistical analyses have been carried out on the locomotor activity scores of the offspring of the control crosses, in the two sexes separately.

The square root transformation has been applied to the data in order to normalize the four frequency distributions (two genotypes × two sexes) of the values obtained for each of the six X-linked and the seven autosomal markers. Even if this transformation turned out to be better than the others, like the log transformation, suggested by the form of the distributions, in a few cases the distributions departed significantly from normality in spite of the transformation applied. We have then resorted to nonparametric methods to compare them. The distributions have been graphically described by Tukey's box-and-whiskers representation (Tukey, 1977), and have been compared, between sexes and between genotypes, by Mann-Whitney U-test.

The analyses performed for the detection of linkage are based upon the model developed by Jayakar (1970) for one autosomal di-allelic locus influencing the quantitative trait and a di-allelic marker locus. However, it can be easily shown that the model is valid for X-linked genes in either sex, if appropriately modified to take into account the mode of inheritance of sex-linked characters.

The assumptions made are that the frequency distribution of the quantitative character has different means but equal variances in the three genotypes of the "quantitative" gene, the distributions being normal. The variance within genotype, besides the environmental component, includes a component of variability due to the rest of the genome. The same model has been adopted also by Hill (1975).

A complete theoretical analysis of all possible backcross families of the marker locus and their progeny shows that, in the presence of linkage, the mean value of the trait is expected to be different in the two segregating classes of those families in which a proportion *r* of the offspring is derived

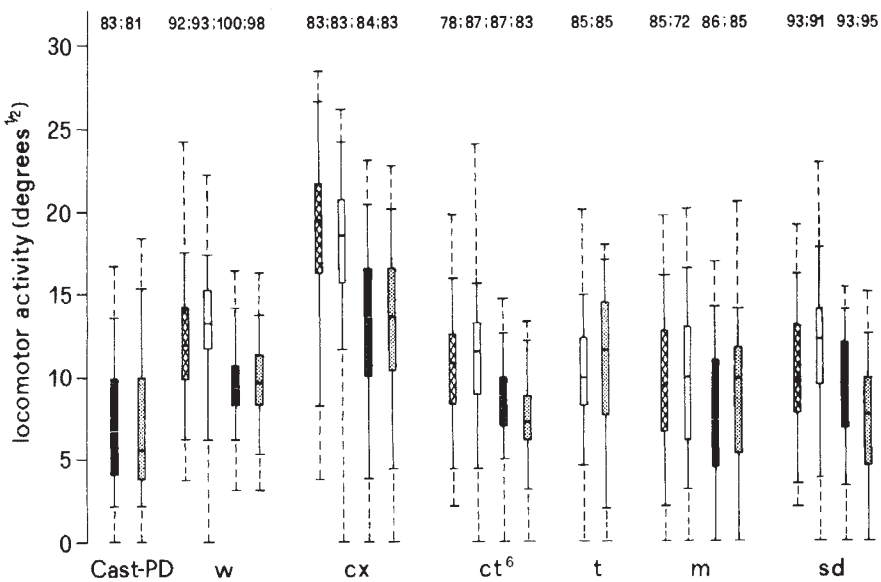

Figure 3 Box-and-whiskers representation of the distributions of locomotor activity scores in the offspring of control crosses for the wild type and the X-linked marker stocks. For each distribution, besides the lowest and the highest values, the 5th, 25th, 50th, 75th and 95th percentiles are indicated. The sample size is also given. +0 males, *m*0 (or wild type) males, *m*+ females, *mm* (or wild type) females. See also legend to Table 1.

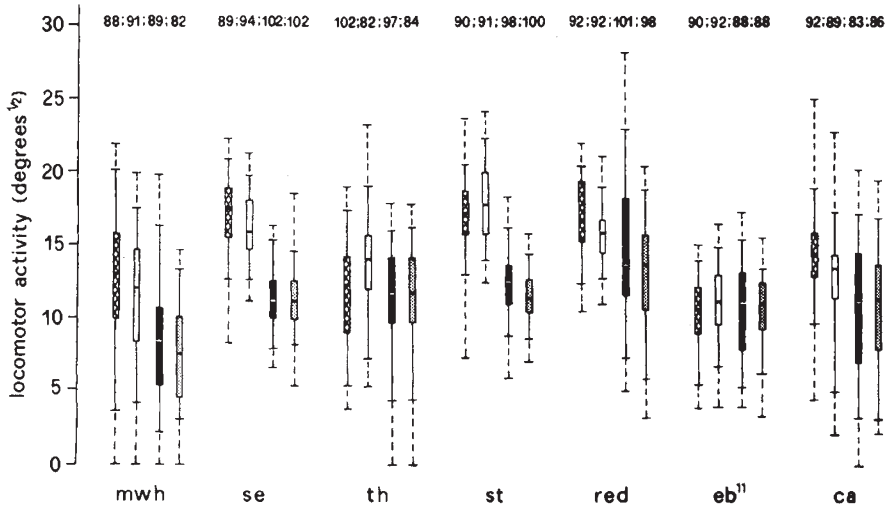

Figure 4 Chromosome 3 markers. See legend to figure 3. *m*+ males, *m*+ females, *mm* males, *mm* females.

from recombinant gametes. Therefore, while some families have identical means in the segregating classes independently of linkage, some do show differences which are a function of the recombination frequency (Jayakar, 1970). The probability of finding these informative sibships in a sample is determined by the allelic frequencies at the "quantitative" locus, with a maximum of 50 per cent of informative families when the two "quantitative" alleles are equifrequent. The first approach has been that of comparing the mean locomotor activity in the two segregating classes for all the families of the marker locus, by means of Mann–Whitney U-test.

Furthermore, for the detection and estimation of linkage we have applied the index (I_2) derived by Jayakar and colleagues (Jayakar *et al.*, 1977), which is based on the difference between the trait means of the two segregating classes within sibship. In fact, while the expected value of this difference over all sibships is zero, its variance among families is a function of the recombination frequency r of the form $\text{var} = F_1 + F_2(1-2r)^2$, with a maximum value for $r = 0$ and a minimum for $r = 0{\cdot}5$. F_1 and F_2 are constants which depend upon the parameters of the model. The estimate of this variance is quite complicated; however, the variance of the differences observed in the segregating classes of a sibship can be a useful approximation. The index I_2 is then computed as $I_2 = \sum_{i=1}^{n} (x_1 - x_2)^2/(n-1)$ where x_1 and x_2 indicate the trait means of the two segregating classes, and n the number of families.

To avoid the influence of extreme values on the estimate of the mean in the segregating classes, the midmean has been used, that is the average of the values which are between the 1st and the 3rd quartiles. While it is not easy to assess the statistical significance of index I_2, it has the advantage to provide a linkage map when a battery of marker loci is used to explore a whole chromosome and only one "quantitative" gene is present on it: in fact since I_2 is a function of $1-2r$ (r = recombination frequency), it is expected to be maximum for the marker nearest to the "quantitative" gene and to decrease monotonically for markers farther away. It has to be remembered that some flaws in the map might occur, when by mere chance no informative family is included in the backcrosses of a marker.

RESULTS

1. Controls

A "box-and-whiskers" description of the distributions of locomotor activity scores in the controls is given in figs. 3 and 4, separately for the two sexes and the genotypes at the marker locus, with the corresponding number of individuals tested. In figure 3 the information for the wild type stock Cast-PD has been included. A few individuals (1·12 per cent) show complete lack of activity: it has to be noted that this phenomenon is not present in all the stocks (see figures 3 and 4). On the whole, the distributions are quite different from one another with respect both to location and dispersion. In general, hybrids are more similar to the marker stock they are derived from than to the

Table 1 Significance of Mann–Whitney U-test performed between sexes (MM males; FF females) and between genotypes among the offspring of the control crosses. + and *m* refer to wild type and mutant allele respectively. 0 indicates the hemizygous condition for X-linked markers

Chromosome X				
	+0 vs. *m*0	+*m* vs. *mm*	+0 vs. +*m*	*m*0 vs. *mm*
w	***	***	*	ns
cx	***	***	ns	ns
ct^6	***	***	ns	*
t	—	*	—	—
m	**	ns	ns	*
sd	ns	***	*	**

Chromosome 3				
	+*m* vs. *mm*		MM vs. FF	
	MM	FF	+*m*	*mm*
mwh	***	***	ns	ns
se	***	***	***	ns
th	ns	***	***	ns
st	***	***	ns	***
red	***	***	***	ns
eb^{11}	ns	ns	ns	ns
ca	***	***	***	ns

ns = $P > 0{\cdot}05$.
* = $0{\cdot}01 < P < 0{\cdot}05$.
** = $0{\cdot}001 < P < 0{\cdot}01$.
*** = $P < 0{\cdot}001$.

Table 2 Number of backcrosses set up for each marker (NF) and total number of flies tested for each sex and genotype. See also legend to table 1

Chromosome X					
	NF	+0	+*m*	*m*0	*mm*
w	10	181	187	178	163
cx	11	266	277	194	234
ct^6	9	170	164	180	157
t	15	—	379	—	286
m	11	168	195	179	189
sd	10	138	198	141	164

Chromosome 3					
		+*m*		*mm*	
	NF	MM	FF	MM	FF
mwh	10	205	208	175	181
se	10	172	175	146	143
th	10	162	153	112	129
st	10	132	145	143	137
red	10	153	174	161	161
eb^{11}	16	268	277	175	193
ca	9	217	261	129	169

wild type, even when significantly different from either parental stock (table 1). While very similar results have been obtained for the two sexes in the wild type stock, a variable pattern has been observed in the marker stocks. In general though there is less difference between sexes than between genotypes within sex (table 1).

In order to verify whether the fly length could affect the locomotor activity score, the relationship between this and thorax-abdomen length was analyzed in some of the stocks. In all the cases no significant relationship has been found.

2. Backcrosses

Table 2 shows for each marker the number of backcrosses set up and the total number of flies in the sibships analyzed within sex and genotype; midmeans of locomotor activity scores are given in tables 3(a) and 3(b) for all sibships and markers.

With respect to chromosome X markers, in 53 out of 66 sibships heterozygous females are more active than all their sibs; in 58 sibships heterozygous females are on the average more active than their homozygous sisters, and in 43 families wild type males are more active than their mutant brothers. As far as chromosome 3 is concerned, the heterozygotes show the highest activity independently of sex; in fact which sex is more active seems to depend upon the stock, that is females in *mwh*, *th*, eb^{11} and males in *se*, *st*, *red*, ct^6 marker stocks.

The first step in the statistical analysis aimed to the detection of linkage between the marker loci and genes affecting locomotor activity was to test the significance of the difference of the average locomotor activity scores between the two segregating classes in the two sexes separately. Midmeans and the significance level of the U-test between genotypes are given in table 3. Very different patterns are shown in these results, from no significant value (see eb^{11} for males, *red* and *ca* for females) to almost all significant values (see *se* for both sexes). In all the other cases a high variability is seen among sibships for both chromosomes. Jayakar's I_2 index, which is an estimate of this variability, provides the profiles shown in fig. 5.

DISCUSSION

The results of our analyses clearly show that the expression pattern of spontaneous locomotor

Table 3a Mean locomotor activity score for sex and genotype in each backcross sibship. The significance of U-test for the comparison between genotypes within sex is also shown. See also legend to Table 1

Chromosome X

white				curlex			
+0	*m*0	+*m*	*mm*	+0	*m*0	+*m*	*mm*
11·19	10·40	11·51	12·68	16·43	17·28	21·80	16·62*
10·39	10·73	12·12	12·36	20·12	20·48	20·54	17·99*
10·79*	8·90	12·59	11·50	18·63	17·23	18·62	18·01
10·67	10·23	13·62	10·02**	19·47*	14·62	17·71	14·99*
11·30	10·58	15·05	10·98***	15·88	17·81	18·20	16·77
10·94	11·67	13·79	14·01	18·14**	14·38	19·03	16·03**
11·33	10·70	14·46	11·56**	19·36*	16·76	19·82	16·94*
11·43	10·48	12·26	12·24	18·84	17·53	21·27	18·18*
12·15	10·49	14·53	11·94	19·23	17·52	21·14	21·07
11·59	10·18	12·35	11·44	17·93	17·19	20·65	16·77**
				17·79	16·53	17·71	18·07
cut^6				tan			
+0	*m*0	+*m*	*mm*	+*m*	*mm*	+*m*	*mm*
9·42**	6·10	11·95	5·93***	10·18	8·20	10·20	9·90*
10·44**	6·76	11·69	7·08**	12·67	10·91	9·28	8·95
9·82**	6·70	10·29	6·18*	12·86	12·72	10·77	11·14
8·55	7·26	9·80	7·03*	9·99	9·82	10·67	11·40
10·94***	6·70	11·54	6·16**	13·67	10·26	10·86	11·18
10·83*	7·71	12·89	7·05**	11·70	11·56	13·38	12·27
10·76	8·36	12·13	6·74***	8·20	9·94	11·62	10·52
10·55**	5·67	11·34	8·04	13·41	12·09		
9·25	8·65	11·17	7·96**				
miniature				scalloped			
+0	m0	+m	mm	+0	m0	+m	mm
12·00**	9·24	12·86	10·06	8·91	8·37	9·20	7·20
10·46	7·82	13·85	8·38**	12·07*	9·56	14·40	11·34
12·36*	9·34	12·37	9·01**	10·87	10·52	13·81	9·30*
9·76	8·16	11·07	9·56	10·14	9·98	11·82	11·53
10·28	6·13	14·45	10·43	8·00	8·35	10·43	7·90
14·48*	9·32	14·17	8·15***	9·50	7·12	12·51	9·58
12·53***	6·76	13·17	8·52***	12·15	9·37	13·48	12·04
13·20	10·57	13·85	11·59**	11·11	9·86	13·35	10·90
11·53*	9·24	11·61	9·35	10·28	8·47	11·04	10·01
13·35***	8·34	13·09	9·94**	10·74*	8·17	10·51	6·03**
13·06**	9·41	12·22	12·03				

activity differs from stock to stock. Noteworthy is also the clustered distribution of null-activity individuals observed among the marker stocks. The above remarks suggest that quite a lot of genetic variability exists among marker stocks for the control of locomotor activity, which is not surprising because of their different historical origins: in fact the stocks we have used come from different laboratories, and may be characterized by different arrangements of alleles for the genes affecting locomotion. The higher locomotor activity generally shown by the *m*/+ heterozygotes, which are in fact hybrids of two different stocks, could be explained by the fact that crosses between individuals from a marker stock and wild Cast-PD may yield increased genetic variability accompanied by positive effects on locomotion.

Previous findings (van Dijken *et al.*, 1979; Burnet *et al.*, 1988) suggesting a clear influence on locomotor activity exerted by chromosome X are confirmed by our results, since significant differences in activity between sexes within the same genotype have been observed for chromosome 3 markers, with the exception of *eb*^{11}.

Table 3b Mean locomotor activity score for sex and genotype in each backcross sibship. The significance of U-test for the comparison between genotypes within sex is also shown. See also legend to Table 1

Chromosome 3

multiple wing hair				sepia			
MM		FF		MM		FF	
$+m$	mm	$+m$	mm	$+m$	mm	$+m$	mm
5·44	5·14	10·68	5·77***	17·39*	14·84	17·01	14·26***
6·48	7·59	11·03	6·28*	19·62***	15·64	17·60	14·75***
8·54	5·89	8·66	5·09**	18·36***	15·40	16·32	14·37**
7·72	4·93	6·01	4·20	16·99**	15·09	16·98	14·52***
8·03*	5·79	8·84	4·69**	17·92	17·56	15·74	13·94
9·65	6·90	8·61	6·18*	20·80***	15·17	16·78	13·47***
9·42**	4·58	9·67	6·83*	19·58***	13·72	16·51	13·95***
8·00	5·82	8·48	4·32***	18·51***	14·02	17·05	14·34***
6·41	3·78	7·61	5·65	19·04***	14·45	16·84	15·09***
6·10	6·21	6·36	6·38	19·52***	14·72	14·74	15·15**
thread				scarlet			
MM		FF		MM		FF	
$+m$	mm	$+m$	mm	$+m$	mm	$+m$	mm
12·43	11·60	15·32	10·43	16·35	15·56	16·17	14·52
12·90	11·79	13·56	13·11	17·95*	16·18	13·65	14·79
10·30	9·40	13·16	10·98**	15·79	17·16	16·40	15·30
12·44***	8·43	14·85	10·31***	17·50	16·75	16·46	14·13**
15·43*	10·60	14·20	13·96	17·73	16·40	17·50	15·88***
11·16	11·78	15·03	14·03	18·84*	16·20	17·76	15·16
11·71*	9·04	12·76	9·42*	17·70*	15·36	17·62	15·57*
12·32***	9·57	14·43	10·47***	17·20	15·64	17·07	15·15*
9·83	9·33	13·24	12·48	18·09	16·78	17·66	16·47
13·26	8·70	13·54	13·54	17·86	17·86	16·17	14·68
ebony[11]							
MM		FF		MM		FF	
$+m$	mm	$+m$	mm	$+m$	mm	$+m$	mm
9·04	9·37	10·10	10·24	10·86	9·72	11·82	11·56
9·38	8·48	10·25	9·12	8·48	7·81	10·43	7·24**
10·20	9·56	11·62	8·87**	9·47	9·05	11·10	10·23
9·85	8·74	10·68	9·58	9·47	8·99	10·45	9·74
8·51	8·71	9·25	9·04	6·60	9·85	10·77	9·11
9·59	9·42	10·10	9·36	9·35	9·86	10·67	11·20
8·72	9·82	10·77	9·89	9·49	9·54	9·70	10·15
9·35	8·10	8·48	9·10	8·64	8·21	9·50	10·18
red				claret			
MM		FF		MM		FF	
$+m$	mm	$+m$	mm	$+m$	mm	$+m$	mm
17·81	20·34	15·82	15·60	14·23	13·29	13·62	12·22
18·93	18·14	15·21	14·82	12·80	11·58	12·71	11·01
16·42	14·98	14·47	15·34	13·80	13·61	13·73	13·56
18·45	16·05	16·53	15·12	13·19	13·17	12·82	14·27
19·59*	16·26	15·34	15·23	15·50	16·17	14·76	15·27
18·98***	15·49	14·48	14·42	13·70	14·47	13·27	13·89
19·02***	15·32	14·74	13·05	13·25*	15·11	13·61	13·62
17·04	16·82	15·79	14·59	13·33	12·20	12·25	12·28
17·48	17·31	15·22	14·77	14·60	14·04	13·03	12·52
17·58	17·78	15·72	15·85				

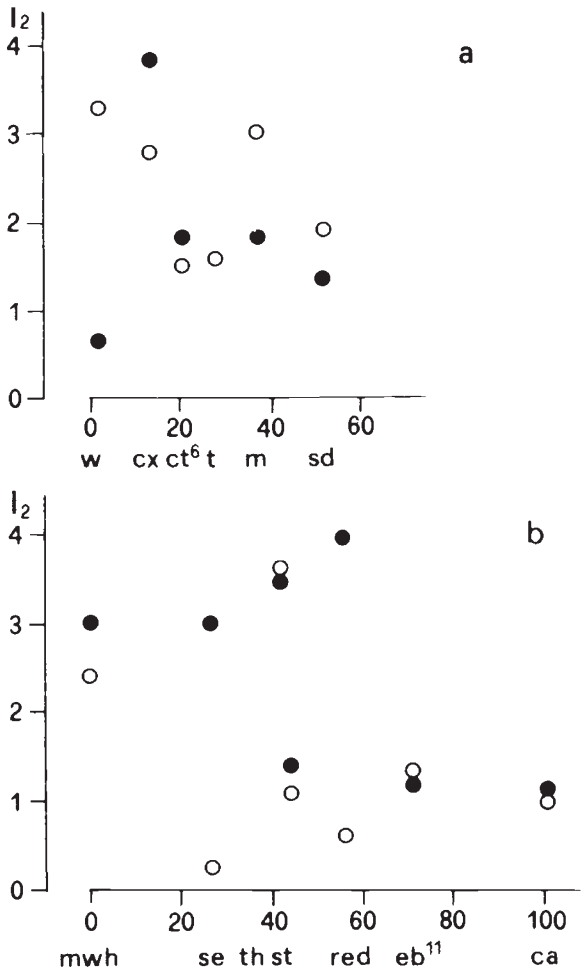

Figure 5 I_2 index values are shown according to the chromosome map separately for the two sexes (● males; ○ females). a, chromosome X; b, chromosome 3.

Considering the results of backcross experiments, the tendency towards higher locomotor activity shown by almost all of the $m/+$ heterozygotes, only for females in the case of sex linked markers, suggests that the chromosomal region carrying the genetic marker does affect locomotion. As a matter of fact, a common randomized genetic background is expected in the $m/+$ and m/m individuals of a sibship, so that the differences in the expression of the quantitative character are likely to be due either to the marker locus itself or to genes tightly linked to it. Unfortunately, the greater differences in the genetic background between $+/m$ and m/m controls prevented us from applying a correction for the effect of the marker locus of the type $(x_1 - x_2) - (x_{c1} - x_{c2})$—where x_1 and x_2 are the means of the two segregating classes and x_{c1} and x_{c2} are the means of the controls of the corresponding genotypes—suggested by the experimental scheme of fig. 2 (Jayakar *et al.*, 1977). According to Jayakar's model, only the whole set of comparisons for several sibships of a marker may be informative on the presence of linkage between this marker locus and a "quantitative" gene. The heterogeneity of the U-test values among sibships (tables 3(a) and 3(b)) for the different markers indicate that several genes influencing locomotor activity are present on both chromosomes. Jayakar's I_2 index has been applied as an estimate of the variability among sibships, since it reveals the presence of linkage from the variance among sibships of the difference of the trait means of the segregating classes within sibship. The I_2 values calculated for all markers have then been mapped along the two chromosomes. The I_2 profiles we have obtained for both chromosomes X and 3 are quite complex and once more suggest the presence of several genes affecting locomotor activity on both chromosomes. Furthermore, because the value of I_2 depends also on the parameters F_1 and F_2 which can differ in the different marker stocks, a univocal interpretation of the linkage map is not possible. An improvement in the experimental approach could be achieved using enzymatic or restriction fragment length polymorphisms rather than morphological markers. The overall I_2 picture suggests that there should be at least one locus significantly affecting adult locomotor activity near to each marker locus tested. It is not particularly surprising that a very complex quantitative trait as adult locomotor activity could be affected by at least a dozen major genes distributed on different chromosomes. As for which chromosomes are involved, our results are consistent with those of van Dijken *et al.* (1979) and Burnet *et al.* (1988). Our measure of locomotor activity does not discriminate between amount of locomotion and speed, but since Burnet *et al.* (1988) demonstrated significant effects of chromosome 3 on the former component and of chromosomes X and 2 on the latter, it is likely that some of the major genes affecting locomotor activity detected by our analysis influence specifically one of the above components.

Differences at the biochemical levels have been hypothesized to account for different levels of locomotor activity in strains of *D. melanogaster* selected for different values of this character. van Dijken *et al.* (1979) found differences in NADH dehydrogenase activity between high and low locomotor activity lines. Tuncliff *et al.* (1969) found

a significant influence on locomotion of dopamine and noradrenaline levels, implying that a control can be exerted by the balance existing between the two; on the contrary, he did not detect significant differences in serotonin levels and cholinesterase activity. More recently, Meehan and Wilson (1987) studying the dopamine-deficient *Tyr-1* mutant, and giving separate measures for different components of locomotor activity, demonstrated that *Tyr-1* flies have "normal" levels of spontaneous activity or reactivity but higher levels of "stimulated activity". It seems interesting to note that some of the morphological markers we used are linked to loci coding for enzymes playing a relevant role in the energetic metabolism of the flies and known to be polymorphic in the wild. This is the case of *w* linked to *Pgd*, of ct^6 linked to *Fum*, of *se* linked to *Idh*, of *th* and *st* linked to *Pgm*, of *red* linked to *Ace*, of *ca* linked to *Tpi*. Nevertheless whether or not the above biochemical loci are really involved in significantly influencing locomotor activity remains at the moment merely a fascinating hypothesis.

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