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The Genetics of Resistance to Long-term Exposure to CO_2 in *Drosophila melanogaster*; an Environmental Stress Leading to Anoxia

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Summary. Genetic heterogeneity in populations of *D. melanogaster* has been described for resistance to long-term exposure to CO_2 (4 to 5 hours). Crosses between inbred strains, and between strains set up from single inseminated females collected in the wild show the importance of additive genes. Genetic activity for resistance and sensitivity was found on the X, 2 and 3 chromosomes.

The mechanism of resistance was shown to be an anoxia effect since the effect of an N_2 atmosphere was the same as that of CO_2. A study of 18 strains collected in the wild revealed a positive correlation between metabolic rate as measured by O_2 uptake and mortality under CO_2, and negative correlations were found between body weight, and both mortality under CO_2 and metabolic rate. These results are consistent with an anoxia effect. A further variable correlated with body weight is resistance to desiccation. Thus the anoxia effect is correlated with factors determining the distribution of the species in the wild.

Introduction

From work done on the genetic architecture of resistance to acute stresses, chemical and otherwise, it is clear (see Parsons, 1973) that resistance in *Drosophila* populations is generally under additive genetic control, indicating traits peripheral to fitness under stabilizing selection (see Mather, 1966). Such stresses include D.D.T. (Crow, 1954, 1957), P.T.U. (phenylthiourea) (Deery and Parsons, 1972a), chloroform and ether (Deery and Parsons, 1972b), and resistance to acute levels of irradiation with ^{60}Co γ-rays (Parsons, MacBean and Lee, 1969; Westerman and Parsons, 1973). This probably applies to many other stresses not normally present in the environment. In addition to being additive, at least some of the genes involved are of fairly large effect, since it is not difficult to locate regions of chromosomes and even loci involved in the control of resistance (and sensitivity).

Mortalities can be assessed in various ways, namely percentage of deaths after the lapse of certain time periods after exposure to the stress, or by assessing mean longevities following the stress. Both approaches have been used but the latter is more tedious than the former. The latter approach was, however, used by Westerman and Parsons (1973) in a study of genetic architectures found after exposure to a range of doses of ^{60}Co γ-rays namely 40, 60, 80, 100 and 120 krads. The last two doses can be described as acute, as death occurred rapidly following exposure, most flies dying within 24 hours. For the other doses longevities are greater, and of course increase as the dose decreases. These doses can perhaps therefore be described a sub-acute. The acute doses show additive genetic control, whereas the sub-acute doses show additive effects to be relatively unimportant and indeed non-additive effects turn out to be relatively more important. An analogous result was found for data on flies exposed to the high temperature of 29.5 °C for 24 hours (Parsons, 1966), where longevity is reduced to that found for the 40 to 80 krad range. Acute temperature stresses have yet to be tested.

So far, therefore, the results indicate additive genetic control of resistance to extremely acute environmental stresses, but less additivity if the stresses are less acute. Longevity *per se*, as assessed in control flies, shows additive and non-additive variation in some, but not all cases, and in any event the effects are not of the same order of magnitude as found under ^{60}Co γ-irradiation. This is reasonable, as longevity is probably not subject either to very intense stabilizing selection which leads to additivity, or directional selection which leads to dominance effects in the direction of increased longevity.

In this paper, we will discuss a further stress, namely exposure to a CO_2 atmosphere over a number of hours. This differs from the situation described by L'Héritier and Teissier (1937), who found flies sensitive to even a thirty second exposure to CO_2, which is due to a cytoplasmic particle with virus-like properties. Such strains are common in *D. melanogaster* from Europe and some areas of South America at least (Kalmus, Kerridge and Tattersfield, 1954), but we have not detected any locally. The virus, referred to as sigma, can grow normally and produce sensitivity after inoculation into several other species of *Drosophila* but not other genera (L'Héritier, 1951, 1957). In *D. affinis* and *D. athabasca*, cyto-

plasmic CO_2 sensitivity seems to follow the *D. melanogaster* pattern (Williamson, 1961).

The preliminary result upon which this study was based comes from Matheson and Arnold (1973), who found variation among eighteen strains referred to as LM strains, set up from single inseminated females from a wild population at Leslie Manor, Victoria, after 6 hours of exposure to CO_2. This result indicates that the founder females differ genetically, and hence that the population in the wild is polymorphic for genes controlling the trait. This is so, not only for the other stress traits mentioned above, but also for a number of other morphological and behavioural traits (Parsons, Hosgood and Lee, 1967). Like the other stress traits referred to, CO_2 is rarely, if ever, encountered in the environment at the concentrations used, even though it is ubiquitious in the biosphere in low concentrations. Six hours of exposure to CO_2 does, however, form an acute stress as defined above with major mortality occurring within 24 hours of exposure.

Method

Flies were exposed to CO_2 by placing them in a plastic desiccator which was then flushed with CO_2 for one minute before being sealed for $4^1/_2$ hours. Variations from this time will be noted where necessary. In order to control humidity, wet filter paper was placed in the bottom of the desiccator. In any case, mortality effects due to desiccation with a really effective desiccant only begin after fourteen to sixteen hours exposure (Parsons, 1970), and so the possibility of desiccation effects was regarded as unimportant in this study. For treatment, flies were shaken into $2'' \times 1''$ glass vials whose open ends were then covered with cheesecloth secured with a rubber band. After treatment, flies were placed into vials with food, and mortality scored after twenty four hours. This technique differs from that used by Matheson and Arnold (1973), who exposed flies in a plastic gas chamber with CO_2 bubbling through water continuously into the chamber for $5^1/_2$ to 6 hours. However, both techniques gave comparable results.

Experiments were carried out two to four days after eclosion, since a preliminary experiment showed a marked increase in mortality with age, but over the two to four day period there was little change. It is an any case reasonable to control ageing, since it is known that considerable changes in metabolic rates occur with age in flies; older flies generally having lower metabolic rates than younger flies. In fact Lints and Lints (1969) observed that maximal respiration occurred between five and ten days after eclosion and declined steadily thereafter, until at fifty days oxygen consumption was one or two sevenths of the maximal rate. Since a stress trait such as CO_2 must interfere with metabolic processes, age is a variable that must be standardized for genetic analyses.

Flies were tested at 25 °C except for one experiment at 20 °C.

The results were scored as percentage mortalities. The angular transformation was applied before analyses to avoid a dependence of the variance on the mean.

In general in the interests of space, data for female mortalities only will be presented. With few exceptions the male mortality data gave a similar picture, however overall mortality in males was slightly greater than in females, as is usual for stress traits.

Table 1a. *Mean mortalities of females after exposure to CO_2 based on three replicates. The data are for four inbred strains and the hybrids between them*

		Male parent			
		Y_1	Y_4	N_4	OR
Female parent	Y_1	64.44	39.32	47.58	57.26
	Y_4	49.93	78.80	79.18	71.61
	N_4	46.79	84.00	65.03	57.16
	OR	58.19	72.48	77.11	74.97

Table 1b. *Combining ability analysis of hybrid female mortalities in Table 1a*

Source of variation	d.f.	m.s.	F
General combining ability	3	566.11	11.18***
Specific combining ability	2	204.43	4.04*
Reciprocal	6	44.54	0.88
Error	64	50.66	

* $P < 0.05$; *** $P < 0.001$

Results

a. *Inbred Strains and Hybrids*

Four inbred strains that had been sib-mated for at least three hundred generations were crossed in all possible combinations. Mean mortalities over three replicates are given in Table 1 arranged as a 4×4 diallel cross. The data were analysed using Griffing's (1956) model omitting the inbred strains themselves, since they differ in genetic architecture from the hybrids. A large significant general combining ability effect was found which indicates additive genetic differences between strains. The specific combining ability was smaller but significant at the 5% level indicating some dominance effects, but even so, additive genetic control is much more important. The lack of significance of reciprocal effects indicates the lack of importance of maternal of cytoplasmic effects, in contract with the cytoplasmic CO_2 sensitivity discussed in the introduction.

b. *Strains set up from Single Inseminated Females Collected in the Wild*

Four strains set up from single inseminated females collected at Leslie Manor (LM strains) which based on Matheson and Arnold's (1973) results were extreme, two being sensitive and two resistant, were crossed in all possible combinations in two replicates to give a 4×4 diallel cross (Table 2). (*Note*: data analogous to the original data of Matheson and Arnold appear in Table 6, and an analysis of variance in Table 7b shows clear differences between strains.) In this case the model of Griffing (1956) including the strains themselves was used, as there is no reason to suspect that the genetic architectures of the strains themselves should differ greatly from the genetic architectures of crosses between strains.

Table 2a. *Mean mortalities of females based on two replicates. The data are for four LM strains and crosses between them*

		Male parent			
		LM22	LM29	LM34	LM3
Female parent	LM22	86.49	84.77	70.83	79.12
	LM29	80.53	55.90	74.33	48.36
	LM34	63.00	54.90	59.63	54.95
	LM3	76.87	54.85	56.61	53.50

Table 2b. *Combining ability analysis of female mortalities in Table 2a*

Source of variation	d.f.	m.s.	F
General combining ability	3	2338.37	19.77***
Specific combining ability	6	290.09	2.45
Reciprocal	6	336.78	2.84*
Error	15	118.26	

* $P < 0.05$; *** $P < 0.501$

The general combining ability was large and significant, again indicating additive differences between strains. The specific combining ability was not significant indicating the lack of importance of interactions in the determination of the trait. Reciprocal effects were just significant at the 5% level, but this isolated result cannot be taken as any indication of reciprocal effects of general importance. The high level of additive genetic control in the two experiments just described points to the likelihood of fairly readily finding out more about the genetic architecture of resistance to CO_2.

c. *Chromosomal Analyses*

General. The first step was to examine briefly the situation at the chromosomal level by attempting to locate genetic activity to chromosomes using the technique of Kearsey and Kojima (1967). This technique consists of taking two strains A and B, and by the use of a triple marker stock marking each of the three major chromosomes, eight true breeding substitution lines AAA, AAB, ABA, ABB, BAA, BAB, BBA and BBB can be built up where the sequence of the letters corresponds to the source of the X, 2 and 3 chromosomes. Two pairs of strain contrasts were carried out, namely strains LM22 and LM34, and strains LM29 and LM3 (Table 3); the former strain in each pair being sensitive and the latter resistant. As can be seen in Table 3, significant additive differences occurred between strains LM22 and LM34 for chromosomes X and 2, and there was a significant dominance effect in chromosome 2. The other pair only gave a dominance effect for the X, however, the male data (not presented) gave additive effects for the X and 2 chromosomes. These preliminary results taken with the diallel cross data, although not conclusive, at least confirmed the need to look at each of the major chromosomes in detail in various strains.

Detailed intrachromosomal analyses. Experiments were designed to test for the effects of different regions of the three major chromosomes on CO_2 resistance. The tester stock was crossed to a stock marked with three or four recessive mutants on a given chromosome, and the F_1 females backcrossed to the marker stock, so giving a number of progeny genotypes. Analyses of variance, splitting the strain effects into 1 d.f. components indicate the effects of the regions of chromosomes marked by given mutants.

A summary of results for four LM strains and two inbred strains is given in Table 4 for the X chromosome using the marker stock *sc cv v f*, the four loci being at 0, 13.7, 33.0, and 56.0 respectively. Different regions of significant sensitivity were found in three strains indicating the likelihood of a number of genes controlling CO_2 resistance.

For the chromosome 2 analysis the stock *al cn bw* was used, the loci being at 0, 57.5 and 104.5 respectively, and the F values obtained are given in Table 5a, showing mainly resistant regions in contrast with the X chromosome, especially in the *al cn* region of the chromosome.

For the chromosome 3 analysis the stock *ru st ca* was used, the loci being at 0, 44.0 and 100.7 respectively, and the F values are given in Table 5b, showing mainly resistant effects especially for the *ca* region. The *ru* region shows sensitivity in one strain and resistance in another

Table 3. *F values for female data from analyses of variance of percentage mortalities for two pairs of strains LM22 and LM34, and LM29 and LM3 using the chromosome assay technique of Kearsey and Kojima (1967). All F values are for one degree of freedom, and entries are made in the table only where $P < 0.05$*

Source of variation	Chromosome	Strain contrast	
		22−34 (over 6 replicates)	29−3[a] (over 3 replicates)
Additive (a)	X	7.41**	—
	2	8.93**	—
	3	—	—
Dominance (d)	X	—	8.45**
	2	9.35**	—
	3	—	—
d × d[b]	2 × 3	3.99*	

* $P < 0.05$, ** $P < 0.01$

[a] Male data show additive effects significant at $P < 0.001$ for the X and 2 chromosomes

[b] All other interactions are insignificant.

Table 4. *F values for female data from analyses of variance of percentage mortalities for four LM strains, and two inbred strains to determine the regional effects of the X chromosome. All F values are for one degree of freedom, and entries are made in the table only for $P < 0.05$*

Region of chromosome	LM strain[a]				Inbred strain[b, c]	
	LM22	LM23	LM25	LM34	Y_2	N_4
sc	—	—	—	—	—	—
cv	—	—	6.71***(−)	—		
v	—	5.86* (−)	—	—	—	—
f	—	—	—	—	—	6.85* (−)
sc × *cv*[d]	5.16* (+)	—	—	—		

* $P < 0.05$; *** $P < 0.001$; + signifies resistance; − signifies sensitivity.

a Two of the LM strains in Tables 4 and 5 differ from those in Tables 2 and 3, but extreme strains were chosen in all cases.

b Some of the inbred strains used differ from Tables 1 and 5 because of difficulties in breeding inbred strains at various times.

c For these tests the *cv* region was ignored because of insufficient flies.

d All other interactions were insignificant.

Table 5. *F values for female data from analyses of variance of percentage mortalities for four LM strains[a] and three inbred strains[a] to determine the regional effects of chromosomes 2 and 3. All F values are for one degree of freedom, and entries are made in the table only for $P < 0.05$*

a. Chromosome 2

Region of chromosome	LM22	LM23	LM25	LM34	Y_1	Y_2	N_4
al	—	—	—	6.02* (+)	7.35***(+)	6.98* (+)	30.30***(+)
cn	—	14.35***(+)	13.43**(+)	21.56** (+)	5.95** (+)	8.93** (+)	—
bw	—	—	—	—	—	—	—
al × *cn*[b]	—	—	—	6.02* (+)	—	11.18***(+)	6.33* (+)
cn × *bw*[b]	—	—	—	—	—	—	6.14* (+)
al × *cn* × *bw*[b]	—	—	—	—	—	—	9.23** (+)

b. Chromosome 3[c]

ru	—	4.38* (+)	—	—	5.49* (−)	—	—
st	—	—	—	—	—	—	—
ca	5.73*(+)	12.16***(+)	—	12.96***(+)	—	22.96***(+)	—

+ signifies resistance; − signifies sensitivity; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

a See footnotes a and b to Table 4.

b The *al* × *bw* interaction was not significant.

c No significant interactions were found.

with no significant effects associated with the *st* region.

Finally, an analysis of the very short chromosome 4, using markers *spa*, *ci* and *sv*^{n}, revealed no significant effects.

The conclusion to be drawn is rather similar to Deery and Parsons' (1972b) analysis of ether resistance, in that there are indications of genes controlling variations in resistance and sensitivity in various parts of the three major chromosomes, and that the various strains differ between each other in regard to regions of resistance or sensitivity. The results are therefore consistent with Mather's (1942, 1943) postulate that different strains should carry combinations of genes producing a net effect which is relatively intermediate. The present results show variations in specific chromosomal regions, which suggest that there are not a multiplicity of polygenes each having a small effect on CO_2 resistance scattered throughout the genome. Rather the situation is one of genes or gene complexes with differing but reasonably large additive effects. Even so, the impression is that areas of CO_2 resistance and sensitivity are not as clear cut as for more specific chemical stresses such as D.D.T. and ether. It is of interest that the sensitive regions are mainly on the X chromosome and the resistant regions on chromosomes 2 and 3, but more data on more strains are needed for generalizations, especially as somewhat different strains were used in different experiments (see footnotes to Table 4).

d. *The Mechanism of Resistance*

From studies on various insects there has been some controversy as to whether the anaesthetic effect of CO_2 is specific to CO_2, or whether it is just a gener-

alized anoxia effect (Edwards and Patton, 1965; Brooks, 1965). During anoxia, certain changes take place in metabolism which normally result in the building up of an oxygen debt (Gilmour, 1965), which occurs by switching to metabolic pathways which are not oxygen dependent but are usually less efficient in energy production enabling survival for a time. The survival time depends on the energy requirements of particular organs in the individual concerned, and these may be measured in a general way as the overall metabolic rate before anoxia. The higher this is, the greater the energy requirement during anoxia and the shorter the length of time that the organism would be expected to survive. Assuming an anoxia effect, it would be expected that exposure to the inert gas N_2 should mimic CO_2, whereas if CO_2 acted as a direct poison this would not necessarily be so. Figs. 1a and 1b give percentage mortalities of females after exposure to various doses of CO_2 and N_2 for four LM strains, from which it can be seen that regressions on time are significant, and furthermore that the equations of the regression lines

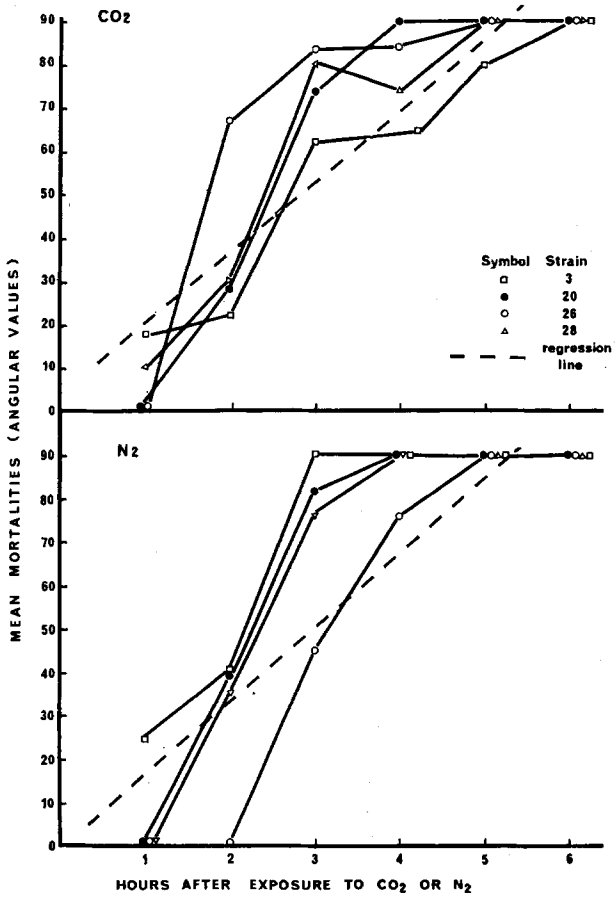

Fig. 1. Mean female mortalities (angular values) after exposure to periods of CO_2 and N_2 for periods up to 6 hours for strains LM3, 20, 26 and 28. The equation of the overall regression line for CO_2 is $y = 62.57 + 16.19\,(x - \bar{x})$, and for N_2 $y = 61.86 + 17.49\,(x - \bar{x})$

are very similar. The regression coefficients in fact do not differ significantly. This would lend support to the anoxia hypothesis.

The similarity of effects of CO_2 and N_2 is given further support by a test of mortalities of 15LM strains to CO_2 and N_2 respectively. Correlation coefficients between the mortalities to the two gases, strain by strain, were +0.623 in females and +0.744 in males, the former being significantly different from 0 at the 5% level and the latter at the 1% level. Therefore over a series of strains considerable similarities were found.

A further confirmatory experiment on four strains, LM3, LM22, LM29, and LM34, was carried out by exposing the strains to CO_2 and N_2 for a total of 5 hours as follows:

1. 5 hours in CO_2 only
2. 1 hour in CO_2 then 4 hours in N_2
3. 2 hours in CO_2 then 3 hours in N_2
4. 3 hours in CO_2 then 2 hours in N_2
5. 4 hours in CO_2 then 1 hour in N_2
6. 5 hours in CO_2 only.

No differences in treatments were observed, since 5 hours of exposure lead essentially to 100% mortality in all cases as might be expected, since most tests leading to differential mortalities were carried out for $4^1/_2$ hours of exposure.

Therefore the anoxia effect seems more likely than a specific poisoning effect. If this is so, then there should be a relationship between metabolic rate and mortality. This can be looked at first by considering fly sizes since this may be important in determining the rate of penetration of CO_2. Further, metabolic rate and body weight are related approximately by the equation:

$$\text{Metabolic rate} = k\,W^b,$$

where k = constant, W = body weight and $b = 0.73$ for ectotherms including insects (Prosser and Brown, 1961). Any genetic differences in body weight should then affect metabolic rate, and as a consequence resistance to anoxia, so that if CO_2 kills by anoxia, then mortality should be related to body weight.

A direct way of assessing metabolic rate is to measure oxygen uptake in a Warburg respirometer according to the method outlined in Umbreit, Burris and Stauffer (1964). In Table 6, mean body weights (of 10 flies per sex per strain) and mean mortalities are given for flies grown at a density of 25 larvae per vial, as well as metabolic rates expressed as microlitres of oxygen consumed per hour per milligram of body weight grown at two larval densities, 25 and 50 per vial. Data for both sexes are presented for 18 LM strains. Analyses of variance of these data are given in Table 7a, b and c. In all cases a sexes effect occurs as would be expected. The strains effects are highly significant for mean body weights and CO_2 mortalities, but only border

Table 6. *Mean body weights, CO_2 mortalities, and metabolic rates of 18 LM strains*

Strain	Mean body weights (mgm) (based on 10 flies)		CO_2 mortalities (%)		Metabolic rates — expressed as microlitres of O_2 consumed per hour per mg body weight.			
					25 larvae per vial		50 larvae per vial	
	Females	Males	Females	Males	Females	Males	Females	Males
1	1.02	0.76	100	100	0.50	0.62	0.36	0.55
2	1.44	0.94	42	96	0.26	0.38	0.27	0.35
3	1.44	0.97	84	89	0.52	0.42	0.25	0.38
20	1.43	0.85	86	100	0.44	0.42	0.39	0.34
21	1.48	0.87	39	82	0.39	0.33	0.68	0.38
22	1.26	0.83	97	94	0.63	0.45	0.68	0.45
23	1.51	0.94	12	47	0.43	0.36	0.60	0.25
24	1.08	0.73	79	100	0.61	0.36	0.54	0.36
25	1.48	0.84	93	100	0.51	0.54	0.82	0.32
26	1.48	0.81	88	100	0.49	0.43	0.50	0.36
27	1.65	0.88	48	84	0.41	0.55	0.60	0.45
28	1.47	0.90	62	100	0.47	0.35	0.47	0.28
29	1.45	0.87	57	97	0.45	0.57	0.40	0.58
30	1.06	0.75	54	100	0.65	0.51	0.56	0.44
31	1.65	0.95	58	93	0.44	0.36	0.62	0.54
32	1.58	0.97	91	100	0.34	0.33	0.56	0.38
33	1.49	0.90	58	65	0.50	0.37	0.51	0.60
34	1.42	0.84	54	84	0.42	0.34	0.51	0.28

Table 7. *Analyses of variance of the data in Table 6*

a. Mean body weight

Source of variation	d.f.	m.s.	F
Strains	17	0.20	14.13***
Sexes	1	25.26	1804.45***
Strains × Sexes	17	0.24	16.95***
Error	324	0.01	

b. CO_2 mortalities

Strains	17	757.39	7.51***
Sexes	1	5673.26	26.27***
Replicates	1	180.88	1.79
Strains × Replicates	17	212.67	2.11*
Error	35	100.82	

c. Metabolic rates

Strains	17	0.0178	1.60+
Sexes	1	0.1139	10.26***
Larval density	1	0.0028	0.25
Error	52	0.0111	

d. Correlation coefficients over 18 strains (the probabilities refer to the significance of deviations from 0)

	Females	Males
Body weight — CO_2 mortality	−0.52*	−0.52*
Body weight — metabolic rate[a]	−0.43+	−0.66**
CO_2 mortality — metabolic rate[a]	+0.48*	+0.62**

+ $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] Based on mean values over the two competition levels used.

on significance for metabolic rates. These results show genetic heterogeneity for these traits in natural populations as expected (see introduction).

Turning to the correlations between the traits (Table 7d), there is a positive correlation between metabolic rates and CO_2 mortalities as predicted for an anoxia effect, and negative correlations occur between body weights and both CO_2 mortalities and metabolic rates as also is predicted. It is clear that the evidence favours an anoxia effect.

A final point in confirmation comes from testing flies with CO_2 or N_2 at 20 °C and 25 °C, since it is well-known that metabolic rates of insects increase over the non-lethal range (Prosser and Brown, 1961), and this has been specifically demonstrated in *D. melanogaster* (Hunter, 1964). Therefore at 25 °C the anoxia effect would be expected to be more severe. The mortality was greater at 25 °C than 20 °C for four LM strains (Table 8a) for exposure to both gases for 4 hours, and again there are no overall differences between gases (Table 8b) as predicted.

Discussion

The genetic basis of CO_2 resistance is in good agreement with other chemical stresses in showing a high level of additive genetic control as discussed in the introduction. However, CO_2 is a stress of a more general kind than chemicals such as ether, D.D.T. and P.T.U., which presumably act by interacting in various ways with specific metabolic pathways (see Deery and Parsons, 1972a, b). Because of this, it seems likely that obtaining a detailed overall idea of the genetic architecture of CO_2 resistance will not be easy. Even so, regions of chromosomes involved in CO_2 resistance were easily located. Furthermore, genes have been located for body size (Thoday, Gibson and Spickett, 1963)

Table 8a. *Mean mortalities (over 4 replicates) in four LM strains after treatment with CO_2 or N_2 at either 20 °C or 25 °C*

Gas	CO_2				N_2			
Temperature	25 °C		20 °C		25 °C		20 °C	
Strain	Females	Males	Females	Males	Females	Males	Females	Males
LM3	90.00	89.05	49.11	90.00	82.66	90.00	68.53	83.52
LM22	75.74	79.91	58.18	76.51	77.56	75.56	65.06	90.00
LM29	66.09	83.84	25.52	58.76	77.10	90.00	44.29	62.70
LM34	72.25	83.44	60.04	61.42	65.89	73.92	64.39	78.75

b. *Analyses of variance of the data in Table 8a*

Source of variation	Females			Males		
	d.f.	m.s.	F	d.f.	m.s.	F
Strains	3	1135.21	3.42*	3	212.40	1.22
Temperatures	1	7412.13	22.34***	1	2045.30	11.73**
Gases	1	589.21	1.78	1	300.53	1.73
Strains × Temperatures	—			3	710.74	4.07*
Temperatures × Gases	—			1	1340.66	7.69**
Error	58	331.77		54	174.43	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

which are largely additive in effect. Presumably there will be a high level of correlation between the genetic architecture of body size and the anoxia effect as indicated by the high correlations between the two traits in the strains under study. The situation may not, of course, be quite as simple as this, since there may be mechanisms affecting anoxia independent of body weight.

CO_2 resistance, therefore, like body size is largely under stabilizing selection. CO_2 is normally found in small concentrations outside the body, and in higher concentrations inside the body. In any aerobically respiring organism, CO_2 is produced in respiring tissues, and is transported to specialized organs for release to the atmosphere. Where the atmosphere consists entirely of CO_2, as in the experimental conditions, transport will take place in the reverse direction. Under normal conditions this would not occur in *Drosophila*, as CO_2 is not found in such concentrations in the natural environment. Variability in resistance to CO_2 (and N_2) must therefore be related to other factors, and body size is clearly one. Therefore in this case the observation that the population is polymorphic for anoxia, a stress not encountered in nature, is not surprising, considering the association with body weight.

It is thus worth considering body weight and its possible relationship with other environmental stresses. Parsons (1970) showed that there is genetic variability in natural populations for the ability to tolerate desiccation as measured by mortality over 16 hours. He found a high correlation between body wet and dry weights, with resistance to desiccation. Desiccation is, of course, a stress to which natural populations are exposed in the wild for which variability might be expected in natural populations.

Using the same genetic approach as described in this paper, Deery and Parsons (1972b) studied resistance to ether as measured by mortalities under standardised experimental conditions. Additive genetic control was found, and some chromosomal regions for resistance and sensitivity detected, but for this trait no association with body weight was detected. Similarly, there seemed to be no association with body weight for chloroform resistance, which in itself showed no association with ether resistance. This latter observation is not surprising since the two anaesthetics are chemically different and therefore may be expected to interfere with different metabolic pathways. Traits such as ether and chloroform resistance, being more specific chemical stresses than CO_2, are worthy of detailed study from the genetic architecture point of view because of the possibility of extrapolating any general principles found to chemical stresses found in the environment such as insecticides. The additive genetic architecture found for many chemical stresses including insecticides has implications for insect control, since the application of the stress is a form of directional selection favouring the survival of the carriers of resistant genes. Since these genes are fairly large in effect and additive, rapid responses to selection would be expected to specific chemical stresses, as has been found for D.D.T. (Crow, 1954; King and Sømme, 1958). Thus it is not surprising that the rapid development of insecticide-resistant strains occurs in nature. The more generalized the stress (and the anoxia effect fits this category), the less rapid would be

any response to selection, because the genetic architecture of such a trait is likely to be more complex, since many more physiological processes are involved.

Generalized stresses are not therefore likely to be of any significance in insect control as compared with specific stresses. On the other hand the likelihood is that genetic heterogeneity for a generalized stress may reflect something of ecological or physiological significance. The anoxia effect is of ecological significance in being associated with body weight, and most importantly, resistance to desiccation. Variations in resistance to anoxia are therefore associated with factors determining the distribution and survival of the species in different environments.

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