fraglichen Kröte aus dem Variationsbereich beider Arten gegeben. Das Pherogramm erscheint für die Globuline eines Wechselkröten-Kreuzkröten-Bastards hingegen weniger kennzeichnend. Infolge der saisonabhängigen quantitativen Veränderungen kann sich in diesem Bereich die bei Vorhandensein der Eiweissbanden beider Elternarten (entsprechend der Situation bei Fischbastarden[10]) resultierende Bastardkurve in stärkerem Masse verschieben; weiterhin treten in der für eine natürliche Bastardierung in Frage kommenden, hauptsächlich mitteleuropäischen Überschneidunszone der Verbreitungsgebiete beider Arten bei *Bufo calamita* zwei verschiedene Globulinmuster auf (Figuren 2 und 4). Mit diesen Befunden lässt sich die von den Verfassern bereits früher[11] ausgesprochene Vermutung bestätigen, dass es sich bei in einer Population bei Gensingen häufig gefundenen Wechselkröten mit Rückenband nicht um F1-Bastarde handelt. Als solche angesprochene Kröten[9] aus Freilandpopulationen waren nach der Erarbeitung des hier mitgeteilten Befundes nicht in lebendem Zustand zur Überprüfung mit dieser Methode verfügbar.

Die Blutentnahme für derartige Bestimmungen ist in für die angewandte Methode der Serumgewinnung[12,13] voll ausreichender Menge ohne dauerhafte Schädigung der Kröten aus der Vena angularis möglich[14]. Das Serumeiweissbild kann daher ohne Schwierigkeit auch zur Diagnose von solchen Individuen Verwendung finden, deren weitere Lebendhaltung von Interesse ist.

Summary. Electrophoretic investigations of the serum protein patterns of natterjacks (*Bufo calamita* Laur.) from the Iberian Peninsula and from Western and Central Europe, and of green toads (*Bufo viridis* Laur.) from Central Europa, Asia Minor and North Africa show that the two species differ disctinctly in the position of their albumin fractions. The identification of problematic specimens can result from the production of mixed serum with specimens of both species. As the albumin fraction of hybrids is doubled according to this method, it is also suitable for demonstrating the existence of such hybrids in natural populations.

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Resistance to Carbon Dioxide, an Anoxic Stress in *Drosophila melanogaster*

Variation in response to carbon dioxide in *D. melanogaster* was first reported by L'HERITIER and TEISSIER[1] who discovered sharp segregations between strains which were capable of surviving short exposure to CO_2, and those which were not. Further work[2] showed that this variation was caused by a virus called sigma which was passed between generations in the cytoplasm of the eggs and sperm. Individuals carrying the virus are incapable of withstanding even a 30-sec exposure to CO_2. Populations of *D. melanogaster* carrying sigma seem to be widespread[3], and the virus has been discovered in at least two other species[4].

In the present work variation in response to longer exposure to CO_2 is reported. 18 strains derived from single inseminated female *D. melanogaster* collected at Leslie Manor near Camperdown in Victoria in December 1963 and December 1964 were tested for their mortality levels after exposure to CO_2. There was no significant mortality after 15 min exposure during a pilot trial which indicated that a 6 h exposure allowed adequate discrimination between strains for mortality. A plastic gas chamber was used for administration of CO_2 which bubbled through water, passed into the chamber, and passed out again through small holes drilled in the lid. For this experiment, the 6 replicates involved were tested separately. Mortalities were scored as percentages 1 day later, and were transformed using the angular transformation for analysis. This transformation is commonly used for fractional, proportional or percentage data which typically are distributed binomially, and whose variance depends on the magnitude of the proportion, and on the size of the sample[5].

The analysis of variance showed highly significant ($P < 0.005$) differences between strains, both for males and for females. Table Ia) shows that the mortalities among strains, far from being sharp and discontinuous, as in L'HERITIER and TEISSIER's[1] experiments, show a continuous distribution.

A diallel cross was undertaken to analyse in more detail the genetic basis of differences and in particular to test for maternal effects, a characteristic of cytoplasmic inheritance[6]. 4 strains (22, 29, 34 and 3) were selected to represent the range of mortalities. 2 collections of F_1 progeny were made and tested separately for 6 h. Resulting female mortalities (Table IIa) were analysed using GRIFFING's[7,8] Method 1 Model No. 1 combining ability analysis (Table IIb). Male data are not given as they add no further information, and the analysis is essentially similar. Significant reciprocal effects were found, but were probably due solely to the strain pair 34–29 whose reciprocal F_1s were different. There were no systematic reciprocal differences, and hence no evidence of maternal effects or cytoplasmic inheritance.

The highly significant general combining ability effects indicate the presence of additive genetic variation among the strains used in the diallel. This implies the existence of such variation among all the L.M. strains, which in turn implies genetic polymorphism in the original popula-

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tion at Leslie Manor. Such a result is not unexpected in view of MATHER's[9] predictions and more recent results on quantitative characters in populations[10–13].

The nature of the stress was investigated by comparison with mortality after exposure to nitrogen. In this case, because nitrogen is slightly less dense than air, a desiccator flushed with nitrogen and then sealed was used for nitrogen administration. Mortalities after exposure were compared strain by strain over 15 of the 18 strains mentioned in Table I. The correlation coefficient between the 2 stresses for females was $+ 0.6230$ ($P < 0.02$) and for males $+ 0.7442$ ($P < 0.01$).

A comparable result indicating similarity of effect between CO_2 and N_2 was found in another experiment. Mortalities following sequences of treatment times in one gas followed by the other, adding to a total of 5 h (minimum exposure for 100% mortality in CO_2 using the desiccator) in each case, were compared with mortalities in differing periods of time up to 5 h in one or other gas separately. For this experiment the desiccator method of administration was used not only for nitrogen, but also for CO_2. Regression coefficients for mortalities in CO_2 and N_2 on length of exposure to one gas on its own were 24.93 and 24.22, both of which differ significantly from O as $P < 0.001$. These regression coefficients were not significantly different from each other ($P > 0.90$), indicating similarity of effect. However, where the single or sequential treatments covered a total of 5 h, there were no mortalities less than 100%. This indicates that the effect of the first period (in CO_2) was added to that of the second (in N_2), and hence that the 2 gases affected the same system. The possibility of hyperadditivity is not considered likely as this action of nitrogen is mechanical.

D. melanogaster responds similarly in both CO_2 and N_2 atmospheres. This is reason enough to suppose that the effect of CO_2 when administered over a prolonged period is that of an anoxic gas. It is not possible at this stage to determine which organs by their early loss of function under CO_2 are responsible for death. It is concluded that CO_2 acts mainly as an anoxic gas when administered over prolonged periods, and that populations of *D. melanogaster* are genetically polymorphic for resistance to it.

Table I a) Mean mortalities of Leslie Manor strains of *D. melanogaster* after 6 h exposure to carbon dioxide

Female strain	Mortality (%)	Male strain	Mortality (%)
23	4.90	34	16.75
3	11.87	2	27.43
32	15.43	21	28.45
29	15.51	3	29.37
21	15.56	23	34.50
34	16.11	20	36.75
2	18.02	1	38.02
24	21.63	29	40.19
28	24.60	28	40.82
31	25.22	32	47.08
33	29.31	31	51.41
30	32.49	24	51.73
1	32.58	33	52.16
27	34.50	22	54.34
22	39.21	26	56.46
20	43.50	30	63.01
25	48.34	27	66.22
26	56.46	25	69.53

b) Analyses of variance of mortalities in Table Ia.

Source of variation	D.F.	Female M.S.	Female F	Male M.S.	Male F
Replicates	5	1,927.51	20.59[a]	242.58	1.94
Strains	17	1,238.22	13.23[a]	1,087.33	8.71[a]
Error	84	93.60		124.78	

[a] Significant at 0.001 level.

Table II. a) Mean female mortalities of diallel cross using 4 Leslie Manor strains (after application of the angular transformation)

Female parent	Male parent 22	29	34	3
22	86.49	84.77	70.83	79.12
29	80.53	55.90	74.33	48.36
34	63.00	54.90	59.63	54.95
3	76.87	54.85	56.61	53.50

b) Combining ability diallel analysis of percentage mortalities (after application of the angular transformation)

Source of variation	D. F.	Mean square	F
GCA	3	2338.3703	19.77[b]
SCA	6	290.0940	2.45[c]
Reciprocal	6	336.7805	2.84[a]
Error	15	118.2557	

Significance: [a] (5%) [b] (0.1%) [c] N.S.
GCA = General Combining Ability, SCA = Specific Combining Ability.

Résumé. Des lignées de *D. melanogaster* formées à partir d'une femelle fécondée naturellement, sont polymorphiques par raport au degré de mortalité résultant de leur maintient prolongé dans une atmosphère de CO_2. Il est établi que ces différences proviennent de variations dans les gènes additifs. On observe que la réaction au CO_2 est presque identique à celle que provoque le N_2, un gaz biologiquement inerte, causant l'asphyxie. L'auteur conclut que le CO_2 entraîne l'asphyxie lorsqu'on l'administre durant des périodes prolongées et que les populations naturelles de *D. melanogaster* sont polymorphiques quant à leur résistance à l'asphyxie.

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