within a few cell generations. In certain rare instances, they may be retained even after many divisions where a breakage-fusion-bridge cycle is in operation[13,14], or in those cases in which 1 of the 2 centromeres assumed dominant centromeric activity and the other remained suppressed during anaphase movement, as reported by Sears and Camara in *Triticum*[3] and, more recently by Niebuhr in the human[5]. However, in our material it is difficult at present to suggest accurately what is the exact mode of separation and the mechanism of migration of the dicentric chromosome during anaphase stage of cell division. It is well known that the mouse cell material is not at all a suitable tool for anaphase study. Moreover, due to hypotetraploid condition, it becomes more difficult to obtain a clear view of individual chromosomes during anaphase separation. Since no variation in the lenght of the dicentric chromosome has been noticed even after successive in vivo passages of the tumour (figures 6–8), it may be assumed that McClintock's breakage-fusion-bridge cycle is not in action in this particular cell-line. This idea is further strenghtened by the fact that no dicentric bridge has been encountered in anaphase stages studied so far.

In their material, both Sears and Camara[3] and Niebuhr[5] have noted an unequal size of centromeres and assumed that during division 1 of the 2 centromeres remained attached with the spindle fibre and the other remained inactive. But in contrast to this finding in our material we have noticed 2 almost equal sized centromeres (figures 6–8). However, in PMG stained late anaphases of MS-180 the existence of 2 protruded chromosomal elements on either side of the separating cells led us to suggest that probably in this case also 1 of the 2 centromeres have taken dominant activity and the other remained suppressed (figure 4).

Again an isolated peripheral placement of the dicentric in C-metaphases and a peculiar protruded orientation in spontaneous metaphases have produced another confusing situation. Several workers have reported the occurrence of nuclear projections in the interphase nuclei of various tumours in association with long chromosome markers[15,16]. However, in MS-180 cells such protrusions are visible only in well-flattened spontaneous metaphases and in late anaphases (figure 4) in relation to the dicentric marker. In interphase stage, on the other hand, no such nuclear projection has been recorded. The occurrence of the unusual dicentric element in most of the C-metaphases, and the detection of several spontaneous metaphases with protruded chromosomes corresponding to the dicentrics, is in support of the view that abnormal dicentric markers encounter an apparently normal division during mitotic process; but their behaviour during anaphase and in other stages of cell division still requires further elucidation.

1 Grateful acknowledgment is made to Dr N. Chatterji, CNCRC, Calcutta, for supplying the cell-line to the first author. Sincere thanks are due to Prof. Prasanta Ghosh, Hooghly Mohsin College and to Dr A.K. Roy, Department of Zoology of the same college for constant encouragement.

2 B. McClintock, Cold Spring Harb. Symp. quant. Biol. *16*, 13 (1951).
3 E.R. Sears and A. Camara, Genetics *37*, 125 (1952).
4 R. Angell, F. Giannelli and P.E. Polani, Ann. human Genet. *34*, 39 (1970).
5 E. Niebuhr, Humangenetik *16*, 217 (1972b).
6 D. Warburton, A.S. Handerson, L.R. Shapiro and L.Y.F. Hsu, Am. J. human Genet. *25*, 439 (1973b).
7 A. de la Chapelle and K. Stenstrand, Hereditas *76*, 259 (1974).
8 M.W. Shaw and T.R. Chen, in: Chromosomes and Cancer, p. 135. Ed. J. German. John Wiley, New York 1974.
9 S. Chakrabarti and A. Chakrabarti, Experientia *33*, 1296 (1977).
10 S. Chakrabarti, Curr. Sci. *44*, 630 (1975).
11 S. Chakrabarti and A. Chakrabarti, Experientia *33*, 175 (1977).
12 A.T. Sumner, Expl Cell Res. *81*, 223 (1973).
13 C.D. Darlington and A.P. Wylie, Heredity *6*, suppl. 197 (1953).
14 A. Bajer, Chromosoma *14*, 18 (1963).
15 N.B. Atkin and M.C. Baker, Acta cytol. *8*, 431 (1964).
16 G.L. Castoldi, G.D. Grusovin, M. Gualandi and G.L. Scapoli, Experientia *32*, 856 (1976).

Inbreeding effect: Embryonic development and fecundity of *Drosophila melanogaster* offspring

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Summary. Inbreeding depression observed on fecundity of adult *Drosophila* depends on the effect observed during development of the eggs laid by their parents. This depression does not then depend on the homozygosity per se of the adult genome. It is mainly due to the deleterious effect observed primarily during embryogenesis.

Inbreeding depression is believed to result from expression of lethal recessive genes ordinarily concealed in the genome. Because of a large number of loci at which lethality may occur[2–5], development is perturbed at different stages up to adulthood. However, by studying egg hatchability and egg-to-adult survival, one of us has shown that mortality during embryonic development of a batch of inbred eggs leads to a correlative depression at the larvo-pupal stage[6]. Some embryos do, however, develop normally from fertilisation to adulthood even when inbreeding depression occurs during embryogenesis. We can therefore ask if lethality during development may be associated with a correlative effect in the resulting offspring. We now compare fecundity among inbred offspring from different sib couples. We show that inbreeding depression during development in batches of inbred eggs leads to adults with reduced egg production.

Flies from a wild stock of *Drosophila melanogaster* were reared in an axenic maize-dried yeast-agar medium[7] at 25 °C. P_0 couples, randomly mated, were set up in small boxes with medium to reproduce. The F_1 siblings descended from each P_0 female were crossed. The ensemble of F_1 sib couples thus obtained from a single P_0 couple is called a family. An F_1 couple was considered 'sensitive' to inbreeding when some of its eggs showed blocking during development. These eggs generally exhibited normal embryogenesis but the larvae failed to hatch. Only 1 F_1 family which produced both sensitive and insensitive couples was observed intensively. 3 F_1 sib couples from this family and their F_2 offspring were studied. The 1st F_1 couple (a, table) laid eggs some of whose embryos died during embryonic and larvo-pupal stages. Embryos which developed successfully gave F_2 flies whose egg production was recorded. The 2nd F_1 couple (b, table) laid eggs that developed normally

Mean daily egg production of F_2 females during a 15-day laying period. These females were offspring of 3 F_1 sib couples, all of the same parents (P_0)

F_1 couple	F_1 characteristics			F_2 characteristics						
	Egg hatchability (%)	Egg-to-adult survival (%)	Viability* (%)	Mean daily egg production per couple N	All couples	N	Couples which laid eggs with normal development	N	Couples which laid eggs showing partial blocking	
a	69.4	86.3	59.9	37	48.8±2.4	0	-	37	48.2± 2.4	
b	94.6	70.7	66.9	40	54.7±3.6	29	57.1±3.6	11	48.6± 9.4	$p<0.001$
c	96.1	95.0	91.4	38	61.9±4.4	30	64.0±4.8	8	53.4±10.5	$p<0.001$
Controls	95.4	96.1	91.7	31	67.7±1.6	31	67.7±1.6	0	--	

For a, b and c F_1 couple characteristics, see text. The ensembles of F_2 couples were compared by the Mann-Whitney U test[12]. N, number of F_2 couples. * Viability is the product of egg hatchability and egg-to-adult survival of the eggs laid by the F_1 female.

but then high larvo-pupal mortality occurred. The 3rd couple (c, table) laid eggs which apparently developed normally from fertilisation to adulthood.

The F_2 adults, obtained from the above F_1 couples, were crossed between brother and sister. About 30–40 such sib couples were set up in small boxes renewed daily to determine egg production. These F_2 couples were followed throughout a 15-day laying period. Afterwards, females were dissected and their ovarioles inspected. All couples which produced unfertilised eggs were discarded since unmated females lay few eggs[8]. P_0 couples served as controls.

The table shows the F_2 average daily egg production. Production of the inbreeding sensitive a-family was smaller than that of the b- and c-families and of the controls. The b- and c-families, however, showed egg production lower than that of controls. There was considerable variation in egg production among the F_2 couples within a family. By this approach, we have been able to distinguish F_2 couples which lay eggs that may be blocked during embryogenesis from those which lay eggs that develop normally. In the couples subject to blocking, egg production was lower (table, $p < 0.001$).

These results agree with previous observations of an inbreeding effect on egg production in *Drosophila*[9,10]. However, here only certain females were responsible for the decrease. They originated from batches of inbred eggs which showed low hatchability and/or low egg-to-adult survival. A strong correlation exists between viability (table) of eggs laid by the F_1 sib couples and egg production of the F_2 offspring. Furthermore, a small egg production of the F_2 females is associated with blocking in development of some of their eggs.

Everything takes place as if lethal factors acting throughout embryonic and larvo-pupal development can also act as imaginal factors in the resulting adults. We believe these factors, which we of course observe by their physiological effects, may exert a pleiotropic effect in the ovaries of inbred adults and result in abnormalities in ovariole development. Indeed, as we observe in the present work that low egg production relates to weak vitellogenesis, non-functional or degenerated ovarioles or atrophied ovaries. Likewise, F_2 inbred females with high fecundity also have good vitellogenesis and no abnormalities.

Such effects due to brother-sister inbreeding imply that greater care is necessary in the interpretation of inbreeding depression on quantitative characters related to fitness. Indeed, inbreeding depression of egg production[9], a quantitative trait with a polygenic genetic determinism[11], is mainly due, in our opinion, to the deleterious effects observed primarily during embryogenesis.

1 We thank R. Grantham for his criticism, J.M. Legay for reading the manuscript and Mrs A. Heizmann and S. Lardon for their help. The laboratory is associated with C.N.R.S. No. 243.
2 Th. Dobzhansky, B. Spassky and T. Tidwell, Genetics *48*, 361 (1963).
3 R. Greenberg and J.F. Crow, Genetics *45*, 1153 (1960).
4 N.E. Morton, J.F. Crow and H.J. Muller, Proc. natl. Acad. Sci. *42*, 855 (1956).
5 R.C. Lewontin, in: The genetic basis of evolutionary change. Columbia University Press, New York, London 1974.
6 C. Biémont, Mech. Age Devt *8*, 21 (1978).
7 J. David, Bull. Biol. Fr. Belg. *93*, 472 (1959).
8 J. Bouletreau-Merle, Thesis 7516, Lyon 1975.
9 D. Marinkovic, Genetics *56*, 61 (1967).
10 C. Biémont, C.r. hebd. séanc. Acad. Sci. Paris *278*, 1095 (1974).
11 J.F. Kidwell, Can. J. Genet. Cytol. *11*, 547 (1969).
12 S. Siegel, in: Nonparametric statistics. McGraw-Hill, New York, Toronto, London 1956.

Inhibition of the regulatory ability of stomata caused by exhaust gases

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Summary. It is demonstrated that very low concentrations of exhaust gases from a combustion engine inhibit the regulatory ability of stomata. However, when gas treatment was stopped, plants showed a quick recovery of the ability to close stomata.

The stomata represent the pathway to the gas exchange for the green plant. One of the factors that influences the extent of the opening of the stomata is the prevailing CO_2-concentration in the environment. Low CO_2-concentrations result in the opening of the stomata[2], while high concentration causes their closing[3,4]. It would therefore be expected that the presence of exhaust gases from combustion engines, with their high content of CO_2, should cause the