Zur Frage der genetischen Änderung eines Ascites-Hepatoms der Ratte durch Heterotransplantation auf chinesische Hamster

Die Transplantation des Zajdela-Hepatoms der Ratte auf Mäuse ergibt eine Tumorlinie, die spontan auch auf chinesische Hamster (*Cricetulus griseus*) überimpfbar ist. Der chinesische Hamster ist wegen seiner geringen Chromosomenzahl für cytogenetische Untersuchungen besonders geeignet[1]. Die Angehrate des Tumors betrug bei ihm bisher 100% (15 Passagen).

Im Ausstrichpräparat fand sich kein Unterschied in der Morphologie der Geschwulst auf Ratten (H), Goldhamstern (HH), Mäusen (HHM) und chinesischen Hamstern (HHC). Das Rattengenom blieb auch durch alle Wechsel der Trägertierarten unverändert erhalten. Das Hepatom wuchs auf der Ratte über mehrere Jahre konstant im pseudotriploiden Bereich[2]. Die Stammlinie lag bei Ratten zunächst bei 67, später (zwischen der 200. und 300. Passage) bei 68 Chromosomen; beim Goldhamster bei 68, bei der Maus und beim chinesischen Hamster bei 67. Auffallend war im Zusammenhang mit dem heterologen Wachstum die Zunahme der Plusvarianten von H = 6% und HH = 5% auf 23% bei HHM und 16% bei HHC. Die Minusabweicher lagen bei allen untersuchten Linien um 65%. Das Chromosomenkomplement der Ratte blieb formal erhalten. Die Analysen für diese Geschwulst zeigten unabhängig vom Trägertier keine echte Triploidie, da die Gruppen gegenüber dem normalen Komplement stark abweichen. Die Gruppen 12, 14 und 15–17 sind überbesetzt, während in anderen Chromosomen fehlen. Veränderungen im Bereich der Markerchromosomen ergaben sich nicht.

Das Karyogramm des Hepatoms auf der Maus zeigt Zellen mit nur 40 telozentrischen Chromosomen, die offenbar vom Wirtstier stammten. Bei den Untersuchungen des Tumors auf chinesischen Hamstern wurden im Verhältnis 1:100 Mitosen mit einer für den chinesischen Hamster charakteristischen Chromosomenzahl von 22 gefunden.

Es werden demnach infolge der charakteristischen Chromosomenzahl Zellen des Trägertieres im heterolog wachsenden Tumorascites beim chinesischen Hamster nachweisbar.

Summary. Heterotransplantation of the rat hepatoma of Zajdela into golden hamsters, mice and Chinese hamsters has induced virtually no change in the neoplasm. The host tissues have shown, however, a 1% increase in their own mitoses, which is attributed to growth stimulation by the tumor.

O. WIESER und U. MOHR

Deutsches Krebsforschungszentrum, Institut für Experimentelle Pathologie, 69 Heidelberg (Deutschland), 5. Dezember 1966.

1 E. STUBBLEFIELD, *Cytogenetics of Cells in Culture* (Ed. R. J. C. HARRIS; Academic Press New York and London 1964).
2 H. WRBA, J. EICKE, M. L. MEINERS und A. EMMINGER, Z. Krebsforsch. *66*, 349 (1964).

Genetics and Activity in *Drosophila melanogaster*

Activity levels have been measured in a variety of animals including mice (BRUELL[1]), rats (STRONG[2]), blowflies (BARTON BROWNE and EVANS[3]) and *Drosophila* (ROBERTS[4]). A number of quite different techniques have been employed in obtaining these measures. However no clear definition of activity has evolved and it is probable that many of the measures have little in common. After attempting to select for changed activity levels in *D. melanogaster* EWING[5] concluded that it is only relevant to discuss activity in relation to the exact experimental conditions under which this character is measured. Thus experimental situations which appear similar to the experimenter may provide quite different stimulus situations for *Drosophila*. One criterion for testing the equivalence of different measures of activity is to look for common genetic control. This can be done by examining the performance of 2 inbred lines and of the crosses between them under different experimental or environmental conditions. If the measures are equivalent then the inbred lines and crosses will bear the same quantitative relationship to one another. The fewer the genes that the measures have in common the more dissimilar will the patterns of inheritance tend to be. This method allows one to compare measures of activity with differing units of measurement. As an example this paper shows how a comparison between 3 measures of activity was carried out. 2 of these employed the same units of measurement but were carried out under different environmental conditions while the third used a different test situation.

Materials and methods. 2 inbred lines of *D. melanogaster* (PA and PB) which had been derived from geographically separate base populations and subjected to well over 200 generations of brother-sister mating were used. The stocks were reared and all experiments carried out at 26 ± 1 °C. Details of the experimental procedure are otherwise as previously reported (EWING[6]). Female flies only were employed in these experiments.

The following measures of activity were used. Flies were introduced singly into a 13 cm diameter circular runway of 0.3 cm internal cross section (see CONNOLLY[7]). This apparatus was designed in an attempt to provide a

1 J. H. BRUELL, in *Roots of Behaviour* (Ed. BLISS; Harper, New York 1962), p. 48.
2 T. STRONG, J. comp. physiol. Psychol. *50*, 596 (1957).
3 L. BARTON BROWNE and D. R. EVANS, J. Insect. Physiol. *4*, 27 (1960).
4 S. K. DE F. ROBERTS, Science *124*, 172 (1956).
5 A. W. EWING, Anim. Behav. *11*, 369 (1963).
6 A. W. EWING, Anim. Behav. *9*, 93 (1961).
7 K. CONNOLLY, Anim. Behav. *14*, 444 (1966).

constant stimulus situation for the fly. The runway was marked off at 0.6 cm intervals and the number of divisions crossed during 2.5 min was scored. A preliminary examination showed that the flies ran at a fairly uniform speed throughout the test period.

The second apparatus consisted of a series of 6 vials 5 · 2.2 cm connected by funnels with exit diameters of 0.3 cm and arranged in such a way that the flies could migrate through the vials in one direction only. This is fully described in a previous publication[5].

2 measures of activity were obtained from this apparatus. In the first flies were introduced into the apparatus singly and in the second in multiples of 25. Activity was expressed as the number of moves between vials per 50 flies in 30 min which was the duration of the test. Crosses were set up so that samples from the 2 parental lines, F1 and F2 and the 2 backcrosses could be tested each generation. This ensured that there was no bias due to variability between generations.

Results and conclusions. It is possible to demonstrate graphically the relationship between 2 inbred lines and of the crosses between them so as to show dominance and any deviation from the expected values (MATHER[8], BRUELL[1]). The inbred lines and crosses are disposed along the horizontal axis and activity scores on the vertical axis. The degree of dominance is shown by the deviation of the F1 from the mid-parent value (M). The backcrosses, BA and BB, should theoretically be intermediate between the F1 and parental values PA and PB respectively, while the F2 should lie between BA and BB. It is often necessary to transform the data so that the segregating populations (the F2 and backcrosses) do not deviate from the expected positions before values for dominance are calculated. However the following results are presented in the untransformed state to enable a direct comparison to be made of patterns of inheritance of the different measures of activity.

Figures 1 and 2 are diagrams of this type for activity in the one-way vial apparatus using single flies and multiples of 25 respectively. The pattern of inheritance is very similar in both cases and only PA and the F1 show significantly heightened activity over the single fly level when tested in multiples of 25, ($P < 0.01$ in both cases using Wilcoxon's test (QUENOUILLE[9]) although the means of both BA and the F2 are also higher. It is difficult to interpret this result but it does suggest that reactivity, that is the tendency of flies to repel one another, is controlled by rather few alleles which are absent in PB. Certainly the speed with which this character responds to selection supports the view that it is controlled in a genetically simple manner[5].

While there is no significant difference between PA and PB in the activity levels of flies tested singly this similarity is phenotypic only and does not imply that the genes controlling activity are the same in the 2 inbred lines. If they were so then one would expect the 2 backcrosses to show similar levels of activity. In fact BB is probably more active than BA ($P < 0.03$).

Figure 3 illustrates the pattern of inheritance as measured in the circular runway. It is not possible to compare these results with those of the previous experiments quantively as the apparatus used and consequently the units of measurement are different. However from a comparison of the relative positions of the inbred lines and their crosses it is obvious that the pattern of inheritance is very different for this measure of activity. Indeed in this case the relative position of the inbred lines is different, PB being significantly more active than PA ($P < 0.001$). This suggests that the 2 types of apparatus are measuring very different aspects of activity with few

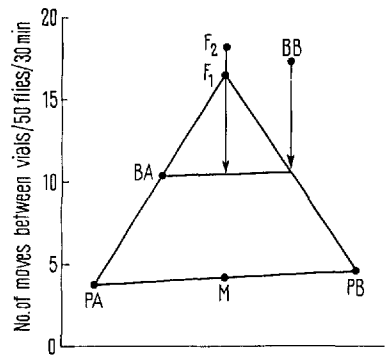

Fig. 1. Pattern of inheritance for activity as measured in the one-way vial apparatus using single flies. Arrows show the deviation of the segregating populations from the predicted values.

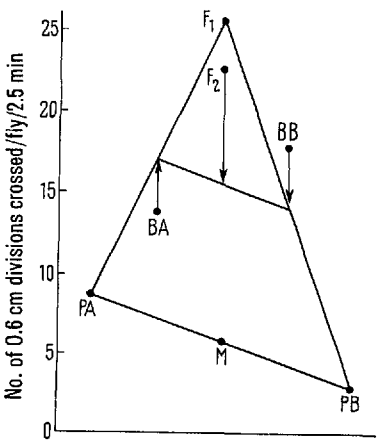

Fig. 2. As for Figure 1 but using multiples of 25 flies in the one-way vial apparatus.

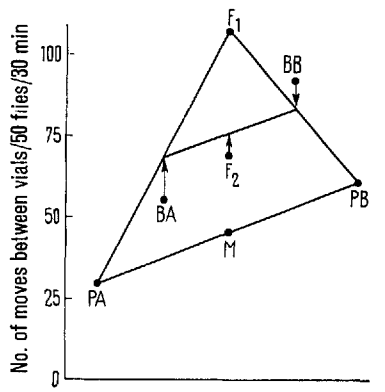

Fig. 3. Pattern of inheritance for activity as measured in the circular runway.

[8] K. MATHER, *Biometrical Genetics* (Methuen, London 1949).
[9] M. H. QUENOUILLE, Rapid Statistical Calculations (Griffin, London (1959).

alleles in common, or, if the same genes are involved, then they are being expressed in quite a different fashion.

One possible explanation for the different modes of inheritance could be different time scales used in the 2 experimental situations. However activity in the one-way vial apparatus does not change significantly during the test period. Both types of apparatus provide measures of the amount of walking done by flies introduced into a novel situation. Despite this superficial similarity the results show that the stimulus situation presented to the flies in the 2 instances must be quite different.

The purpose of these preliminary studies was behavioural rather than genetical and the data was collected in such a manner as to facilitate scoring rather than in a form from which the maximum amount of genetical information could be extracted. For example in the one-way vial apparatus the activity of groups of flies rather than that of individuals was scored. It is not therefore possible in this case to estimate the number of segregating units involved in these behavioural characters. It is however possible to design experiments so that a full analysis could be made. Further, animals could be tested in the same apparatus under a variety of experimental conditions. This would allow one to assess the genetical contribution towards each of the variables in terms of direction of action, mode of interaction and possibly also the number of segregating units. The technique outlines in this paper could be used to analyse behavioural characters other than activity[10].

Zusammenfassung. Eine Methode zur Abschätzung dreier Maßstäbe von Lokomotionsaktivität bei *Drosophila melanogaster*, die unter gemeinsamer genetischer Kontrolle stehen, wird beschrieben. Es wird wahrscheinlich gemacht, dass die Kontrolle des einen Maßstabes von einem besonderen Teil des Gen-Komplementes ausgeübt wird, während die andern mehrere kontrollierende Gene gemeinsam besitzen.

A. W. EWING

Department of Zoology, University of Edinburgh (Scotland), 28th October 1966.

[10] *Acknowledgments*: My thanks are due to Dr. A. MANNING and Dr. B. CLARKE for their helpful criticism of the manuscript.

The Control of Wheat Gluten Synthesis at the Genome and Chromosome Levels

Since aneuploid wheat samples have become available, a powerful tool has been added to studies on the genetic control of wheat gluten synthesis. It is well known that gluten is a complex mixture of proteins all characterized by their carrying a relatively low charge over a wide range of pH. That genotype determines the distribution and identity of the individual gluten proteins has been known for some years. No genes have been specifically associated with individual protein components and little has been done at the chromosome level.

One of the most powerful tools for the study of gluten protein composition ('profile') is gel electrophoresis. This communication reports the results of starch gel electrophoretic studies on some aneuploid lines of the wheat variety 'Chinese Spring', on another hexaploid wheat 'Canthatch' and a tetraploid derivative of it, together with 3 reconstituted Hexaploids.

Materials and methods. Wheat samples used included ditelocentric lines of the variety 'Chinese Spring' where one pair of arms of one of each of the 21 pairs of chromosomes had been removed. Of the 42 possible ditelocentrics only 22 were available for study. Samples of the variety 'Canthatch', a hexaploid ($2n = 42$) with A, B, and D genomes and a tetraploid derivative, 'Tetracanthatch' ($2n = 28$) of it with the D genome removed (KERBER[1]) were also examined together with reconstituted hexaploids in which 3 varieties of *Aegilops squarrosa* L. ($2n = 14$) had contributed a D genome to the A and B genomes of 'Tetracanthatch'. Protein was extracted from crushed single grains of wheat with 0.4 ml $2M$ aqueous urea. Starch gel electrophoresis in aluminium lactate buffer, pH 3.1, containing $2M$ urea was conducted according to the method of GRAHAM[2]. Only gluten or storage proteins were examined.

Results. The ditelocentric lines of 'Chinese Spring' showed the most dramatic change from the parent when one arm of the 1D chromosome was removed. The effect of this is shown in Figure 1. Only 7 of the 22 samples

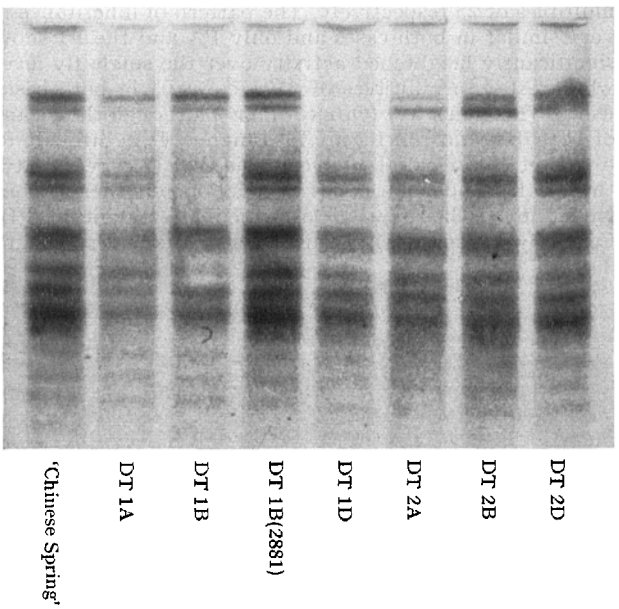

Fig. 1. Starch gel electrophoretic patterns of gluten proteins from ditelocentric lines of the hexaploid wheat variety 'Chinese Spring'.

[1] E. R. KERBER, Science *143*, 253 (1964).
[2] J. S. D. GRAHAM, Aust. J. biol. Sci. *16*, 342 (1963).