this case the use of the discutable IQ, which is finally rather subjectively defined, can be replaced by much more precisely formulated data in the labyrinth experiments. The sequence of the generations can also be thoroughly checked in this case. But, in the first instance, it would be particularly important to carry out future investigations on a considerably larger sample of persons.

5. *Final remark*. It is hardly necessary to emphasize that our findings are only concerned with correlation and the overall situation, but say nothing whatever about the 'mikro-caused' background, which ought to be investigated by biological methods.

Zusammenfassung. Zur Erhärtung der oft formulierten Vermutung, dass hochbegabte Kinder meistens von älteren Eltern abstammen, wurde eine Serie von Untersuchungen durchgeführt an verschieden zusammengestellten Listen hervorragender Persönlichkeiten. Dabei stellte sich als wertvoll heraus, neben dem Alter des Vaters auch die Alter der beiden Grossväter heranzuziehen und die Summe dieser 3 Alter, die «väterliche Dreierzahl» zu benutzen. Im Resultat ergibt sich, dass die Alter der Väter unter 30 nur in weniger als 20% aller Fälle und zugleich «Dreierzahlen» unter 90 in weniger als 5% aller Fälle vorkommen. Die Hypothese, dass allgemein der IQ im Durchschnitt monoton mit der «Dreierzahl» wächst, wurde an einer Gruppe von 87 Schülern eines Gymnasiums getestet und durch den «Spearman rank correlation test» als signifikant mit der Fehlerwahrscheinlichkeit unter 4% nachgewiesen. Eher unerwartet, scheint das Alter des väterlichen Grossvaters stärkeres Gewicht zu haben als das des leiblichen Vaters.

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PRO EXPERIMENTIS

An Improved Giemsa C-Banding Procedure for Plant Chromosomes

The Giemsa banding techniques that have been developed for animal and human chromosomes are in general not directly applicable to plants. In the former systems they made possible the recognition of constitutive heterochromatin (C-banding) as well as allowing a discrimination between other chromosome regions (G-banding). In some monocotyledonous plant species of the genera *Trillium* and *Fritillaria*, a simple method consisting of 4 steps has been developed for the preferential staining of heterochromatin[1]. However, this technique gave variable results or failed when applied to other species, such as *Vicia faba* or *Pisum sativum*.

A method has now been developed which is an improvement on the earlier technique and adds as an essential stage a pretreatment of the preparations with hot barium hydroxide. This step has been introduced in a method for demonstrating human centromeric heterochromatin[2]. The new procedure was tested with pea and rye and then successfully applied to *Anemone* and *Hepatica*[3].

Colchicine pretreated root tips were employed and several alcoholic fixatives commonly used for plant chromosomes[4] were found to be compatible with Giemsa C-banding. Fixed root tip meristems were placed in 45% acetic acid on a slide previously coated with a thin layer of HAUPT's adhesive dried with ethanol[5]. 'Subbing' with HAUPT's adhesive is superior to albumen-glycerine because there is less material loss during subsequent steps. Root tip meristems were crushed and dispersed into a very fine suspension and squashed in 45% acetic acid, applying gentle heat to the slide. The coverslip was prised off using the dry ice method or liquid nitrogen. The slide was plunged into 2 changes of 90% ethanol followed by two changes of absolute ethanol prior to air drying. Preparations were then usually stored for a day or more at room temperature. For pretreatment, slides were placed in a ridged coplin jar in a waterbath and a freshly prepared prewarmed aqueous solution of barium hydroxide was added. Optimal barium hydroxide concentration and temperature were determined in a preliminary experiment; incubation time was varied only within the range of 10 to 20 min. The alkali treatment was stopped by adding distilled water to the coplin jar so that the skin of $BaCO_3$ which had formed was washed away. After thorough rinsing with distilled water, preparations were then incubated at 65 °C for 1–2 h, or in a few instances overnight, in 2×SSC (0.3 *M* NaCl plus 0.03 *M* trisodium citrate, pH 7.0). Preparations were washed briefly with distilled water and stained in buffered Giemsa (G. T. Gurr's R66 'improved' stock solution diluted about 50× with *M*/15 Sörensen phosphate buffer, pH 6.9). Precipitated stain on the squashes is largely avoided by tilting the staining jar so that the preparation is on the underside of the slide. Staining was repeatedly monitored; the time required for optimum differential staining varied widely between preparations. Fine detail was often best seen in slowly developing preparations which were left in stain overnight. Lastly preparations were rinsed with distilled water, air dried and mounted directly in DePeX (G. T. GURR; Searle Scientific Services, High Wycombe, Bucks., England).

Two main difficulties arise in using the Giemsa C-banding technique on plants. Firstly, a method of fixation and preparation has to be found which yields cytologically acceptable chromosome figures and which is compatible with the subsequent Giemsa procedure. Secondly, modifications of the known Giemsa techniques may have to be devised to take into account the properties of the particular plant used and the conditions created by the fixation/preparation method employed.

Direct preparation of fixed root tips as conventional 45% acetic acid squashes[6] led to good chromosome

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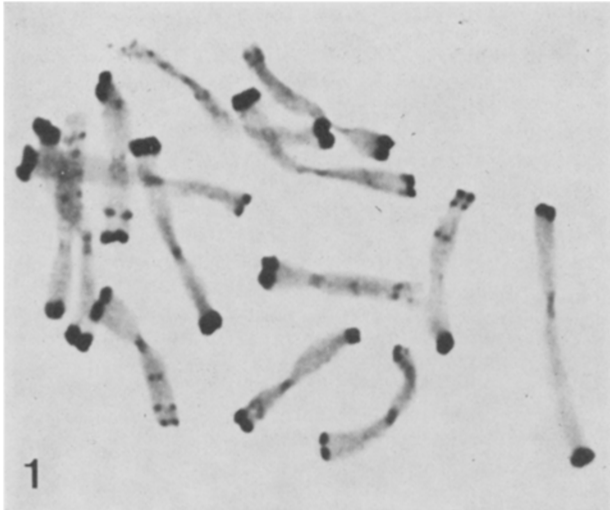

Fig. 1. *Secale cereale* ($2n = 14$), root tip metaphase showing terminal, intercalary and centromeric C-banding. Fixation: ethanol/acetic acid (3:1) overnight. Pretreatment: 4.5% $Ba(OH)_2.8H_2O$ at 50°C for 10 min; ×1700.

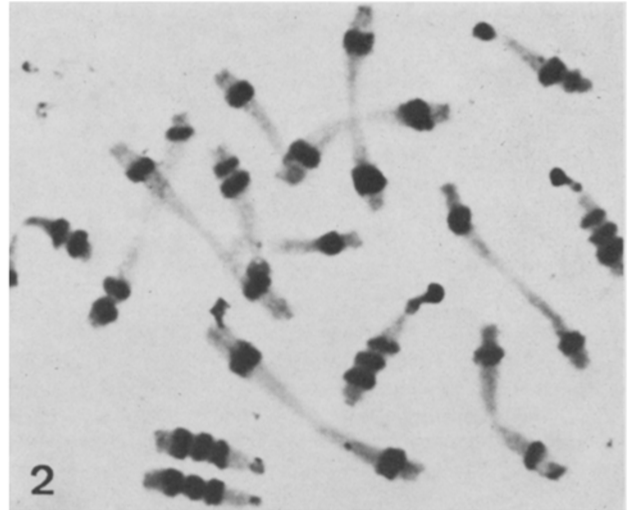

Fig. 2. *Anemone blanda* ($2n = 16$), root tip metaphase; large heterochromatic blocks are apparent in all chromosomes. Fixation: ethanol/acetic acid (3:1) for $6^1/_2$ h followed by storage in 90% ethanol overnight. Pretreatment: 6% $Ba(OH)_2 \cdot 8H_2O$ at 60°C for 20 min; ×1700.

figures with such different plants as pea, rye, and species of *Anemone* and *Hepatica*. The quality of the squash preparations obtained with a particular plant apparently depended on both the fixative and the conditions. With rye, for example, differences in the preservation of chromosome morphology were apparent between such fixations as ethanol abs./acid acetic glacial (3:1), Carnoy (ethanol/chloroform/acetic acid 6:3:1), or BATTAGLIA[7] (ethanol/chloroform/aceticacid/formalin 5:1:1:1), applied at room temperature or cold for various durations with or without 90% ethanol posttreatment, and a suitable treatment could be found. On the other hand with some plants, complete metaphase figures are very seldom obtained in direct squashes, but it has been shown to be possible in some species to macerate the fixed tissue prior to squashing and to use such preparations for Giemsa banding[1,8,9].

Hot barium hydroxide treatment of the preparations proved to be superior to all the pretreatments tested earlier[1]. Even in the pea, C-bands could be revealed, whereas our previous attempts had resulted in the chromosomes being stained overall. However, generally applicable conditions cannot be indicated. In the first place they depend on the plant used. For example, in *Secale cereale*, which can be recommended as a test system (Figure 1), C-banding in ethanol/acetic acid fixed chromosomes was readily revealed with a relatively mild barium hydroxide treatment, whereas similarly prepared specimens of *Anemone blanda* (Figure 2) required stronger conditions. In addition, the barium hydroxide sensitivity of a plant species seemed to depend on factors which are all assumed to influence the fixation state of the chromatin such as fixative and fixation conditions, the manner in which the squashing was carried out and the duration and conditions of storage of the dried preparations prior to barium hydroxide treatment. For example, it was found that through addition of formalin to the fixative[7], the alkali resistance of rye chromosomes was increased. Moreover, in all series there was some variation of the differential staining between preparations, even with optimal conditions for barium hydroxide treatment. The origin of such variation could be the squashing process which is difficult to standardize. The variability of the method is demonstrated by the following experiment: 89 preparations of *Anemone* and *Hepatica* were treated in 10 separate batches at different times with barium hydroxide (6 g $Ba(OH)_2 \cdot 8H_2O$/100 ml distilled water) at 60°C for 20 min. In 9.0% of the preparations the nuclei and chromosomes were stained overall, in 70.8% of the preparations the heterochromatin in nuclei and chromosomes was preferentially stained; 20.2% of the preparations did not stain, nuclei and chromosomes appearing ghost-like. Amongst those preparations showing bands and dark chromocentres, only 25.4% ,or 18.0% of all preparations, were cytologically satisfactory and worth examining. If preparations of *Pisum sativum* in which the chromosomes were stained overall were subjected to a further stronger barium hydroxide treatment followed again by incubation and staining, then C-banding was sometimes seen. It is thought that for both the heavily staining preparations and those which do not stain any more, the conditions of barium hydroxide are incorrect; this would be due for the former group to too low a concentration, temperature or too short a treatment. Non-staining preparations have been irreversibly altered by too high a concentration and temperature or too long a treatment with alkali[10].

Zusammenfassung. Es wird ein für Pflanzenchromosomen modifiziertes Verfahren zur präferentiellen Giemsa Färbung von Heterochromatin mitgeteilt.

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