ter[7] according to the method of growing cells in vitro of Echalier and Ohanessian[2].
2 cultures were set up for each line, while 2 control cultures per line were maintained in D225 medium.
When inosine replaced YE (medium 1), growth rate was lower than in D225 medium for 2 or 3 passages. Afterwards it increased and was comparable to that in D225 medium. At present cells in medium 1 are at the 20th passage. Medium 2, in which an amino acid mixture replaced LH, could not support cell proliferation and cells stopped multiplying after 4 or 5 passages.
When using medium 3 (insulin replacing 15% FCS), cells grew as well as in medium D225. Cells in medium 3 are now at the 24th passage. Media 4,5 and 6 caused a sudden reduction in growth rate in every line. In fact, the doubling time was almost trebled. Furthermore, subculturing of these cells required an inoculum size 4 times larger than the inoculum size in D225 medium. It is of note that inosine and insulin, when present in the same medium (medium 5) cause a considerable reduction in cell proliferation; on the contrary, when present in different media (medium 1 and 3) they enable cells to grow at the same rate as in D225 medium. Medium 7, completely synthetic, dramatically reduced the growth rate in all 4 lines and cells generally stopped multiplying after 2 passages.
No differences in the behaviour of the 4 lines were noticed when they were grown in the same medium (table 2).
In conclusion, the experiment failed to demonstrate the validity of a chemically defined medium: medium 7 was unable to support cell growth. Nevertheless, one very interesting result was achieved. The findings indicate that insulin can completely replace serum in *D. melanogaster* cell cultures in vitro. This is important for the following reasons: different batches of FCS stimulate cell growth differently and some of them may even be toxic to cells; the cost of FCS is very high and sometimes it is not available. The use of insulin instead of FCS will overcome these difficulties.

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Q-banding and A-T rich DNA in *Ornithogalum montanum* (Liliaceae)[1]

P. Barsanti, T. Marazia and F. Maggini

Istituto di Genetica dell'Università, Via Amendola 165/A, I-70126 Bari (Italy), 28 September 1980

Summary. An A-T rich component was detected by CsCl-actinomycin D centrifugation in nuclear DNAs from three natural populations of *Ornithogalum montanum* (Liliaceae). No correlation was found between the Q-banding pattern and the amount of the A-T richest fractions of the genome.

Quinacrine (Q) bands are a common feature of the karyotype in animal species. In plants, although in several species no Q bands can be demonstrated[2], polymorphism for the banding pattern is frequent within species that do show Q bands[3–5].
Considerable evidence indicates that a base composition of the chromosomal DNA with a preponderance of A-T pairs is the primary determinant of the brightly fluorescing Q^+ bands[6–8]; other authors, however, put the emphasis on differential chromosome condensation[9], on the effects of chromosomal proteins on quinacrine binding[10] or on the pattern of interspersion of G-C between A-T pairs[11]. In this context it would be of interest to know whether intraspecific variation for the Q-banding pattern is correlated with variation in the base composition of nuclear DNA. In the present study nuclear DNAs from 3 morphs of *Ornithogalum montanum* (Liliaceae) showing extensive variation in the number of Q^+ bands per diploid genome were characterized by equilibrium centrifugation in CsCl and in CsCl-actinomycin D preparative gradients.
Materials and methods. Ornithogalum montanum bulbs were collected by Prof. P. Marchi (Institute of Botany, University of Rome, Italy) from 3 natural populations at 3 widely separated locations in South Italy, i.e. A) Pollino (North Calabria); B) Gargano (Apulia) and C) Aspromonte (South Calabria). As previously reported[12] the number of Q^+ bands varies from 0 to 3 within population (A); from 11 to 18 within population (B) and from 20 to 25 within population (C). Q-banded karyotypes of the *Ornithogalum montanum* bulbs used for this study were obtained according to Vosa[13] and kindly provided by Prof. P. Marchi and G. F.

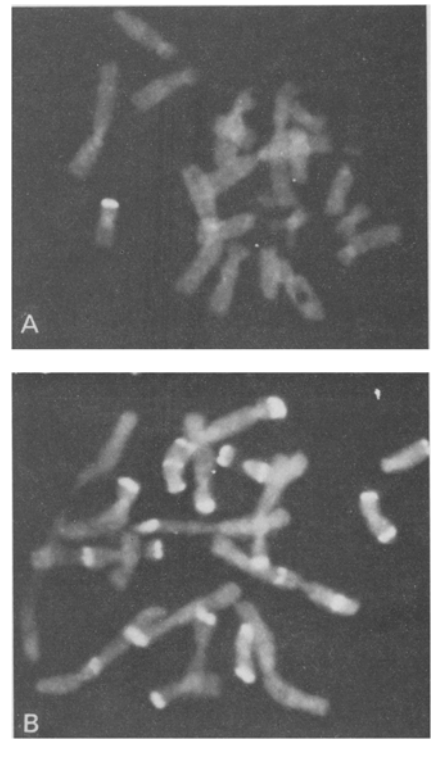

Fig. 1. Q-banded chromosomes from *Ornithogalum montanum* bulbs showing 1 (A) and 24 (B) Q^+ bands.

D'Amato (Institute of Botany, University of Rome, Italy). In figure 1 are presented Q-banded chromosomes from a bulb showing a single Q^+ band (population A) and chromosomes from a bulb showing 24 Q^+ bands (population C). *Ornithogalum montanum* bulbs were grown under sterile conditions for 36 h in distilled water containing 50 µCi/ml ^{3}H-thymidine (Radiochemical Center, Amersham, sp. act. 29 Ci/mmole). DNA was extracted as previously described[14] from the growing roots of each bulb. Specific activity of the purified DNA preparations varied from 48,000 to 66,000 cpm/µg. Unlabeled *Allium cepa* DNA was obtained by the same procedure.

Equilibrium centrifugation in CsCl or CsCl-actinomycin D was performed according to Hemleben et al.[15]; conditions for each run are reported in legends of figures 2 and 3. After fractionation of the gradients, aliquots of each fraction were dried on filter paper discs for radioactivity counting or used for refractive index and A_{260} measurements.

Results and discussion. In preparative neutral CsCl gradients *Ornithogalum montanum* DNA bands as a single peak whose buoyant density we found to be 1.695 g/ml both by determination of the refractive index of the relevant fractions and by comparison with the position in the same gradient of *Allium cepa* DNA (p = 1.691 g/ml, Ingle et al.[16]). No density satellites are detectable (fig. 2A). DNAs from several bulbs differing for the Q-banding pattern (see materials and methods-section), were also analyzed by centrifugation in CsCl-actinomycin D. In a typical experiment, shown in figure 3, DNA samples were from bulbs characterized respectively by 0 (3A), 11 (3B) and 24 (3C) Q^+ bands. In each DNA preparation examined, a 'cryptic' satellite appeared as a prominent shoulder on the heavy side of the main band (fig. 3). When rerun in neutral CsCl after elimination of actinomycin, DNA from the heavy shoulder (fractions indicated in figure 3) banded as a single peak at the density of 1.691 g/ml (fig. 2B); it is therefore richer in A-T pairs than main band DNA and should in fact contain the A-T richest fractions of the *Ornithogalum montanum* genome. Although we did not attempt exact quantitation of this DNA component, it represented about 15–20% of the total DNA in all samples studied. According to our results, in *Ornithogalum montanum* dramatic variation in Q-banding pattern (from 0 to 24 Q^+ bands per diploid genome) does not entail gross variation in nuclear DNA composition as characterized by equilibrium density centrifugation. This finding strongly suggests that in *Ornithogalum montanum* relative A-T composition of chromosomal DNA is insufficient to account alone for the production of the Q-banding pattern.

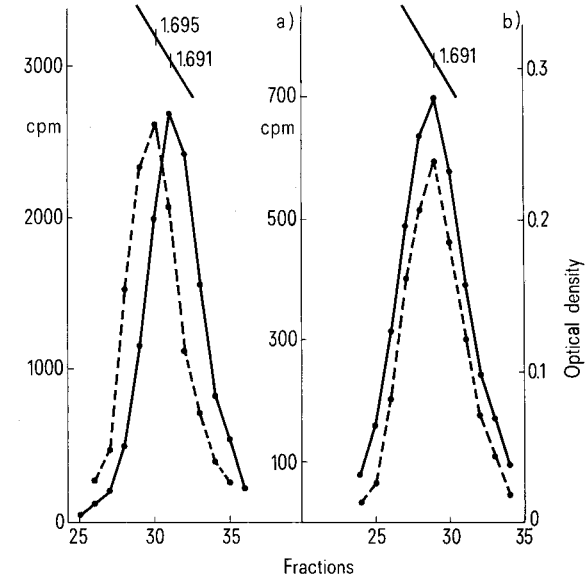

Fig. 2. Analysis of *Ornithogalum montanum* DNA in preparative CsCl gradients. *a*) 3 µg of *Ornithogalum montanum* ^{3}H-DNA (cpm, broken line) and 100 µg unlabeled *Allium cepa* DNA (Optical density$_{260}$, solid line) were centrifuged together in a 50-SW Spinco rotor at 38,000 rpm for 48 h at 20 °C. *b*) ^{3}H-DNA (cpm, broken line) from the heavy shoulder fractions of the 3 CsCl-actinomycin D gradients shown in figure 3 was pooled and, after extraction of actinomycin D, centrifuged together with 100 µg *Allium cepa* unlabeled DNA (Optical density$_{260}$, solid line). Run conditions as in 2A.

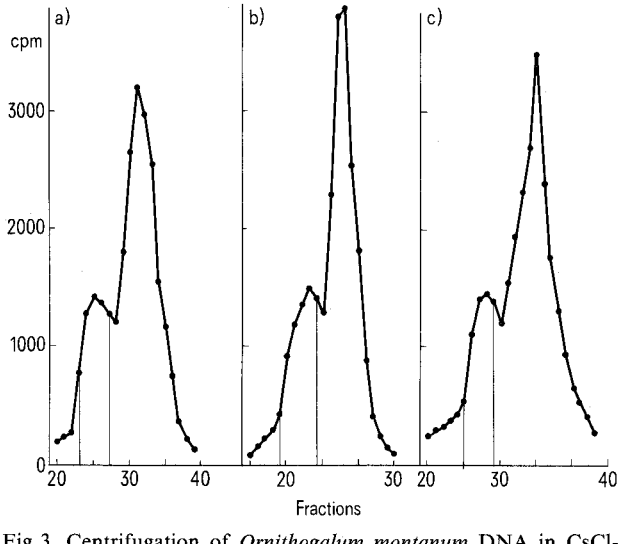

Fig. 3. Centrifugation of *Ornithogalum montanum* DNA in CsCl-actinomycin D gradients. 3 µg ^{3}H-DNA from pooled roots of single bulbs showing respectively 0 (A), 11 (B) and 24 (C) Q^+ bands were centrifuged in a 50-SW Spinco rotor at 35,000 rpm for 60 h at 4 °C in 0.025 M sodium tetraborate buffer pH 9.2, together with 100 µg *Allium cepa* unlabeled DNA (not shown). Actinomycin was 100 µg per 100 µg total DNA. Vertical lines indicate the fractions used for heavy shoulder DNA characterization in neutral CsCl, as in figure 2B.

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