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NEUTRALITY VERSUS NATURAL-SELECTION - WHY IS THERE SO MUCH POLYMORPHISM

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## Abstracts of papers presented at the eleventh meeting of the Scandinavian Association of Geneticists June 3–6, 1984, Leangkollen, Asker, Norway

**R. AALEN and W. BLIX GUNDERSEN:** Gene products from recombinant plasmids containing pBR322 and phenotypically cryptic plasmids from *Neisseria gonorrhoeae* (poster presentation)

Two phenotypically cryptic plasmids (p31788K and pG5449K) with slightly different restriction endonuclease patterns, from two different strains of *N. gonorrhoeae*, have been integrated in the HindIII site of pBR322 and transformed into the minicell strain *E. coli* DS410. The protein profile of two Ap<sup>r</sup>Tc<sup>r</sup>-transformants with recombinant plasmid (pTA9, pTA11) containing p31788K, and one transformant with a plasmid (pTBe) containing pG5449K, have been investigated by the minicell method. p31788K and pG5449K are integrated in the same orientation in pBR322.

In addition to pBR322-coded proteins (mature and premature beta-lactamase), the strains with the recombinant plasmids all express proteins of 25,000 and 10,020 dalton. The strain with pTBe expresses two additional proteins of 22,300 and 10,070 dalton. It is reasonable to suppose that the difference in protein profile reflects differences in DNA sequence between the two gonococcal plasmids.

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**K. AASTVEIT:** Induced barley mutants for developmental stability

It is well known that individual genotypes within a species may be more or less buffered against environmental variation. This phenomenon has variously been named as developmental flexibility, developmental stability, phenotypic stability, developmental homeostasis, canalization, auto-regulation, phenotypic developmental flexibility and individual adaptability. Since this character tends to show a continuous variation within populations, the author prefers to talk about various grades of developmental stability. There are good reasons to believe that genetic variation in developmental stability plays an important role in natural adaptation to changing environments. In addition, developmental stability is of great importance in plant breeding.

Data will be presented showing that homozygous stability mutants for grain yield in barley have been selected after X-ray and  $\gamma$ -ray treatments. Crosses between different stability mutants and their respective mother lines followed by selection have shown that induced variation for stability can be recombined and give rise to homozy-

gous segregants more extreme than the original mutants or segregants from the mother lines.

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**H. C. ANDERSSON, B. A. KIHLMAN and M.-B. KARLSSON:** Effects of G<sub>2</sub>-treatments with methylated oxypurines on mutagen-induced chromosome damage and mitotic inhibition in human lymphocytes cultured in vitro (poster presentation)

1,3,7-trimethylxanthine, or caffeine, is known to interfere with many of the effects produced by mutagenic agents in proliferating eucaryotic cells. Post-treatments with caffeine have been shown to reduce the mutagen-induced inhibition of DNA synthesis and G<sub>2</sub>-delay, and to increase the aberration frequency and cell death. It has not been possible to explain these effects in molecular terms. The facts that post-treatments with caffeine reduce the G<sub>2</sub>-delay and, thereby, the depression of the mitotic activity induced by ionizing radiation, and at the same time increase the yield of radiation-induced aberrations, have been interpreted by PAINTER (*J. Mol. Biol.* 143, 1980, 289–301) in the following way; Caffeine exerts its effect by preventing the cells from going through delays that would allow them to repair DNA damage before it can be expressed. This hypothesis, which was tested in whole blood cultures of human lymphocytes, would be supported if methylated oxypurines (MOPs) that do not counteract mutagen-induced mitotic inhibition as efficiently as caffeine, also are less efficient potentiators of chromosome damage.

The blood cultures were either irradiated with 50 rad X-rays at 68 ½ h after stimulation with phytohaemagglutinin and harvested 3 ½ h later, or treated for 2 h with 4 × 10<sup>-5</sup>M thiotepa before stimulation and then cultured for 52 h. In both types of experiment, various MOPs were added together with colcemid 3 h before harvesting. The MOPs tested were caffeine, theophylline, theobromine, paraxanthine, 8-chlorocaffeine, 8-methoxycaffeine and 1,3,7,9-tetramethyluric acid.

In general, the X-ray data agreed quite well with the hypothesis tested, but some observations did not. Thus, even though paraxanthine and 8-chlorocaffeine increased the radiation-induced reduction in mitotic frequency, they still potentiated the induced aberration frequency, 3.5 and 2.5 times, respectively. Furthermore, 1,3,7,9-tetramethyluric acid decreased the mitotic inhibition as efficiently as caffeine, but in contrast to caffeine, it had no marked

influence on the radiation-induced frequency of chromosomal aberrations.

No MOP reduced the TT-induced depression in mitotic activity. The observation that caffeine, theophylline and theobromine, in contrast to the other MOPs tested, are capable of increasing the TT-induced aberration frequency without reducing the mutagen-induced mitotic inhibition shows that these two effects are not always related, even after treatments with chemical mutagens.

In conclusion, our data show that a correlation does not necessarily exist between the ability of ( $G_2$ -treatments with) MOPs to enhance mutagen-induced chromosome damage and to counteract the induced mitotic inhibition.

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**P. A. ANDRESEN and B. H. LINDQVIST:** Distribution and state of P4-DNA homologies in natural isolates of *E. coli*

P4 is a naturally occurring *E. coli* plasmid. As such P4 has three modes of propagation. It can propagate as an autonomous replicating plasmid, it may be propagated as a prophage and in the presence of a helper such as P2, it uses the morphogenic components of the helper for propagation as a virus particle. P4 was originally isolated as virus from the *E. coli* strain K235 using a P2 lysogen as indicator (Six 1963).

To learn about the incidence of P4 or related DNA elements in natural populations of *E. coli*, we tested 100 different clinical isolates of *E. coli* for P4 DNA homologies by colony hybridization using radioactively labelled P4-DNA as probe. Twenty-five per cent of the strains turned out to contain P4 DNA homologies. Twelve of these were characterized with regard to the state of the homologies. As measured by hybridization, none of the homologies were present in the plasmid or bacteriophage DNA's isolated from the strains. This result indicates that the homologies must reside in the chromosomes of the isolates. To demonstrate this, total DNA of the cells was isolated, treated with a restriction enzyme, separated electrophoretically, transferred to hybridization membranes and hybridized, with  $^{32}P$  labelled P4-DNA as probe.

This type of analysis shows that the homologies present in the isolates vary between 5–8 Kb in size and they are thus considerably smaller than the genome of P4 (11.3 Kb). The restriction analysis suggests that the homology-containing strains share a common P4 related sequence of about 600 bases.

*Reference:*

SIX, E. W. 1963. — *Bacteriol. Proc.* 138

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**Ú. ÁRNASON:** Highly repetitive DNA in cetaceans

Studies on highly repetitive DNA in cetaceans (whales, dolphins, porpoises) have shown the presence of some

highly conserved sequences. A 1730 base pair unit characterizes all cetacean families except the Delphinidae, in which the length of the repeat is 1580 bp. The 1730 and 1580 bp units hybridize with each other under stringent hybridization conditions. In the 1730 bp repeat identical relative localizations of several restriction sites have been demonstrated in both mysticetes and odontocetes. This conservation is quite striking when it is considered that mysticetes and odontocetes separated more than 40 million years ago.

Another sequence, about 420 bp long, occurs among mysticetes. This component, too, is conserved.

A third component characteristic for the mysticete genus *Balaenoptera* shows great differences between the three balenopterids studied.

Restriction patterns of the two conservative components show that they are organized in tandem. The restriction pattern of the variable component does not indicate a tandem organization.

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**F. J. AYALA:** Neutrality versus natural selection: Why is there so much polymorphism?

Electrophoretic studies have established that natural populations of most organisms possess large stores of genetic variation. The frequency of heterozygous loci in an average individual is about 6.0 percent for vertebrates and 13.4 percent for invertebrates. Plants, even those reproducing by self-fertilization, have levels of variation similar to those of animals.

Sequential electrophoresis, protein denaturation, 'fingerprinting' of soluble peptides, and other methods suggest that, at the protein level, the heterozygosity may be two to three times greater than estimated by electrophoresis. At the level of the DNA nucleotide sequence, however, every individual is heterozygous at virtually every locus, if introns as well as exons are taken into account.

What is the evolutionary significance of this wealth of protein and DNA variation? The neutrality theory suggests that at the molecular level most of the variation is adaptively neutral. Two general arguments have been adduced. One is a direct argument that relies on the apparent existence of a molecular clock. Although there is a rough molecular clock, it provides no direct support for the neutrality theory. A second argument is negative: if the number of gene loci subject to natural selection is very large, populations would withstand an enormous genetic load and be unable to survive. Experiments designed to examine this argument show that it is not valid. Natural populations carry genetic loads sufficient to maintain thousands of polymorphic loci by natural selection.

Recently obtained DNA sequences show that the rates of nucleotide substitution are significantly heterogeneous in different parts of the genome, even when they do not yield amino acid substitutions in the encoded proteins. This is inconsistent with the neutrality theory, or can only be explained ad hoc.

When particular enzyme loci are experimentally tested, it is generally found that they have significant effects on fitness, at least under some environmental conditions. Overdominance is only one of the selection mechanisms contributing to the maintenance of molecular variation; other, perhaps more important, processes are frequency-dependence, overcompensation, and alloprooptic selection.

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**B. O. BENGTSSON:** The flow of genes through a genetic barrier

Genetic differences can add to the effect of a geographical barrier in reducing the gene flow between two populations. If, for example, the populations differ by a translocation for which heterozygotes have fitness  $1-s$  compared to a normal value of unity, then the effective gene flow between the populations is reduced by a factor  $(1-s)/(1+s)$ . The strength of such genetical barriers depends on the number of factors building them, their linkage relationships and their fitness interactions. Barriers built by independently acting factors can never become very strong unless the fitness of the first generation, between-population hybrids, is severely reduced.

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**N. K. BIRKELAND, R. NIELSEN and B. H. LINDQVIST:** In vitro deletion analysis of a gene controlling late gene expression of Phage P2

The late genes of phage P2 code for proteins involved in head and tail synthesis. These genes are organized into four operons. During infection the expression of P2 late genes depends upon P2 DNA replication genes A and B and requires the host RNA polymerase. A mutant of *E. coli* called *gro109* blocks P2 late gene expression. This is due to an altered  $\alpha$ -subunit of the host RNA polymerase.

Certain P2 mutants are able to overcome the *gro109* block by restoring P2 late gene expression. These mutants are called P2 *ogr* (over grow). The *ogr* mutations map between the early and late genes of P2, and the mutants complement a P2 wildtype in a *gro109* host. This means that the *ogr* gene codes for a diffusable product. This product is presumed to be a positive regulator of P2 late transcription.

In order to study the organization and control of the *ogr* gene and to identify its product, we have cloned and performed an in vitro deletion analysis of the *ogr* gene region. The deletions have been characterized by marker rescue, restriction mapping and to some extent by sequence analysis. Several interesting features have emerged from this analysis, e.g. one of the deletants appears to over-produce the *ogr* product. By using this overproducer

the *ogr* gene product has tentatively been identified as a 9.5 Kd protein.

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**K. BLOCK:** Structural rearrangements observed in connection with TE — a large transposon in *Drosophila melanogaster* (poster presentation)

TE (= transposing element) represents a large transposon carrying the two structural genes white and roughest. It was discovered by Gunnar Ising many years ago and more than 200 positions for TE have been localized, many of them both genetically and cytologically.

Preliminary investigations have indicated that TE might cause structural rearrangements. The present investigation is an attempt to estimate the frequency of newly arisen structural rearrangements in one particular TE stock.

The stock contained TE in 100A in chromosome 3R. In 2,500 larvae the polytene chromosomes were investigated as to structural rearrangements. Four different newly arisen structural rearrangements were observed, each of them with one break point identical with or close to the insertion point of the TE, which indicates a frequency for these events of about  $10^{-3}$ .

Another screening method for new structural rearrangements in the same stock will be presented.

Thus, it will be possible to get a more accurate estimation of the frequency of chromosomal rearrangements and also an estimation of breakage distribution.

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**M. BOSNES and O.-A. OLSEN:** Developmental mutants in barley — a tool for the study of gene regulation in higher plants? (poster presentation)

Due to the presence of both hormonal and tissue specific gene control, the developing cereal seed is one of the more attractive systems for the study of gene regulation in higher plants. In order to isolate potential mutants in regulatory genes, seeds from the barley cv. Bomi were treated with Na-azid. After this treatment, two different screening strategies were followed, i.e. screening for changes in specific gene products, and screening for alterations in seed anatomy.

The present paper deals with the latter approach, in which  $M_1$ -plants were screened for heads segregating 3:1 into plump and shrivelled seeds. A high number of such mutants were isolated, out of which 14 have been characterized further by means of allelism testing, light and transmission electron microscopy as well as electrophoresis of extracted hordeins.

Out of the 14 mutants, 9 had seeds that germinated readily, while 5 were lethal. Dry seed weights at maturity ranged from 10–60% and 10–40% compared with normal among the viables and lethals, respectively. In the

combinations tested so far, no allelic mutants have been found.

From studies of longitudinal (scutellum and embryo) and transverse sections (endosperm) it can be concluded that the induced single gene mutations affect different tissues differently, but, in general, the magnitudes of the effects in the endosperm and embryo were comparable. In the lethals embryo development was arrested at different early stages, some already at the 16-cell stage. A wide range of effects were observable in the starchy endosperm, ranging from complete lack of aleuron cell differentiation to mutants with little or no accumulation of starch. In one of the mutants (B9) examination with transmission electron microscopy revealed the mutant phenotype in the embryosac already two days post fertilization, the mutant having a more condensed cytoplasm compared with the wild type. Among the viable mutants, all except one had well developed embryos and a varying degree of impairment in the starchy endosperm and aleuron.

Further efforts to characterize protein synthesis in these mutants by means of two-dimensional gel electrophoresis and recombinant DNA-technology are in progress.

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#### A. BRØGGER: Cytogenetic monitoring of environmental mutagens

The aim of cytogenetic monitoring in public health is to protect the population against genetic hazards from environmental agents. By genetic hazard is understood any possibility that a primary change in the quality or the quantity of the genetic material in any of the cells of an individual results from the influence of an environmental agent, which then acts as a mutagen.

Monitoring may be focused upon the environment or upon people. Cytogenetic monitoring plays a minor role in environmental surveillance (such a short-term testing of air or water samples), but is mainly aimed at human subjects.

Targets for environmental mutagens are *germ line cells* with the possibility of a *hereditary effect* (genetic disease in the offspring) and *somatic cells* with the possible development of *cancer*. The effects upon germ line cells and somatic cells are correlated with each other.

The cytogenetic parameters are (1) structural or numerical chromosome aberrations, (2) sister chromatid exchange frequencies, and (3) occurrence of micronuclei.

Hansteen studied the chromosomes of all newborns in Telemark, Norway during one year (HANSTEEN et al. 1982; HANSTEEN 1983). The parents were classified according to residence as 'exposed' (neighborhood of factories), 'medium exposed' (urban areas) and 'unexposed' (rural areas).

The highest number of de novo chromosome aberrations, congenital malformations and spontaneous abortions was found in the 'exposed' areas. If the material had been twice as large with the same trend, the increase would have been statistically significant.

A working party engaged by the Nordic Council of Ministers has prepared a report on Chromosomal Changes Caused by Occupational Factors (available from Nordiska ministerrådets sekretariat, St. Olavsgt. 29, 0166 Oslo 1). From their survey of 72 published reports it is concluded that the following occupational factors are identified with certainty to induce chromosome damage in exposed persons: cytostatic agents, benzene, epichlorohydrin, ethylene oxide, ionizing radiation, styrene, vinyl chloride.

It is stated that "A significant increase in the frequency of chromosomal changes in the peripheral blood lymphocytes of an exposed group of workers should be considered an indicator of the existence of genotoxic agents in the working environment and, consequently, may represent an increased risk of malignancies and of reproductive ill-health in the exposed population. The clinical consequences of such findings at the individual level cannot be evaluated at present."

In cytogenetic monitoring care must be taken to evaluate possible confounding factors, such as the individual variation in the response to clastogenic agents, viral infections, use of clastogenic drugs, smoking habits and radiation exposure. The selection of a proper reference group is also important. Laboratory standards differ throughout the world, and even if there is an international nomenclature for chromosome aberrations, aberrations in chromosome preparations are scored differently in different laboratories. Efforts are being made to develop a better standard for work with clastogenic effects.

Cytogenetic methods are expensive. Monitoring must therefore be restricted to small groups with high risks.

In case of a chemical accident specific emergency plans are needed. Among the immediate measures are the identification of possible genotoxins. If genotoxins are involved, blood samples for cytogenetic studies should be taken as soon as possible.

#### References:

- HANSTEEN, I.-L. 1983. Arvestoffskader og miljøpåvirkning. Rapport 83.03 A and B from Genetics Laboratory, Department of Occupational Medicine, Telemark Central Hospital, N-3900 Porsgrunn, Norway. Pp 110 + tables. (In Norwegian).
- HANSTEEN, I.-L., VARSLØT, K., STEEN-JOHNSEN, J. and LANGÅRD, S. 1982. Cytogenetic screening of a newborn population. — *Clin. Genet.* 21: 309–314

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#### A. BRØGGER<sup>1</sup>, R. BECK NICOLAYSEN<sup>2</sup>, R. NYGAARD<sup>3</sup>, H. WAKSVIK<sup>1</sup>, P. J. MOE<sup>3</sup> and J. COHN<sup>2</sup>: Long-term cytogenetic effects of cytostatic therapy of cancer patients (*poster presentation*)

Cytogenetic studies were made of 25 children (11 girls and 14 boys) with acute lymphoblastic leukemia, treated successfully with cytostatic agents, and a reference group of

children matched for age, sex and residence. The cytostatic treatment involved vincristine, prednisone, l-asparaginase, methotrexate, 6-mercaptopurine for two to three years, and, in a few cases, cyclophosphamide, daunomycin and cytosinearabioside. No irradiation was given. Chromosome breakage and sister chromatid exchange were not increased at one to five years after end of therapy. More cells with cytologically stable aberrations (translocations, deletions) were found among the patients although an unexpected amount of aberrant cells was also observed in the reference group.

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**F. B. CHRISTIANSEN and H. R. SIEGISMUND:** Selection component analysis of biochemical polymorphisms in *Gammarus oceanicus* (poster presentation)

The resolution of reproductive selection components in males by population studies of mother-offspring combinations is limited in organisms where the females are multiple-inseminated, as optimal information is gathered from mother-offspring combinations with one offspring per female. The selection component analysis in populations of *Zoarces viviparus* provides an example of this (CHRISTIANSEN, FRYDENBERG and SIMONSEN, *Hereditas* 87: 129–150, 1977). However, population samples of mother-offspring combinations with three or more offspring per female provide complete information on the male reproductive selection components in organisms where a brood is sired by one male only (CHRISTIANSEN, *Hereditas* 92: 199–203, 1980). For each female genotype the genotypic proportions among the population of their mates may be extracted from the mother-offspring data in addition to information on the segregation in male heterozygotes. Thus, compared with the selection component analysis applied to *Zoarces viviparus* a selection component analysis on data from a monogamous species supplies additional information on gametic selection and sexual selection in males. Further, a more detailed description of the mating pattern is gained from complete mother-offspring data.

The amphipod *Gammarus oceanicus* shows a mating behaviour that strongly suggests that a brood is sired by one male only: before mating the male carries the female in a precopula and awaits her moult which is the time for mating. The female carries the developing eggs until the juveniles hatch, so populations of *G. oceanicus* are available for mother-offspring investigations of selection components. A population sample of 809 individuals including 361 mother-offspring combinations with five offspring per female was investigated for two polymorphisms, at the *Gpi* and *Mpi* loci, in *G. oceanicus*. In addition, the data comprised a sample of 400 precopulas. At the *Gpi* locus no evidence of selection was found. At the *Mpi* locus no evidence of reproductive selection components was found, but the genotypic proportions among adults showed a deficit of heterozygotes. This deficit, however,

could be accounted for by the segregation of a rare recessive allele at the *Mpi* locus.

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**M. DEGERMAN:** Nutritional evaluation of mature pea seeds (poster presentation)

Yellow and coloured peas are traditionally used for human and animal nutrition, respectively. They are known to have a difference in palatability and the latter are therefore of minor use in animal feed. Three pea varieties of different genotypic origin were chosen to investigate if there were differences according to both the biochemical contents and the nutritive value of the mature pea seeds. One of the peas was a yellow cultivar used for human consumption, another was a coloured cultivar used for animal consumption and the third was a subspecies of *Pisum*, *Pisum sativum*, ssp *elator* which is not an ordinary cultivar. The material was grown in the field and then analyzed for the contents of amino acids, tannins, trypsin inhibitors and cyano-glucosides. Animal experiments with rats were used to determine the nutritional quality as biological value, true digestibility and net protein of utilization.

In spite of the genotypic differences these three varieties showed no significant differences in their amino acid contents, nor in their chemically determined nutritive value, the amino acid score. On the other hand differences in antinutritional substances, especially tannins, were found and even the biologically determined nutritional values (biological value, true digestibility and net protein of utilization) showed variations. There may be a coupling between the content of tannins and the biologically determined nutritional values. On the other hand, it was found that the nutritional values could be considerably increased when synthetic amino acids were supplemented to the raw pea feed.

The results indicate that the peas can be cultivated as a very good resource for nutritional improvement, but further investigations are needed for a better understanding of the mechanisms behind the results.

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**A. EICHE, U. HAGLUND and G. ERICSSON:** An alternative to the spot test in the mouse? (poster presentation)

A spontaneous new mutation in the mouse was previously described (EICHE and HAGLUND 1982). This colour mutation, which occurred in an inbred subline of C57BL, is morphologically dominant but acts as a recessive lethal at late fetal stage. The carriers are grey and white. The colour pattern is distributed similarly in all animals. The heterozygous animals are as viable and fertile as nonmutant animals from the inbred subline.

Revertant black spots of spontaneous origin are found in 3–5 per cent of the heterozygotes. These spots are of

varying size and location. This unstable mutation has been studied for its usefulness in mutagenicity tests. Females carrying heterozygous fetuses have been treated with ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS) and procarbazine on days 8–10 of pregnancy. For all three substances, a significant increase was found in the frequency of young carrying spots. Thus, this mutation may offer a good alternative to the spot test after further evaluation.

*Reference:*

EICHE, A. and HAGLUND, U. 1982. — *Hereditas* 97: 316–317

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C. FLYG, G. DALHAMMAR and H. G. BOMAN: Humoral immunity in *Drosophila*

In vivo experiments have earlier indicated that *Drosophila melanogaster* has an inducible, cell-free immune system. In adult flies, induction of the system is best obtained by injection of freeze-thawed cells of *Enterobacter cloacae*. Induction was also obtained by ultrasonic or with microwave treatment, but at lower levels. In both cases, the exposure was only 15–20 seconds. Flies to be exposed to ultrasound could be anaesthetized in ether but those to be induced with the microwave cooker had to be kept awake.

To identify the immune factors induced, the flies were homogenized in Eppendorf tubes together with PMSF (phenylmethylsulfonyl fluoride), soy bean trypsin inhibitor, and PTU (phenyl thio urea) in 1 M acetic acid. The tubes were then centrifuged, boiled for 2 min, and then recentrifuged. Electrophoresis at pH 4 combined with an overlay of *Escherichia coli* has previously been used to identify the Cecropins and Attacins, two main classes of antibacterial immune proteins in the *Cecropia* moth. The same method now showed that both induced and uninduced *Drosophila* contain substances with mobilities resembling cecropin A and the attacins. The substances were electrotransferred to nitrocellulose filters and treated with antibodies specific for cecropin A and the attacins. The induced *Drosophila* substances were found to cross-react only with the cecropin-specific antibodies.

We conclude that *Drosophila melanogaster* flies have an immune system which contains cecropin-like factors. This is now being studied at the gene level.

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I. FRYKMAN: Electrophoretic analysis of a karyotypic hybrid zone in *Sorex araneus*

A karyotypic hybrid zone, represented by eight populations, from a region in the north of Sweden where the central and the northern karyotypic races of the common shrew come in contact, has been electrophoretically analyzed. Three of the populations belong to the northern race and four to the central race while in one population kary-

otypic hybrids and also animals of both chromosomal types were found.

The changes in allele frequencies over the hybrid zone have been analyzed for the three genetically variable enzyme loci, *Ada*, *EsB1* and *Mpi*. The coincident clines shown by the *Mpi A*, *B* and *C* alleles over the investigated region indicate that gene flow occurs between the two karyotypic races. This conclusion is supported by the finding of ten out of eleven alleles from the three investigated loci in animals belonging to both races. The genetic exchange appears to go in both directions, but has not been sufficient to equalize the allele frequencies between the populations in the contact region.

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H. HED: Fertility and mortality: a study on a number of Swedish populations

Mortality and female fertility data from five Swedish populations have been analysed. The populations are from different parts of the country, representing the counties of Norrbotten, Malmöhus, Södermanland, Uppland, and, the largest sample, the counties of Östergötland and Småland (the diocese of Linköping). The material for the first four populations covers the time period 1800–1850, and the fifth population, the years 1600–1850.

Both the mortality and the fertility data reveal substantial differences between populations and, for the Linköping population, significant changes over time. Each population is shown to consist of three parts as regards reproductive age: (1) those women who die before the reproductive age; (2) the childless women; (3) the childbearing women. The sibship size distributions are in good agreement with the expected theoretical distribution; with one important exception: the proportion of zero sibships (=number of childless women). This group is much larger than expected. The group of childbearing women are a reproductively successful group, but the reproductive achievements of this group are not always large enough to raise the net reproduction of the population above the zero growth limit.

When followed during two and a half centuries the results show a sharply declining child mortality over time, a significant rise in mean age at first childbirth, a reduction in mean sibship size and range, and an increasing proportion of childless married women. The result of this is that the opportunity for selection will depend more and more on differences in reproductive success and that childhood mortality will become less important as a selective factor.

A comparison with other studies places my results within the observed range. However, my population concept is different from that usually applied, being neither a tribe nor a state or nation. The present material differentiates subpopulations within a nation, and reveals a significant regional, and possibly social, heterogeneity.

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**A. HOIKKALA:** Evolution of the male courtship sounds in the species of the *Drosophila virilis* group.

The male courtship sounds of the 12 species of the *D. virilis* group consist of polycyclic sound pulses with a frequency of about 300 Hz. Six species of the group have species-specific sounds, which can be distinguished by the number of cycles in a sound pulse, by the length of an interpulse interval (ipi) and/or by the number of pulses in a pulse train. The sounds of the other six species of the group consist of dense pulse trains being very similar to each other or differing by the number of pulses in train.

Genetic control of different traits in the courtship sounds of the *D. virilis* group species has been traced from the genetic variation in different sound traits within the species and by analysis of the sounds of several  $F_1$ s and backcross hybrids of *D. virilis* and other species of the group and of the species having similar courtship sounds. The "most important" sound traits, the number of cycles in a pulse, the length of an ipi and the number of pulses in a train seem to vary quite independently within the sounds of the species studied. Also differences between species in these traits seem to be determined, at least partly, by different genetic factors, the first two traits being affected by genes on the X chromosome and the autosomes, and the last trait, by autosomal genes.

The male courtship sounds do not seem to be very important in mating for the females of most species of the *D. virilis* group, as the females of these species accept the courtship quite easily even without any sounds. The males seem to be, in many cases, mainly responsible for maintaining the sexual isolation between the *D. virilis* group species. If the species-specific courtship sounds have evolved for strengthening the sexual isolation between species, the role of the males and the females in maintaining sexual isolation must have changed during the evolution of the isolating mechanisms.

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**G. ISING:** Gene-transposition mediated by mobile genetic elements in *Drosophila melanogaster*

Some years ago the so called "spontaneous" structural chromosome changes in *Drosophila* as well as other organisms were regarded as caused by several uncontrolled sources. Among these were: natural radiation, effects of chemicals in the food and specific instability of some chromosome regions. This complex picture has to some extent lost some of its unknown components after the discovery of a number of so called mobile genetic elements, constituting as much as 10–12% of the *Drosophila* DNA. These elements are capable of moving around at a low rate, which has not yet been possible to influence by physical means. What is more, one or two such elements can cause deficiencies, inversions and translocations. Thus, they may be responsible for a large part (maybe the majority) of the rearrangements that have been called "spontaneous".

Also transposition of one or a few genes into new chromosome regions may be considered as a phenomenon usually caused by transposable elements. One of the questions we are studying in Lund is whether these transposed genes can become stable in their new environment if the mediating mobile structures are lost or changed.

During the last two years the so called P elements, which constitute the background for the "hybrid dysgenesis" phenomenon observed in specific crosses, have been successfully utilized in transformation experiments by a number of workers. The first two were RUBIN and SPRADLING in 1982 (*Science* 218: 348–353). These transformed genes usually do not enter the normal position when incorporated, but seem to be more or less randomly inserted over the chromosomes.

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**R. BAGGER JØRGENSEN:** Evolutionary relationships in the *Hordeum* (Barley) genus. An electrophoretic examination of proteins

The wild relatives of cultivated plants have been the subject of increasing interest in recent years. Wild species are a potential source of new genetic variation which could be used in plant breeding. The genus *Hordeum* (barley) comprises 32 species. All species except *H. guatemalense* were examined electrophoretically in six enzyme systems, viz. Got, 6-Pgd, Mdh, Idh,  $\alpha$ - and  $\beta$ -amylase. Eleven loci were scored for in these systems. Numerical taxonomic methods applied to the electrophoretic data divided the genus into three groups. The cultivated barley, *H. vulgare* ssp. *vulgare*, and its wild form, *H. vulgare* ssp. *spontaneum*, were grouped together with the Eurasiatic species *H. bulbosum* and *H. murinum*. Another group consisted of one species, *H. marinum*, with two subspecies, which might be given status as separate species. The third group was comprehensive, including the rest of the species; however, as to protein composition the group was well defined. The enzyme phenotypes suggested that the main part of the polyploid cytotypes in the genus originated as allopolyploids. An evolutionary theory for the genus *Hordeum* is proposed, with two centres of differentiation, in South-West Asia and South America, respectively.

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**U. KAUFMANN, K. CHRISTENSEN and B. AVERY:** Chromosome mapping in domestic pigs (*Sus scrofa*) (poster presentation)

To study the chromosome organisation in swine must be considered as a prerequisite for application of the new DNA-technology in breeding work.

The chromosome mapping has been done by means of somatic cell hybridisation. Swine cells have been fused with mice myeloma cells. The fused cells preferentially lose swine chromosomes and 24 hybrid cell lines have been



derived which contain from one to ten swine chromosomes.

The analysis to identify the retained swine chromosomes has been done by means of normal Giemsa staining and R-banding. Isozyme analyses were carried out for 12 systems to determine if enzyme activity belonging to swine was present or not. If a swine isozyme is present in a cell line, it must be encoded by one of the remaining swine chromosomes in that particular cell line.

Until now the swine isozymes for MPI (mannose phosphate isomerase) and NP (nucleoside phosphorylase) have been present in several of the hybrid cell lines. They are linked and have been mapped to swine chromosome No. 7. PK (pyruvate kinase) from swine is found by us not to be linked with MPI and NP contrary to the findings of other groups. Our mapping of this enzyme tentatively points to chromosome No. 8. The chromosome numbering given is in accordance with GUSTAVSSON et al. 1972, *Exp. Cell Res.* 70: 471–472.

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**K. KLINGA: Aneuploid frequency and competition in an autotetraploid population of *Festuca pratensis* (poster presentation)**

Autotetraploid populations usually consist of a number of aneuploids among the euploids. Irregularities at meiosis result in some gametes with chromosome numbers deviating from the normal  $n=14$ . If these gametes are viable, aneuploid plants can be produced.

In the investigated autotetraploid population of *Festuca pratensis*, there were 29.3% aneuploids, with 11.8% hypoploids and 17.5 hyperploids. The relation between seed size and aneuploid frequency has been studied and it turns out that the frequency of hypoploids is higher among small seeds, while hyperploidy frequency is independent of seed size.

Studies in many species indicate that aneuploids, especially hypoploids are less vigorous and almost always less fertile than euploids. This investigation, on individual plants in pots, gives further evidence of these indications.

The effect of different plant densities on survival and yield of euploids and aneuploids has also been studied. The experiment was carried out for three seasons, the year of sowing included. Seeds were sown at four different densities. On five occasions during the experimental period aneuploid frequency, survival, and yield were determined.

The aneuploid frequency changed with age of seed, from about 30% to about 12%. Survival of euploids is much higher than that of aneuploids. It seems that survival of euploids is density dependent, while among aneuploids, there is just a slight dependence.

The yield of aneuploids is usually lower than that of euploids, but there are few significant differences. Dry matter yield, per unit area, is density dependent at the

beginning of the experiment. Later, variation in sowing densities is highly compensated by variation in growth of individual plants.

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**K. KOIVISTO and P. PORTIN: Induction of mortality mutations by ethyl methane sulfonate (EMS) in *Drosophila melanogaster***

The genetic control of adult life span has been studied in the fruit fly, *Drosophila melanogaster*, by investigating mortality mutations which markedly decrease life span. The present study is limited to X-linked genes which bring about life-shortening. Two strains of *D. melanogaster* were used: yellow and Basc (Muller-5), the latter being a mutant strain commonly employed to the detection of X-linked lethal mutations.

200 males from the yellow stock were exposed to EMS. Adult males were fed on Kleenex saturated with 0.025 M solution of EMS in sterile 1% sucrose solution. Adult males (50) were added and the bottle was tightly stoppered with a large wad of cotton. Males were fed on the EMS solution for 24 hours in 25°C, after which they were shaken into a fresh culture bottle containing standard food medium.

Males were then mated singly to Basc virgin females. The F<sub>1</sub> females from these matings were collected as virgins and mated individually with their Basc brothers. Yellow type males among the progeny of each of these crosses would then possess an X chromosome derived from one EMS-treated sperm. A large number of these yellow type males were tested for duration of adult life, along with control flies from the untreated yellow and Basc strains.

Testing was done as follows: several populations of 10 newly hatched males of each stock were housed in a shell vial with food, and kept in an incubator at 25°C. Surviving males were transferred to fresh vials every 10 days. The number of survivors was recorded each day until all had died. Mean adult life spans were computed for the control groups and for EMS-treated strains. Those EMS-treated strains whose mean adult life span was significantly shortened have been selected as subjects for further study. From 51 X chromosomes thus tested, 13 produced strains which were found to have significantly shortened adult life spans. Homozygous females from each of these strains were also bred and their mean adult life spans determined.

We have elected to use the method (Muller-5) to screen for mortality mutants whose effective phase is during the adult stage of development, and whose phenotypic effect is a decrease in life span. In each of the EMS-treated mortality mutations strains there was a clear life-shortening effect as compared to the control flies. The effective phase was confined to a quite short period (mortality crisis) of adult life span, as shown by steep slope of the curves. Moreover, almost each of the mortality mutants had a different survivorship curve.

It seems that obviously the mode of action of the morta-

lity mutations is different and perhaps none of the mortality mutations was an allele of another.

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**H. G. KÖLMARK and S. VEENHUIZEN:** Microconidial mutants with high fertility in *Neurospora crassa*

*Discovery and origin.* — In a cross between the standard wildtype strain 74-OR23-1A and an auxotrophic mutant, *inos* (37401), was found a large number of offspring with microconidia. This was unexpected since both of the parental strains were known to produce macroconidia. We named the microconidiating type *blo* (bleak orange) since it was devoid of the orange colour of *Neurospora macroconidia*, while it had retained a bleaker orange colour due to pigment in the hyphae. Some of these strains were noticed to produce a profusion of protoperithecia and were tested to be highly fertile as protoperithecial (female) parents in crosses with strains of the complementary mating type.

The origin of the *blo* type was traced to the parent strain 74-OR23-1A since a sample of reisolates from single vegetative spores (conidia) from this strain yielded 29 and 57 cultures with macro- resp. microconidia. Seven of the *blo* reisolates were tested for mating type and all were found to be *A*, the same as the 74 *A* strain. Comparison of the growth patterns of the 7 reisolates revealed that hardly any two of them were entirely identical with regards to morphologies. This makes it unlikely that the *blo* type could have been introduced as a contaminant from the outside. On the other hand it raises the question whether they originated from repeated mutations due to some unstable genetic condition in the wildtype strain.

*Genetics.* — Several of the *blo* isolates had similarities with previously known double mutant strains of *pe fl* (peach, fluffy), which produce only microconidia. We have tested ascospore isolates from 5 different crosses between *blo* and *fl* strains and found no wildtype or *pe* recombinants among a total of 269 isolates. The offspring thalli were either *blo* or *fl* phenotype, indicating that *blo* is allelic with or closer than 0.4 c.o. units from the *fl* locus on the right arm of linkage group II. The c.o. distances to other markers on the right arm of L.G. II were determined as follows: *blo* is distal to *ure-3* ( $14.3 \pm 4.7\%$ ) and to *ace-1* ( $7.2 \pm 1.9\%$ ), while it is proximal to *trp-3* ( $7.2 \pm 3.9\%$ ) similar to the order of *fl* with respect to these markers, and in fair agreement with the distances from the markers to *fl* (PERKINS, RADFORD, NEWMEYER and BJÖRKMÄN. 1982. — *Microbiol. Rev.* 46: 426–570).

*Mating type tester strains.* — Alleles of *fl* are often female sterile but some highly fertile *fl* strains are known (LINDEGREN. 1939. — *Bot. Gazette* 100: 592–599) and have been used as mating type testers (PERKINS. 1974. — *Genetics* 77: 459–489). The *blo* mutants are similar to this latter type of *fl* and we have found that they are very convenient to use as mating type testers in Petri plates since they produce no macroconidia and the microconidia have no tendency to airborne spreading when the lids are removed.

*Combination of blo with crisp.* — We have combined *blo* with various other auxotrophic and morphological markers. The combination *blo cr* is interesting because it grows colonially while it has still preserved the high fertility of the parental *blo* strain. The *crisp* phenotype is suppressed since the double mutants are bleak orange and microconidiating.

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**H. KORPELAINEN:** Genic differentiation of *Daphnia magna* populations

Enzyme gene variation and differences in fecundity between genotypes were examined in 22 natural populations of the cyclically parthenogenetic *Daphnia magna* (Crustacea: Cladocera) on the coast of southern Finland and in two Hungarian populations. All Finnish populations studied are intermittent: Pools freeze every winter and dry up periodically. Populations are regenerated entirely from diapausing ephippial eggs which represent the sexual phase.

Allozyme variation was detected at 7 of 13 loci examined. The number of polymorphic loci varied from one to five, and the average degree of genic polymorphism was 0.257. The average heterozygosity across all populations was revealed to be low, only 0.085. On the basis of genetic distances, populations near each other tended to be more similar than populations far apart. However, several exceptions were found, which are suggested to result from founder effect. In most populations, the genotype frequencies deviated significantly from the Hardy-Weinberg equilibrium. These deviations were mainly due to a deficiency of heterozygous individuals, which is believed to be caused by different selection coefficients acting against particular genotypes. Considerable temporal changes in gene and genotype frequencies were also found.

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**S. LAKE:** Recombination in proximal regions in X/X·Y<sup>L</sup> or S females of *Drosophila melanogaster* during temperature shocks of three stages of life cycle

Former tests of recombination in hybrids of inbred *Drosophila melanogaster* have, in the *f*-centromere region, shown:

- A. Temperature sensitivity.
- B. Specific brood pattern of recombination; high in 1–3rd day and lower recombination in later broods.
- C. Variable sensitivity to temperature in meiosis, occurring in different stages of the life cycle.
- D. Eggs laid 1–3rd day after eclosion correspond to egg development during early pupal stages, and eggs laid 8–10th day, to egg development in newly eclosed females, 0–48 h old.

In former tests was used a Y<sup>L</sup>B<sup>S</sup> arm attached to the centromere in order to score the recombination between *f* and the centromere. X–2·Y<sup>L</sup>B<sup>S</sup>.

The following questions were raised: Was the level of recombination frequency and sensitivity to temperature shocks different in hybrids from early and late egg laying according to the results of B and C? Would  $Y^S y^+$  or  $Y^L B^S$  attached to the centromere alter the proximal recombination and/or the temperature sensitivity with regard to the result of A, B and C?

To investigate these problems temperature shocks were given to three stages, two in pupal and one in adult. The temperature shocked pupae and adult females were collected from two egg laying periods, 1–3rd and 9–10th day after eclosion. Three types of X-chromosomes were used:  $X-2.Y^L B^S$ ,  $X-K.Y^L B^S$  and  $X-2.Y^S y^+$ . "K" and "2" indicate two inbred lines.

The results showed effects of temperature in the three stages shocked. The responses to the temperature were similar within the shocked stage but different between stages in the three tested X-chromosomes. Differences between egg laying periods were obtained in the  $Y^S$  serie only, indicating higher recombination in the 1–3rd day egg laying. In the two  $Y^L$  series no differences were detected. In periods not affected by the temperature shock the recombination frequencies were different between females carrying  $Y^L$  or  $Y^S$ .

The recombination frequency in the *f*-centromere region and the effects of the  $Y^L$  and the  $Y^S$  arm attached to the centromere will be discussed.

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**G. LEVAN, P. SANDBERG, F. STÅHL, B. DAHLÖF, T. MARTINSSON and Y. WETTERGREN:** Selective gene amplification in mammalian cells

Selective gene amplification in mammalian cells is now recognized as a common cellular response to selection in a number of different toxic drugs, such as methotrexate (MTX), coformycin, PALA, hydroxyurea (HU), vincristine (VCR), colcemid (COL) and actinomycin D (AMD). Recently, we have studied SEWA murine tumor cells in culture exhibiting the pleiotropic drug resistance (PDR) phenotype. Cells subjected to stepwise selection in AMD, VCR or COL all develop double minute chromosomes (DM), which are a cytogenetic expression of gene amplification. These lines overproduce a 21 K acidic soluble protein and show a high degree of cross resistance, which is typical for the PDR phenotype. Other workers have shown that cells with this phenotype exhibit a shift in membrane-bound glycoproteins from 90–100 K to 150–170 K. Thus, it is likely that several genes are involved in the development of the PDR phenotype.

We have isolated a fraction highly enriched in DM from an AMD-resistant SEWA subline. DNA was extracted from this fraction, and several DM-specific DNA-probes were developed. These probes were used to study independently derived SEWA sublines resistant to AMD, VCR, COL, MTX and HU. The results showed that the investigated amplified DNA-segments in AMD-, VCR-, and COL-resistant lines exhibited a high degree of sequen-

ce homology, indicating that basically the same segment was amplified in the 3 inductions. In contrast, the amplified DNA-segments in MTX- and HU-resistant lines that do not show the PDR phenotype, displayed no sequence homology to the probes used.

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**G. LEVAN, J. SZPIRER, C. SZPIRER, C. HANSON and Q. ISLAM:** The gene map of the rat, *Rattus norvegicus* (poster presentation)

Both the rat and the mouse are commonly used as experimental animals. Compared with the mouse, however, the rat has a very low number of mapped genes, which severely limits the usefulness of the rat in genetic experimentation. We have used rat-mouse somatic cell hybrids to map rat genes and present the following results:

Table 1. Chromosomal assignment of 9 rat genes

Rat gene		Chromo- some	Comment
<i>Alb</i>	albumin	14	New assignment
<i>Afp</i>	$\alpha$ -fetoprotein	14	"
<i>c-Hras1</i>	Harvey sarcoma oncogene 1	1	"
<i>c-Hras2</i>	Harvey sarcoma oncogene 2	X	"
<i>c-Kras</i>	Kirsten sarcoma oncogene	4	"
<i>c-myc</i>	myelocytomatosis oncogene	7	"
<i>Hprt</i>	hypoxanthine-guanine phosphoribosyl transferase	X	Confirmatory
<i>IgK</i>	kappa light chain	4	New Assignment
<i>Tg</i>	thyroglobulin	7	"

(1) Nine rat genes have been assigned to individual chromosomes (Table 1). Several additional genes have been tentatively localized.

(2) There are obvious homologies between rat and mouse chromosomes in their G-banding patterns. These homologies appear to prevail also on the genic level, in the few instances studied so far. Out of 15 genes mapped in both organisms, 11 proved to be concordant with expectations from the similarities in G-band chromosome morphology. Furthermore, when 2 or more genes were syntenic in the rat, they were also syntenic in the mouse in 5 out of 6 cases.

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**Ø. LIE, M. SYED and H. SOLBU:** Genetic studies on bovine non-specific immunity

Lysozyme activity (LZM), total haemolytic complement levels (HC) and levels of complement component C<sub>3</sub> have been determined in the blood of 180 testing station bulls. In addition, serum and colostrum LZM have been assessed in 336 first lactating cows. The bulls comprise 14 sire families, whilst the cow material consisted of 20 half sib groups.

The main object of the study was to make preliminary evaluation of the trait as potential breeding tools. This evaluation embraced parameters like genetic control, i.e. heritability (h<sup>2</sup>), mode of inheritance and possible interrelationships, i.e. correlations.

The specificity of the lytic assays (for LZM the lytic rate of *Micrococcus lysodeikticus* (M.1.), and for HC the lytic activity on sheep amboceptor sensitized rabbit red blood cells (RRBS) is not very high. Therefore, to confirm that we were dealing with a lysozyme, electrophoretic and chromatographic methods besides heat stability tests were employed with the following result:

The lytic activity on M.1. was elicited by a katanioic element with isoelectric points (pI) between 9 and 10, with molecular weight of approximately 15,000 and showing heat stability at acidic pH, and lability at alkaline pH. These are properties in accordance with those of lysozymes. The confirmation that HC reflected the complement enzyme cascade was based on the findings that RRBC lysis was heat labile, that it was specifically inhibited by rabbit anti-bovine C<sub>3</sub> antibodies, and finally that a significant correlation of  $r=0.30$  existed between HC and C<sub>3</sub>. C<sub>3</sub> levels were determined by single radial immunodiffusion.

The results from our studies can be summarized as follows. LZM is controlled by a single dominant gene for high activity. This simple Mendelian inheritance is reflected in sera of bulls and cows as well as in lactal secretions (colostrum) of cows. The genetic influence on serum HC is very high (h<sup>2</sup>=0.75) and a correlation of  $r=0.30$  exists between HC and C<sub>3</sub>.

The above results contribute to a better understanding of non-specific immunity and genetics, as well as contributing to the future preparation of indirect selection schemes for improved resistance in cattle employing marker traits.

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**V. LUND and B. H. LINDQVIST:** Cloning and analysis of promoters from Phage P4

P4 is a helper dependent *E. coli* phage which can replicate its DNA genome of 11.3 Kb by its own but requires help to form virus particles. Because P4 contains no morphogenic genes it is totally dependent on all the known late genes (head and tail) of a helper phage such as P2. The P4 genomes are thus matured into particles by the presence of

all the known P2 head and tail components. Of special interest is the fact that P4 can obtain help from a P2 prophage. Under such conditions P4 activates the genes of P2 by at least two different mechanisms (derepression and transactivation). As a result of this activation P2 now interacts with the P4 genome, leading to stimulation of P4 "late" gene expression. Hence, this relatively simple two-chromosome system shows a complex interaction of gene expression.

As part of our effort to define the interacting elements of P2 and P4 we are searching for promoter activities of P4 and P2 by cloning DNA fragment into the promoter cloning systems of MCKENNEY et al. (1981) The insertion of promoter-containing fragments into the vectors will result in expression of galactokinase (galK) after transformation of a galK<sup>-</sup> host. The galK<sup>+</sup> colonies are easily distinguished from galK<sup>-</sup> bacteria on galactose-containing indicator plates. By these procedures we have isolated several promoter-containing fragments and obtained a preliminary map of functional promoter activities of P4. One of the regions containing a promoter activity which is likely to reflect the P4 *int* gene promoter has been analysed in detail by DNA sequencing.

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**J. MAC KEY:** Developmental correlative growth and yield structure in cereals

Today, plant breeding programmes are often led by an ecoideotype concept, i.e. some kind of simulation model based on knowledge of importance of morphological and physiological traits for a given agricultural situation as to climate, soil, water, nutrients and management. The value of such a more or less concrete goal depends heavily on the profoundness of the functional analysis behind.

The green revolution, characterized by an expansion in Asia of dwarf type cereals over 13.5 million hectares within only four years, was based on a revised view of the optimal architecture of the shoot. An analysis of the relative contribution of assimilating organs to yield of grain had indicated 75 per cent to rely on the green surface above the top stem internode. From a generative point of view, the vegetative production was apparently overemphasized by an earlier, evolutionary need for competitive capacity in a mixed stand. The lower part of the plant could obviously be reduced drastically when merely grain yield in a monoculture became decisive. In addition, a shortening of the stem should offer resistance against lodging, which is essential for maximal utilization of nitrogen fertilizers and water.

The reduction of vegetative growth for benefit of generative productivity had unforeseen correlative effects on

root development. An investigation of a worldwide collection comprising 89 different spring wheat cultivars showed a plant height — root depth interrelation of  $r=0.51\pm 0.04^{***}$ , an interdependence between number of tillers and of crown roots of  $r=0.73\pm 0.04^{***}$  and a correlation between dry weight at heading time for shoot and root of  $r=0.85\pm 0.01^{***}$ . The social tension caused by dwarf cultivars being adapted only on irrigated land and unable to provide sufficient root development in many rainfed areas proved the risk of setting up too simplified an ideotype.

The introduction of densely sown monocultures offering interplant competition on equal terms and with reduced tillering of each plant allowed more emphasis on generative growth. As a correlative consequence, an original crown root dependent system lost in selective value. The adaptation had to proceed through developing a seminal root system instead, independent of tillering but now dependent on endosperm size, another feature of domestication.

Breeding for increased grain yield in cereals is not only a matter of improved partitioning between vegetative and generative phase. Grain yield is a joint effect of number of plants per unit area of land, number of fertile tillers per plant, number of spikelets per head, number of florets per spikelet, seed setting and seed size. The interrelation between these components of grain yield has proved to be very strong, highly restricting transgressions. The emphasis on one or the other has, however, agroecological consequences, since they are not completely able to compensate for each other.

In general terms, the developmental correlation in plants can be summarized in the following way: The size of an organ is proportional to the size of the meristem from which it develops (SINNOTT'S law). Plasticity is inversely proportional to ontogenetic proximity (GRAFIOUS' corollary 1). Number and size tend to have an inverse relationship (GRAFIOUS' corollary 2).

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**B. J. MIFLIN, B. G. FORDE, M. KREIS, J. FORDE, R. FRY, M. WILLIAMSON, H. LEWIS and P. R. SHEWRY:** Prolamin genes of cereals; multigene families with unusual molecular structure

About 40 to 50% of the endosperm proteins of cereal grains are prolamin storage proteins. They are therefore one of the quantitatively most important group of proteins

in our agriculture biosphere and they also influence the quality of cereal grain. We have classified the prolamins of the Triticeae into three major groups: the sulphur-rich, the sulphur-poor and the high molecular weight prolamins. We have mapped the loci encoding these proteins in barley and rye. We have further investigated the nature of these by isolating cDNA and genomic DNA clones related to each of the major sub-groups of proteins from either barley, rye or wheat. Southern blotting has shown that each group is encoded by a multigene family. Sequencing of the DNA clones allied to direct sequencing of the prolamin polypeptides has revealed that each family is made up of genes that contain within them a number of repeated sequences. The relationships between the families and some speculations regarding the origin of the genes will be presented.

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**O. MYKLEBOST, S. ROGNE, S. WALLIS, S. HUMPHRIES and H. PRYDZ:** Isolation and characterization of recombinant clones coding for proteins suspected to be of importance for the development of lipidaemias and cardiovascular disease. I.

We have cloned cDNAs coding for the apolipoproteins E and CII, variants of which may be important for hereditary hyperlipidaemia and cardiovascular disease.

The clones were isolated from a human liver cDNA library by screening with mixed oligonucleotide probes made to be complementary to all combinations of codons coding for part of the known amino acid sequences. The identities of the cloned cDNAs were confirmed by sequence determination.

Two overlapping apoE clones span 1 kb of mRNA sequence from 30 bases 5' to the NH<sub>2</sub>-end of the mature apoE protein to the 5' end with its poly A tail. Sequencing of the areas corresponding to the known amino acid substitutions in the protein variants determined that these variants can be accounted for by C (methyl-C ?)→T or T→C point mutations.

The largest of the apoCII clones contained 440 bp of mRNA sequence, including the whole 3' untranslated sequence (146 bases) and the sequence coding for the mature protein and 17 amino acids of a hitherto unknown signal peptide, 80% of which are hydrophobic.

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**B. V. NIELSEN:** Selection in mice for increased and decreased growth on a normal and a low protein diet

Genotype-environment interaction may be important in the choice of environment in animal breeding. To investigate this a model experiment on growth rate in mice was performed.

Two-way selection for weight gain from 3 to 9 weeks of age was carried out in two environments: a normal protein diet (19.3% protein) and a low protein diet (5.1% protein). Control lines were maintained in both environments. Each selection experiment was made in three replicates each maintained by minimal inbreeding with 8 single-pair matings. After 6 generations of selection, half of the mice selected on the normal protein diet were tested on the low protein diet and vice versa for mice selected on the low protein diet.

Responses were obtained in both environments. Genotype — environment interactions were significant in both sexes, both when selecting for increased and for decreased growth. This suggests that in animal breeding the selection should be performed in an environment equal to the environment in which the improved breed is going to be reared.

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**G. NIELSEN and H. JOHANSEN:** Identification of barley varieties by means of isoenzymes and hordein (*poster presentation*)

The barley varieties on the 'Danish List of Varieties of Agricultural Crops, 1983/84' comprising 59 spring barley and 7 winter barley varieties have been investigated for variants of enzymes and hordein. Of the 35 examined isoenzyme loci 14 showed variation between varieties. The isoenzymes could separate the 66 varieties into 60 different groups, 55 of which comprised only one variety each. Three groups comprised two and one group three varieties. The two hordein loci could separate the varieties into 32 groups, 22 of which comprised one variety each. Four groups comprised two, one group four, one group nine, and one group twelve varieties. The isoenzymes combined with the hordein could separate the varieties. The isoenzymes combined with the hordein could separate the varieties into 64 groups, 63 of which comprised one variety each. The remaining three varieties were placed in one group. Twenty kernels of each variety were analysed for hordein and for the isoenzymes showing variation. Twenty-one varieties showed polymorphism in one or more loci.

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**K. NIELSEN, H. HOPPE and A. GROPP:** Two new established mouse cell lines originating from balanced heterozygous cells (*poster presentation*)

Two mouse cell lines, HB 774 and HB 775, have separately been established from cells originating from balanced heterozygous,  $2n=39$ , mouse embryos 14–15 days old. In both cases one chromosome No. 12 was involved in a Robertsonian translocation, furthermore the cells had a C-band marker, No. 1 with an interstitial C-band; both characteristics which could be followed during the evolution of the cell lines.

The cells were growing as monolayer cultures and cytogenetically investigated in the passages 20 and 26. It was observed in the 20th passage that both HB 774 and HB 775 spontaneously had transformed to hypotetraploid cell lines with  $S=77$  and  $S=74-75$  respectively. Both new cell lines had a few cells in the hypohexaploid region. Six passages later HB 774 still had approximately the same S-number,  $S=78$ , but HB 775 had evidently changed, and around 40% of the cells had 101–125 chromosomes, and more than 30% had chromosome numbers between 126 and 250.

The distribution of the C-band marker and the banded chromosomes in the two cell lines during their evolution was examined. Remarkable was the appearance of minute chromosomes in passage 26, where they were regularly present in HB 775 and randomly present in some of the HB 774 cells. No minutes were detected before ( $P_{20}$ ).

If the spontaneous transformation of the cells to cell lines also was connected with development to malignancy is not known.

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**K. NIELSEN and K. D. ZANG:** Cytogenetical investigations of human brain tumors

Around fifty human intracranial tumors, excluding meningiomas, were cytogenetically examined by us between December 1981 and January 1984. With a few exceptions, the cells were analyzed in stationary particle cultures. When these cultures were not successful, cells were collected from one of the first passages of monolayer cultures. The quality and the number of the metaphases in the particle cultures were mostly inferior to those from the corresponding monolayer cultures. Our opinion was, however, that these negative sides were redeemed by our possibility of avoiding selection by trypsinization and adaptation to the monolayer environment.

Parallel cultures were stained with haematoxylin-eosin for control of the histological pattern in vitro in order to exclude cultures with stromal overgrowth.

The predominant tumor types were astrocytomas and glioblastomas. Several other types were also represented, as for instance plexus papillomas and neurinomas, but with considerably fewer cases. Most of the tumors had chromosome numbers in the diploid region ( $46\pm 1-2$ ), and the cells were accordingly diploid, pseudo-, hyper- or hypodiploid. Very few regularly present chromosome aberrations, gains or losses or markers, except loss of the Y chromosome were observed. The most extreme chro-

mosomal disturbances were revealed among the glioblastomas and the anaplastic astrocytomas.

The number of cases from the different intracranial tumors we have analysed cytogenetically and present here represents around 25% of the until now published intracranial tumors, excluding the meningiomas, which are the most investigated brain tumors.

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**O. F. NIELSEN, A. H. ANDERSEN, B. J. BONVEN, K. CHRISTIANSEN, E. GOCKE, H. R. JENSEN and O. WESTERGAARD:** Involvement of a site- and strand-specific topoisomerase activity in regulation of gene expression in eukaryotic cells

An enzyme activity resembling the eukaryotic type of topoisomerase I has been detected in the protozoan *Tetrahymena thermophila*. The enzyme has been found to cleave the non-coding strand of the spacer regions flanking the rRNA genes at DNase hypersensitive sites. Three cleavage sites were mapped at position -1000, -600 and -150 relative to the transcription initiation point, and a fourth cleavage site immediately down-stream of the transcription termination point. By sequence analysis the enzyme was found to have a specific 16 bp recognition sequence. The finding that all cleavages are confined to the non-coding strand demonstrates a strong polarity of the putative topoisomerase with the DNA at the hypersensitive sites. The location of the cleavage sites in regions framing the coding sequences indicates a function of the enzyme in the transcriptional process, perhaps by providing a swivelling mechanism conferring rotational freedom to the transcribed DNA segment.

Comparative reconstitution studies using topoisomerase extracted from human cells and the strand- and site-specific topoisomerase from *Tetrahymena* show that the same enzyme activity is present in human cells.

Computer analyses or published DNA sequences have shown the presence of the specific 16 bp sequence in regions flanking a number of genes in various eukaryotic organisms including man. Considered together the pieces of information indicate a possible general function of a topoisomerase I activity in regulation of gene expression in eukaryotic cells.

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**Ø. NILSSEN and B. H. LINDQVIST:** Genetic analysis of the head size-determining gene in the P2-P4 phage system (*poster presentation*)

P4 is a satellite phage which depends on all the late genes of its helper P2 (SIX 1975). The genome of P4 is 1/3 in molecular weight (and length) compared with the P2 genome.

Despite the fact that P4 utilizes the very same morphogenetic components as P2, its head size is about one third of that of P2. Hence, an interesting feature of P4 is its

ability to direct the synthesis of a novel head size. This ability is a genetic property of P4 since it has been possible to isolate a mutant of P4 which is unable to make P4 size heads. This mutant, which is called Sid (*size determination*), makes a P2 size head (SHORE et al. 1978). There was recently isolated a helper independent P4::P2-hybrid (Hy 19) with the essential gene region of P4 linked to the late genes of P2 by in vitro recombination techniques (LINDQVIST 1981). This hybrid expresses a P4 Sid<sup>-</sup> phenotype since it, by necessity, makes large heads.

In order to investigate whether the *sid* gene is mutated in Hy19 or simply made inactive by the novel P2::P4 genome arrangement, we have reconstructed the P4 genome from Hy 19 by in vitro recombination as well as by in vivo crosses. The resulting P4 particles were scored for their head size. In all cases the reconstructed P4 makes small heads (Sid<sup>+</sup> phenotype).

These results demonstrate that the *sid* gene in Hy 19 is undamaged and the failure of Hy 19 to express the Sid<sup>+</sup> phenotype must therefore be due to the novel P2::P4 genome arrangement.

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**V. PARROW, P. ALESTRÖM and K. M. GAUTVIK:** 5-azacytidine stimulates hormone synthesis and causes chromosome alterations in cultured rat pituitary tumour cells (*poster presentation*)

Two rat pituitary tumour cell lines GH<sub>1</sub> and GH<sub>4</sub>, were cultured in the presence of 3 μM 5-azacytidine. After 6 and 9 days there was a 2–5 fold increase in both prolactin and growth hormone synthesis as measured by immunoprecipitation of the culture medium with specific antisera and by mRNA dot hybridization. In addition, total protein synthesis (cpm/mg cell protein) as measured by TCA precipitation was also increased.

Samples for chromosome studies were collected four days after removal of the drug. Compared with chromosomes in control cells, the chromosomes of 5-azacytidine treated cells were abnormal, with occurrence of fragments and atypical chromosomes. The cell morphology became also altered from epithelial to spindle formed with rapid growth pattern and cessation of contact inhibition.

Our data suggest that 5-azacytidine has different effects on GH cells. Short-term treatment induces an increased protein synthesis, which may be mediated by gene activation via a demethylation of DNA. In addition 5-azacytidine induces changes in the chromosomes as well as the cell phenotype and growth characteristics.

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**E. J. PAULSSEN<sup>1</sup>, P. ALESTRÖM<sup>1</sup>, V. GAUTVIK<sup>1</sup>, M. KRIZ<sup>1</sup>, E. HAUG<sup>3</sup>, K. SLETTEN<sup>2</sup>, L. PHILIPSON<sup>4</sup> and K. M. GAUTVIK<sup>1</sup>:** Prolactin is made from different mRNAs in a rat pituitary tumour cell line (*poster presentation*)

A rat pituitary adenoma cell line (GH<sub>4</sub>C<sub>1</sub>) produces and secretes the polypeptide hormone prolactin (PRL). The level of PRL mRNA and the rate of PRL synthesis are increased when thyroliberin (TRH) and/or estradiol (E<sub>2</sub>) is added to the culture medium. In cytosol from cells that are cultured with <sup>35</sup>S-methionine in the presence or absence of TRH and E<sub>2</sub>, immunoprecipitable <sup>35</sup>S-PRL forms of 80, 65, 55, 40 and 24 kilodalton (K) are demonstrated. Pulse chase experiments show a shift in the specific radioactivity from the big molecular forms to the intact <sup>35</sup>S-PRL of 24 K. TRH and E<sub>2</sub> increase the synthesis of all these precursor-like PRL forms.

In parallel experiments *in vitro*, translation of poly (A)-rich RNA from GH<sub>4</sub>C<sub>1</sub> cells reveals a 90 K PRL immunoprecipitated polypeptide, which is translated from a 28S mRNA, whereas intact prePRL is made from a 14S mRNA. An immunoreactive PRL form of 30 K is also detected.

RNA blot hybridization to a <sup>32</sup>P labelled cDNA<sub>PRL</sub> probe shows the existence of multiple mRNA species from GH<sub>4</sub>C<sub>1</sub>-cells. The existence of heterogenous PRL-mRNA species is also demonstrated in fertile rat pituitaries, but not in rat liver.

In conclusion, rat PRL is apparently synthesized on multiple mRNA species. The big PRL forms have the characteristics of being a hormone precursor.

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**B. RASMUSON:** How insertions regulate gene expression in *Drosophila melanogaster*

Different strains of *Drosophila melanogaster*, characterized by mutational instability in the white locus, have been analyzed by means of probing cloned parts of the white locus to restriction enzyme digested nuclear DNA. The instability gives rise to shifts between different eye colour phenotypes associated by integration or excision of a transposable genetic element of about 1 kb in the white locus region. Other phenomena associated to the presence of this fragment are generation of white mutants, all shown to be short deletions, and transpositions of the intact white locus to positions spread over the entire genome, with or without this 1 kb element. It has been found that the element is not a part of the white locus but its effect is to regulate the phenotypic expression of the gene.

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**M. RASMUSON:** Selection for sexual dimorphism in *Drosophila*

In a selection experiment using *Drosophila* and with sternopleural bristles as the observed character, females with low bristle number were always crossed to males with high bristle number. The result was an inversion of the normal sexual dimorphism, so that males on the average obtained more bristles than females. This change was highly specific and did not include groups of bristles on other parts of the body.

The genetic background of the new dimorphic pattern was analysed in outcrosses to different wild type and marker stocks. It could be shown that the pattern partly is due to recessive genes on the X-chromosome which depress the female bristle number, and that, mostly dominant, genes on the autosomes have a slight modifying effect.

Since the inverted sexual dimorphism is caused mainly by genes on the X-chromosome a change in dosage compensation might be the explanation. However, none of the current models for dosage compensation can be shown to fit the observations.

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**A. RIIHIMAA:** The inheritance of the facultative diapause of *Chymomyza costata*

*Chymomyza costata* is a holarctic species. In Europe it is very common in Lapland, rare in southern Finland and has been recorded only occasionally in Central Europe.

The diapause and overwintering stage of *C. costata* is the third instar larva. *C. costata* has both a facultative and an obligatory diapause. The facultative diapause is controlled by photoperiod. If there is a diapause stage in every generation independent of photoperiod and temperature, the diapause is obligatory.

Photoperiodic reaction curves at 16°C were determined from population samples collected between latitudes 62°–68° N in Finland. The proportion of larvae diapausing in the temperature of 23.5°C and continuous light was regarded as a measure of the proportion of obligatory diapause in the population samples.

There is a South-North cline both in the facultative diapause (critical daylength) and in the proportion of obligatory diapause. The range of critical daylengths in Finland is from 19 hours (62°N) to 22 hours (68°N). The proportion of larvae having an obligatory diapause ranges from 1% to 44%.

Response to selection for facultative or obligatory diapause or for shorter critical daylengths was rapid. This indicates that the genetic system controlling diapause in *C. costata* is based on only a few genetic factors.

Crossing experiments show that there are at least two genetic factors controlling the critical daylength of the facultative diapause reaction. One of them is located on the X chromosome and the other is autosomal.

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**K. H. RØED:** Transferrin variability and reindeer management (*Poster presentation*)

Interest has been focused on genetic effects of different management strategies of wildlife populations. In Norway, the reindeer, *Rangifer tarandus* L., are separated into a number of both wild and semi-domestic populations. The semi-domestic animals are selectively bred to improve different traits as for example body weight and tameness. Among the wild reindeer, the population size and composition is almost completely controlled by man through the regular hunt. One aim of this study is to test possible genetic effects of different management strategies of reindeer.

Blood samples of both semi-domestic and wild populations of reindeer were analyzed for transferrin variability by polyacrylamide gel electrophoresis. A total of 12 alleles were detected, and in all populations the numbers of alleles were high, ranging from eight to eleven. The pattern of allele frequency distribution indicates high degree of genetic heterogeneity in the transferrin locus. In a hierarchical approach, almost half the heterogeneity between populations was explained by dividing into semi-domestic and wild animals. The results suggest that the different selection strategies in the management of semi-domestic and wild reindeer influence the transferrin allele frequencies.

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**S. ROGNE, O. MYKLEBOST, S. WALLIS and H. PRYDZ:** The use of recombinant clones to study the organization and expression of genes which may be of importance for the development of lipidaemia and cardiovascular disease. II (*poster presentation*)

By using cloned cDNAs as probes, apolipoprotein E and apo CII mRNA was detected in samples from different human tissues by the RNA blotting technique. An apoCII mRNA of approximately 600 bases was present in liver and intestine, but not in kidney, whereas an apoE mRNA of about 1.2 kb was detected in liver and kidney but not in intestine. In leukocytes two different apoE mRNA bands of about 1.4 and 2.7 kb were detected, but not the band at 1.2 kb.

The cloned cDNAs were used to detect recombinant phages containing the respective genes in Maniatis' genomic library. At present we are studying the organization of the apoCII gene.

The apoCII cDNA can be used to detect a polymorphic restriction enzyme site adjacent to the apoCII gene. This DNA polymorphism was used to follow the inheritance of apoCII alleles in families informative for other apolipoprotein protein variants. In this way, the apoCII locus was shown to be closely linked to the apoE locus on chromosome 19.

At present, we are looking for association between apolipoprotein alleles and lipidaemias and cardiovascular diseases. We are furthermore attempting to clone cDNAs

for factor VII and thromboplastin, two proteins which are suspected to be important for thrombosis as well as hemostasis. The cloning of thromboplastin will involve construction of an endothelial cell cDNA library and direct or indirect immunological detection of clones containing thromboplastin cDNA.

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**E. SEEBERG:** DNA repair mechanisms

Both pro- and eucaryotic cells have evolved elaborate enzymatic DNA repair mechanisms specifically designed to maintain the integrity of their DNA. Damage to DNA occurs quite frequently in living cells, either formed spontaneously by hydrolysis at physiological pH and temperature, or induced by exogenous agents such as ultraviolet irradiation, ionizing radiation or numerous chemicals. Formation of DNA damage is efficiently reversed by DNA repair, and is therefore only very rarely (less than 1 in a thousand) expressed as a mutation or leading to cell death.

Clear evidence for the importance of DNA repair for the normal life existence comes from studies of certain rare hereditary human diseases caused by defective repair, e.g. Xeroderma pigmentosum (XP) or Ataxia telangiectasia (AT). Patients suffering from such diseases generally develop cancer at an early age. Cells from XP patients are extremely sensitive to sunlight and the repair defect in such cells has been identified as a deficiency in an enzyme activity initiating repair of UV-damage. The existence of such diseases is the best evidence for DNA damage being a direct cause of cancer.

Essentially all we know about the molecular basis for DNA repair comes from model studies of bacteria. However, both genetic and biochemical studies of eucaryotic cells have shown that analogous processes exist in higher organisms, although it has so far proven difficult to elucidate chromatin repair in molecular terms. The various repair pathways as we understand them today will be reviewed, with special emphasis on excision repair of DNA damaged by UV and adduct-forming mutagens (e.g. benzo(a)pyrene and acetylaminofluorene).

Excision repair of UV-induced pyrimidine dimers and large base-adducts appears in most organisms to be initiated by a complex type of repair endonuclease composed of several different subunits. This enzyme is, in *E. coli*, controlled by the *uvr* genes (ABC), in yeast cells by the *rad* genes (1 through 4, and 10), and in human cells by the XP genes (A through E). The biochemical nature of this type of repair is known so far only in *E. coli*, where the subunits of the enzyme (*uvrA*, *B* and *C* proteins) now have been purified and the repair reconstructed in vitro from purified components. It appears that the endonuclease releases the damage in fragments 12 nucleotides in length. Cuts are made at both sides of the lesion, 7 undamaged nucleotides away to the 5'-side and 4 nucleotides away to the 3'-side. The fragment is displaced from the DNA by DNA helicase II (*uvrD* protein). Repair is then completed by repair synthesis with DNA polymerase I, and the strand is re-joined with ligase. This type of excision repair mechanism is much more complicated than was predicted from the cellular studies. It seems likely that XP-type repair in mammalian cells must be even more complicated and that additional factors are required to make the DNA within chromatin accessible for repair.

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**H. SKINNES:** Genetic investigations on seed dormancy in barley

Crosses were made between Scandinavian lines of barley with a high and a low level of seed dormancy. Results from glasshouse, field and phytotron experiments revealed:

1. Seed dormancy is governed by several recessive genes.
2. No effects of cytoplasmatic factors have been detected.
3. No close associations between seed dormancy and other agronomic characters have been found.
4. The heritability of seed dormancy is high, even for single seeds of early segregating generations.

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**S. SLETNER, A. H. DEGGERDAL and O. A. OLSEN:** An electrophoretic and morphometric study of the phenotypic effects of two high lysine genes in barley (*poster presentation*)

In spite of more than 15 years of studies of barley high lysine mutants, no satisfactory hypothesis to explain the primary effect of such genes has been advanced. In an attempt to characterize the phenotypic effect further, we have studied the interaction between two different high lysine genes as well as compared phenotypic effects in the starchy endosperm and in the embryo. In the first part of the present work, homozygous plants from the Bomi mutants M1508 and M527 and the double mutant M527/1508 (seeds received from Dr. H. Doll, Risø), were compared

with those from Bomi by means of morphometric analysis and electrophoretic techniques. In addition, in order to isolate maternal effects as well as background mutations in the homozygous lines, seeds from F<sub>1</sub> (M1508 × Bomi), F<sub>1</sub> (M527 × Bomi), B<sub>1</sub> (M527/1508 × M1508) and B<sub>1</sub> (M527/1508 × M527) were included.

In the starchy endosperm, M1508 and M527 consisted of cells of significantly smaller size than those of Bomi. The double mutant exhibited an additive effect. In F<sub>2</sub>-seeds there was no significant difference between M527 and Bomi, indicating the presence of a background mutation in the M527 line used. Alterations of large starch granule size follow the pattern described above for cell sizes. The alcohol soluble starchy endosperm protein fraction was run on SDS-PAGE. In accordance with data published by others, we found that M1508 was changed in the A-, B- and C-hordeins. We also found that two high molecular A-hordein bands of M527 were reduced. The modification in SDS-PAGE pattern of M527/1508 was more extreme than that of any of the single mutants.

In the scutellum, TEM on the apical parenchyma cells revealed an increased amount of starch grains in M1508 and the double mutant. While these cells in M1508 and the double mutant were significantly larger than those of Bomi, M527 did not have a similar effect. In seeds from homozygous M527/1508 plants, an additive effect on cell size was apparent. When B<sub>2</sub>-seeds were thus analysed, difference between M1508 and the double mutant could not be recognized, again indicating the involvement of background mutation. In order to further characterize embryo scutellum differences, 2-D electrophoreses were run on total proteins extracted from 21 day old embryos. In summary, changes in 8 proteins (2 albumins, 5 globulins and one gluteline) were detected in the mutants as compared with Bomi. Work is presently in progress to characterize these proteins further.

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**L. SØNDERGAARD:** Mating in artificial populations of *Drosophila melanogaster*

It is well-known that the *ebony* (*e*) mutant of *Drosophila melanogaster* has the extraordinary feature of stabilizing itself in artificial populations. The stable level of *e* is dependent upon the light regime: in constant light about 2% of the population is homozygous *ebony* and in darkness it is 6.5%. Stable levels are reached after 16 generations irrespective of the starting *e* gene frequency. When the populations in constant darkness were transferred to constant light the frequency of *e/e* flies dropped after 9 generations to the level observed in constant light (2% *e/e* flies). Taking into consideration the partial blindness of *e/e* flies these results indicate a behavioural background for this stabilization, i.e. differences in selective mating under light and dark conditions. To investigate this, mating competition experiments were designed which simulated the conditions of artificial populations as closely as possible. Experiments using +/+, *e/+* and *e/e* flies under conditions

of strong male competition showed that in light only 90% of the  $+/+$  ♀♀ were inseminated, whereas 94% of the  $el+$  and the  $e/e$  ♀♀ were inseminated. In darkness, 86% of the  $+/+$  ♀♀, 91% of the  $el+$ , and 94% of the  $e/e$  ♀♀, respectively, were inseminated. In light,  $el+$  ♂♂ preferred  $e/e$  and  $+/+$  ♀♀ to  $el+$  ♀♀, whereas both  $e/e$  and  $+/+$  ♂♂ preferred  $el+$  ♀♀ to  $e/e$  and  $+/+$  ♀♀. In darkness, neither  $e/e$ ,  $el+$  nor  $+/+$  ♂♂ showed preference to any female genotype. The mating success of  $+/+$  ♂♂ was independent of the light regime, whereas the mating success of  $el+$  ♂♂ diminished and that of  $e/e$  ♂♂ increased in darkness compared to light. The conclusion from these experiments is that in a mixed population of  $e/e$ ,  $el+$  and  $+/+$  flies mating is not random and the mating pattern is dependent upon the light regime (i.e. light or darkness).

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**R. STEEN:** Genetic organization of Tn7 (*poster presentation*)

The 14 kilobases transposon Tn7 mediates resistance to trimethoprim and streptomycin/spectinomycin. This transposable element is spread among the enteric bacteria and is commonly found in trimethoprim resistant clinical isolates in most parts of the world.

The two resistance genes carried by TN7 were by restriction enzyme analysis closely mapped within the left third of the transposon, and their transcripts were studied by RNA-DNA hybridization. The allelic state of the *rho* gene of the host influenced the expression of dihydrofolate reductase from Tn7. This could be related to the observation of two overlapping transcripts with a common starting point in the genetic area preceding the structural gene for dihydrofolate reductase and with lengths of 170 and 840 bases, respectively. Their occurrence could reflect an attenuation mechanism.

From DNA-DNA hybridization studies the Tn7-borne streptomycin resistance gene was found to be homologous to the streptomycin adenylyltransferase gene of R plasmid R1. The formation of the adenylyltransferase was furthermore observed to be influenced by the expression of the dihydrofolate reductase gene which is located about 600 base-pairs before the streptomycin adenylyltransferase gene. A deletion of a 2.5 kb *Hpa* I fragment, which abolished the dihydrofolate reductase synthesis, would thus decrease the adenylyltransferase formation to about 40% of its normal level. These results suggest that the two resistance genes encoded by Tn7 are organized in a transcriptional unit under the influence of an attenuation mechanism.

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**A.-L. STEINUM and E. SEEBERG:** Nucleotide sequence of the *tagA* gene from *Escherichia coli* (*poster presentation*)

We have sequenced a DNA fragment of 895 nucleotides

containing the *tagA* gene from *Escherichia coli*. The *tagA* gene encodes 3-methyladenine DNA glycosylase I (TagI), which removes 3-methyladenine from DNA and is involved in repair of cells treated with simple alkylating agents such as N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and methyl methane-sulfonate (MMS). The DNA fragment has only one open reading frame and there is only one initiation codon of translation within that frame which has a ribosomal binding site "GAGGGAAAG" (Shine-Dalgarno sequence) in front. The amino-acid sequence of TagI is deduced from the nucleotide sequence and TagI appears to contain 187 amino-acids and has a molecular weight of 21,178. It is unusually rich in cysteine. A putative initiation point of transcription is located 24 nucleotides upstream from the initiation codon with a -10 region sequence of "TGAAAT" and a -35 region sequence of "TTCACC". This corresponds reasonably well to the consensus promoter of *E. coli* being "TATAAT" at -100 and "TTGACA" at -35. The coding sequence of the *tagA* gene is followed by an inverted repeat sequence which may serve as a transcriptional stop signal.

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**Y. VIINIKKA:** Neocentric activity in rye

Neocentric activity is a secondary kinetic activity of the chromosomes. Spindle fibers attach to certain telomeres simultaneously with the normal centromere, usually at both meiotic divisions, but not at mitosis. As a result the neocentric active sites move first towards the poles, coorientate with the centromeres and other neocentric active sites in the same bivalent, and produce tension in the chromosomes at both meiotic divisions. In rye, neocentric activity is known to occur in certain inbred lines (Weihestephaner Winterroggen O<sub>1</sub> and Stålråg). The intensity of the activity varies among lines, plants and even among the anthers of the same plant. A high-intensity line from the variety Stålråg has been examined with the aid of the C-banding technique and compared with a line without the activity. The formation of the bouquetlike attachment plate is normal, usually only one attachment plate is present at leptotene-zygotene cells in both lines. Neocentric activity was discernible for the first time at the first prometaphase. The most suitable stages for the analysis of the frequency of the phenomenon and identification of the chromosomes were the first anaphase and second metaphase. The frequency of cells with neocentrics varied from 32 to 68%. Usually only one telomere showed the activity, but also cells with two, three and four active sites have been observed. It seems that there is a primary site at the telomeric heterochromatin of the short arm of chromosome 4R. When two or more active sites were present, the secondary sites of activity were located at the telomeric heterochromatin of the metacentric chromosomes (2-3-7R). It has been suggested that some of the telomeric sequences, which are active during the bouquet formation, remain active at the two meiotic divisions, e.g. acting

as additional accumulation sites for the kinetochoic proteins.

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**K. H. YTTERBORN:** Hybrid dysgenesis: I-R condition of related strains of *Drosophila melanogaster*

Female fertility is one of the traits affected in the I-R system. In crosses between females from a reactive (R) strain and males from an inducer (I) strain the daughters, called SF females, are more or less sterile because of a reduced hatching rate of the eggs. In the reciprocal cross the daughters, called RSF females, have normal fertility. In intrastain crosses and crosses involving neutral (N) strains, no sterility effects appear. The SF sterility is considered to be due to an interaction between a cytoplasmic (but chromosomally dependent) R factor and an I factor linked to any chromosome. In SF and RSF females the chromosomes of reactive origin can acquire the I factor, probably by insertion of a mobile genetic element.

In the present work the genome from a moderately reactive strain with long generation intervals was introduced into six different maternal inducer strains by backcrosses for at least 19 generations. 16 lines divided into six groups of two to four parallel lines were kept with short generation intervals. Two lines from different groups were still inducer while all others were strongly reactive. Three parallel lines with long generation intervals were reactive but on different levels. Another three parallel lines, started from RSF females and reactive males and kept for 10 generations of endogamous mating, were classified as neutral.

In order to get some information about the population dynamics of the I-R system five strains with a common origin and kept apart for 80–100 generations were tested. The results showed that evolutionary processes had occurred. Two strains were inducer, one was reactive and two were classified as neutral.

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