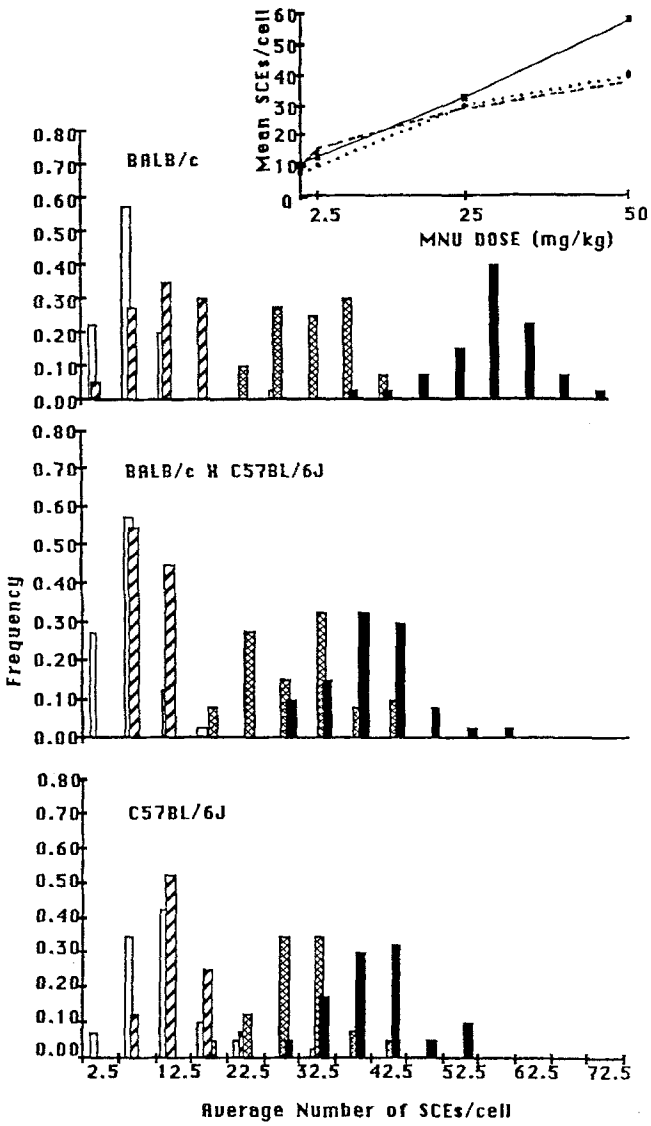

Figure 3. Distribution of SCEs/cell in BALB/c, C57BL/6J and their F_1 hybrid (BALB/c ♀ × C57BL/6J ♂) at four doses of MNU (□ 0 mg/kg; ▨ 2.5 mg/kg; ▩ 25 mg/kg; ■ 50 mg/kg) in young mice (10 ± 1 weeks). Insert shows SCEs/cell (mean ± SEM) as a function of MNU dose. — BALB/c; ··· BALB/c × C57BL/6J; - - - C57BL/6J.

It may be pointed out that the observed heterogeneity probably represents the interaction of a number of biological and non-biological factors. The known biological components may have genetic predispositions and include the ability to protect the DNA from damage directly or through detoxification of the mutagen, and the ability to repair any induced damage, among others. It is difficult, if not impossible, to identify all of the biological components of this heterogeneity. However, in general, these results follow earlier reports that older individuals are generally more sensitive to chromosomal damage by physical and chemical agents [13, 14] and less able to repair any induced damage [7–9, 14]. The data set presented here points to a complex pattern of causations for in vivo SCE induction, in particular, and relative mutagenic sensitivity in general. Although it is not possible to evaluate the actual genetic determinants involved in relative sensitivity through the data set presented, these conclusions have implications in two areas. First, towards the understanding of the process of SCE formation, it suggests involvement of a number of biological components that are prone to genotype-dependent aging (i.e. DNA repair). Second, towards the use of SCEs in mutagenesis testing, it offers reason for caution and selecting animals of comparable age and genetic background to be used.

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Chromosome aberrations in cattle raised on bracken fern pasture

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Summary. Thirteen cows maintained on natural bracken fern (*Pteridium aquilinum*) were analyzed cytogenetically. The frequency of structural chromosome aberrations detected in peripheral blood cells was significantly higher when compared to that detected in animals raised on pasture containing no bracken fern. We discuss the clastogenic action of fern and its synergistic action with infection by type 2 and 4 papilloma virus in the same animals.
Key words. Pteridium aquilinum; bovine papilloma virus; chronic enzootic hematuria; alimentary cattle cancer; chromosome aberrations; clastogenicity.

The occurrence of bladder and digestive tract tumors in cattle seems to be related to the presence of the fern *Pteridium aquilinum* in the pasture and to infection with bovine papilloma virus. The radiomimetic and carcinogenic action of fern

has been observed experimentally in cattle [2,3], rats [4,5] and mice [6]. The carcinogenic action of *P. aquilinum* has been attributed to quercetin [7], one of the flavonoids present in the plant. More recent data, however, have suggested that both the mutagenic and carcinogenic activities of fern may be related to an intermediate substance in the biosynthesis of pterosides, denoted aquilide A [8]. The etiologic relationship of the papilloma virus with bladder and digestive tract tumors was first suspected on the basis of simple observations: 1) non-induced bladder tumors induce similar lesions when injected into healthy cattle [9,10] and 2) animals with digestive carcinoma show a high incidence of papillomas at the tumor sites [11]. Type 2 bovine papilloma virus (BPV2) has been detected in bladder tumors [12] and BPV4 has been isolated and identified from papillomas in the digestive tract of animals raised on pasture infested with bracken fern [13].

We are currently developing an integrated multidisciplinary project aiming at a better understanding of the mechanisms involved in the development of these tumors, where chemical and viral factors seem to interact in a multifactorial oncogenic process. In the present paper we report preliminary results obtained in a cytogenetic study of 13 cows raised on pasture and chronically intoxicated with the fern *Pteridium aquilinum*.

Materials and methods. Thirteen *Bos taurus taurus* × *Bos taurus indicus* cows aged 3–8 years were studied. The animals were raised on pastures naturally infested with *Pteridium aquilinum*. Animal selection was based on symptoms of chronic bracken fern poisoning disease. Diagnosis was confirmed by clinical examination, by hematological tests [14] (red blood cell and differential leukocyte counts, hemoglobin and hematocrit determination) and urinalysis. Twenty-eight healthy cows of the same breed from regions in which bracken fern does not occur naturally were used as controls.

For cytogenetic analysis, blood was collected from both groups and short-term (68-h) peripheral lymphocyte cultures were performed [15]. Fifty metaphases/animal were scored for structural chromosome aberrations such as gaps, breaks, fragments and rearrangements. After natural death or sacrifice of affected animals, a detailed pathological study was performed with special emphasis on the urinary and digestive tracts. Bladder and digestive tract fragments were obtained and fixed in 10% formol for histological analysis. The fragments were embedded in paraffin, cut into 6-µm sections and stained with hematoxylin-eosin for examination under the light microscope.

Results and discussion. Eleven of the thirteen animals studied showed changes in the urinary tract manifesting as macrohematuria, and one showed digestive symptoms manifested as regurgitation, coughing and spasmodic movements. The 13th animal showed simultaneous involvement of the urinary (microhematuria) and digestive tracts. Urinalysis confirmed the presence of varying degrees of hematuria in 12 cases; in 2 cases, no histopathological lesions were detected despite the presence of blood in urine.

Anemia was detected in 11 animals, ranging from 4.5×10^6 erythrocytes/mm^3 (slight) to 1.5×10^6 erythrocytes/mm^3 (intense), and was classified as normochronic, normocytic or macrocytic. Leucocyte counts revealed 5 cases of normoleucocythemia ($6.7-11.9 \times 10^3$ leucocytes/mm^3) and 8 cases of leucocytopenia due to lymphocytopenia ($2.7-4.9 \times 10^3$ leucocytes/mm^3). These data agree with the classical descriptions of chronic *P. aquilinum* poisoning symptoms [2] for varying degree and intensity.

The histopathological data presented in table 1 indicate the typical lesions detected in cases of bracken fern intoxication. About 90% of the animals showed digestive tract lesions represented by neoplasias in 80% of cases (40% papilloma, 40% carcinoma, and 20% papilloma plus carcinoma). Urinary lesions were detected in 75% of the animals and were represented by neoplasias in 40% of cases (75% carcinoma and 25% carcinoma plus hemangioma).

The severity of signs and symptoms did not necessarily correspond to the evolution of the pathological lesions. The clinical condition of the intoxicated animal is sometimes critical enough to lead to death before the onset of detectable histo-

Table 1. Histopathological lesions detected in 13 *Bos taurus* × *Bos indicus* cows raised on pasture infested with bracken fern (*Pteridium aquilinum*).

Animal number	Digestive tract		Urinary tract
	Oral-pharinx	Esophagus	Bladder
1	PA	PA	Ca, He
2	–	PA	UH
3	CA	–	CIP
4	CA	–	Ca
5	CA	PA	Ca
6	CA	PA	Ca
7	–	CA	FCC
8	CA	CA	–
9	CIP	–	–
10	CIP	–	CIP
11	CIP, PA	–	CIP
12	CIP	PA	UH
13	–	–	–

CA, Epidermoid carcinoma; Ca, Transition cell carcinoma; FCC, Focal chronic cystitis; He, Hemangioma; PA, Papilloma; CIP, Chronic inflamatory process; UH, Ulceration and hemorrhage.

Table 2. Frequency of cells with structural chromosome aberrations in 13 *Bos Taurus* × *Bos indicus* cows raised on pasture infested with the bracken fern *Pteridium aquilinum*.

Animal	Cells presenting Gaps		Breaks		Fragments		Rearrangements		Total No. of analyzed cells	Total of abnormal cells		Total of chromosome aberrations	
No.	No.	%	No.	%	No.	%	No.	%		No.	%	No.	%
1	12	24.00	5	10.00	1	2.00	0	0.00	50	18	36.00	33	66.00
2	4	13.33	2	6.67	1	3.33	1	3.33	30	8	26.67	9	30.00
3	9	18.00	2	4.00	2	4.00	1	2.00	50	14	28.00	19	38.00
4	2	14.29	2	14.29	0	0.00	0	0.00	14	4	28.57	5	35.71
5	11	22.00	2	4.00	1	2.00	3	6.00	50	17	34.00	21	42.00
6	7	14.00	2	4.00	2	4.00	1	2.00	50	12	24.00	16	32.00
7	4	13.33	2	6.67	0	0.00	1	3.33	30	7	23.33	9	30.00
8	6	12.00	0	0.00	0	0.00	1	2.00	50	7	14.00	7	14.00
9	6	12.00	3	6.00	1	2.00	0	0.00	50	10	20.00	10	20.00
10	4	8.00	4	8.00	1	2.00	0	0.00	50	9	18.00	9	18.00
11	7	14.00	2	4.00	0	0.00	1	2.00	50	10	20.00	12	24.00
12	6	12.00	2	4.00	4	8.00	0	0.00	50	12	24.00	13	26.00
13	7	14.00	2	4.00	1	2.00	0	0.00	50	10	20.00	10	20.00
Total	85	14.81	30	5.23	14	2.44	9	1.57	574	138	24.04	173	30.14

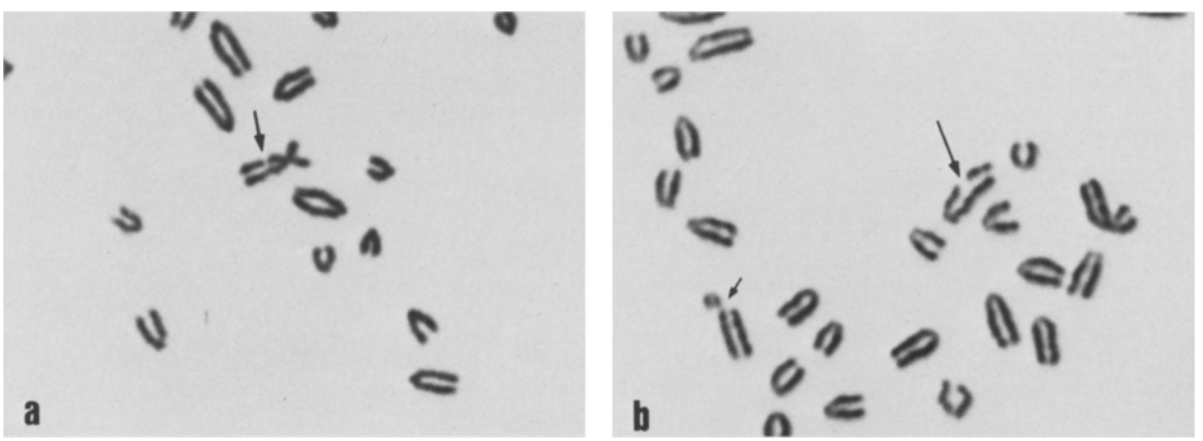
pathological damage or before malignant transformation has occurred. This situation suggests that the clinical signs and symptoms and the cytogenetic alterations observed may be more related to the amount of bracken fern ingested, while the pathological lesions seem to be more related to the time of exposure to the plant. It should be pointed out that in this retrospective study the animals were intoxicated in the field by ingesting variable amounts of bracken fern over variable periods of time.

The frequency of structural chromosome aberrations in animals from infested pastures was significantly higher (table 2) than in the controls (table 3) even though we did not analyze only the first metaphases in culture. The chi-square test applied to the data to determine within-group homogeneity was nonsignificant at the $p = 0.05$ level, demonstrating that both groups were homogeneous. When the same test was used to compare groups, the rate of chromosome aberrations in the affected group was found to be significantly higher than in the control ($p \leq 0.01$).

The high frequency of aberrations detected in the 13 affected animals, which mainly consisted of gaps (60%) and breaks (20%) (fig.), (14.81% and 5.23%, respectively of the total of 24.04% of aberrant cells, table 2), may have been the result of the direct mutagenic action of bracken fern on circulating lymphocytes and/or bone marrow, respectively. Considering that lymphocytes are target cells for latent papilloma viral

Table 3. Frequency of cells with structural chromosome aberrations in 28 *Bos taurus* × *Bos indicus* cows raised on pasture free from the bracken fern *Pteridium aquilinum*.

Animal	Cells presenting Gaps		Breaks		Fragments		Rearrangements		Total No. of analyzed cells	Total of abnormal cells		Total of chromosome aberrations	
No.	No.	%	No.	%	No.	%	No.	%		No.	%	No.	%
1	1	2.0	0	0.0	0	0.0	0	0.0	50	1	2.0	1	2.0
2	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
3	0	0.0	0	0.0	0	0.0	1	2.0	50	1	2.0	1	2.0
4	0	0.0	0	0.0	0	0.0	1	2.0	50	1	2.0	1	2.0
5	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
6	1	2.0	0	0.0	0	0.0	0	0.0	50	1	2.0	1	2.0
7	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
8	0	0.0	0	0.0	0	0.0	1	2.0	50	1	2.0	1	2.0
9	1	2.0	2	4.0	0	0.0	0	0.0	50	3	6.0	3	6.0
10	0	0.0	1	2.0	0	0.0	0	0.0	50	1	2.0	1	2.0
11	0	0.0	0	0.0	0	0.0	1	2.0	50	1	2.0	1	2.0
12	0	0.0	1	2.0	0	0.0	1	2.0	50	2	4.0	2	4.0
13	1	2.0	0	0.0	0	0.0	0	0.0	50	1	2.0	1	2.0
14	1	2.0	2	4.0	0	0.0	1	2.0	50	4	8.0	4	8.0
15	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
16	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	3	6.0
17	1	2.0	0	0.0	0	0.0	0	0.0	50	1	2.0	1	2.0
18	4	8.0	0	0.0	0	0.0	0	0.0	50	4	8.0	4	8.0
19	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
20	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
21	0	0.0	1	2.0	0	0.0	0	0.0	50	1	2.0	1	2.0
22	2	4.0	1	2.0	0	0.0	0	0.0	50	3	6.0	3	6.0
23	2	4.0	0	0.0	0	0.0	1	2.0	50	3	6.0	3	6.0
24	1	2.0	1	2.0	0	0.0	0	0.0	50	2	4.0	2	4.0
25	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
26	1	2.0	1	2.0	0	0.0	0	0.0	50	2	4.0	2	4.0
27	4	8.0	0	0.0	0	0.0	0	0.0	50	4	8.0	4	8.0
28	2	4.0	0	0.0	0	0.0	0	0.0	50	2	4.0	2	4.0
Total	38	2.71	10	0.71	0	0.0	7	0.50	1400	55	3.93	56	4.0



Most frequent structural chromosomal aberrations detected in bracken fern fed animals: *a* arrow indicates a chromatid gap; *b* long arrow indicates a chromatid break and short arrow indicates an isochromatid break.

infection [12], it is reasonable to suggest that the virus may be contributing to the production of chromosomal anomalies in these cells.

An important role has been attributed to the papilloma virus in the development of epithelial neoplasias both in animals and in man [16–20]. The transformation of benign lesions induced by the virus into malignant tumors seems to depend on an interaction with other factors. If the epithelial cells of the digestive and urinary tracts are also exposed to the clastogenic action of *P. aquilinum*, then the plant may induce chromosome rearrangements in these cells. Perhaps the induction of chromosome rearrangements in cells infected by the papilloma virus is one of the cofactors needed for the malignant transformation of papillomas through oncogenic activation. Alternatively, the transformation of cultured cells infected with bovine papilloma virus is known to be induced by 12-O-tetradecanoyl-phorbol-13 acetate (TPA). The use of TPA as a chemical promoter induces transcription of the viral genome both in vivo and in vitro [21]. It is possible that substances present in bracken fern may act in a similar manner by promoting genome expression of the virus and thus contribute to the malignant transformation of the papillomas.

The bladder and digestive tract tumors of cattle represent a convenient model for the study of papilloma virus (BPV2 and BPV4) in terms of the induction of benign neoplasias that may turn malignant through the direct or indirect action of chemical agents present in bracken fern. While the virus seems to play a role only in tumor initiation [22], bracken fern may contribute to the maintenance and progression of these tumors either by inducing mutations and/or chromosome aberrations or by promoting viral gene activity in the infected cells.

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Photoperiodic diapause induction suppressed at low temperatures in a long-day insect

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Summary. The long-day insect *Yponomeuta vigintipunctatus* was subjected to various combinations of temperature and photoperiod. The photoperiodic induction curve at 10 °C resembled the one at 20 °C, but with a shift of the critical photoperiod towards the shorter day-length. Such unusual averting of diapause at lower temperatures in combination with intermediate long-day photoperiods has still been described in only few insect species of the temperate zone.
Key words. Yponomeuta vigintipunctatus; Lepidoptera; Yponomeutidae; diapause; photoperiodic induction curves; low temperature influence.

In most long-day insects it has been found that the photoperiodic induction curve shifts to longer day-length when the temperature at which they are reared is lowered [2]. Preliminary laboratory studies with the small ermine moth of orpine, *Yponomeuta vigintipunctatus* Rez., however, revealed the opposite effect of low temperatures on diapause induction: at LD 16:8 almost all the pupae enter diapause between 20 °C and 13 °C; diapause induction is suppressed to a large extent within the range from 12 to 7.5 °C. In this paper I report the effects of various combinations of larval rearing temperatures and photoperiods on the incidence of diapause in the small ermine moth of orpine. This species has two generations a year and hibernates as a pupa in diapause.

Material and methods. All experiments were performed with larvae of the F1 generation obtained from parents collected in the field in the autumn of the foregoing years. They originated from three different localities in The Netherlands. Copulation and oviposition took place under long-day conditions (LD 17:7, 20 °C) [3]. The larvae were equally distributed over each of the three groups of treatments (in total 19) presented in the figure. They were fed until pupation with leaves of orpine, *Sedum telephium* L., the only host-plant of this monophagous species [4].

Immediately after pupation all individuals were placed at 20 °C, LD 17:7 to secure diapause termination. For each of the 19 combinations the percentages diapausing and non-