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# Genetic studies on nucleolus organizer regions (NORs) in cattle

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**Summary**

Experimentally produced monozygotic twins, natural opposite sex blood chimeras (freemartins), and several pedigrees were used to evaluate the genetic influences on the nucleolus organizer region (NOR) patterns in cattle. In monozygotic twins, the NOR patterns of both twins are extremely similar. In chimeras, NOR patterns of genetically identical, peripheral blood lymphocytes (PBL) from the two partners resemble each other. In contrast, genetically different PBL (sib organ) differ significantly in the same environment. A high heritability of the individual NOR patterns is also demonstrated in our 23 pedigrees. In conclusion, our data demonstrate that variation in NOR expression is predominantly due to genetic factors.

## 1. Introduction

Nucleolus organizer regions (NORs) harbour the genes for 18S and 28S RNA. Active NORs can be demonstrated by the use of silver staining (Goodpasture & Bloom, 1975; Howell *et al.* 1975; Miller *et al.* 1976). Silver staining of the NORs in a chromosome is the result of the binding of silver to the acidic NOR proteins rather than to DNA (Howell, 1977; Busch *et al.* 1982). In cattle, the NORs are localized telomerically on chromosomes 2, 3, 4, 11, and 28 (Di Berardino *et al.* 1979; Henderson & Bruére, 1979; Mayr & Czaker, 1981). In a recent NOR/G-band study on AI (artificial insemination) bulls NOR patterns were found to be characteristic for an individual (Mayr *et al.* 1987). We have continued these studies using monozygotic twins, chimeras and pedigree analysis in cattle to measure the extent to which NOR pattern is under genetic control.

## 2. Materials and Methods

### (i) *Monozygotic twins*

Five sets of identical twin calves were produced experimentally by microsurgery at the 7th day of pregnancy. Three sets (twin pairs 1, 2, and 3) resulted from transferring two demi-embryos per recipient and thus were born twins; the other two sets (twin pairs 4 and 5) were the result of transferring demi-embryos to two dams, and thus were born singly. Recipients were Fleckvieh (Simmental) heifers, donors were cows of the same breed.

### (ii) *Chimeras*

The 10 chimeras studied represented 5 natural opposite sex Fleckvieh twin pairs. The ratio of male to female cells in 4 chimeric twin pairs was about 50:50, but the fifth chimeric twin pair (pair 11) contained only female cells.

### (iii) *Pedigree studies*

Six Fleckvieh calves and their parents (1 bull and 6 cows), 15 Braunvieh calves and their parents (3 bulls and 15 cows), and 2 calves from the Tuxer breed with their parents (1 bull and 2 cows) were included in the pedigree study.

Parentage testing in all three groups was carried out using serological examination of the following blood group systems: A, B, C, F, J, L, S, Z, R′, T′. Electrophoretic testing of transferrin, posttransferrin and hemoglobin was also carried out. This leads to an exclusion security of 98%.

### (iv) *Chromosome preparations*

Chromosome preparations were made according to standard methods and subjected to a sequential silver

Table 1. *NOR frequencies and their mean values in monozygotic twins*

| Monozygotic twin | $n$ | Chromosome pair 2 | | | | Chromosome pair 3 | | | | Chromosome pair 4 | | | | Chromosome pair 11 | | | | Chromosome pair 28 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0x | 1x | 2x | $\bar{x}$ | 0x | 1x | 2x | $\bar{x}$ | 0x | 1x | 2x | $\bar{x}$ | 0x | 1x | 2x | $\bar{x}$ | 0x | 1x | 2x | $\bar{x}$ |
| 1a | 21 | 14 | 7 | 0 | 0·33 | 4 | 17 | 0 | 0·81 | 0 | 2 | 19 | 1·9 | 0 | 4 | 17 | 1·81 | 0 | 2 | 19 | 1·9 |
| 1b | 42 | 20 | 22 | 0 | 0·53 | 8 | 34 | 0 | 0·81 | 0 | 5 | 37 | 1·88 | 0 | 15 | 27 | 1·64 | 0 | 5 | 37 | 1·88 |
| 2a | 38 | 3 | 35 | 0 | 0·94 | 38 | 0 | 0 | 0·0 | 0 | 3 | 35 | 1·92 | 0 | 14 | 24 | 1·63 | 0 | 38 | 0 | 1·00 |
| 2b | 17 | 2 | 15 | 0 | 0·88 | 17 | 0 | 0 | 0·0 | 0 | 2 | 15 | 1·88 | 0 | 6 | 11 | 1·65 | 0 | 11 | 0 | 1·00 |
| 3a | 40 | 5 | 35 | 0 | 0·88 | 0 | 34 | 6 | 1·15 | 0 | 6 | 34 | 1·85 | 0 | 8 | 32 | 1·8 | 0 | 21 | 19 | 1·48 |
| 3b | 36 | 8 | 28 | 0 | 0·78 | 1 | 32 | 3 | 1·06 | 0 | 6 | 30 | 1·83 | 0 | 12 | 24 | 1·67 | 0 | 21 | 15 | 1·42 |
| 4a | 62 | 0 | 62 | 0 | 1·00 | 3 | 59 | 0 | 0·95 | 0 | 15 | 47 | 1·76 | 0 | 8 | 54 | 1·87 | 0 | 29 | 33 | 1·53 |
| 4b | 36 | 0 | 34 | 2 | 1·06 | 2 | 34 | 0 | 0·94 | 0 | 14 | 22 | 1·61 | 0 | 9 | 27 | 1·75 | 0 | 32 | 4 | 1·11 |
| 5a | 46 | 10 | 35 | 1 | 0·8 | 2 | 44 | 0 | 0·96 | 0 | 16 | 30 | 1·65 | 5 | 41 | 0 | 0·89 | 0 | 1 | 45 | 1·98 |
| 5b | 48 | 11 | 37 | 0 | 0·77 | 1 | 47 | 0 | 0·98 | 0 | 13 | 35 | 1·73 | 4 | 44 | 0 | 0·92 | 0 | 1 | 47 | 1·98 |

$n$, number of metaphases examined.

NOR (Howell & Black, 1980) and trypsin-Giemsa staining for identification of NOR-carrying chromosomes. Photographs were taken on a Reichert Polyvar. Repeated chromosome preparations in intervals of weeks to months did not show differences in the individual NOR pattern so that the culture and staining methods can be regarded as stable and reliable. The nomenclature followed the system of the Reading Conference (1980).

### (v) *Statistical methods*

The NOR patterns of each chromosome pair were examined separately. Then 10–50 complete metaphases of each animal were judged with respect to NOR pattern and chromosome identification by sequential staining.

In the case of each pair of homologous chromosomes, let $n_1$, $n_2$ and $n_3$ be the number of pairs with 0, 1 and 2 NORs in the sample of $n = n_1 + n_2 + n_3$ metaphases. Then the mean frequency of NORs is $\bar{x} = (n_2 + 2n_3)/n$. As the data are categorial, NOR frequencies were compared by contingency tables. Analysis of variance of mean NOR frequencies was applied to all three systems (monozygotic twins, chimeras, pedigrees), and a $t$ test was used to compare the data from the chimeric twins.

## 3. Results

### (i) *Monozygotic twins*

A very high similarity in NOR pattern was found in identical twin pairs (Table 1), both for twins in single recipients (same uterine environment; pairs 1, 2, and 3) and such twins in separate recipients (different uterine environment; pairs 4 and 5). In the monozygotic sibs, the NOR patterns and their percentage profiles showed almost no differences. Only chromosome 28 in twin pair 4 (D.F. = 1; $\chi^2 = 15{\cdot}47$; $P < 0{\cdot}01$) gave a significant difference. The analysis of variance of mean NOR frequencies (Table 2) reveals that there is far more variation between the twin pairs than within the twin pairs ($F = 20{\cdot}27$; D.F. = 4). Moreover, the analysis shows that there are characteristic NOR

Table 2. *Analysis of variance of mean NOR frequencies in monozygotic twins*

| Source of variation | D.F. | S.S. | M.S. | $F$ |
|---|---|---|---|---|
| Chromosome pairs | 4 | 9·2 | 2·3 | 306·67** |
| Twin pairs totals | 4 | 0·61 | 0·15 | 20·27** |
| Chromosomes × twin pairs | 16 | 3·88 | 0·24 | 32·4** |
| Scores of twins (remainder and error) | 25 | 0·19 | 0·008 | |
| Total | 49 | 13·88 | | |

** $P < 0{\cdot}01$.

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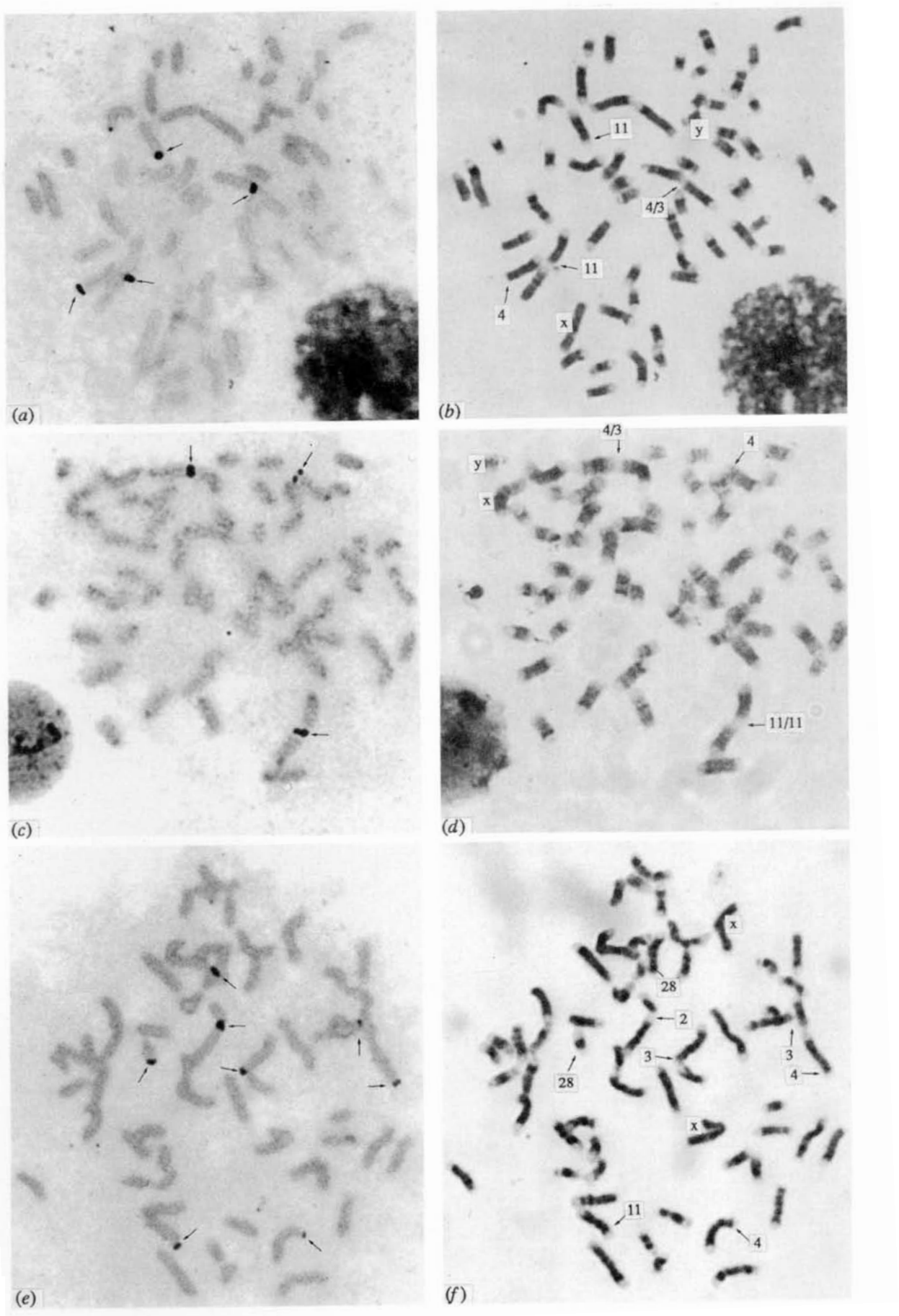


Fig. 1. Identical NOR patterns in the monozygotic twin pair no. 2. Arrows indicate the Ag-NORs on the respective chromosomes. (*a*) Silver stained metaphase of monozygotic twin no. 2a. (*b*) The same metaphase after trypsin-Giemsa-staining for identification of the chromosomes. (*c*) Silver stained metaphase of monozygotic twin no. 2b. (*d*) The same metaphase after trypsin-Giemsa-staining.

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Table 3. *NOR frequencies and their mean values in chimeric twins*

| Chimeric twin-cell pair | | *n* | Chromosome 2 | | | | Chromosome 3 | | | | Chromosome 4 | | | | Chromosome 11 | | | | Chromosome 28 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ |
| 7 | ♀♀ | 34 | 34 | 0 | 0 | 0·0 | 0 | 7 | 29 | 1·85 | 0 | 2 | 32 | 1·94 | 0 | 8 | 26 | 1·76 | 0 | 6 | 28 | 1·82 |
| | ♀♂ | 14 | 14 | 0 | 0 | 0·0 | 0 | 14 | 0 | 1·00 | 0 | 1 | 13 | 1·93 | 0 | 1 | 13 | 1·93 | 3 | 9 | 2 | 0·93 |
| | ♂♀ | 15 | 14 | 1 | 0 | 0·07 | 1 | 0 | 14 | 1·87 | 0 | 4 | 11 | 1·73 | 1 | 4 | 10 | 1·6 | 0 | 3 | 12 | 1·8 |
| | ♂♂ | 14 | 14 | 0 | 0 | 0·0 | 0 | 11 | 3 | 1·21 | 0 | 0 | 14 | 2·00 | 0 | 2 | 12 | 1·86 | 5 | 6 | 3 | 0·86 |
| 8 | ♀♀ | 7 | 7 | 0 | 0 | 0·00 | 1 | 6 | 0 | 0·86 | 0 | 5 | 2 | 1·29 | 0 | 1 | 6 | 1·86 | 1 | 3 | 3 | 1·29 |
| | ♀♂ | 7 | 0 | 7 | 0 | 1·00 | 7 | 0 | 0 | 0·00 | 0 | 0 | 7 | 2·00 | 0 | 6 | 1 | 1·14 | 0 | 0 | 7 | 2·00 |
| | ♂♀ | 17 | 17 | 0 | 0 | 0·00 | 2 | 15 | 0 | 0·88 | 0 | 3 | 14 | 1·82 | 0 | 7 | 10 | 1·59 | 0 | 6 | 11 | 1·65 |
| | ♂♂ | 21 | 1 | 19 | 1 | 1·00 | 20 | 1 | 0 | 0·05 | 0 | 4 | 17 | 1·81 | 0 | 18 | 3 | 1·14 | 0 | 5 | 16 | 1·76 |
| 9 | ♀♀ | 14 | 0 | 2 | 12 | 1·86 | 6 | 8 | 0 | 0·57 | 0 | 5 | 9 | 1·71 | 0 | 3 | 11 | 1·79 | 1 | 3 | 10 | 1·64 |
| | ♀♂ | 10 | 1 | 9 | 0 | 0·90 | 0 | 1 | 9 | 1·90 | 0 | 2 | 8 | 1·80 | 0 | 2 | 8 | 1·80 | 1 | 4 | 5 | 1·40 |
| | ♂♀ | 12 | 0 | 2 | 10 | 1·83 | 5 | 7 | 0 | 0·58 | 0 | 2 | 10 | 1·83 | 0 | 4 | 8 | 1·67 | 0 | 5 | 7 | 1·58 |
| | ♂♂ | 4 | 0 | 4 | 0 | 1·00 | 0 | 0 | 4 | 2·00 | 0 | 1 | 3 | 1·75 | 0 | 1 | 3 | 1·75 | 1 | 2 | 1 | 1·50 |
| 10 | ♀♀ | 6 | 0 | 0 | 6 | 2·00 | 0 | 5 | 1 | 1·17 | 0 | 3 | 3 | 1·50 | 0 | 2 | 4 | 1·67 | 0 | 4 | 2 | 1·33 |
| | ♀♂ | 6 | 0 | 6 | 0 | 1·00 | 4 | 2 | 0 | 0·33 | 0 | 1 | 5 | 1·83 | 0 | 4 | 2 | 1·33 | 0 | 0 | 6 | 2·00 |
| | ♂♀ | 10 | 0 | 4 | 6 | 1·6 | 0 | 10 | 0 | 1·00 | 0 | 3 | 7 | 1·70 | 0 | 6 | 4 | 1·40 | 0 | 1 | 9 | 1·9 |
| | ♂♂ | 13 | 3 | 9 | 1 | 0·85 | 9 | 4 | 4 | 0·31 | 0 | 5 | 8 | 1·62 | 0 | 13 | 0 | 1·00 | 0 | 5 | 8 | 1·62 |
| 11 | ♀♀ | 29 | 3 | 26 | 0 | 0·90 | 0 | 29 | 0 | 1·0 | 0 | 4 | 25 | 1·86 | 3 | 8 | 18 | 1·52 | 0 | 8 | 21 | 1·72 |
| | ♂♀ | 22 | 3 | 16 | 3 | 1·00 | 1 | 21 | 0 | 0·95 | 0 | 3 | 19 | 1·86 | 4 | 6 | 12 | 1·36 | 2 | 6 | 14 | 1·55 |

*n*, number of metaphases examined.

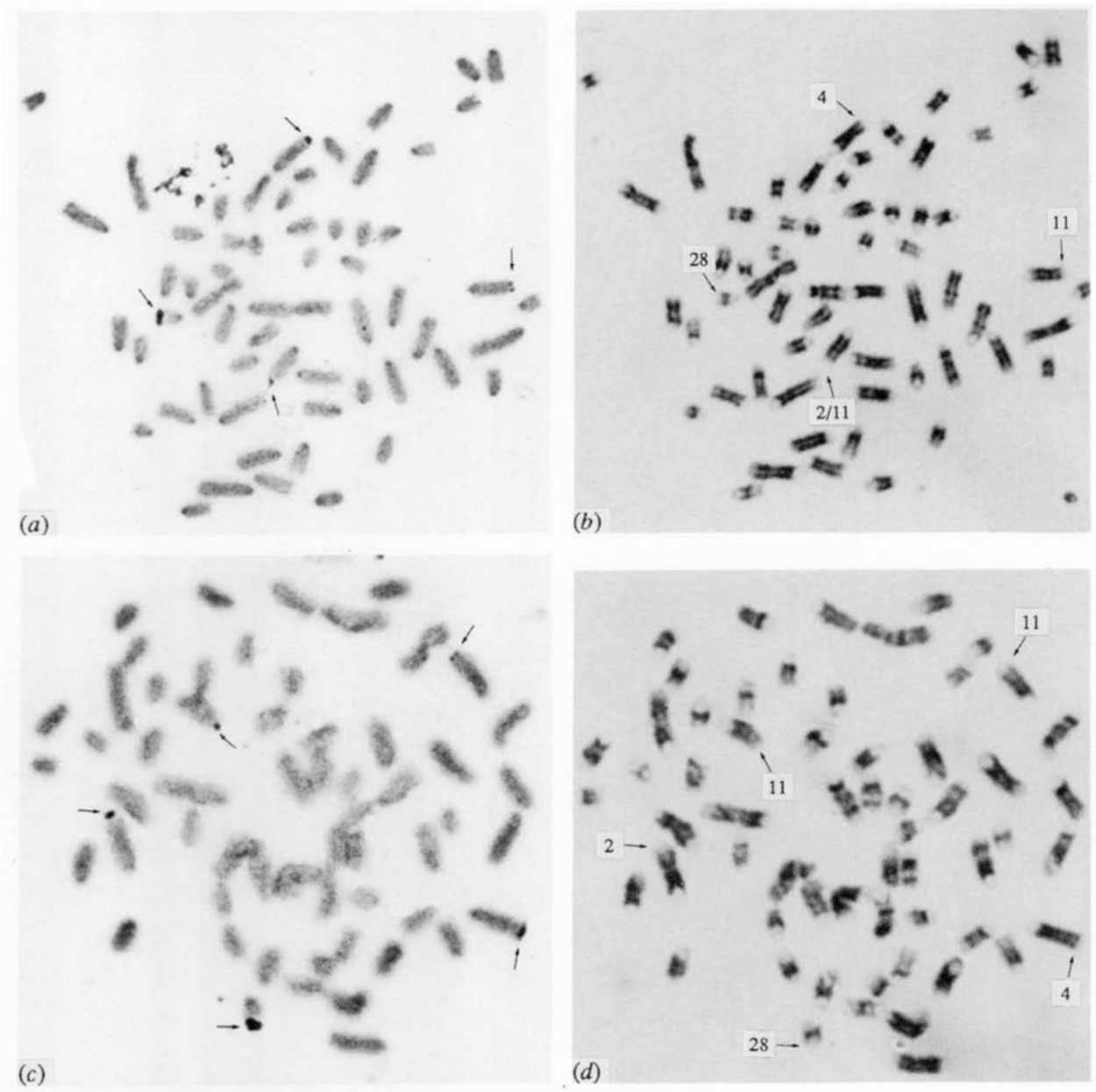


Fig. 2. NOR patterns of cells in the chimeric twin pair no. 7. Arrows indicate the Ag-NORs on the respective chromosomes. (*a*) Silver-stained metaphase of the ♂ partner, ♂ cell. (*b*) The same metaphase after trypsin-Giemsa-staining. (*c*) Silver-stained metaphase of the ♀ partner, ♂ cell. Note that the Ag-NOR pattern is identical with that shown in *a*/*b*. (*d*) The same metaphase after trypsin-Giemsa-staining. (*e*) Silver-stained metaphase of the ♂ partner, ♀ cell. Note that the Ag-NOR pattern is different from ♂ cells shown in *a*/*b* and *c*/*d*. (*f*) The same metaphase after trypsin-Giemsa-staining.

frequencies for each chromosome pair which are in most cases consistent for the different sets of animals.

(ii) *Chimeric twins*

The results show a marked similarity of NOR pattern in genetically identical cells (i.e. cells of the same sex) from the male and female calves in chimeric twins (Table 3). On the other hand, different sex cells in the same animal resemble each other much less. A good example is chromosome 2 in chimeric twin pair 8. In this case, the male cells showed mean values of 1·00 in both sibs. The female cells had mean values of 0·00 in both sibs. There are only negligible differences between

Table 4. *NOR frequencies in blood cells of chimeric twins (comparison of same sex cells from different environments with different sex cells from the same environment)*

| | Differences of mean NOR frequencies between | |
|---|---|---|
| | Same sex cells from both chimeric twins | Different sex cells from the single chimeric twin |
| $\bar{x}$ | 0·15 | 0·53 |
| S.D. | 0·14 | 0·4 |
| *n* | 40 | 40 |

*t* test (chimeric pairs 7–10). $t = 5{\cdot}66$; $P < 0{\cdot}01$.

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Table 5. *NOR frequencies and their mean values in some cattle matings*[a]

| Animal | *n* | Chromosome pair 2 | | | | Chromosome pair 3 | | | | Chromosome pair 4 | | | | Chromosome pair 11 | | | | Chromosome pair 28 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ | 0*x* | 1*x* | 2*x* | $\bar{x}$ |
| Family 1 (Fleckvieh) | | | | | | | | | | | | | | | | | | | | | |
| Bull 12 | 20 | 18 | 2 | 0 | 0·1 | 20 | 0 | 0 | 0·0 | 0 | 9 | 11 | 1·55 | 1 | 19 | 0 | 0·95 | 0 | 2 | 18 | 1·9 |
| Dam B | 6 | 6 | 0 | 0 | 0·0 | 0 | 6 | 0 | 1·0 | 0 | 4 | 2 | 1·33 | 0 | 5 | 1 | 1·17 | 0 | 4 | 2 | 1·33 |
| Calf B | 8 | 8 | 0 | 0 | 0·0 | 0 | 8 | 0 | 1·0 | 0 | 1 | 7 | 1·88 | 8 | 0 | 0 | 0·0 | 0 | 4 | 4 | 1·5 |
| Dam F | 14 | 0 | 7 | 7 | 1·5 | 0 | 2 | 12 | 1·86 | 0 | 6 | 8 | 1·57 | 1 | 12 | 1 | 1·0 | 0 | 13 | 1 | 1·07 |
| Calf F | 13 | 0 | 12 | 1 | 1·08 | 0 | 13 | 0 | 1·0 | 0 | 1 | 12 | 1·92 | 7 | 6 | 0 | 0·46 | 7 | 6 | 0 | 1·46 |
| Family 4 (Braunvieh) | | | | | | | | | | | | | | | | | | | | | |
| Bull 15 | 14 | 0 | 14 | 0 | 1·0 | 14 | 0 | 0 | 0·0 | 0 | 3 | 11 | 1·79 | 0 | 5 | 9 | 1·64 | 0 | 4 | 10 | 1·71 |
| Dam B | 9 | 0 | 9 | 0 | 1·0 | 9 | 0 | 0 | 0·0 | 0 | 0 | 9 | 2·0 | 0 | 0 | 9 | 2·0 | 0 | 9 | 0 | 1·0 |
| Calf B | 7 | 0 | 7 | 0 | 1·0 | 7 | 0 | 0 | 0·0 | 0 | 0 | 7 | 2·0 | 0 | 0 | 7 | 2·0 | 0 | 7 | 0 | 1·0 |
| Dam C | 6 | 0 | 0 | 6 | 2·0 | 0 | 6 | 0 | 1·0 | 0 | 2 | 4 | 1·67 | 0 | 6 | 0 | 1·0 | 0 | 6 | 0 | 1·0 |
| Calf C | 9 | 0 | 0 | 9 | 2·0 | 1 | 8 | 0 | 0·89 | 0 | 1 | 8 | 1·89 | 0 | 9 | 0 | 1·0 | 0 | 9 | 0 | 1·0 |
| Family 5 (Tuxer) | | | | | | | | | | | | | | | | | | | | | |
| Bull 16 | 14 | 10 | 14 | 0 | 0·29 | 11 | 3 | 0 | 0·21 | 0 | 10 | 4 | 1·29 | 0 | 14 | 0 | 1·0 | 0 | 5 | 9 | 1·64 |
| Dam A | 14 | 0 | 2 | 12 | 1·86 | 8 | 6 | 0 | 0·43 | 0 | 6 | 8 | 1·57 | 1 | 12 | 1 | 1·0 | 0 | 2 | 12 | 1·86 |
| Calf A | 17 | 0 | 2 | 15 | 1·88 | 17 | 0 | 0 | 0·0 | 3 | 7 | 7 | 1·24 | 6 | 11 | 0 | 0·65 | 0 | 1 | 16 | 1·94 |
| Dam B | 17 | 5 | 11 | 13 | 0·76 | 2 | 15 | 0 | 0·88 | 0 | 2 | 15 | 1·88 | 0 | 1 | 16 | 1·94 | 0 | 3 | 14 | 1·82 |
| Calf B | 20 | 12 | 8 | 0 | 0·40 | 8 | 11 | 1 | 0·65 | 0 | 8 | 12 | 1·60 | 0 | 4 | 16 | 1·8 | 0 | 3 | 17 | 1·85 |

*n*, number of metaphases examined.
[a] Complete lists are available from the authors upon request.

Table 6. *Analysis of variance of mean NOR frequencies in 5 cattle families*

| Source of variation | D.F. | S.S. | M.S. | *F* |
|---|---|---|---|---|
| Chromosome pairs | 4 | 31·93 | 7·98 | 33·69** |
| Families | 4 | 1·49 | 0·37 | 1·58 |
| Animals | 46 | 4·65 | 0·1 | 0·43 |
| Chromosomes × families | 16 | 18·26 | 1·14 | 4·82** |
| Remainder and error | 108 | 42·64 | 0·24 | |
| Total | 250 | 98·97 | | |

** $P < 0{\cdot}01$.

the cells of the same sex ($\chi^2 = 0{\cdot}26$ and 0·18, respectively; D.F. = 1; confidence limit for $P < 0{\cdot}05 = 3{\cdot}84$). We compared the similarities between cells of the same sex from the two partners (different environments) and cells of different sex in one partner (same environment) by a *t* test (Table 4). We pooled the differences of mean NOR frequencies between same sex cells from both chimeric partners for all chromosomes and compared them to the differences between opposite sex cells for all chromosomes from the single chimeric twins. In the first case, the mean difference in NOR frequency between same sex cells from different animals was 0·15 (S.D. = 0·14); in the second case the mean difference between male and female cells harboured by a single animal was much higher at 0·4 (S.D. = 0·53). The difference between the two groups was highly significant ($t = 5{\cdot}66$; $P < 0{\cdot}01$).

Genetically identical cells in different individuals, therefore, produce very similar NOR patterns, while genetically different cells in a single animal may have very different NOR patterns. Thus, the environment does not affect the mean NOR values significantly.

(iii) *Family studies*

The pedigree studies also demonstrate a strong genetic component in expression of NOR pattern. Table 5 shows the actual NOR numbers and mean NOR frequencies in some of our matings. For example, in bulls 12 and 15 there were no NORs on chromosome 3. None of their 11 calves had a mean value greater than 1·00. Likewise, all parents with mean values of 2·00 or close to 2·00 or even significantly exceeding 1·00 did not give rise to calves with values lower than 1·00.

However, one of the 23 pedigrees did not conform to simple chromosomal inheritance. This case was chromosome 2 in the pairing of sire 16 (mean value 0·29) with cow A (mean value 1·87) resulting in a calf with a value of 1·85. Table 6 shows the analysis of variance of the mean NOR frequencies in the 5 cattle families. There is strong evidence for an overall chromosome specific expression of NOR patterns ($F = 33{\cdot}69$; D.F. = 4; $P < 0{\cdot}01$) and for highly significant interactions between families and chromosomes ($F = 4{\cdot}82$; D.F. = 16; $P < 0{\cdot}01$). This leads to the conclusion that there exist inherited NOR patterns that are characteristic for the members of a cattle family.

## 4. Discussion

With a single exception, the monozygotic twin comparisons show a very close similarity in the NOR pattern of peripheral blood cells from identical twins, whether these are grown in the same or in different mothers, and this indicates the overwhelming influence of genotype compared with uterine environment on NOR patterns. This influence clearly controls the distribution of NOR frequency between the different pairs of homologous chromosomes, as well as the number in each pair. The one exception (chromosome 28 in pair 4) is probably due to a technical error resulting in understaining of some cells.

The chimeric twin studies show essentially the same picture. Most twins in cattle (about 90%), it will be remembered, are blood chimeras because of placental anastomosis between the twins *in utero*. Cells of the same sex (therefore of the same genotype) from the two chimeric partners have almost identical NOR patterns, while male and female cells (therefore of different genotypes) from a single chimeric individual show no such similarity in NOR pattern.

The family pedigree studies also indicate a strong genetic component in the correlation between the NOR patterns of parents and progeny, but it has not been possible to express this in a form which would give an estimate of the heritability of NOR pattern.

The general conclusion is that genetic factors within the cell very largely control the NOR pattern of its chromosomes, regardless of environmental effects.

Our results of a high genetic influence on bovine NOR patterns are well compatible with the NOR data in humans including monozygotic twins (Morton *et al.* 1981; Zakharov *et al.* 1982; Weltens *et al.* 1975) and pedigrees (Mikelsaar *et al.* 1977; Markovic *et al.* 1978).

Technical problems affecting NOR measurements may partly contribute to intraindividual NOR variations. The idea that certain NORs have sizes close to the limits of light microscopy and, therefore, might be detected in some cases and not detected in others is reasonable and might therefore, to a moderate extent, contribute to the variation. Technical factors in the silver staining and subjective microscopical evaluation may become critical in this special situation. Nonetheless, NOR activation mechanisms in different cell types (e.g. in distinct peripheral lymphocyte subsets) are generally believed to account for the major part of variation within the individuals.

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