Table 2. Allelic frequency with standard errors of concerned genes in the gene pool of the population surveyed

	Populations				
	Brahmin	Kayastha	Rajput	Bania	Muslim
p	0 28 ± 0 012	0 30 ± 0 010	0 38 ± 0 018	0 19 ± 0 015	0 07 ± 0 005
q	0 21 ± 0 016	0 13 ± 0.015	0.31 ± 0 016	0 36 ± 0 023	0 16 ± 0 018
r	0 51 ± 0 020	0 57 ± 0 021	0.31 ± 0 018	0 45 ± 0.017	0 77 ± 0 012
D	0 90 ± 0 016	1 00 ± 0 053	0 86 ± 0 018	0 86 ± 0 020	0 80 ± 0 021
d	0 10 ± 0 016	0 00 ± 0 053	0 14 ± 0 018	0 14 ± 0 020	0.20 ± 0 021
T	0 47 ± 0 026	0.47 ± 0 025	0 44 ± 0 026	0 41 ± 0.290	0 42 ± 0 026
t	0 53 ± 0 026	0 53 ± 0 025	0 56 ± 0 026	0 59 ± 0 290	0 57 ± 0 026

Table 3. Estimate of Nei's measure of genetic distance (D) among five population groups of Purnia, Bihar

	Brahmin	Kayastha	Rajput	Bania
Kayastha	0 0071			
Rajput	0 0193	0 0382		
Bania	0 0131	0 0324	0 0184	
Muslim	0 1300	0 1388	0 1927	0 1400

population on the basis of their ability to taste PTC have been classified as tasters and non-tasters. The distribution of PTC among the Brahmin, Kayastha, Rajput, Bania and Muslim was bimodal. The antimode in them lies between the solutions nos 5 and 6. The frequency of non-tasters gene in all the five populations was high (Table 2).

Genetic distance can be regarded as a function of the difference in gene frequencies. It is simply a tool to investigate the relationship among a set of population, but it may not necessarily establish any exact phylogenetic relationship among them[6]. Besides, the genetic distance is used to measure the degree of gene differentiation among local populations and sometimes it can be related to geographic distance, linguistic differences or historical pattern of migrations of populations and thereby one can study the mechanism of microevolution[7]. Out of several so-called 'genetic distance' measures available in the literature, the one proposed by Nei[8] has been employed in the present study, since it has a biological meaning. In the present study the lowest genetic distance (D) (0.0071) has been observed between Brahmin and Kayastha and highest (0.1927) between Rajput and Muslim (Table 3 and Figure 1). From the genetic distance (D) of the population surveyed it is quite clear that there are two main clusters i.e., one for Muslims and the other for non-Muslims (Rajput, Brahmin, Bania, and Kayastha). In spite of common ancestry Muslims form a separate group which might be due to consanguinity. Further on the basis of genetic distance it may be concluded that the Rajput, Bania, Kayastha and Brahmin differ from each other in their gene pool.

1 Balgir, R S , *Man in India*, 1992, **72**, 293–301
2 Singh, M and Sinha, S P., *Biol Bull India*, 1988, **10**, 105–111
3 Karve, I , *Hindu Society and Interpretation*, Dasmukh and Co , Poona , 1974
4 Malhotra, K C , *A Review in Human Population Genetic in India* (eds Sanghvi, L. D , Balkrishnan, V , Bhatia, H M , Sukumaran, P K , Undevia J V), Orient Longman, New Delhi, 1974, vol. 1, pp 157–172
5 Harris, H and Kalmus, H , *Ann Eugen* , 1949, **15**, 124–131
6 Bhasin, M K. and Khanna, A , *India J. Hum Ecol* , 1992, **3**, 193–219
7 Nei, M , in *Genetic Structure of Populations* (ed. Morton, N E), Honolulu Univ Press of Hawaii, 1973, pp 45–54
8 Nei, M , *Am Natur* , 1972, **106**, 283–292.

ACKNOWLEDGEMENTS We thank Prof. S P Sinha, Department of Zoology, Bhagalpur University and Prof M. K Bhasin of Department of Anthropology, Delhi University, for valuable suggestions

Received 15 April 1993, revised accepted 30 September 1993

Cytophotometric DNA estimation in *Luzula* species

Sumona Mukherjee*, J. Sen and A. K. Sharma

*Postgraduate Department of Botany, Darjeeling Government College, Darjeeling 734 101, India
Centre of Advanced Study in Cell and Chromosome Research, 35, Ballygunge Circular Road, Calcutta 700 019, India

***In situ* DNA amount in eight species of *Luzula* is quite constant, ranging from 5.89 to 7.77 pg. The chromosome number in these species ranges from diploid $2n = 6$ to polyploid $2n = 48$. Evolution in these species has probably been due to transverse fragmentation of chromosomes as well as polyteny.**

THE species of *Luzula* do not have a centromere of the normal type in their chromosomes. The centromere is diffused or of multipolar type[1]. This characteristic of *Luzula* spp makes it an interesting case in the study of *in*

situ DNA content as associated with polyploidy. With this in mind, eight species of *Luzula* with chromosome number ranging from $2n = 6$ to 48 were subjected to cytophotometric analysis.

Seeds of *Luzula elegans*, *L. campestris*, *L. nivea*, *L. pediformis*, *L. luzuloides*, *L. spicata*, *L. pedemontana* and *L. sudetica* were germinated aseptically. The method for cytophotometric DNA estimation is based on the application of Feulgen reaction for microscopic localization and quantitative cytochemical determination of deoxyribonucleic acid by cytophotometry[2–7].

For cytophotometric DNA estimation in *Luzula* species, root tips were cut into 2 mm segments and fixed in Newcomers fluid for an hour. The optimum hydrolysis time giving maximum staining of nuclear DNA was 20 min at 60°C in 1N HCl. The root tips were washed and stained in Feulgen solution prepared from BDH-Gurr basic fuchsine (No. 42510). Maximum stainability was obtained after an hour at 20°C. Stained root tips were squashed in a drop of 45/propionic acid. From 50 metaphase plates 4C nuclear DNA was estimated. A Lietz Wetzlar Aristophot with microspectrophotometer was used for all quantitative cytophotometric determinations in this study. All DNA measurements were accomplished using Feulgen cytophotometric procedure employing the single wavelength 550 nm method[8]. All values recorded as arbitrary units were converted to absolute picogram values by using Van'tHof's[9] 4C nuclear DNA estimation of *Allium cepa* as a standard. Statistical *t* test was carried out between the mean nuclear DNA values obtained in the eight *Luzula* species.

The 4C DNA values show an extremely narrow range from 5.89 to 7.77 pg, although the range of chromosome number is quite wide from $2n = 6$ to 48 (Table 1). The six-fold increase in chromosome number is associated with only 1.3 times increase in DNA amount. Mello-Sampayo[10] considered the haploid $n = 3$ to be the ancient and original species from which the rest of the *Luzula* species are derived. Hence, *L.elegans* is a diploid species with $2n = 6$ chromosomes and the rest of the seven species are polyploids. Statistical *t* test between the 4C DNA values of all the polyploid species studied here has been made with the diploid *L. elegans*. Except *L. spicata*, all the others show a non-significant difference with *L. elegans* (Table 1). This difference in DNA content indicates that the type of polyploidy found in *Luzula* species is attained by transverse fragmentation of chromosomes instead of by multiplication of the chromosome sets in the nucleus, since a high chromosome number is reached without any increase in chromatin mass or chromatid length[11]. The diploid *L. elegans* has much longer chromosomes than the polyploid species[11]. The chromosomes of *Luzula* spp have diffused centromere so that the centromeric function is distributed all over the surface of the chromosome. When such chromosomes are broken into pieces, each individual fragment seems to have similar characteristics as the intact chromosome[12].

Similar centrometric behaviour is present in certain algae, mosses, fungi and in the invertebrate *Ascaris megalocephala*. Polyploidization by fragmentation of chromosomes and not duplication has been termed 'agmatoploidy' and can be detected by cytophotometric studies[12,13]. In the present study cytophotometric analysis confirms that agmatoploidy has played a role in the evolution of *Luzula* species. Despite polyploidization, the nuclear DNA amount remained nearly constant during evolution of *Luzula* species because the increase in chromosome number is due to fragmentation of chromosomes accompanied by a concomitant decrease in chromosome size. However, agmatoploidy is not the only cause of speciation in *Luzula* species. The significant difference in 4C DNA content between *L. elegans* and *L. spicata* indicates that in addition to fragmentation, duplication of chromosomes has also taken place. Within the tetraploid *Luzula* species significant difference in DNA content has been noted between *L. campestris* and *L. luzuloides* which could be due to differential polyteny[14]. Thus cytophotometric DNA estimation indicates that evolution in *Luzula* species has been accompanied mostly by transverse fragmentation of chromosomes as well as duplication and polyteny.

Table 1. *In situ* DNA amount and chromosome number in *Luzula* sp.

Species of *Luzula*	Chromosome number (2n)	4C DNA per cell ± sem (pg)	*t* value
L. elegans	6	6 17 ± 0 45	–
L. campestris	12	5 89 ± 0 29	0 523
L. nivea	12	6 05 ± 0 28	0 226
L. pediformis	12	6 46 ± 0 24	0 569
L. luzuloides	12	7 03 ± 0.22	1 717
L. spicata	24	7 77 ± 0 28	3 019*
L. pedemontana	30	7 01 ± 0 25	1 632
L. sudetica	48	6 88 ± 0 28	1 339

*Significant ($P < 0\ 05$)

1. LaCour, L. F., *Heredity*, 1952, **6** (Suppl.), 77–81
2. Pollister, A. W. and Leuchtenberger, C., *Proc Natl Acad Sci USA*, 1949, **35**, 66–71
3. Ris, H. and Mirsky, A. E., *J. Gen Physiol*, 1949, **33**, 125–156
4. Lessler, M. A., *Int. Rev Cytol.* 1953, **2**, 231–245
5. Kasten, F. H., *Int. Rev Cytol*, 1960, **10**, 1–100
6. Mcleish, J. and Sunderland, N., *Exp Cell Res*, 1961, **24**, 527–540
7. Yemma, J. J. and Penza, S. L., *Cytobios*, 1987, **50**, 13–28
8. Sharma, A. K. and Sharma, A., in *Chromosome Technique Theory and Practice*, Butterworths, London, 1980
9. Van't Hof, J., *Exp Cell Res*, 1965, **39**, 48–58
10. Mello-Sampayo, T., *Genet. Iber*, 1961, **13**, 1–22.
11. Sen, J., Mukherjee, S. and Sharma, A. K., *Genome*, 1990, **33**, 143–147.
12. Nordenskiold, H., *Hereditas*, 1951, **37**, 323–355
13. Halkka, O., *Hereditas*, 1964, **52**, 81–88
14. Mukherjee, S. and Sharma, A. K., *J. Genet Breed*, 1993 (in press).

ACKNOWLEDGEMENTS We are grateful to Professor M. Mota, Department of Genetics, Estacao Agronomica National, Oerias, Portugal, for providing the seeds

Received 1 March 1993, revised accepted 24 September 1993

An ancient Egyptian pregnancy test extended to cattle

T. Veena and R. Narendranath

Department of Veterinary Physiology, Veterinary College, University of Agricultural Sciences, Bangalore 560 024, India

Based on clues provided in papyrii from ancient Egypt (2100–2200 BC), we attempted to devise a simple test to diagnose pregnancy in cattle. The test relies on the differential response in germination and shoot growth of wheat seeds to the urine of pregnant and non-pregnant cows. Our results show that germination and shoot growth of wheat seeds were suppressed by the urine of pregnant cows and this persisted for 2–3 months after parturition. However the urine of non-pregnant cows did not cause such inhibition. Such a differential response was not found to be due to pH. We suggest that these results would be important in developing a simple test to diagnose pregnancy in cattle.

IN a set of papyrii brought by Flinders Peterie to London during 1898 from excavations of Kahun in Egypt, there are details of several gynecological diseases and the diagnostic tests apparently being practiced during 2100–2200 BC[1] One among these suggests that the germination response of barley and wheat seeds to woman's urine could indicate the state of her pregnancy and even the sex of the growing foetus. It is stated that the germination of seeds and shoot growth of both the species serve as a positive test for pregnancy and a relatively enhanced growth of barley suggests female sex and that of wheat male sex[1].

Though there has been an attempt in the past to test the validity of this ancient practice[1], results are neither clear nor well known. We were prompted to extend this test to diagnose pregnancy in cattle and hence the present study.

Urine was collected from pregnant ($n = 9$) and non-pregnant ($n = 15$) cows between 0630 and 0830 hrs from the UAS Dairy, University of Agricultural Sciences, Bangalore. The pregnant cows were those that tested positive for pregnancy by rectal examination 40 days after insemination. The non-pregnant cows were grouped into (a) those that had calved within the past three months and (b) those for which at least three months had elapsed after parturition. These two groups were not inseminated after their last parturition. Five ml of diluted urine (1 ml urine made to 5 ml with distilled water) was added to each petri dish containing 15 wheat seeds; four such petri dishes were maintained for each cow and the percentage germination was recorded after two days and shoot growth after five days. A control group was maintained using distilled water. The pH of the diluted urine samples was also determined by a digital pH meter.

The germination and shoot growth of wheat seeds treated with cow urine reduced significantly compared to those treated with water (Table 1). However, the urine of the pregnant cows suppressed the seed germination (46.48%) and shoot growth (0.93 cm) significantly more than that of the group (b) of non-pregnant cows (75.04% and 4 cm respectively). Urine of

Table 1. Germination (%) and shoot growth (cm) of wheat seeds treated with water, urine of pregnant and of non-pregnant cows The pH of the urine of these cows is also provided#

		Germination (%)		Shoot length (cm)		pH	
Treatments	*n*	Mean	SE	Mean	SE	Mean	SE
Water	13	87 70[a]	5 13	6 43[a]	0 34	6 95[a]	0 75
Urine							
Pregnant cows	19	46 48[b]	4 24	0.93[b]	0 83	7 38[a]	0.40
Non-pregnant (> 3 months elapsed after calving)*	7	75 40[c]	6.99	4 00[c]	0 47	7 08[a]	0 37
Non-pregnant (within 3 months after calving)**	8	49 51[b]	6.54	1.27[b]	0 44	7.30[a]	0 39

* group (b) in the text, ** group (a) in the text, # values with same superscript are not significantly different (one way analysis of variance)