with this, antigen H-2.4 was not found in a single wild animal despite the fact that it is one of the strongest H-2 antigens and could hardly be missed in typing. The same holds for antigens H-2.23 and H-2.30 which were also absent in our sample of wild mice. Antigens H-2.2, 9, 16, 17, 19, and 22, which according to the H-2 chart can also be considered as private, were found in the wild mice, but their occurrence was limited to only one farm. On the other hand, antigens H-2.1, 3, 5, 7, and 8, which are widely distributed in the H-2 chart of laboratory mice and are therefore considered public, were also widely distributed in the wild population.

The strength of reaction of red blood cells from wild mice with antiserums against antigens H-2.1, 3, 5, and 8 was comparable with that of red blood cells of the positive inbred strains. The reactions of antiserums against antigens H-2.11, 16, 17, 19, and 22 were usually weaker with red blood cells of wild mice than with red blood cells of the positive inbred strains. To what extent cross-reactivity is responsible for this difference we have no way of knowing.

The cells of some wild mice did not react with any of the 23 antiserums and some others reacted only with one antiserum (mice from farm GA). This could be due either to reduced expression of the H-2 antigens on red blood cells of these mice or to the absence of known H-2 antigens and their replacement by others as yet undetected.

All 40 wild mice were also typed with a rabbit antimouse serum for the presence of the serum protein substance (Ss) controlled by the H-2 region (*10*). The immunodiffusion tests revealed a high level of Ss in all mice. The Ss^h/Ss^h genotype of all males and two females was confirmed by progeny tests. The rest of the females, from whom no progeny were obtained, could have been either of Ss^h/Ss^h or of Ss^h/Ss^1 genotype, since the heterozygous phenotype cannot be reliably distinguished from the high homozygous phenotype on an undefined genetic background.

The direct hemagglutination typing of wild mice with antiserums produced in laboratory animals has, of course, its drawbacks, most obvious of which are the following:

1) A negative result does not necessarily mean an absence of an antigen, since such phenomena as dosage effect and differential tissue distribution play an important role in the typing.

2) The combination of antigens found in an individual animal does not necessarily represent an *H-2* allele, since the animal could be a heterozygote.

3) A positive reaction of a monospecific antiserum does not mean that the detected antigen must be identical with the one against whom the antiserum was prepared, since cross-reactivity with a related antigen cannot be excluded.

4) Antiserums produced against laboratory mice and then applied to the study of wild mice can probably detect only a small part of the antigenic polymorphism of the wild animals, since there are probably many other presently undetected antigens in the wild population.

However, at least some of these drawbacks can be counterbalanced by addition of absorption analysis, progeny studies, genetic analysis, and cross-immunizations of the wild mice. Experiments along this line are already in progress. We hope that the results of such combined studies will allow us to estimate rather precisely the extent of the H-2 polymorphism. Since the *H-2* system is such a close homolog of the *HL-A* system in man, this might have some important bearing on the perspectives of clinical transplantation. However, even with this limited knowledge about the H-2 antigens in wild mice, it is obvious that antigens can be extremely useful in such areas as the study of population dynamics and ecology of this species.

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Indian Muntjac, Muntiacus muntjak: A Deer with a Low Diploid Chromosome Number

Abstract. *The Indian muntjac* (Muntiacus muntjak) *has a diploid chromosome number of 7 in the male and 6 in the female, the lowest number yet described in a mammal. Its near relative, Reeve's muntjac* (Muntiacus reevesi) *has a diploid number of 46, and the karyotypes of the two species are very different.*

The deer family, Cervidae, probably arose from traguloid ancestors in the Oligocene. The main line evolved in Eurasia, eventually spread to the New World and culminated in the two largest cervid subfamilies, the Odocoileinae and the Cervinae (*1*). These subfamilies contain 34 species (*2*). There are two primitive cervid subfamilies, the Muntiacinae (muntjacs and tufted deer) and the Moschinae, both of which are solely Asian. The former is represented by six species, and the latter by a single species, the musk deer, *Moschus moschiferus*. The Muntiacinae and Moschinae are considered to be representatives of an early branch from the main cervid line that has evolved with little or no divergence (*3*).

Mammalian chromosome numbers vary from 10 in the female, and 11 in the male of the marsupial *Protemnodon bicolor* (*4*) to 84 in the black rhinoceros, *Diceros bicornis* (*5*). Thirteen species of the Odocoileinae and Cervinae have diploid numbers of 56 to 70 but a relatively constant fundamental number (FN) of 70 to 74, that is, number of major chromosomal arms. Of the two primitive deer subfamilies the Reeve's muntjac, *Muntiacus reevesi*, has a diploid number of 46 (FN of 46), and its near relative, the Indian muntjac, *M. muntjak*, was reported to have a diploid number of 6 (*6*). We now confirm, after the study of two male and one female muntjacs, that this species has this unusually low diploid number of 6 for the female and 7 for the male (Fig. 1).

The karyotype consists of two large metacentric and four acrocentric autosomes. The X chromosome, which constitutes 9.3 percent by weight of the haploid complement, is fused to the centromeric end of the smallest pair of acrocentric autosomes. The Y chromosome, a tiny metacentric, equals 3.1 percent by weight of the haploid complement. The procedure for determining the mass of the X chromosome relative to that of the haploid complement was as described (*7*) except that the images were cut from a photograph enlarged 3600 times rather than from a tracing on paper (*8*). Morphological characteristics of note are (i) an achromatic gap in the proximal region of the long arms of the autosome which bears the X chromosome, (ii) a narrow elongate neck appearing to be a compound, segmented centromeric region in the X chromosomes and in the large metacentrics, (iii) an achromatic gap in the distal portion of one arm of the large metacentrics, (iv) pronounced telomere formation, and (v) overall large size of the chromosomes.

The heterochromatic X is visible in the female interphase nucleus as a single Barr body (Fig. 1). Terminal labeling with tritiated thymidine shows the following DNA replication pattern —the short arms of the larger acrocentrics, the attenuated centromeric regions of the metacentrics, and the X-autosome element replicate early. The rest of the chromosomal material replicates almost synchronously; there are, however, small sections in all elements that are somewhat earlier labeling which produces a segmental pattern at a later stage. The Y is not late replicating.

These findings in the Indian muntjac are pertinent to basic physical mechanisms of chromosomal evolution. Since DNA content of the nucleus of any of the few mammals investigated is roughly the same, regardless of the number of chromosomes present (*9*), karyotype evolution involves chromosomal rearrangement rather than duplication or deletion of material. There are three theories of how these rearrangements occur: (i) the theory of chromosomal fusion holds that primitive mammals had a large number of acrocentrics, and that the highly evolved and specialized species attained a lower diploid number through fusion of acrocentrics to form metacentrics. In support of this theory is the frequent spontaneous occurrence of such fusions in natural populations of animals, in humans (translocations of acrocentric chromosomes) and in tissue cultures. Each fusion event leads to the loss of one centromere. (ii) The theory of fission proposes that the primitive mammals had a low diploid number composed mainly of metacentrics. Karyotypes evolved by periodic fission which produced higher diploid numbers and more acrocentrics. This scheme is convenient to explain the transformations that have occurred in many puzzling variations of karyotypes among species. There is, however, no detectable spontaneous occurrence of fission in vivo or in vitro nor is there a satisfactory explanation of a source of new centromeres that would be needed to create new elements after fission. (iii) The third theory involves the operation of both fusion and fission. By using both mechanisms, and invoking inversions where needed, one can indeed begin with any karyotype and arrive eventually at any other.

The Indian muntjac and Reeve's muntjac are quite similar in appearance. The Indian muntjac has the wider and more southerly distribution (*10*). The two are said to have hybridized in a number of cases (*11*) with allegedly fertile offspring (*12*). A third species, *M. rooseveltorum*, has characteristics intermediate to those of *M. reevesi* and *M. muntjak* (*10*).

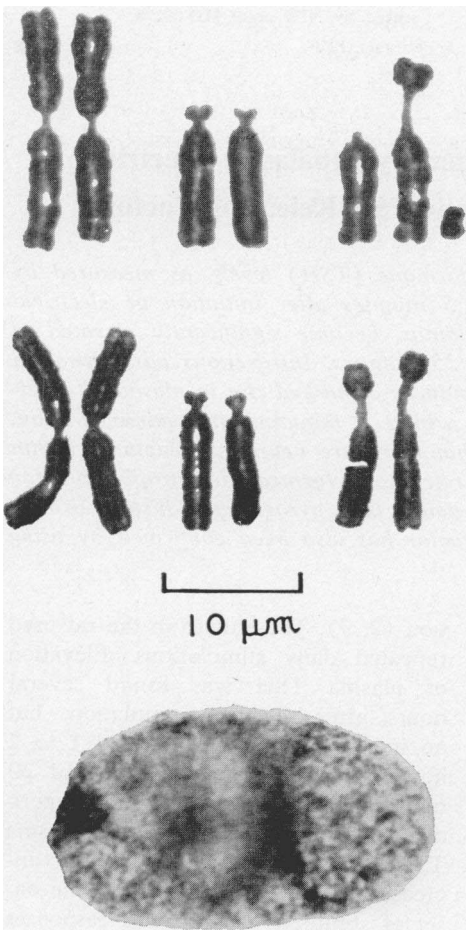

Fig. 1. Karyotypes of Indian muntjac; (top row) male; (middle row) female. The X is the upper portion of pair 3 (unpaired in the male) and the Y is the tiny metacentric. (Bottom) Nucleus of female cell showing Barr body; stained with carbol fuchsin (× 1500).

The chromosomes possibly have compound centromeric regions that could represent either centromere storage resulting from condensation of chromatin material or potential donors of centromeres for fission. Segmental labeling patterns and telomere formation suggest compound chromosomes, and centromere formation by means of telomeric structures as a possible mechanism for the formation of new acrocentric elements. Telomeric structures have been seen in this laboratory in a number of other mammalian species as well as on the ends of broken chromosomes. Whether these can develop the capacity to serve as centromeres is unknown.

The unusually large X chromosome of the Indian muntjac is divisible into two sections, the distal portion which appears similar to a small acrocentric and the proximal portion which is a long, thin "neck." This whole X chromosome is equal to 9.3 percent by weight of the haploid complement. The neck alone amounts to 2.6 percent by weight of the haploid complement; the distal portion alone accounts for 6.7 percent by weight, a figure which is comparable to the 6.78 percent by weight accounted for by the X chromosome found in the near relative, the Reeve's muntjac (*13*). It may be that only the distal portion is active X material.

A similar type of X-autosome translocation has been described in natural populations of three marsupials—*Protemnodon bicolor*, $2n = 11/10$ (*14*), *Potorous tridactylus*, $2n = 13/12$ (*15*), and *Macrotis lagotis*, $2n = 19/18$ (*16*). The female of each of the former two species reduplicates the DNA of one entire X chromosome asynchronously (*17*), thereby indicating a relative mass of about 5 percent ["original" type X (*18*)] of the haploid complement. The X chromosome in one karyotype of *Protemnodon bicolor* (supplied by D. L. Hayman) equals 5.5 percent by weight of the haploid complement. The synchronization of the DNA reduplication in adjacent portions of the fused autosomes was not affected by association with the heterochromatic X.

Some other genera also show rather large ranges of chromosome numbers among sibling species, for example, *Equus* $2n = 32$ to 66, *Microtus* $2n =$ 17 to 62, *Gerbillus* $2n = 30$ to 66, and *Sigmodon* $2n = 22$ to 52 (*19*). Sibling rodent species of the tribe Ellobini, subfamily Microtinae also vary; *Ellobius talpinus* has $2n = 52$ to 54 and *E. lutescens* has $2n = 17$ in both sexes (*20*).

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Thyrotropin Secretion in Rats after Hypothalamic Electrical Stimulation or Injection of Synthetic TSH-Releasing Factor

Abstract. *Plasma thyroid-stimulating hormone (TSH) levels, as measured by radioimmunoassay, begin to rise within 5 minutes after initiation of electrical stimulation of the medial-basal hypothalamus, become significantly elevated at 10 minutes, and reach a peak at 10 to 25 minutes. Intravenous administration of synthetic thyrotropin-releasing factor induces a marked rise in plasma thyroid-stimulating hormone which is maximal within 5 minutes after administration. These data are interpreted to indicate that there are neuronal structures within the medial-basal hypothalamus which release preformed thyrotropin-releasing factor. The claim (based on bioassay data) that pyroglutamyl-histidyl-proline amide is a potent thyrotropin-releasing factor has also been confirmed by using a highly specific immunoassay system.*

A number of authors have claimed that electrical stimulation of the hypothalamus causes an increase in pituitary thyroid-stimulating hormone (TSH) secretion. Most of these studies have measured changes in thyroid function as an index of TSH release (*1*). Certain of these experiments suffer from the criticism that non-TSH changes such as vasopressin release (in the rabbit), epinephrine release (in the dog), or other unknown factors such as vascular changes due to sympathetic stimulation might have accounted for the observed results. Increases in plasma TSH as measured by bioassay have been reported in the rat and the rabbit following hypothalamic stimulation (*2, 3*). The studies in the rat used repeated daily stimulations. Elevation of plasma TSH was found several hours after the last stimulation, but no increases could be detected 1 to 2 hours after a single stimulation of 20 minutes' duration (*2*). In the experiments performed in the rabbit, plasma TSH levels were shown to be increased within 15 minutes after the onset of stimulation, with peak responses occurring in some animals between 15 and 45 minutes. This is the only report of the time course of plasma TSH response after hypothalamic stimulation.

The development of a sensitive and specific radioimmunoassay for TSH in the rat (*4*) has provided the methodology for determination of TSH in small samples of plasma, permitting repeated sampling from the same animal. With this method we have made detailed time-course studies of the effect of electrical stimulation of the hypothalamus. Since it has been hypothesized that the regulation of TSH release is dependent upon the secretion of a hypothalamic "releasing factor" (thyrotropin-releasing factor, TRF), changes in plasma TSH following electrical stimulation were compared with those obtained following the intravenous administration of "synthetic" TRF. Recently two laboratories have reported that a tripeptide amide, pyroglutamyl-histidyl-proline-amide, has thyrotropin-releasing activity identical with that of TRF isolated from hypothalamic extracts and is virtually indistinguishable chemically from the isolated native material (*5, 6*).

Male Sprague-Dawley rats weighing 250 to 450 g were used. Plasma samples were obtained under pentobarbital (50 mg/kg) or ether anesthesia either by puncture of the external jugular vein or from indwelling jugular cannulas. For electrical stimulation, bipolar .032 gauge Nichrome electrodes, insulated with Insl-X except at the tip, were placed stereotactically into various areas of the medial-basal hypothalamus. The electrodes were soldered to a connector which was affixed to the skull with screws and dental cement. Electrical stimulations were performed 10 to 14 days later. Biphasic square waves from a constant-current stimulator were delivered for 5 or 10 minutes in trains of 4 seconds on and 4 to 10 seconds off, with current of 0.5 to 1.0 ma, frequency of 60 cycle/second, and pulse duration of 1 to 2 msec.

Two separate experiments were undertaken. In the first, electrodes were placed in various regions of the basal hypothalamus extending from the anterior hypothalamus to the posterior ventromedial nucleus. Plasma samples were taken prior to and at several intervals for a period of 1 to 2 hours after stimulation. In the second experiment, electrodes were implanted in the median eminence [de Groot coordinate (*7*): anterior (+)5.6, lateral 0.5, depth (—)3.0], and samples were taken at 0, 5, 10, 15, and 25 minutes.

Two kinds of controls were used. Five sham-stimulated animals with electrodes in place were anesthetized, and blood samples were withdrawn