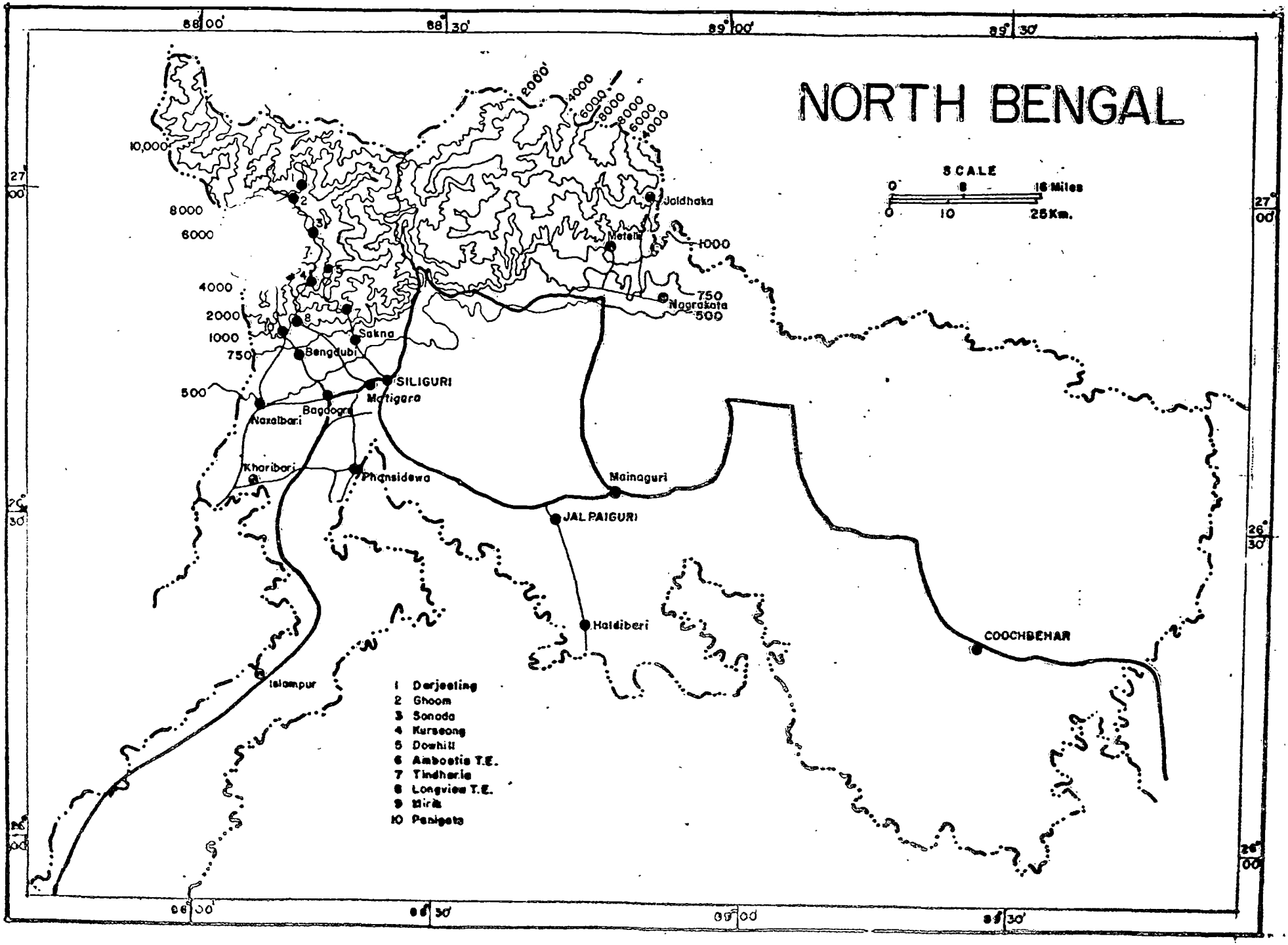
PART I

CONSTITUTIVE HETEROCHROMATIN POLYMORPHISM


NORTH BENGAL
SCALE
0 8 16 Miles
0 10 25Km.
88°00'
88°30'
89°00'
89°30'
27°00'
26°30'
26°00'
10,000
8000
6000
4000
2000
1000
750
500
Jaldhaka
Metelli
Nagrakata
Sakna
Bengdubi
SILIGURI
Matigara
Naxalbari
Bagdogra
Kharibari
Phansidewa
Mainaguri
JAL PAIGURI
Haldibari
COOCHBEHAR
Islampur
1 Darjeeling
2 Ghoom
3 Sonada
4 Kurseong
5 Dowhill
6 Ambootia T.E.
7 Tindharia
8 Longview T.E.
9 Mirik
10 Panighata

1 INTRODUCTION

Since 1965, the steady decline in the progress of researches in human cytogenetics, in comparison to the explosive way of development during the early 'sixties, may be mainly due to the limitation of techniques which remained unchanged except for minor refinements. However, in recent years remarkable advances have been made in techniques for the study of linear differentiation of chromosomes which have revolutionized the cytogenetic researches.

A new era was opened when Caspersson group (1968) observed reproducible pattern of fluorescence in the chromosomes of Vicia fava and Trillium erectum following quinacrine mustard fluorochroming and UV illumination. The extreme usefulness of this discovery has been proved not only in its application in precise identification of individual chromosomes (Caspersson et al. 1971, followed by others) but also in its capability of revealing chromosomal organization at the molecular level. Fluorescence pattern is still a reliable analytical tool. Pardue & Gall (1970) demonstrated that following denaturation and renaturation of chromosomal DNA, the region of centromeric heterochromatin in mouse chromosomes could be heavily stained with Giemsa. Because of inherent technical difficulties of fluorescent banding procedures for routine exploitation, Pardue & Gall's discovery led to modifications of their technique for use in producing Giemsa bands.

Arrighi & Hsu (1971), Yunis et al. (1971), and Summer (1972) developed staining techniques called the C-banding techniques which predominantly stain the centromeric heterochromatin. Besides centromeres, acrocentric heterochromatin associated with the short arm and satellite regions of the D and G group chromosomes, secondary constriction heterochromatin

located in the proximal long arms of chromosomes 1, 9 and 16 and heterochromatin on the distal portion of the long arm of the Y chromosome are also stained (Craig-Holmes et al. 1973). Since the introduction of these techniques specifically demonstrating constitutive heterochromatin, careful examinations have revealed the existence of quantitative variations of this moiety of chromosome (Craig-Holmes & Shaw 1971, Paris Conference 1971).

Genetic polymorphism has been demonstrated in man for many characteristic features including blood groups, serum proteins, tissue enzymes and haemoglobins. Before the discovery of banding techniques, the length of the Y chromosome was known to vary from person to person and from one ethnic group to another (Cohen et al. 1966, Cohen & Shaw 1967, Ghosh & Singh 1973). Some autosomal regions were also found to differ in various racial groups (Starkman & Shaw 1967, Lubs & Ruddle 1971). The advent of C-banding techniques have added a new dimension to this area of cytogenetic research. This has led to the discovery of a class of chromosomal polymorphism involving constitutive heterochromatin which can be demonstrated especially by CBG and CBG methods established by Arrighi & Hsu (1971) and Sumner (1972) respectively.

The constitutive heterochromatin or C-band represents perhaps the most notable chromosome heteromorphism because of its variation in length and stability in size from one generation to another (Geraedts & Pearson 1974, Robinsons et al. 1976). Besides the well known size variations, the C-bands can also present a position heteromorphism. Excepting the Y-terminal C-bands, all others have a pericentromeric localization. The C-bands of the extensively studied chromosomes 1, 9 and 16 are generally located on the long arm (q), though the pairs 1 and 9 may also reveal part or all of the heterochromatin on the short arm (p), which is commonly classified as pericentromeric inversion.

Polymorphism involving C-bands has been found to occur at a relatively high frequency in man. Although such heteromorphisms were initially reported in clinical material from retarded and / or dysmorphic subjects (Wang & Hamerton 1979), later works have demonstrated that heteromorphisms occur with equal frequency among phenotypically normal controls. Constitutive heterochromatin polymorphism of chromosomes 1, 9 and 16 have been shown to be relatively frequent in clinically normal individuals and the incidence varies in different populations and ethnic groups (Craig-Holmes and Shaw 1971, Lubs &

Ruddle 1971, Craig-Holmes et al. 1973 and 1975, Mckenzie & Lubs 1975, Muller et al. 1975, Buckton et al. 1976, Robinson et al. 1976, Lubs et al. 1977, Brogger et al. 1977, Jacobs 1977, Therapel & Summitt 1978, Matsuura et al. 1978, Schnedl 1978, Verma et al. 1978 and 1981, Ibraimov et al. 1982, and Sisi et al. 1982).

Though the biological and clinical implications of human chromosome polymorphism are poorly understood, yet it has been suggested that these heteromorphisms are limited to certain groups (Craig-Holmes et al. 1975). A possible association of malignant diseases with chromosomal heteromorphisms have been demonstrated (Atkin & Baker 1977, Atkin and Pickthall 1977, Atkin 1977, Shabtai et al. 1978, Shabtai and Halbrecht 1979, Atkin and Brito-Babapulle 1981). It has also been found that a large C-heterochromatin in chromosome 9 occurs at a higher frequency among Blacks with low IQ than in those with high IQ (Lubs et al. 1977). A number of publications have suggested a direct correlation between the increasing seriousness of reproductive disorders and the increasing amount of C-heterochromatin (Boue et al. 1975, Ford et al. 1983).

In other mammals also a great degree of variations in the nature and quantity of constitutive heterochromatin in various populations have been observed (Bradshaw & Hsu 1972, Duffey 1972, Pathak et al. 1973, Sharma & Raman 1973, Markvong et al. 1975, Sharma & Gadi 1977). Besides these, uptil now there have been very few facts that can be considered as a demonstration of heterochromatin function (Podugolnikova and Blumina 1983).

The application of in situ nucleic acid hybridization technique (Gall & Pardue 1969, Jones 1970), the C-banding technique for staining constitutive heterochromatin (Arrighi & Hsu 1971), and subnuclear fractionation of chromatin (Frenster et al. 1963, Yunis & Yasmineh 1971), have demonstrated a general correspondence between the loci of highly repititive DNA, constitutive heterochromatin and the C-banding on metaphase chromosomes. The strong correlation between satellite DNA and constitutive heterochromatin alone warrants justification of studying C-band heteromorphism in man. Moreover, recent studies have revealed a great population and evolutionary stability of C-band heteromorphisms.

In recent past the role of C-heterochromatin has been repeatedly emphasized in karyotypic divergence and speciation in various mammals (l.c.). Of the various speculations made on the role of quantitative variation of C-heterochromatin in mammals, its possible involvement in evolutionary differentiation is most attractive.

The lack of information about constitutive heterochromatin polymorphism on Indian population is surprising, in view of the vastness of the country as well as wide diversity of population. There are quite a few different ethnic groups in North Bengal, a part of one of the north eastern states of India (vide frontispiece), and they are distributed from plain to the high altitude belts with different environmental conditions. The multitudeness of ethnic structure in this area provides a unique opportunity for studying certain biological aspects of human population in general, and C-heterochromatin polymorphism in particular. One of the principal objectives of the present scheme of work is to study and evaluate the variability of these heterochromatic regions and to obtain an estimate of the frequency of C-band variants in the Darjeeling Himalayan population having such racial differences.

In the present study C-band polymorphism has been recorded in the four racial groups of a normal population comprising the ethnic groups of North Bengal of diverse origins living at a similar latitude, but in different climatogeographic conditions and in different altitudes. It would also be interesting to assess the possible selective value of chromosomal C-heterochromatin material in the adaptation of ethnic populations of North Bengal to diverse environmental conditions.