chromatic (fig. 3). One arm of the smallest chromosome is also highly heterochromatic. The present finding poses the question whether the Peninsular Malaysian and Thailand populations of *Megaerops ecaudatus* are conspecific. To answer this, more specimens, collected throughout the distribution range of this bat, need to be studied. Meanwhile, on the basis of large chromosomal differentiation, the 2 populations may be accorded subspecific status, viz. *Megaerops ecaudatus malayanus* for the Peninsular Malaysian taxon and *Megaerops ecaudatus siamensis* for the Thailand taxon.

1 This work is supported by a University of Malaya research grant.
2 Ellerman, J.R., and Morrison-Scott, T.C.S., Checklist of Palaeaearctic and Indian mammals, 1966.
3 Panikawongse, S., Karyotype analysis of some common fruit bats of Thailand. M. Sc. Thesis, Kasetsart University 1975.
4 Harada, M., Minezawa, M., Takada, S., Yenbutra, S., Nunpakdee, P., and Ohtani, S., Caryologia *35* (1982) 269.
5 Yong, H.S., and Dhaliwal, S.S., Cytologia *41* (1976) 85.
6 Yong, H.S., Dhaliwal, S.S., and Teh, K.L., Experientia *27* (1971) 1353.

0014-4754/84/080875-02$1.50 + 0.20/0

Hybridization between Robertsonian karyotypic races of the common shrew *Sorex araneus*

J.B. Searle[1,2]

Department of Genetics, University of Aberdeen, Aberdeen AB9 2TN (Scotland), 6 October 1983

Summary. British common shrews of the Aberdeen, Oxford and Hermitage Robertsonian karyotypic races were hybridized successfully in captivity. Hybrids, both simple Robertsonian heterozygotes and double Robertsonian heterozygotes with monobrachial homology, have been identified in an area of contact between the Oxford and Hermitage races. The relative fertility of these two types of hybrid is considered.

Many closely related taxa, classified as separate species or races, differ by karyotypic rearrangements. Of interest is the extent to which such taxa may interbreed, and in particular the contribution of the karyotypic rearrangements to any reproductive isolation. Such a contribution may be direct, through reduction of fertility in hybrids (e.g. due to nondisjunction at anaphase I of meiosis associated with karyotypic heterozygosity) or indirect, as in the case of assortative mating which has arisen as a consequence of selection against karyotypic heterozygotes because of their reduced fertility.

The common shrew *Sorex araneus*, an insectivore with a Palaearctic distribution, is a spectacular example of a mammalian species that is multiplely subdivided into karyotypic races. Altogether 12 races have been described (reviewed by Searle[3]); each of these races differs by presence or absence of the products of Robertsonian fusion mutations. However, little is known of the extent to which these races may interbreed. The area of contact between the northern Swedish and central Swedish karyotypic races of common shrew has been fairly accurately delimited and 1 hybrid individual has been identified[4]. Hybrids have also been detected in recent studies of the area of contact between the Novosibirsk and Chaldejevo (Tomsk) races of common shrew in Siberia[5]. With regards laboratory studies, no attempts have been made to interbreed karyotypic races of common shrew in captivity.

Three karyotypic races of common shrew have been found in Britain. The Aberdeen race occurs in north-eastern Scotland, the Oxford race in southern Scotland and central and northern England and the Hermitage race in southern England[3]. In terms of the standard nomenclature[4], these races are characterized by the following arm combinations: Aberdeen 3*hi* 4*jl* 5*gm* 6*ko* 7*np* 8*qr*; Oxford 3*hi* 4*jl* 5*gm* 6*kq* 7*no* 8*pr*; Hermitage 3*hi* 4*jl* 5*gm* 6*ko* 7,8 *n/p/q/r*. Robertsonian polymorphism is found in all 3 races such that arm combinations *jl* (Aberdeen); *jl, kq, no* and *pr* (Oxford); and *jl* and *ko* (Hermitage) may occur as metacentrics or twin-acrocentrics.

This paper describes attempts to interbreed the British races in captivity, cases of hybridization between 2 of these races in nature and data on fertility of hybrids in nature.

Materials and methods. Common shrews were trapped in Grampian region (Aberdeen race shrews) and Oxfordshire, Berkshire and Hampshire (Oxford race, Hermitage race, Oxford-Hermitage hybrid shrews). For breeding, shrews were kept as single pairs in large enclosures and fed twice daily on a diet based on offal and cereal with a vitamin supplement[6]. Disturbance to breeding shrews was kept to a minimum until weaning, when young were counted and sacrificed. Direct, air-dried, mitotic chromosome preparations were made from bone marrow and G-banded by a composite method[7] in which the preparations are first treated as in the ASG method[8] but with a trypsinization step[9] included prior to staining. Meiotic chromosome preparations were made by a modification of the Evans method[10].

Results. Successful crosses in captivity between Oxford and Aberdeen race shrews, Oxford and Hermitage race shrews and

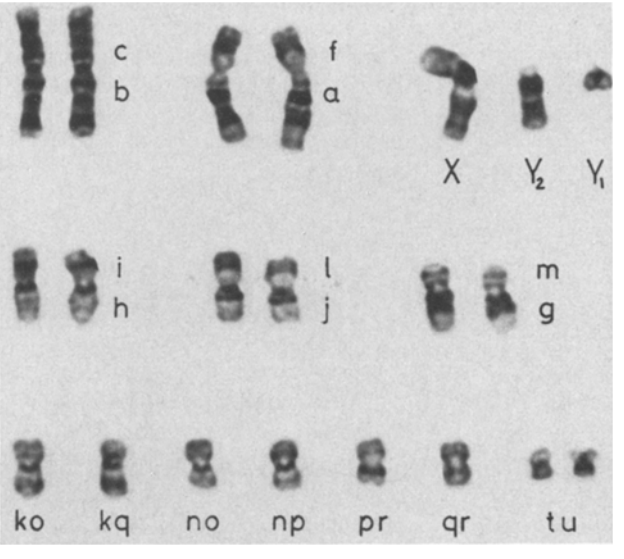


Figure 1. The karyotype of an Oxford-Aberdeen hybrid shrew reared in captivity. Note the 6 unpaired race-specific metacentrics.

Aberdeen and Hermitage race shrews were recorded. In each case, behaviorally normal and healthy young were reared to weaning. A representative karyotype of one of the hybrid offspring is shown in figure 1.

Of the 16 crosses attempted between common shrews of different race, successful fertilization was recorded in 11 crosses (69% of total) and 9 litters (56% of total) were reared to weaning (table 1). The remaining crosses failed; whether no litters were conceived or whether litters died during development is unknown. The mean (±SE) weanling litter size of all successful inter-racial matings, 5.2 ± 0.5 ($n = 9$) did not differ significantly from a series of intra-racial matings (litters conceived in nature or in captivity) reared at the same time, 4.9 ± 0.7 ($n = 10$).

In an area of contact between the Oxford and Hermitage races, around Newbury (Berkshire), inter-racial hybrids (i.e. individuals with karyotypes including metacentrics characteristic of both races) have been detected in nature. Hybrids occurred in all 8 samples of 5 or more individuals from a latitudinal transect of 39 km between East Hendred (Oxfordshire) and Whitchurch (Hampshire). Of 136 individuals trapped along the transect, 24 were hybrids. The karyotypes of these hybrids varied due to Robertsonian polymorphism (table 2). 17 of the hybrids were 'simple Robertsonian heterozygotes', heterozygous for one arm combination or for several arm combinations which lack monobrachial homology (i.e. hybrids heterozygous for arm combination *pr* of the Oxford race and homozygous metacentric or heterozygous for arm combination *ko* of the Hermitage race). Such simple Robertsonian heterozygote hybrids were caught at all sites. Fewer of the hybrids which were caught (7 in total) were double Robertsonian heterozygotes with monobrachial homology (i.e. hybrids with karyotypes including arm combinations *no* or *kq* of the Oxford race and *ko* of the Hermitage race in the metacentric form). This class of hybrids was only trapped over a latitudinal distance of 10 km at the north of the transect.

Of the hybrids recorded (table 2), those which were homozygous metacentric for arm combination *ko* and heterozygous for *pr* must have been backcross, F_2 or other higher generation hybrid progeny. 10 of such individuals were recorded in samples from sites over a latitudinal distance of 29 km at the south of the transect. This is direct evidence that hybrids which are simple Robertsonian heterozygotes can be fertile in nature.

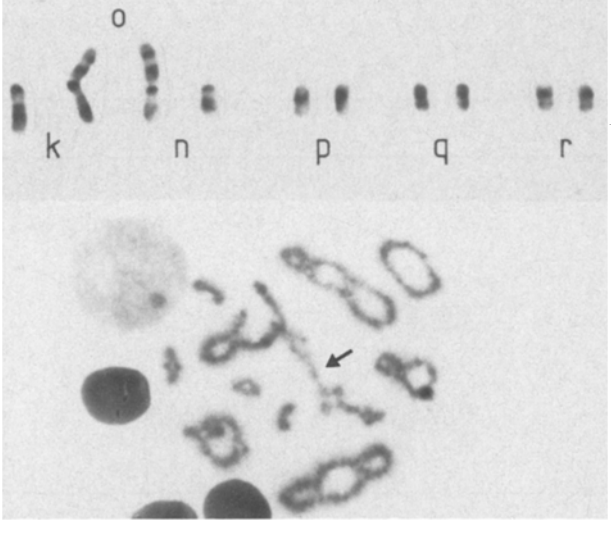


Figure 2. A spread of a late diplotene/early diakinesis cell from an individual heterozygous for arm combinations *ko* and *no* (partial karyotype above). Note the chain quadrivalent (arrow) composed of chromosomes *k-ko-on-n*.

Unfortunately, comparable direct evidence cannot be obtained for hybrids which are double Robertsonian heterozygotes with monobrachial homology. However, one wild adult male hybrid which was heterozygous for arm combinations *ko* and *no* was available for reproductive study. The testes and accessory organs of this animal were normal in external appearance and histological sections of the left testis revealed normal spermatogenesis. Meiotic chromosome preparations also failed to reveal abnormality. Regular chain quadrivalents were observed in spreads of late prophase I and metaphase I cells (fig. 2). In 50 spreads of cells from stages late diplotene to metaphase I, no cases of univalency were recorded.

Discussion. From the breeding studies, no evidence was obtained for a strong pre- or post-mating barrier to hybridization between the British karyotypic races of common shrew. Furthermore, there is direct evidence that hybridization between 2 of the races (Oxford and Hermitage) occurs in nature. It is of particular interest that hybrids which are double Robertsonian heterozygotes with monobrachial homology are produced. While simple Robertsonian heterozygotes have been reported in areas of hybridization between karyotypic races of a wide variety of species (e.g. the rodent *Acomys caharinus*[11], the lizard *Sceloporus grammicus*[12], the grasshopper *Podisma pedestris*[13], the isopod *Jaera syei*[14]), the author knows of only 2 previous reports of naturally-occurring double Robertsonian heterozygotes with monobrachial homology: single specimens of the beetle *Chilocorus stigma*[15] and the mouse *Mus musculus (domesticus)*[16].

Both simple Robertsonian heterozygotes and double Robertsonian heterozygotes with monobrachial homology have been generated and studied extensively in laboratory mice. While simple Robertsonian heterozygotes for one or a few arm combinations may have slightly reduced fertility compared with homozygotes, due to low to moderate rates of anaphase I nondisjunction[17,18], the fertility of double Robertsonian heterozygotes with monobrachial homology is considerably reduced due to high rates of nondisjunction and often, in males, arrest of spermatogenesis[17,19].

Table 1. Inter-racial crosses in captivity

Cross type (female × male)	Number of crosses: No evidence of fertilization	Evidence of fertilization: Not weaned	Evidence of fertilization: Weaned	Total
Oxford × Aberdeen	3	1[a]	4	8
Aberdeen × Oxford	1	1[b]	2	4
Aberdeen × Hermitage	1	0	2	3
Oxford × Hermitage	0	0	1	1
Total	5	2	9	16

[a] Young died post-partum of unknown causes. [b] Female killed during gestation.

Table 2. The karyotypes of the Oxford-Hermitage hybrids[a] trapped in nature

Arm combination *kq*	*no*	*pr*	*ko*	Number of individuals
H	–	H	H	1
–	H	H	H	1
–	H	–	H	5
–	–	H	H	7
–	–	H	M	10
Total				24

[a] Individuals which are heterozygous (H) or homozygous metacentric (M) both for arm combinations characteristic of the Oxford race *(kq, no, pr)* and the Hermitage race *(ko)*.

In the area of hybridization between Oxford and Hermitage races of common shrew, hybrids which are simple Robertsonian heterozygotes were collected over a larger area and at a higher frequency than double Robertsonian heterozygotes with monobrachial homology, despite the fact that the frequency of the metacentric morph of arm combination *pr* is not higher than that of *no* or *kq* away from the area of hybridization[3]. This suggests that there is stronger selection against double Robertsonian heterozygotes with monobrachial homology than simple Robertsonian heterozygotes, presumably due to higher rates of nondisjunction and perhaps male-sterility. Clearly a large series of hybrids needs to be screened to test this hypothesis. The one adult male double Robertsonian heterozygote with monobrachial homology so far examined was apparently fertile and revealed no prophase I-metaphase I abnormalities.

1 I thank Dr C.E. Ford F.R.S.., Prof. F.W. Robertson and Dr A.E. Douglas for reading earlier drafts of this paper, Prof. F.W. Robertson, Dr J.R.K. Savage and Dr J.R. Clarke for laboratory facilities, Mrs J.E. Evans for technical assistance and S.E.R.C. for financial support.
2 Present address: Department of Agricultural Science, University of Oxford, Parks Road, Oxford OX1 3PF (England).
3 Searle, J.B., Syst. Zool., in press.
4 Fredga, K., and Narwin, J., Chromosomes today *6* (1977) 153.
5 Volobouev, V.T., personal communication.
6 Searle, J.B., Lab. Animals, in press.
7 Evans, E.P., personal communication.
8 Sumner, A.T., Evans, H.J., and Buckland, R.A., Nature New Biol. *232* (1971) 31.
9 Seabright, M., Lancet *2* (1971) 971.
10 Evans, E.P., Breckon, G., and Ford, C.E., Cytogenetics *3* (1964) 289.
11 Wahrman, J., and Goitein, R., Chromosomes today *3* (1972) 228.
12 Hall, W.P., and Selander, R.K., Evolution *27* (1973) 226.
13 Hewitt, G.M., and Barton, N.H., Symp. R. ent. Soc. Lond. *10* (1980) 149.
14 Lécher, P., Archs Zool. exp. Génet. *108* (1967) 633.
15 Smith, S.G., and Virkki, N., Animal Cytogenetics, vol.3, Insecta, chapter 5, Coleoptera. Borntraeger, Berlin 1978.
16 Gropp, A., Winking, H., Redi, C., Capanna, E., Britton-Davidian, J., and Noack, G., Cytogenet. Cell Genet. *34* (1982) 67.
17 Gropp, A., and Winking, H., Symp. zool. Soc. Lond. *47* (1981) 141.
18 White, B.J., Crandall, C., Raveché, E.S., and Tjio, J.-H., Cytogenet. Cell Genet. *21* (1978) 113.
19 Gropp, A., Kolbus, U., and Giers, D., Cytogenet. Cell Genet. *14* (1975) 42.

0014-4754/84/080876-03$1.50 + 0.20/0

Chromatin of *h* regions of human chromosomes at high resolution

R.S. Verma*, J. Rodriguez and H. Dosik

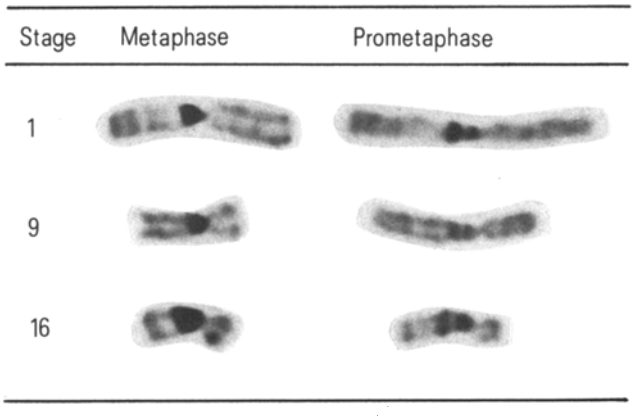
Departments of Laboratories and Medicine, The Jewish Hospital and Medical Center of Brooklyn and The State University of New York Downstate Medical Center, Brooklyn (New York 11238, USA), 29 June 1983

Summary. Segmentation of the secondary constriction region (*h*) of human chromosomes 1, 9 and 16 is demonstrated by a high resolution banding technique. Based on these staining properties, it is suggested that the composition of the *h* region in human chromosomes is heterochromatic as well as euchromatic.

Many structural and functional aspects of human chromosomes have been studied by various banding techniques. The CBG-technique has been used to reveal constitutive heterochromatic material[1,2]. This material is present in the centromere, the secondary constriction regions (*h*) of chromosomes 1, 9 and 16 and the distal portion of the long arm of the Y chromosome. The relative amount of satellite DNA in the C-bands of chromosomes 1, 9 and 16 shows no correlation with the size of the bands[3]. Instead there is a considerable amount of repetitive DNA[4]. It has been shown by the CBG technique that the h regions of chromosomes 1, 9 and 16 are most variable, yet are stable and inherited[5].

Recently, an international system for nomenclature of bands at high resolution has been established[6]. In this system the *h* region has been shown to vary only in size and it is represented by only 1 band. We have used a high-resolution banding technique to demonstrate the further segmentation of *h* regions not noted by ISCN[6]. Further we report that the *h* region is not only heterochromatic (dark staining) but euchromatic (light staining) as well when examined by a high resolution banding technique.

Materials and methods. Chromosome preparations from several individuals were made from cultured peripheral blood lymphocytes that were harvested as usual[7]. First, the CBG technique was carried out as described by Sumner[2] with a few modifications[8] using metaphase chromosomes. There were 3 individuals who had enlarged secondary constriction regions *h* on chromosomes 1, 9 and 16. Therefore the peripheral blood lymphocytes of these individuals were recultured for high resolution banding as follows: Immediately after completion of 68–72 h of incubation, cultures were treated with 5-bromo-2-deoxyuridine (200 µg/ml; Sigma) and 0.3 µg/ml of thymidine (Sigma) for exactly 4.5 h. Prior to harvest, cultures were



Demonstration of segmentation of the regions in chromosomes 1,9 and 16 at prometaphase by the CBG technique; such segmentation is not seen when chromosomes are at metaphase. There is a considerable amount of information lost during photography and printing. However, differentiation of the *h* region is clear.