able. Growth rates obtained from X-radiographic analyses of cores show that very large inshore colonies of massive *Porites* spp. are several centuries old. Thus these colonies contain long records of coastal fluvial history. The implications for regional climatology are wide. Moreover, the use of fluorescent peaks in the skeletal record in conjunction with X-radiographic analysis simplifies the task of determining seasonality of density band formation, which is a matter of controversy[10]. Experimental determination of the nature of the fluorescing agent in corals is being undertaken. Among other possibilities, trace inorganics of fluvial origin may be doping the aragonitic skeleton. Identification of geochemical signatures for particular river systems may contribute to our understanding of water movement over the continental shelf of the Great Barrier Reef.

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1. Isdale, P. J. thesis, James Cook Univ., North Queensland (1981).
2. MacIntyre, I. G. & Smith, S. V. *Proc. 2nd int. Coral Reef Symp.* 2 (GBRC, Brisbane, 1974).
3. Buddemeier, R. W. & Kinzie, R. A. III *Oceanogr. mar. Biol. A. Rev.* **14**, 183–225 (1976).
4. Buddemeier, R. W. *Proc. 2nd int. Coral Reef Symp.* Vol. 2, 259–267 (GBRC, Brisbane, 1974).
5. Dodge, R. E., Aller, R. C. & Thomson, J. *Nature* **247**, 574–577 (1974).
6. Highsmith, R. C. *J. expl mar. Biol. Ecol.* **47**, 55–67 (1979).
7. Queensland Water Resource Commission *Stream Flow Records* 1 (1983).
8. Wolanski, E. *Aust. J. mar. Freshwat. Res.* **34**, 49–63 (1983).
9. Wolanski, E. & Jones, M. *Aust. J. mar. Freshwat. Res.* **32**, 305–319 (1981).
10. Wellington, G. M. & Glynn, P. W. *Coral Reefs* **1**, 215–222 (1983).

Regional localization of sex-specific Bkm-related sequences on proximal chromosome 17 of mice

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Development and fertility in the mouse are known[1,2] to be influenced by loci mapped to the *T/t* complex of chromosome 17. Recent evidence suggests that one or more genes near this region may also be associated with sex determination. Washburn and Eicher[3] recently reported partial to complete sex reversal with the T^{hp} deletion on some genetic backgrounds and suggest that this result may be due to a primary sex-determining locus (*Tas*) that is closely linked to, or a part of, the *T* locus. Sex-specific, Bkm (banded Krait minor satellite DNA)-related sequences are known to have autosomal as well as heterogametic sex chromosomal copies, but specific regions of autosomal localization have not been described[4,5]. We now demonstrate the presence of chromosome Y-related DNA sequences on proximal chromosome 17 in *Sex-reversed* (*Sxr*) and normal mice using *in situ* hybridization of mitotic chromosomes with ^{3}H-labelled pCS316 (ref. 4), a probe that shows major hybridization to the proximal portion of the mouse chromosome Y[6,7]. These data, and those of Washburn and Eicher[3], argue for a gene(s) related to sex determination or differentiation within the proximal portion of mouse chromosome 17.

The probe used, pCS316[4], is cloned into pBR322 and represents a segment of *Drosophila melanogaster* DNA containing Bkm-related sequences. Previous studies[4,7] have shown that Bkm-related DNAs such as pCS316 hybridize to precisely the same sequences as Bkm in mammals and reptiles and that they are concentrated within the sex-determining chromosome of many eukaryotes[4]. Therefore, it has been proposed that Bkm and its related sequences represent a conserved sex chromosome-associated nucleotide sequence[4,7] which may be expressed[8,9].

pCS316 was labelled and hybridized to chromosome spreads from normal and *Sxr* mice. *Sxr* is a sex-limited condition which causes XX mice to appear as phenotypic males. The following six genotypes were analysed: three genotypes (a X/X *Sxr* male, a X/Y *Sxr* carrier male and a X/X famale) were fetal siblings derived from the mating of a normal random-bred (CD-1) female to a 129/SvPas-*Ta-Sxr* X/Y carrier male, two other genotypes (a X/X *Sxr* male and X/Y carrier male) were of the 129/SvPas-*Ta-Sxr* inbred strain, and the final genotype was composed of random-bred (CD-1) normal males. At least 50 metaphase spreads from each of the six genotypes ($n > 300$) were analysed. Regardless of the genetic background, roughly 60% of the metaphases studied showed grain concentrations in bands B to D of chromosome 17. Figure 1 shows these results, with the corresponding idiogram[10].

To determine whether hybridization of pCS316 to autosomal DNA was due to sequence homology or nonspecific binding, the frequency of hybridization within a given chromosome region was calculated for chromosome 17 with chromosome 16 as a control (Fig. 3). Selection criterion for metaphase quantitation was based on a display of hybridization to at least one chromosome of the pair. Grains appeared most frequently in two distinct regions of chromosome 17. One region, extending from 17A3 to the B–C interface, showed grains in 71% of the chromosomes examined. The other region extended from 17D to the D–E1 interface and showed the presence of grains in 38% of the chromosomes. In contrast, the region of greatest binding on chromosome 16, the telomeric end, showed grain localizations in only 7% of the chromosomes studied. The data indicate that the grains seen in the 17B–D region of chromosome 17 in Fig. 1 are due to the specific hybridization of pCS316 to homologous sequences on the chromosome.

As expected, the X chromosome of presumed paternal origin (X^{Sxr}) demonstrated unequivocal hybridization at its distal terminus in X/X *Sxr* males[7]. We have also discovered another hybridization site near the XA5 region in both the X^{Sxr} and the normal maternal X. Figure 2*a*, from a X/X *Sxr* male, illustrates a typical G-banded, fetal liver metaphase spread. The X^{Sxr} and its homologue are shown in Fig. 2*b* with the standard idiotype[10]. The Y chromosome of the X/Y *Sxr* carrier male possessed both proximal and distal grain localization but on grain removal its morphology was similar to that of the normal Y (data not shown). This observation confirms the findings of Evans *et al.*[11] that the Y^{Sxr} chromosome possesses a distal body but otherwise is cytologically normal. The Y chromosome from normal random-bred males showed the expected proximal hybridization[6,7] to bands A to B. On most autosomes, as for chromosome 16, grain localizations were infrequent and random. However, in addition to chromosome 17, we observed another autosome, identified as chromosome 4 (K. K.-M. and R. P. E., unpublished), which seemed to show hybridization to its mid-region in over 50% of the metaphases examined.

Structural and behavioural characteristics of chromsome regions containing concentrations of the evolutionarily conserved Bkm-related sequences suggest that these (or closely linked sequences) are connected with the determination of sex[4]. For example, in the X/Y *Sxr* male, when a region of chromosome showing dense pCS316 hybridization is transferred from the distal end of the Y^{Sxr} to an X chromosome during meiosis, X/X male progeny result. In terms of chromosome 17, the amount of pCS316 hybridization (as judged from grain density) is considerably less than that observed on the Y chromosome. However, it is probable that the probe is pairing with a similar family of DNA sequences. Furthermore, the finding by Washburn and Eicher[3] that a *T*-associated sex reversal trait, *Tas*, results in partial to complete sex reversal in *Tas*/+;XY individuals on certain genetic backgrounds implicates the presence of sex-related gene(s) within the proximal portion of chromosome 17. A locus near this region, which may have a role in sex determination, is the histocompatibility-Y expression locus, *Hye* (refs 12, 13), for which a previous analysis of recombinants demonstrated a location between the *H-2* and *T*

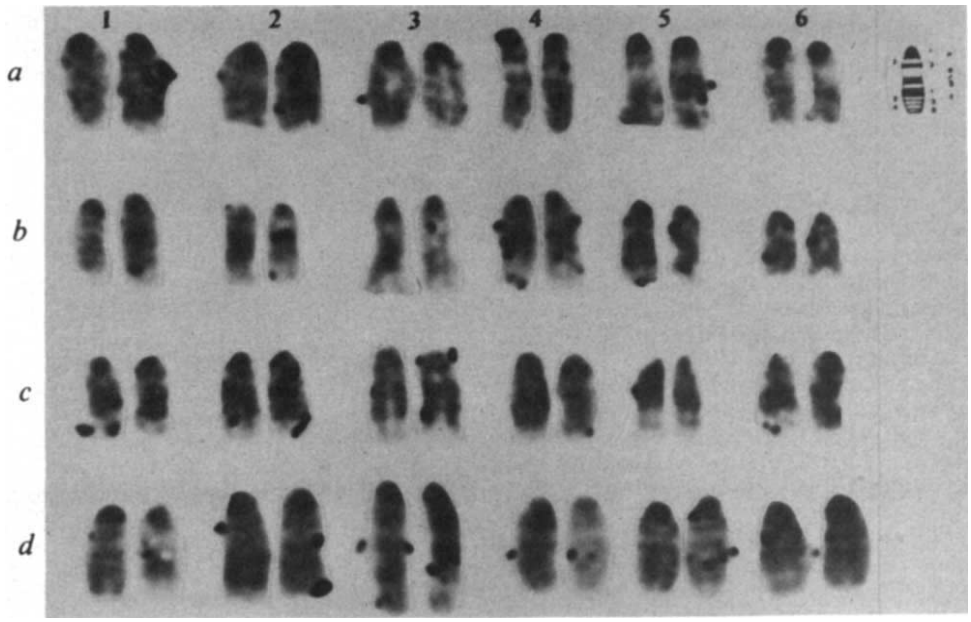

Fig. 1 Representative pairs of chromosomes 17 after *in situ* hybridization with the pCS316 probe followed by G-banding in Wright's stain. *a*, Chromosome 17 from two X/X *Sxr* males. Chromosome pairs 1–3 were obtained from the X/Y *Sxr* male progeny of a CD-1 × 129/SvPas-*Ta-Sxr* X/Y carrier mating. The mitotic spreads were prepared from fetal liver. Chromosome pairs 4–6 were obtained from a X/X *Sxr* male of inbred 129/SvPas-*Ta-SxR* stock and prepared from adult bone marrow. *b*, *c*, Chromosome 17 pairs from X/Y *Sxr* carrier and X/X female, respectively. The chromosomes, prepared from fetal liver, were obtained from the fetal siblings of a CD-1 × 129/SvPas-*Ta-Sxr* X/Y carrier male mating. *d*, Chromosome 17 from X/Y normal (CD-1) male. Bone marrow spreads from three randomly bred CD-1 males were analysed. Only one CD-1 male is illustrated here.

Methods: Mitotic spreads used for analyses were prepared from fetal livers (day 14) or adult bone marrow, as described by Eicher and Washburn[16]. pCS316 DNA was transfected into *Escherichia coli* DH-1. Plasmid DNA was isolated from ampicillin-treated bacteria in CsCl gradients[17] and nick-translated with ^{3}H-TTP (NEN, NEK-005) to a specific activity of 3×10^7 c.p.m. per μg. The probe was precipitated in ethanol and resuspended in 50% formamide/2 × SSC/100 μg ml^{-1} salmon sperm DNA/10% dextran sulphate/10 mM $NaPO_4$ *p*H 7.0 at a concentration of 2.8×10^4 c.p.m. μl^{-1}. DNA was denatured in a 100 °C water bath for 5 min, then quenched on ice. The subsequent hybridization steps and autoradiography process were performed using the procedure of Harper and Saunders[18]. Chromosomes were stained using 0.25% Wright's stain diluted 1:3 with 0.06 M phosphate buffer *p*H 6.8 for 5 min as described by Chandler and Yunis[19]. To achieve banding, the above procedure was modified to include warming the slide sequentially at 31.5 °C for 14 min, 37.5 °C for 14 min, 42 °C for 10 min and 53 °C for 5 min in phosphate buffer. After each warming, the slide was removed from the buffer, stained for 3.5–6 min, examined and destained if necessary, by immersion first in a solution of 95% ethanol–1% HCl for 15 s and then in absolute methanol for 15 s. Samples were destained, heated and restained until satisfactory banding was achieved.

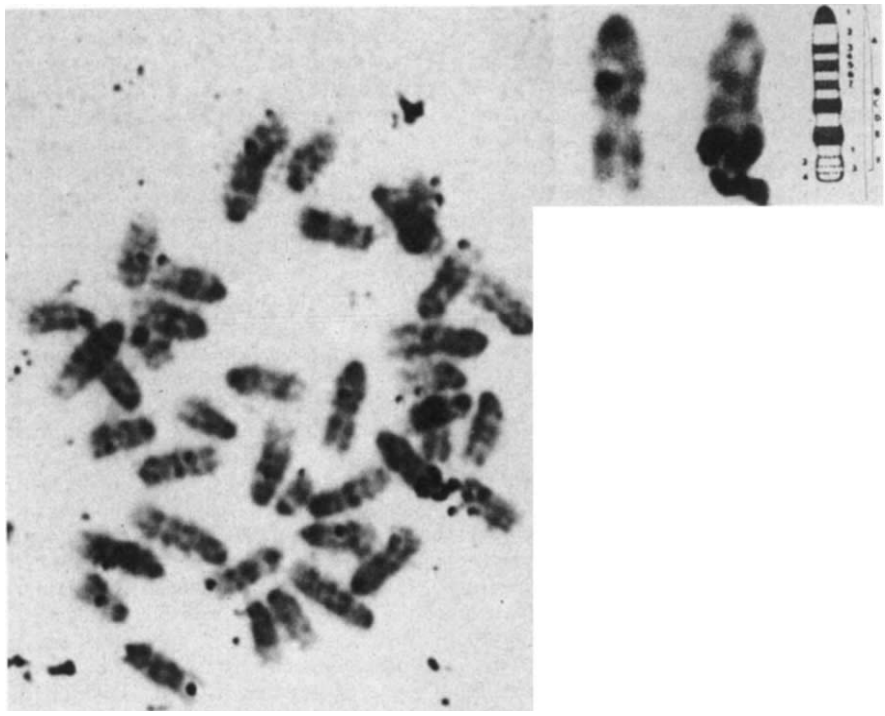

Fig. 2 ^{3}H-labelled pCS316 hybridization to mitotic chromosomes from a sex-reversed X/X *Sxr* male. *a*, Metaphase spread from a X/X *Sxr* male. *b*, The standard idiogram of the mouse X chromosome[10] and the two X chromosomes from the metaphase shown in *a*.

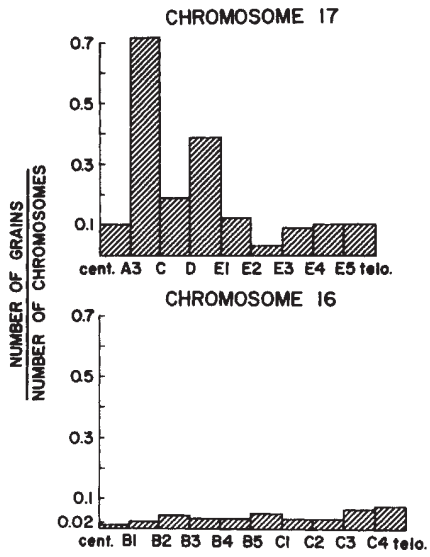

Fig. 3 Histogram frequencies of grain distribution within given chromosome regions on chromosomes 17 and 16, respectively. Normal male, X/X *Sxr* male, X/Y *Sxr* male and X/X female metaphases exhibiting hybridization to chromosome 17 were chosen for quantitation. Chromosome regions shown in Fig. 1 are defined in terms of boundaries (that is, for chromosome 17, A3–C represents the chromosome region extending from A3 to B and terminating at the B–C interface) (see Fig. 1 for idiogram of chromosome 17). Frequencies were determined by dividing the total number of grains per chromosome region by the number of chromosomes examined ($n = 94$).

complexes, closer to the latter[13]. Although *Hye* was originally found to affect the amount of transplantation H–Y, it has been found also to alter the amount of serological H–Y[14]. To the extent that levels of this antigen have a role in sex determination, a decreased expression (as may occur in the T^{hp} deletion) might cause hermaphroditism or sex reversal on some genetic backgrounds.

Intriguingly, translocation studies have mapped a large portion of the *T/t–H–2* complex[15], and therefore most probably the *Hye* locus[13], within a region that shows significant hybridization to the sex-determining-related pCS316 DNA. In light of the previous unsuccessful attempts to locate a structural gene for the H–Y antigen, one might speculate that the H–Y structural locus may itself be located within the *T/t–H–2* complex of chromosome 17 and perhaps, more specifically, in its pCS316-related sequences.

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1. Bennett, D. B. *Cell* **6**, 441–454 (1975).
2. Erickson, R. P., Hammerberg, C. & Sanchez, E. in *Current Research Trends in Prenatal Craniofacial Development* (eds Pratt, R. & Christiansen, R.) 103–117 (Elsevier, Amsterdam, 1980).
3. Washburn, L. L. & Eicher, E. M. *Nature* **303**, 338–340 (1983).
4. Singh, L., Purdom, L. F. & Jones, K. W. *Cold Spring Harb. Symp. quant. Biol.* **45**, 805–813 (1981).
5. Jones, K. W. *Differentiation* **23**(s), 556–559 (1983).
6. Jones, K. W. & Singh, L. *Hum. Genet.* **58**, 45–53 (1981).
7. Singh, L. & Jones, K. H. *Cell* **28**, 205–216 (1982).
8. Epplen, J. T., McCarrey, J. R., Sutou, S. & Ohno, S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3798–3802 (1982).
9. Singh, L., Phillips, C. & Jones, K. W. *Cell* **36**, 111–120 (1984).
10. Nesbitt, M. N. & Franeke, U. *Chromosoma* **41**, 145–148 (1973).
11. Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. *Nature* **300**, 443–445 (1982).
12. Kralova, J. & Demant, P. *Immunogenetics* **3**, 583–594 (1976).
13. Kralova, J. & Lengerova, A. *J. Immunogenet.* **6**, 429 (1979).
14. Shapiro, M. & Erickson, R. P. *J. Reprod. Immun.* **6**, 197 (1984).
15. Forejt, J. *Curr. Trends Histocompatibility* **1**, 103–131 (1981).
16. Eicher, E. M. & Washburn, L. L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 946–950 (1978).
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning: A Laboratory Manual*, 86–95 (Cold Spring Harbor Laboratory, New York, 1982).
18. Harper, M. E. & Saunder, G. F. *Chromosoma* **83**, 431–439 (1981).
19. Chandler, M. E. & Yunis, J. J. *Cytogenet. Cell Genet.* **22**, 352–356 (1978).

Chromosomal localization of the human proto-oncogene c-*ets*

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E26 is an acute leukaemia avian retrovirus which induces myeloblastosis and erythroblastosis *in vivo* and transforms erythroblasts and myeloblasts *in vitro*[1–3]. It contains the oncogene v-*myb* (ref. 4), first described for avian myeloblastosis virus (AMV), as well as a second specific nucleotide sequence, v-*ets* located 3′ to v-*myb* (refs 5, 6). We have reported that v-*ets* has a cellular counterpart (c-*ets*) in chicken and human DNA[5]. Now, using two independent methods—hybridization with human c-*ets* probe of sorted chromosomes and *in situ* hybridization—we report the localization of the *ets* locus on human chromosome 11 at bands q23–q24. This finding may be important, as specific breakpoints around this position have been reported for human malignancies such as acute monocytic leukaemia and Ewing's sarcoma.

Recombinant phages containing nucleotide sequences (c-*ets*) related to the v-*ets* sequences of E26 retrovirus were isolated from a genomic library of normal human DNA[7]. As shown in Fig. 1, a human c-*ets* probe was derived from one such phage, λ *ets* H-3, as a 5.4-kilobase (kb) *Eco*RI fragment which hybridized specifically to a 8.6-kb *Eco*RI genomic fragment of human DNA also detected with a v-*ets* probe. This 5.4-kb fragment, containing no repetitive DNA, was inserted into the *Eco*RI site of plasmid pKH47 to yield the probe used for hybridization experiments on human chromosomes.

Hybridization experiments using sorted chromosomes indicated that the human c-*ets* locus was located on the long arm of chromosome 11. Metaphase chromosomes were separated into nine peaks, immobilized on nitrocellulose filters[8] and hybridized with the c-*ets* probe. With the Raji Burkitt cell line, shown by cytogenetic data to contain normal chromosomes 11,

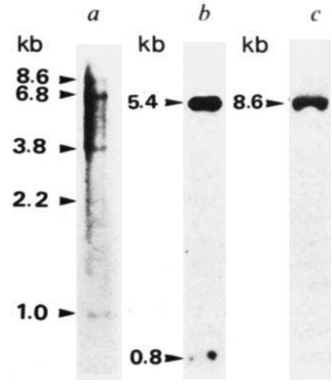

Fig. 1 Characterization of the probe c-*ets* used in hybridization experiments. Recombinant phage λ *ets* H-3 DNA was digested with *Eco*RI, separated by agarose gel electrophoresis and hybridized with ^{32}P-labelled v-*ets* probe. Two *Eco*RI fragments (5.4 and 0.8 kb, respectively) were detected by autoradiography (*b*). Southern blots of placental DNA restricted by *Eco*RI were hybridized in low-stringency conditions with v-*ets* nick-translated probe (*a*) or in high-stringency conditions with 5.4-kb *Eco*RI fragment of phage *ets* H-3 (*c*).