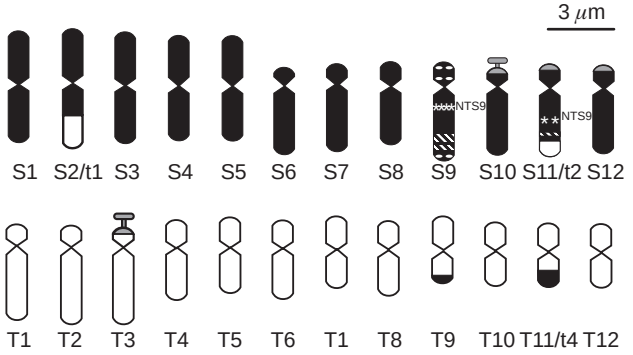


**Figure 3.** Karyotypic diagram of *N. tabacum* cv. Petit Havana SR1. Regions originating from *N. sylvestris* (S subgenome) are black; regions from *N. tomentosiformis* or *N. otophora* (T subgenome) are white. Major and minor NTS9 loci on the S9 and S11/t2 chromosomes, respectively, are indicated by asterisks. The DAPI bands of these two chromosomes are hatched. The complete DAPI banding pattern of *N. tabacum* chromosomes has been published (Moscone *et al.* 1996). Dotted regions show rDNA loci, two of them active in nucleolus formation (S10 and T3).

units 180–182 bp in length and is present in $10^4$ copies per haploid genome (Gazdová *et al.* 1995). The NTRS family comprises monomeric units of 212–219 bp and is present in approximately $8 \times 10^4$ copies per haploid genome (Matyásek *et al.* 1997). The GRS family is present in intercalary sites of five chromosome pairs of the T subgenome (Gazdová *et al.* 1995). The NTRS sequence is specific for the T1 chromosome, where it occupies an intercalary site (Matyáek *et al.* 1997).

In summary, the NTS9 family is distinguished from the three other tabacco tandem repeat families by its sequence, shorter unit length and chromosomal location. To our knowledge, the NTS9 sequence is the first tandemly repeated sequence of tobacco to be localized close to a centromere and to be highly enriched on the S9 chromosome of the S subgenome.

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# Evolutionarily conserved telomeric location of *BBC1* and *MC1R* on a microchromosome questions the identity of *MC1R* and a pigmentation locus on chromosome 1 in chicken

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*MC1R* (melanocortin-1 receptor gene) and *BBC1* (breast basic conserved gene 1) are closely linked in human chromosome (HSA) 16q24.3 (Genome Data Base).

*MC1R* encodes a seven-transmembrane G-protein that regulates pigmentation in vertebrates. Melanocyte proliferation and melanogenesis are stimulated by the binding of melanotropic hormone to this receptor. In mammals, the *MC1R* gene corresponds to the *extension* (*E*) locus (Robbins *et al.* 1993). In chicken, a locus controlling the distribution of eumelanin and phaeomelanin, considered to be *E*, has been tentatively mapped to chromosome (GGA) 1 based on a recombination rate of 43% with *P* (*pea comb*) (Carefoot 1990, 1993; Smyth & Ponce de Leon 1992). *P* had previously been shown to be linked to two rearrangement breakpoints on GGA1 (Zartman 1973; Bitgood & Shoffner 1990). A possible association of *MC1R* mutations with *Extended black* allelic variants has been reported (Takeuchi *et al.* 1996a,b). However, there is no direct evidence that chicken *E* is located on GGA1.

The human *BBC1* was isolated from a cDNA library generated from a breast carcinoma showing significantly higher levels of expression in benign breast lesions than in carcinomas (Adams *et al.* 1992). *BBC1* sequences are highly conserved throughout the eukaryotic kingdoms (Bertauche *et al.* 1994).

A genomic 1.77-kb fragment containing the complete *MC1R* chicken sequence (Takeuchi *et al.* 1996b) and a 717-bp chicken cDNA clone specific for *BBC1* (Sawada *et al.* 1996) were used as probes for screening a gridded chicken cosmid library (Buitkamp *et al.* 1998). The screening experiments resulted in the identification of four and six positive clones for *MC1R* and *BBC1* respectively.

The specificity of cosmid G22-83 for *MC1R* was assessed by the sequencing of 319 nucleotides corre-

```
      027
RI    GGGGCCGGAGGTGCCTGGTGCCAGGGGCTGGACATCCCCAATGAGCTCTTCCTGACGCTG
RI    GlyAlaGlyGlyAlaTrpCysGlnGlyLeuAspIleProAsnGluLeuPheLeuThrLeu
RC    ------------------------------------------------------------
RC    ------------------------------------------------------------
      047
RI    GGGCTGGTGAGCCTGGTGGAGAACCTGCTGGTGGTGGCCGCCATCCTCAAGAACAGGAAT
RI    GlyLeuValSerLeuValGluAsnLeuLeuValValAlaAlaIleLeuLysAsnArgAsn
RC    ------------------------------------------------------------
RC    ------------------------------------------------------------

      067
RI    CTGCACTCGCCCACGTACTACTTCATCTGCTGCCTGGCCGTCTCCGACATGCTGGTGAGC
RI    LeuHisSerProThrTyrTyrPheIleCysCysLeuAlaValSerAspMetLeuValSer
RC    -------------Met---------------------------------------------
RC    -------------T-----------------------------------------------


      087
RI    GTCAGCAACCTGGCCAAGACGCTCTTCATGCTGCTGATGGAGCACGGCGTGCTGGTGATC
RI    ValSerAsnLeuAlaLysThrLeuPheMetLeuLeuMetGluHisGlyValLeuValIle
RC    ----------------Glu-------------------------------------------
RC    ----------------G---------------------------------------------

      107
RI    CGCGCCAGCATCGTCCGCCACATGGACAATGTCATCGACATGCTCATCTGCAGCTCCGTC
RI    ArgAlaSerIleValArgHisMetAspAsnValIleAspMetLeuIleCysSerSerVal
RC    ------------------------------------------------------------
RC    ------------------------------------------------------------

      120           125
RI    GTGTCCTCCCTCTCCTTC
RI    ValSerSerLeuSerPhe
RC    ------------------
RC    ------------------
```

**Figure 1.** Comparison of a partial nucleotide and predicted amino acid sequence (RI, Rhode Island) derived from a cosmid isolated with a *MC1R* cDNA and published sequences (RC, Rock Cornish) (Takeuchi *et al.* 1996a). Matches are indicated by hyphens.

sponding to amino acids 27–125. Two differences relative to the MC1R sequence derived from a Rock Cornish chicken (Takeuchi *et al.* 1996a,b) were identified, both leading to non-conservative exchanges of amino acids, namely Thr-71→Met and Lys-92→Glu (Figure 1). Rock Cornish chicken are assumed to carry the dominant *E* allele at the *E* locus. The *E* allele is thought to be identical to a mutation causing constitutive activation of the melanocortin receptor 1. Takeuchi *et al.* (1996a,b) hypothesized that a negatively charged amino acid (lysine) at position 92 could be responsible for this constitutive activation and thus be the basis for black pigmentation in Rock Cornish. The same point mutation was also found in unrelated chickens from a brown-egg experimental population carrying also the dominant *E* allele (M. Tixier-Boichard, unpublished data). The cosmid library used for the isolation of the MC1R-specific cosmid was derived from a Rhode Island Red chicken with the presumptive $e^y/e^y$ (*recessive wheaten*) genotype at the *Extension* locus. Amino acids 27–125 (Figure 1) are identical to the sequence of Brown Leghorn chicken ($e^+/e^+$, *wild-type*) but deviate at positions 33 and 37 from the sequence of the Nagoya Cortin breed ($e^y/e^y$) as indicated in Takeuchi *et al.* (1996b). The significance of these deviations remains to be elucidated.

The specifity of cosmid F24-220 for BBC1 was confirmed by determining the sequence of 120 nucleotides, showing 100% identity with the published sequence (Sawada *et al.* 1996).

Fluorescence *in situ* hybridization (FISH) with the cosmids specific for *MC1R* and *BBC1* was performed essentially as described by Masabanda *et al.* (1998). Biotin–FITC and digoxigenin–rhodamine detection systems were applied in dual-colour FISH experiments according to Florijn *et al.* (1995). Two experiments of multicolour FISH using the two cosmids were performed. In the first, the *MC1R*-specific cosmid was biotinylated and the *BBC1*-candidate cosmid was subjected to digoxigenin labelling, yielding green and red hybridization signals, respectively, after hybridization and signal detection. The opposite combination was used in a second experiment. In all metaphases analysed, distinct signals were observed at the telomeric region of a microchromosome in size range of GGA15 to GGA20 (Figure 2). The FITC and rhodamine signals

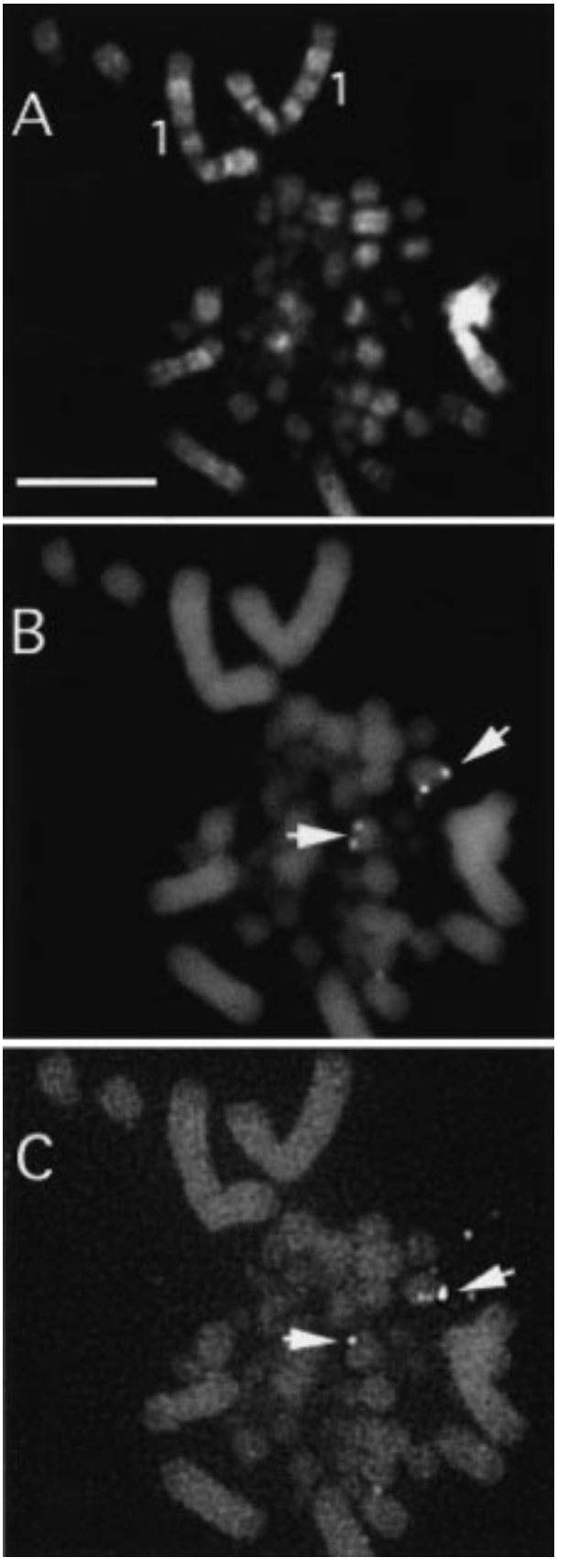


**Figure 2.** Fluorescence *in situ* hybridization of a *MC1R*- and *BBC1*-specific cosmid, on chicken chromosomes. A partial metaphase with chromosomes that were QFQ-banded prior to *in situ* hybridization is shown in **A**. The FITC signals resulting from hybridization of this metaphase plate with the biotinylated *MC1R*-specific cosmid are indicated by arrows in **B**; the rhodamine signal resulting from hybridization with the digoxigenin-labelled *BBC1*-specific cosmid is shown in **C**. Note the absence of a signal on GGA1 (indicated by '1' in **A**). The bar in **A** represents 10 $\mu$m.

were overlapping or located very close to each other. The two loci are not only closely linked in humans and in chicken but also located in a telomeric band in both species. In mouse, *MC1R* may also be telomerically located on chromosome 8, known to be homologous in part to HSA16 (Magenis *et al.* 1994).

The telomeric location of *MC1R* and *BBC1* in both a mammalian and an avian species is another example of synteny conservation in these distant species. The microchromosome carrying *MC1R* and *BBC1* may represent an ancestral telomeric segment corresponding to a 'smallest conserved evolutionary unit segment' as proposed by O'Brien *et al.* (1993).

As indicated above, a locus controlling the relative amounts of eumelanin/phaeomelanin was tentatively mapped to GGA1. However, the evolutionarily conserved location of *MC1R* on a chicken microchromosome and the possibility of variants in *MC1R* being responsible for *E*-specific alleles introduce an apparent contradiction between physical mapping data and genetic mapping data and raise some doubt about the identity of the *E* locus and *MC1R* in chicken. More accurate genetic mapping of the *E* locus with mapped molecular markers would be necessary to clarify the localization of this locus and for its further molecular characterization.

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# A drop technique for flatworm chromosome preparation for light microscopy and high-resolution scanning electron microscopy

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