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Preferential C-banding of Wheat or Rye Chromosomes

A. G. Seal and M. D. Bennett
Plant Breeding Institute, Maris Lane, Trumpington, Cambridge (England)

Summary. Using different stains, wheat chromosomes could be distinguished from rye chromosomes by preferential staining. C-bands of rye chromosomes were preferentially stained with Giemsa while those of wheat chromosomes were preferentially stained with either Leishman or Wright stain. Preferential staining aids the identification of wheat and rye chromosomes and chromosome segments and in particular the recognition of wheat/rye chromosome substitutions and translocations.

Key words: C-banding – Wheat – Rye – Triticale

Introduction

Since its discovery by Pardue and Gall in 1970, C-banding has been a valuable technique, allowing the identification of individual chromosomes and chromosome segments in plants and animals. Numerous techniques have been shown to produce C-bands and the banding pattern revealed by one technique may differ from that shown by another for the same karyotype (Schweizer 1973; Fiskesjo 1974; Stack et al. 1974; Vosa 1976; Yen and Filion 1977). Sometimes different techniques are required to stain C-bands of different karyotypes (Schweizer 1973, 1974). One C-banding technique may resolve different types of C-bands distinguishable by their staining intensity or condensation (Vosa 1974; La Cour 1978).

In general, C-banding techniques have been more successfully and more often applied to rye than to wheat chromosomes. The banding patterns of many rye karyotypes (Singh and Robbelen 1975; Bennett et al. 1977; Giraldez et al. 1979) and some wheat karyotypes (Gill and Kimber 1974; Iordansky et al. 1978a, 1978b; Zurabishvili et al. 1978) have been described. In triticale, much attention has been given to the C-banding patterns of rye chromosomes (Merker 1975; Darvey and Gustafson 1975; Lukaszewski and Apolinarska 1981). The banding patterns of both the wheat and the rye genomes have seldom been described together (Iordansky et al. 1978b). Using most C-banding techniques, rye C-bands stain more intensely than those of wheat (Weimarck 1974; Merker 1975). Wheat and rye chromosomes involved in translocations have been identified by C-banding (Gill and Kimber 1977) and by N-banding (Jewell 1979).

In this paper we describe a technique using different stains to show the banding patterns of wheat and rye chromosomes. Giemsa was used to preferentially stain C-bands of rye chromosomes (Seal and Bennett 1981) while Leishman or Wright stain was used to preferentially stain C-bands of wheat chromosomes (Seal 1982). Preferential staining is especially useful for identifying and delimiting chromosome segments involved in translocations. Those few bands within the wheat and rye genomes which differ from the majority in their response to the stains may provide useful markers for cytological studies in wheat, rye and triticale. Application of the technique to other species and genera is discussed.

Materials and Methods

Materials

The following genotypes were used:

1. Diploids; rye, *Secale cereale* L. cv. King II and cv. UC-90, *S. africanum* Stapf. (R 102); barley, *Hordeum vulgare* L. cv. Sultan (all 2n = 2x = 14) and *Vicia faba* L. (2n = 2x = 12).
2. Hexaploids; breadwheat, *Triticum aestivum* L. cv. Aurora, cv. Chinese Spring (King II rye addition line 3R); triticale, × *Triticosecale* Wittmack cultivars II 75–23b 367, 6TA-876, 274-358, KISS-URSS 2310, URSS-3310, Rosner, Armadillo and Cocorit × UC-90 (all 2n = 6x = 42). The breadwheat cv. Aurora has a 1B/1R translocation (Mettin et al. 1973).

Methods

The C-banding technique of Seal and Bennett (1981) was used in conjunction with different stains. Stain solutions were purchased from BDH/Hopkin and Williams Ltd. and diluted (v/v) in 1/15 M Na_2HPO_4 (pH 9) as follows: Gurr's Improved R66 Giemsa (5%), Leishman (20%), Wright (20%) and Jenner

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(10%). Pinacyanol chloride (1′ diethyl 2,2′ carbo cyanine chloride) from Koch-Light Labs. Ltd. was prepared according to Narayan (1980).

C-banded rye chromosomes were numbered 1R–7R/4R according to Darvey and Gustafson (1975). The identification of wheat chromosomes stained using Leishman or Wright stain has been described by Seal (1982). Long and short arms of chromosomes are referred to by the letters L and S respectively.

Results

(a) Wheat and Rye Chromosomes

(i) Giemsa: All rye chromosomes in rye and triticale had one or two telomeric C-bands (Fig. 1a). Chromosome 1R had an interstitial band near the nucleolar organiser region. These bands were consistently and intensely stained using Giemsa. Many other interstitial rye bands were often found but were less intensely stained. In contrast, in wheat and triticale, only two wheat chromosomes consistently showed intensely stained C-bands with Giemsa (Fig. 2a, c). By restaining with Leishman or Wright stain (see below) these chromosomes were identified as 1B and 6B. A terminal band on 1BL and a proximal band on 6BL were stained besides bands in their nucleolar organiser regions. Occasionally, a subterminal band was found on chromosome 6A. Centromeres and other wheat bands were faintly stained or unstained. Giemsa and pinacyanol chloride gave identical banding patterns.

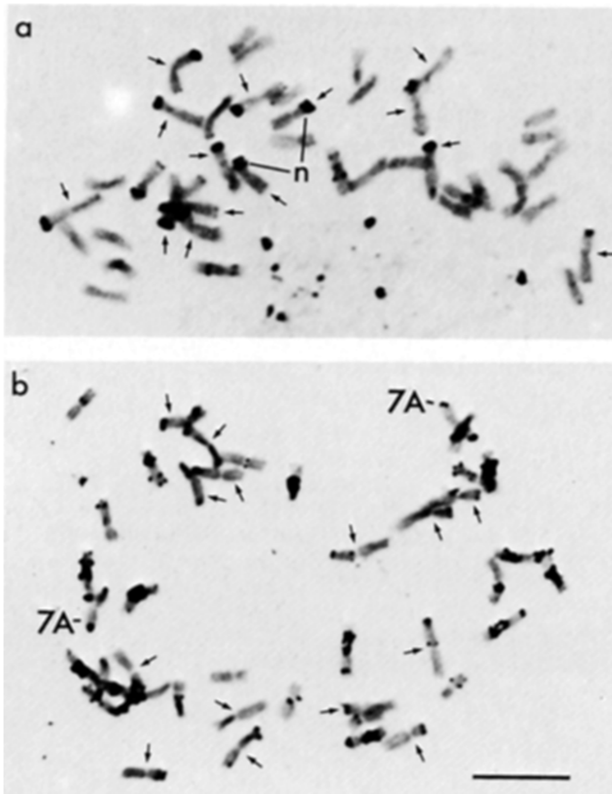


Fig. 1a and b. C-banded chromosomes of triticale II 75–23b 367. **a** stained with Giemsa; **b** stained with Wright stain. Rye chromosomes are arrowed. n = nucleolar organiser region of chromosome 1R. Bar = 10 μ

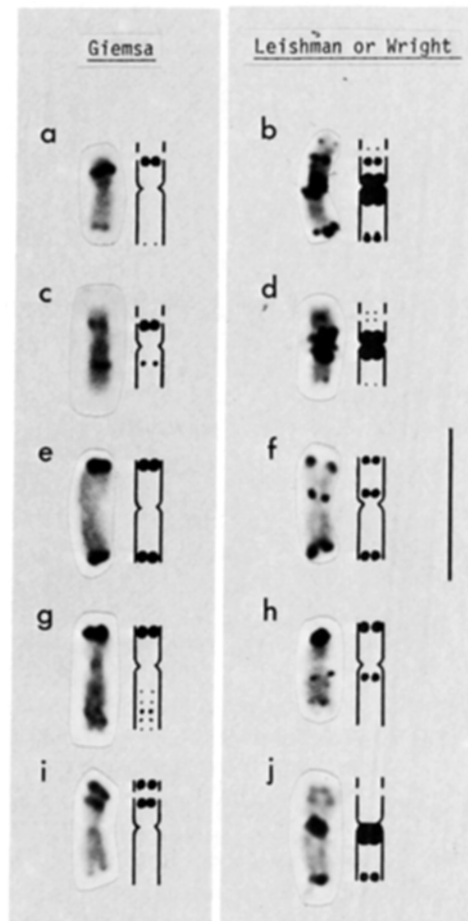
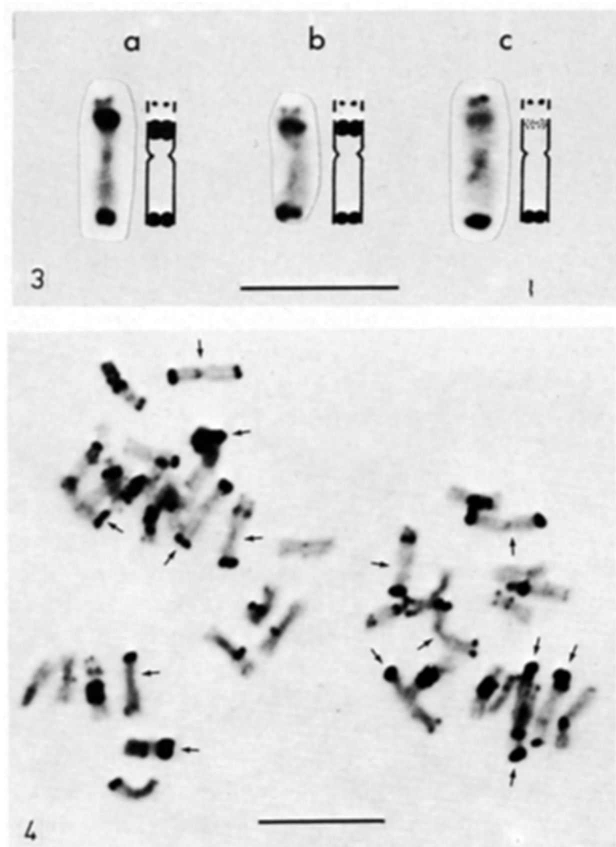


Fig. 2a–j. A comparison of C-banded wheat and rye chromosomes stained with Giemsa and Leishman or Wright stain. **a, b** chromosome 1B from triticale II 75–23b 367; **c, d** chromosome 6B from triticale II 75–23b 367; **e, f** chromosome 3R from *S. cereale* cv. King II; **g, h** chromosome 6R from *S. cereale* cv. King II; **i** and **j** 1B/1R translocation chromosome from *Triticum aestivum* cv. Aurora. Bar = 10 μ

(ii) Leishman or Wright stains: These stains preferentially stained C-bands in the A and B wheat genomes of wheat and triticale (Fig. 1b). Except for 1A, all A and B wheat genome chromosomes were banded. However, chromosomes 1B and 6B had very different banding patterns from those shown by Giemsa staining (Fig. 2a–d). Giemsa showed two bands on 1B and 6B while Leishman or Wright stain showed these and several more. Rye telomeric C-bands stained less intensely (and often a different colour from wheat bands) and the interstitial bands shown by Giemsa

staining were rarely seen. However, two small proximal bands in the *S. cereale* genome, rarely seen using Giemsa, were consistently stained using Leishman or Wright stain (Fig. 2e–h). One was assigned to 3RS using a 3R addition line of rye (cv. King II) to breadwheat (cv. Chinese Spring) and the other to 6RL identified by its length, arm ratio and the presence of a single telomeric band on the short arm. Both bands were found in diploid rye (cv. UC-90 and cv. King II) and in 7 out of 8 triticales (the 3R band was not found in 6TA-876). Using Giemsa C-banding, Vosa (1974) and Giraldez et al. (1979) found interstitial bands on rye chromosomes 3R and 6R in similar positions but did not note any differences in staining between these and other rye bands.

Chromosome 1R in triticale line 6TA-876 stained with Giemsa had an exceptionally large band in the nucleolar organiser region, a small telomeric band on 1RS, and a large telomeric band on 1RL (Fig. 3a).



Figs. 3a–c and 4. 3a–c C-banding pattern of chromosome 1R from triticale 6TA-876 stained with **a** Giemsa, **b** Leishman stain in 1/15 M Na_2HPO_4 pH 9 and **c** Leishman stain in 1/15 M Na_2HPO_4 : 1/15 M KH_2PO_4 pH 6.8. Bar = 10 μ. **4.** C-banded chromosomes of triticale 274–358 stained with Jenner stain (rye chromosomes arrowed). Bar = 10 μ

When stained with Leishman stain made up in 1/15 M Na_2HPO_4 (pH 9) these bands were of similar size to the Giemsa bands although fainter (Fig. 3b). However, if the Leishman stain was made up in 1/15 M Na_2HPO_4 : 1/15 M KH_2PO_4 buffer pH 6.8 at the same concentration then the nucleolar organiser band was very faint compared to the telomeric bands on the same chromosome (Fig. 3c).

(iii) Jenner stain: This stained all large wheat and rye bands simultaneously and intensely (Fig. 4).

(iv) Identification of a translocation chromosome: 'Aurora', a wheat variety with a 1B/1R translocation, was tested to see whether the preferential staining of wheat and rye C-bands could discriminate between wheat and rye chromosome regions combined on the same chromosome. Stained with Giemsa, the translocation chromosome had two prominent bands on the short arm, characteristic of a Giemsa stained 1RS (Fig. 2i), and a faint long arm telomeric band. Stained with Leishman stain, the short arm bands were faint or absent (Fig. 2j). However, two bands in the long arm (one proximal, one telomeric) stained intensely, showing the typical banding pattern of 1BL stained with Leishman stain. This result reconfirmed that 'Aurora' has a 1B/1R translocation (Jewell 1979, 1981) and showed that at least the entire long arm of 1B is present suggesting a whole arm translocation. Moreover, it demonstrated the high selectivity of the stains and their potential for identifying and delimiting chromosome segments involved in translocations.

(v) *Secale africanum:* Giemsa stained *S. africanum* chromosomes showed a banding pattern similar to those published previously (Singh and Robbelen 1975; Bennett et al. 1977). Most large bands were telomeric, one was in the nucleolar organiser region and one was proximal on a metacentric chromosome identified as chromosome 7 by Bennett et al. (1977) (Fig. 5a). Interphase nuclei in these preparations had numerous large chromocentres. However, the banding pattern was very different in preparations stained with Wright stain (Fig. 5b). Only the proximal band on chromosome 7 stained intensely. Others stained faintly or not at all. Interphase nuclei in these preparations usually showed two intensely stained chromocentres (Fig. 5c).

(b) Other Species

Two other species from different genera were tested for preferential staining of C-bands. Chromosomes of barley were unbanded using the present Giemsa technique, but with Leishman or Wright stain they showed numerous intense interstitial bands (Fig. 6). Thus, barley C-bands showed a preferential staining response similar to that of most wheat C-bands.

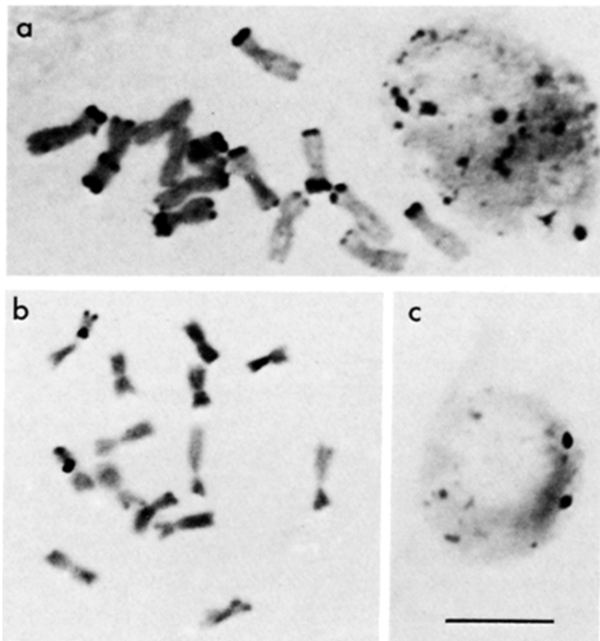
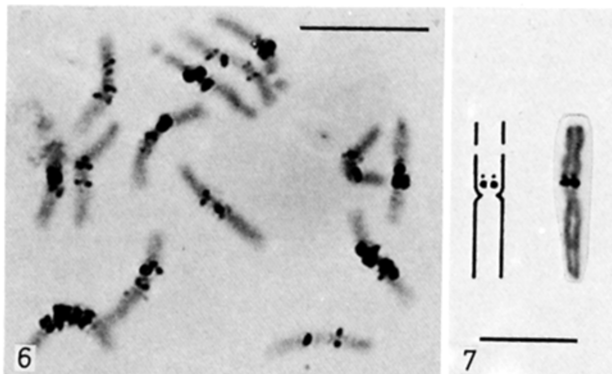


Fig. 5a–c. *Secale africanum.* **a** C-banded chromosomes and nucleus stained with Giemsa; **b, c** C-banded chromosomes and nucleus stained with Wright stain. Bar = 10 μ



Figs. 6 and 7. 6 C-banded chromosomes of *Hordeum vulgare* cv. Sultan stained with Leishman stain; **7** C-banded 'M' chromosome of *Vicia faba* stained with Leishman stain. Bars = 10 μ

7 bands were identified by Vosa (1976) in the M chromosome of *Vicia faba* using different techniques including C-banding. Using the present technique with Giemsa, the M chromosome showed no distinct bands. With Leishman or Wright stain two bands were seen on the short arm corresponding in position with Vosa's bands 3 and 4 (Fig. 7). Thus, within this chromosome certain bands can be preferentially stained.

Discussion

1 Applications of Preferential Staining

Our results show that by using different stain solutions certain C-bands can be preferentially stained. C-banding rye with Giemsa and wheat (A and B genomes) with Leishman or Wright stain permits the identification of all individual chromosomes (Seal and Bennett 1981; Seal 1982). This is the aim of most banding techniques. However, preferential staining, in addition to individual chromosome identification, permits the clear distinction between wheat and rye chromosomes or chromosome segments in hybrid karyotypes such as triticale, wheat/rye substitution lines or wheat/rye translocation lines. The different reactions of barley and rye chromosomes suggest that the present preferential staining technique could usefully be applied to barley/rye hybrids. It is particularly useful for distinguishing between wheat and rye chromosomes with otherwise indistinguishable banding patterns. For example, it is often difficult to distinguish between 7A of wheat and 2R, 3R or 7R/4R of rye in triticale when they are all stained simultaneously and to the same intensity using Jenners stain since their only large C-bands are all telomeric. However, using preferential staining the distinction is clear, since the C-bands of 7A only show using Leishman or Wright stain while those of the rye chromosomes are preferentially stained using Giemsa (Fig. 1a, b). Moreover, a reciprocal translocation between 7A and any of these rye chromosomes involving a chromosome segment including one telomere would probably be undetectable using conventional C-banding techniques but should be more easily recognised using preferential staining.

In most karyotypes some chromosomes had a few bands which differed from the rest in their staining response (e.g. 1B and 6B of wheat, 3R and 6R of *S. cereale* and chromosome 7 of *S. africanum*). Such bands provide additional markers for use in chromosome identification. For example, using Giemsa, chromosomes 2R, 3R and 7R/4R of rye sometimes have indistinguishable banding patterns. However, using Leishman or Wright stain 3R can be regularly distinguished by the preferentially stained proximal band on 3RS.

Since Bennett (1977) proposed that rye telomeric heterochromatin may be responsible for grain shrivelling in triticale, efforts have been made to identify and combine into a single karyotype, several rye chromosomes modified by reduction or loss of such heterochromatin (Bennett and Gustafson 1982; Gustafson and Bennett 1982). However, it is often difficult to distinguish between modified rye chromosomes and substituted D genome chromosomes or wheat/rye translocation chromosomes. The preferential staining

response coupled with the discovery of new interstitial marker bands on rye chromosomes, will help to overcome this problem without recourse to the more time consuming and expensive methods of chromosome identification such as in situ hybridisation (May and Appels 1980).

2 The Structural Basis of Preferential Staining

The normal N-banding procedure (Gerlach 1977) stains bands on wheat but not rye chromosomes and only diffuse bands at wheat nucleolar organiser regions. However, by manipulating the duration and temperature of 1 M Na_2HPO_4 treatment, Jewell (1981) found N-bands at the nucleolar regions of 1B and 6B and on rye chromosomes. The present results confirm and extend these differences between bands. Different stains distinguished between (1) most rye C-bands, the nucleolar bands and two other bands on wheat chromosomes 1B and 6B, and (2) most wheat C-bands, two interstitial rye C-bands and major barley C-bands.

The differential response to the various stains is not simply a reflection of the different distribution of heterochromatin between species. Although most wheat and barley heterochromatin is proximal and most rye heterochromatin terminal in location, five terminal bands were regularly found among wheat A and B genome chromosomes and chromosome 7A often had only terminal bands. All of these bands were preferentially stained with Leishman or Wright stain unlike all rye telomeric bands which were selectively stained with Giemsa. Neither is it due solely to an inherent affinity between each stain and a particular species C-bands. For example, Leishman stain is regularly used in other laboratories for selectively staining rye telomeric heterochromatin (Darvey and Gustafson 1975), and Giemsa has been used for C-banding wheat and barley chromosomes (Gill and Kimber 1974; Linde-Laursen 1975). There is evidence that variation exists in staining reaction between Romanovsky stains of the same name from different companies, and even between batches of stain from the same company (Seal – unpublished). However, it is apparently the use of such stains in combination with a particular C-banding procedure that gives rise to the described effect.

The presence of differently staining bands between and within species and even within individual chromosomes may indicate some kind of structural differentiation between the chromatin comprising banded regions. The distinction C-bands by preferential staining parallels the distinction between heterochromatic regions of wheat and rye chromosomes on the basis of their constituent DNA sequences by in situ hybridisation. Such regions contain highly repeated DNA sequences (Appels et al. 1978; Bedbrook et al. 1980; Dennis et al. 1980; Hutchinson and Lonsdale 1982) arranged in long tandem arrays (Flavell 1980). At least 4 different arrays have been found in single telomeric heterochromatin regions of rye chromsomes (Bedbrook et al. 1980; Hutchinson et al. 1981) and more than one in major $C_0t\ 10^{-2}$ DNA hybridisation sites in wheat (Gerlach and Peacock 1980). The occurrence of different sequences in the same heterochromatic region may explain why some bands on chromosomes 1B and 6B of wheat and 7 of *Secale africanum* were well stained using both Giemsa and Leishman or Wright stain. The nucleolar organiser region of 1R, which in the present work was unique among rye bands in showing a change in banding intensity following changes in the composition of Leishman stain solution, contains other highly repeated sequences, namely those coding for ribosomal RNA (Appels et al. 1980).

Appels et al. (1978) purified two highly repeated DNA sequences from rye. The distribution of one sequence in rye, as shown by in situ hybridisation, coincided with that of the large telomeric blocks of heterochromatin. The other, a polypyrimidine tract sequence, hybridised to two main interstitial sites on two rye chromosomes. Their identity could not be determined by in situ hybridisation using wheat/rye addition lines since the sequence also hybridised to many sites in the wheat genomes. However, the telomeric sequence probe hybridised to both arms of one of the chromosomes involved and to the short arm of the other. The arm ratios of these chromosomes (median and sub-median respectively), and the proximal location of the interstitial sites, suggest that the chromosomes involved were 3R and 6R respectively. If so, the polypyrimidine tract hybridisation sites were probably coincident with the interstitial C-bands stained by Leishman or Wright stain in the present study.

Most repeated sequences in rye heterochromatin are absent from wheat and vice versa (Bedbrook et al. 1980; May and Appels 1980; Hutchinson and Lonsdale 1982). The similarity in staining reaction between wheat and barley chromosomes in the present work parallels the results of Dennis et al. (1980) who extracted biochemically identical satellite DNA sequences from wheat and barley chromosomes. The chromosomal locations of these sequences correspond remarkably with those of the C-bands revealed by Leishman and Wright stain in the present work.

The staining properties of a C-banded region may be related to the AT : GC ratio of its constituent DNA between sequences. Repeated sequences in rye telomeric heterochromatin and in banded regions 3 and 4 of the M chromosome of *Vicia faba* (all of which stained preferentially with Giemsa) are AT-rich (Appels et al. 1981; Vosa 1976), while those of wheat and barley

(which stained preferentially with Leishman or Wright stain) are relatively GC-rich (Dennis et al. 1980; Gerlach and Peacock 1980). However, associations proposed between AT : GC ratio and fluorochrome banding (Weisblum and de Haseth 1972) may be complicated by other factors (Weisblum 1973; Deumling and Greilhuber 1982).

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Dr. A. G. Seal
Dr. M. D. Bennett
Plant Breeding Institute
Maris Lane, Trumpington
Cambridge CB2 2LQ (England)