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Intraspecific variation in C-banded chromosomes of *Aegilops comosa* and *Ae. speltoides*

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Summary. Intraspecific variation in C-banding patterns can be used to differentiate subspecies of *Ae. comosa*, i.e. *eu-comosa* and *heldrechii*, but not those belonging to *Ae. speltoides*. Among the *eu-comosa* accessions, *comosa* E appears to possess a totally different karyotype as well as banding pattern. Two contrasting types of polymorphic changes were found. The first and more common type, observed in the 2 subspecies of *Ae. speltoides* and 2 accessions of *Ae. comosa* ssp. *eu-comosa*, involved variation in C-band size and presence or absence of interstitial, terminal and proximal bands within a basic recognisable pattern. The second type involved complete repatterning of C-bands and could be seen in *Ae. comosa* where the ssp. *eu-comosa* has predominantly interstitial C-bands in contrast to centromeric and telomeric bands in the other ssp. *heldrechii*. Furthermore, the extent of polymorphic variation was found to vary between chromosomes of *Ae. speltoides*. That polymorphic changes have occurred without loss of fertility in hybrids between subspecies of each of the 2 species confirms that these sorts of changes have no effect on chromosome pairing or fertility. Polymorphic changes appear to be widespread within and between diploid *Aegilops* species and their non-random distribution seem to suggest that these changes could perhaps be intimately associated with the processes of speciation and subspeciation.

Key words: C-banding – *Aegilops* – Intraspecific variation

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Introduction

Since C-banding techniques were first applied to the study of chromosome identification in plants by Vosa and Marchi (1972), there has been very little published evidence of polymorphism in C-banding pattern within or between chromosome complements of the same species. This aspect has been largely neglected even in very comprehensive cytotaxonomic studies using interspecific comparisons of C-banding patterns such as those on the genera *Scilla* and *Anacyclus* (Greilhuber et al. 1981; Schweizer and Ehrendorfer 1976). Nevertheless, inter-plant variation does exist in *Allium* species (Vosa 1976) and in barley lines (Linde-Laursen 1978). In recent years inter-varietal variation in C-banding pattern has been reported for wheat and the synthetic triticale (Iordansky et al. 1978; Gustafson and Krolow 1978; Seal 1982). This variation was confined only to polyploids and did not include any of their diploid relatives. However, Teoh and Hutchinson (1983) in their survey of C-banding pattern variation amongst the diploid *Aegilops* species, found that even *Ae. longissima* and *Ae. sharonensis*, which are now considered as a single species (Tanaka 1955; Miller 1981) possess differences in C-banding pattern. Furthermore, with the exception of *Ae. speltoides*, they found that the C-banding pattern was remarkably consistent between cells within each accession of the diploid species investigated. The purpose of this paper, therefore, is to explore further this intraspecific variation in two *Aegilops* species, *Ae. comosa* and *Ae. speltoides*.

Materials and methods

Six accessions of *Ae. speltoides* (2n = 2x = 14, SS) and 5 accessions of *Ae. comosa* (2n = 2x = 14, MM) were investigated from the collection at the Plant Breeding Institute, Cambridge,

Table 1. Sources of origin of the different accessions of *Aegilops speltoides* and *Ae. comosa*

Species	Accession	Specific status	Origin
Ae. speltoides	A	Subspecies *aucheri*	unknown
	D	Subspecies *ligustica*	unknown
	H2	Subspecies *aucheri*	Israel
	M	Subspecies *ligustica*	Israel
	V	Subspecies *aucheri*	Turkey
	S	Subspecies *ligustica*	Turkey
Ae. comosa	A	Subspecies *eu-comosa*	unknown
	B	Subspecies *heldrechii*	Greece
	C	Subspecies *eu-comosa*	Turkey
	D	Subspecies *heldrechii*	Turkey
	E	Subspecies *eu-comosa*	Greece
	G	Subspecies *heldrechii*	Greece

where they have been maintained as open pollinated populations. These accessions, together with their specific status and sources of origin are given in Table 1. All accessions of the same species from a particular country originated from different localities.

Chromosomes were C-banded using the technique developed by Teoh and Hutchinson (1983) where the optimum hydrolysis time and barium hydroxide treatment had been predetermined for each species. For comparative analyses, slides of all accessions of each species were placed together in the same glass dish to ensure equal treatment throughout the whole C-banding procedure. Expression of the maximum number of bands is important if intraspecific comparisons are to be made accurately, otherwise incomplete expression of C-bands could be interpreted erroneously as evidence for polymorphism. On the evidence of the clarity of the C-bands and the expression of more bands than that reported by earlier workers (Gill and Kimber 1974; Gill 1981) it was assumed that maximum expression had been achieved.

C-banded chromosomes were analysed in 5–10 cells at mitotic metaphase for each accession. The karyotypes were obtained from Chennaveeraiah (1960). Because of substantial differences between the karyotype of accession E and those of the other accessions of *Ae. comosa,* a provisional karyotype is provided based only on 2 sets of measurements. The chromosomes have been lettered A to G.

Results

The C-banded karyotypes of all 11 accessions of *Ae. speltoides* and *Ae. comosa* are illustrated in Figs. 1 and 2 respectively. Detailed arrangements of the C-bands within each chromosome are presented in the karyograms shown in Figs. 3 and 4.

Comparisons of C-banding pattern

Aegilops comosa. Accessions A, C and E belong to the subspecies *eu-comosa.* The first 2 accessions, A and C, have virtually identical C-banding patterns and are very similar to the C-banding pattern of *Ae. comosa* reported by Teoh and Hutchinson (1983). Differences between these two accessions are minor and are confined to chromosomes C and F (Fig. 3). In chromosome C of *comosa* C, there is an additional interstitial band whilst the telomeric band is very faintly stained relative to the same chromosome in *comosa* A. Chromosome F of these 2 accessions differs by the absence or presence of 2 interstitial bands in the long arm. What is indeed surprising is the vastly different pattern displayed by the chromosomes of *comosa* E. Distinctive chromosomes of the complement are C, D and F.

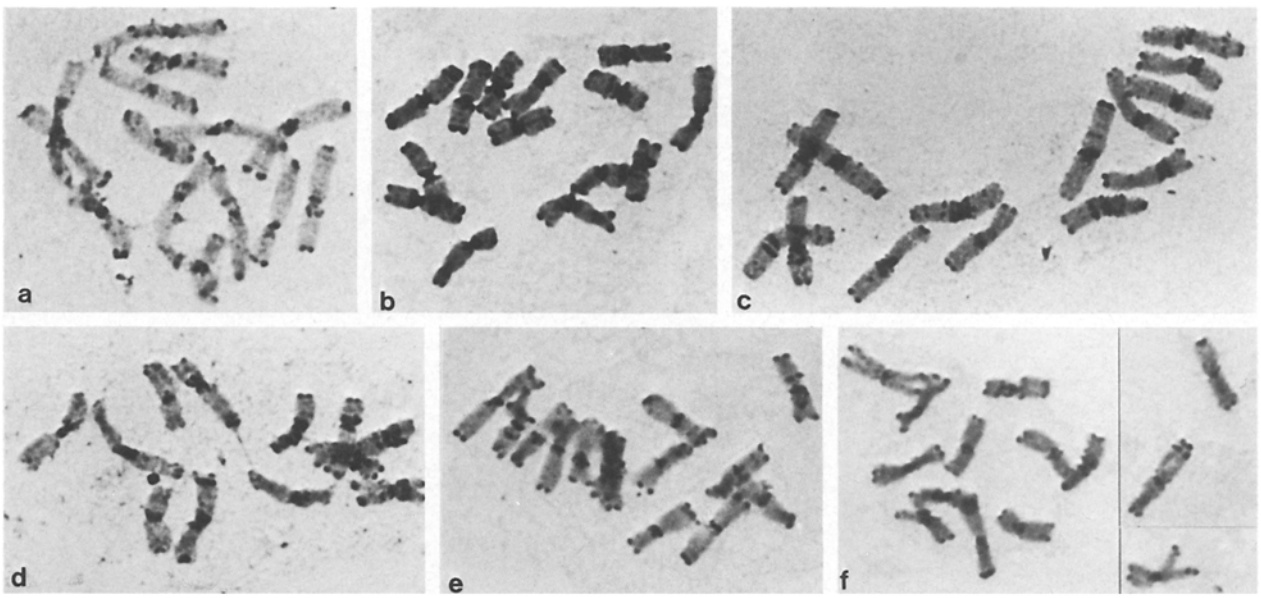

Fig. 1a–f. C-banded mitotic chromosomes at metaphase in accessions of *Aegilops speltoides:* **a** *speltoides* A, **b** *speltoides* D, **c** *speltoides* H2, **d** *speltoides* M, **e** *speltoides* V, **f** *speltoides* S

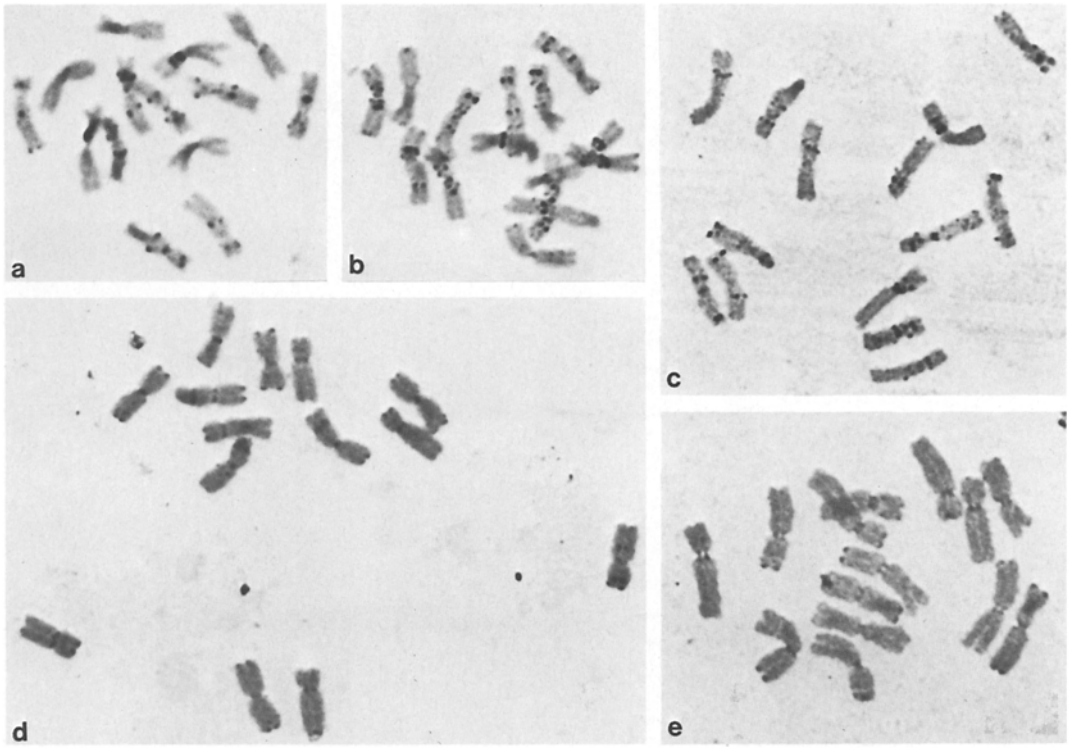

Fig. 2 a–e. C-banded mitotic chromosomes at metaphase in accessions of *Aegilops comosa:* **a** *comosa* A, **b** *comosa* C, **c** *comosa* E, **d** *comosa* G, **e** *comosa* B

Chromosome C, in particular, is very unlike any of the chromosomes of *comosa* A and *comosa* C. In fact, the series of small bands in its long arm is a characteristic feature of some of the chromosomes of *Ae. caudata* and *Ae. umbellulata* (Teoh and Hutchinson, 1983). Furthermore, major differences in karyotype exist between accession E and the other *eu-comosa* accessions. However one common feature shared by these 3 accessions is the lack of telomeric heterochromatin, with the exception of the long arm of chromosome F of *comosa* E. The satellited chromosome G of *comosa* E does bear some resemblance to chromosome F of accessions A and C but the other satellited chromosome, if present, is not identifiable.

Based on their C-banding pattern, accessions B and G are easily identified as belonging to the subspecies *heldrechii.* Their C-banding pattern is in direct contrast to that of the subspecies *eu-comosa.* They possess thin centromeric and telomeric bands with an almost complete absence of interstitial, proximal and distal bands. Furthermore, both nucleolar organizing regions are clearly and prominently banded, unlike those of *eu-comosa.*

Aegilops speltoides. Since *Ae. speltoides* is an obligate outbreeder, it is likely that considerable exchanges of chromosomes have taken place between these 6 accessions. For example, the heterozygous C-banding pattern between some of the homologues in accession D were also found in accession H2. However, not all polymorphic types shown in Fig. 4 were found within each accession and this could be due to the small samples of seeds analysed. Besides it has been shown that the main morphological difference between the sub-species *aucheri* and *ligustica* could be attributed to a single gene (Kihara and Yamashita 1956). They are thus best treated as a single population even though they originated from different countries.

Figure 4 represents the range of polymorphism in C-bands that could be found for each chromosome of the haploid complement. *Speltoides* A and S are not included in this figure as their C-banding patterns are already represented by the range illustrated. All seven chromosomes are individually identifiable.

In general, the basic pattern is retained and recognisable in all accessions. The polymorphic changes involve variation in the size of centromeric and telomeric heterochromatin as well as the presence or absence of interstitial and distal bands. Details of these changes are described below.

Chromosome A. This chromosome is probably the least polymorphic of all the 7 chromosomes. Heterozygosity is found only in the terminal portions of the long arm where either 2 or 3 bands are present.

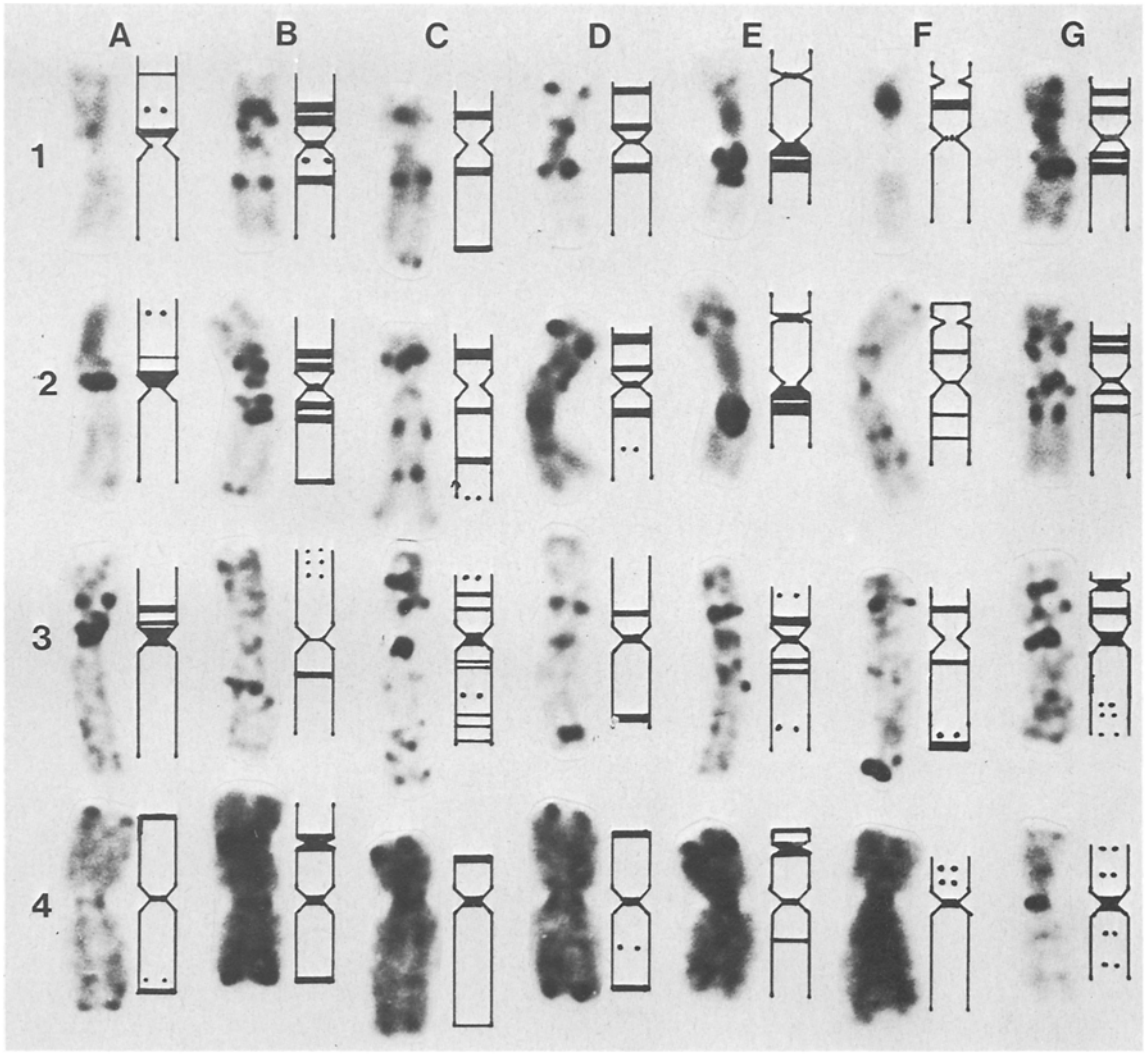

Fig. 3. C-banded karyograms of *Aegilops comosa: 1 comosa* A, *2 comosa* C, *3 comosa* E, *4 comosa* G

Chromosome B. Variation here is confined mainly to the size of both the centromeric and telomeric bands. However, in *speltoides* D, both telomeres have 2 densely stained terminal bands.

Chromosome C. In this chromosome only the interstitial band in the long arm shows heterozygosity. Very often the proximal band in the short arm appears fused to the centromeric band.

Chromosome D. This satellited chromosome shows the most polymorphism involving centromeric, interstitial and telomeric heterochromatin. In *speltoides* M, the short arm has 2 broad interstitial bands and the nucleolar organizing region is not banded. This particular chromosomal type was not found in the other accessions.

Chromosome E. This second satellited chromosome has very minor variations in C-bands compared to the previous satellited chromosome. These involve the number of distal bands in the long arm. There is also variation in the size of the banded nucleolar organizing region.

Chromosome F. The centromeric band and the proximal one close to it in the short arm appear to vary in size. There may be 1 or 2 terminal bands in the long arm.

Chromosome G. The banding pattern appears to be symmetrical in both arms and differs only in the size and number of the proximal bands.

Discussion

Based on C-banding variation, two types of polymorphic changes can be described within these species. The first is where polymorphic changes occur within a

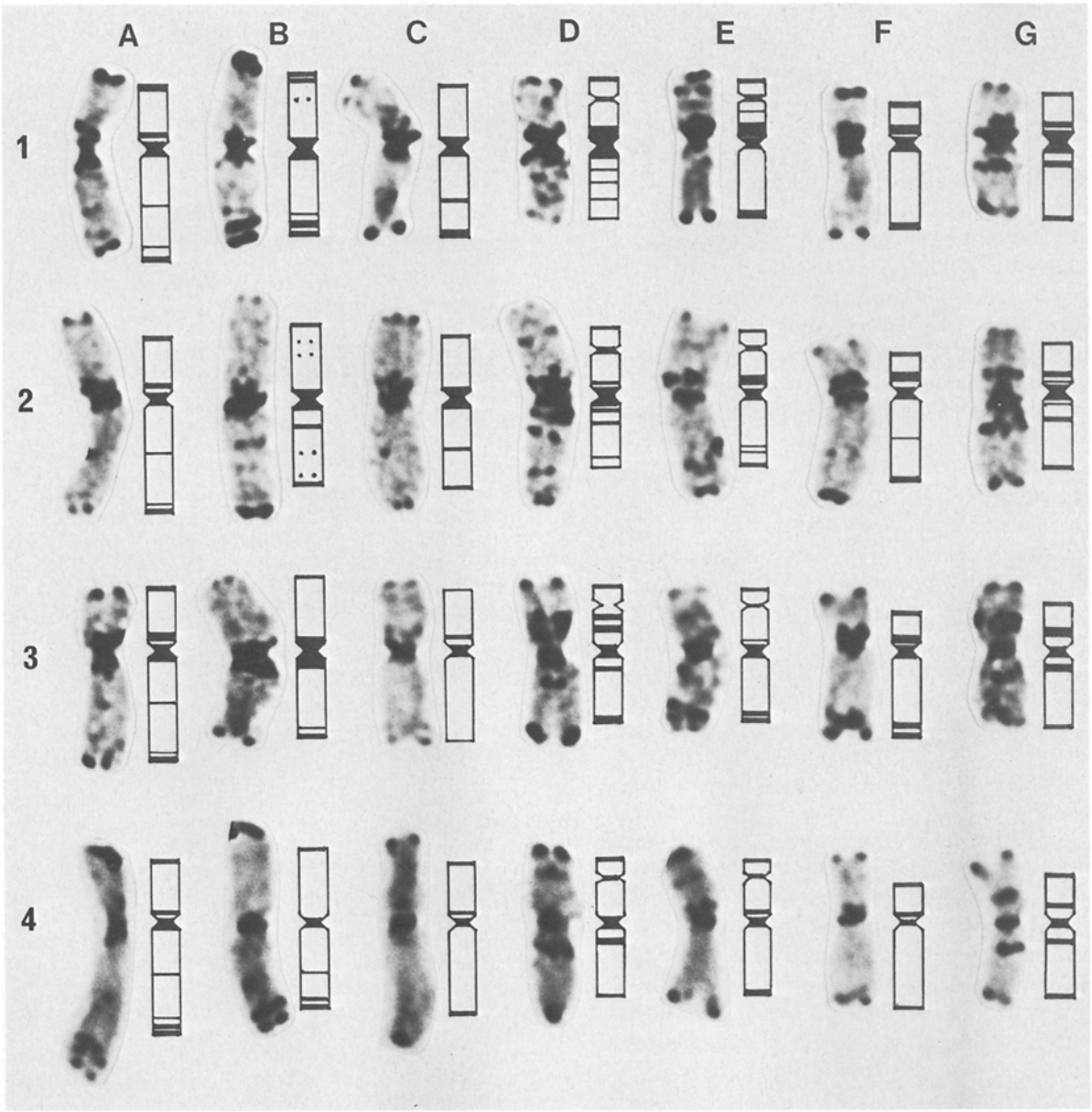

Fig. 4. C-banded karyograms of *Aegilops speltoides: 1 speltoides* D, *2 speltoides* H2, *3 speltoides* M, *4 speltoides* V

basic C-banding pattern which is retained and recognisable and where the inherent heterozygosity involves variability in C-band size and in the presence or absence of terminal, interstitial and proximal bands. This is probably the most common type and has been observed in natural races of species of Australian grasshoppers (John 1981). In plants this type of variation has been found in some *Allium* species (Vosa 1976) and different varieties of hexaploid wheat and triticale (Iordansky et al. 1978; Gustafson and Krolow 1978; Seal 1982). Both the subspecies *aucheri* and *ligustica* of *Ae. speltoides* clearly belong to this category. However *Ae. speltoides* differs from these previous reports in that all chromosomes of the haploid complement are polymorphic; some more so than others.

The second type of intraspecific polymorphic change involves drastic alterations of the C-banding pattern. In a sense this corresponds to the polytypic type as described by John (1981). This type is clearly illustrated by the contrasting C-banding patterns of *Ae. comosa* ssp. *eu-comosa* and ssp. *heldrechii* where the predominantly interstitial heterochromatin has been changed to centromeric and telomeric locations. Whether the same sequences of repetitive DNA are involved in this drastic change is not known. So far these 2 subspecies constitute the only examples of this

type of polymorphic change which resemble the interspecific variation observed in other diploid *Aegilops* species (Teoh and Hutchinson, 1983). However among the *eu-comosa, comosa* E with its vastly different karyotype and positioning of interstitial bands provides clear indications that heterochromatic bands have been rearranged as well as added to the chromosomes. How this is achieved is not yet understood. It is likely that chromosomal rearrangements such as translocations and inversions could have taken place but it is not possible to pinpoint precisely where these have occurred because of the extensive alteration in the positioning of the C-bands as well as the presence of some uncharacteristic C-banded chromosomes.

Except for *comosa* E, subspecies within each of the *Aegilops* species show only minor karyotypic differences (Chennaveeraiah 1960). In *Ae. comosa,* hybrids between the two subspecies are fertile with regular formation of five bivalents and one quadrivalent at meiosis (Kihara 1940) or 18 to 21 bivalents in amphiploid combinations with *Triticum durum* (Maan and Sasakume 1978). Similarly, hybrids of *Ae. speltoides* ssp. *ligustica* and ssp. *aucheri* had normal meiotic behaviour (Kihara 1940; Sears 1941; Riley et al. 1958). From these observations we can deduce that polymorphism in C-bands or heterochromatin variation between subspecies in these 2 *Aegilops* species has no effect on pairing between homologues, which substantiates similar conclusions reached by John (1981) in different races of the Australian grasshoppers, *Cryptobothrus chrysophorus* and *Atractomorpha similis.* On the other hand, in the F1 hybrids of races of the Australian grasshoppers, John (1981) observed pronounced effects on the distribution of chiasma formation which led him to suggest that heterochromatin variation could play a role in regulating recombination. It is possible that similar effects might be observed in hybrids between the subspecies of *Aegilops* but such studies have yet to be made.

Finally, a combination of the interspecific C-banding data of Teoh and Hutchinson (1983) with that of the intraspecific variation reported here shows that both speciation and subspeciation have involved both types of polymorphic changes. Furthermore, the localisation of these C-bands in all chromosomes of the complement in species such as *Ae. uniaristata, Ae. mutica, Ae. squarrosa* and *Ae. comosa* ssp. *heldrechii* suggests that these changes cannot be attributed to random factors. Rather they seem to indicate that these changes are intimately associated with the processes of speciation and subspeciation. Whether these changes are causal factors in the process or the consequences of other changes is still a matter of conjecture.

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References

Chennaveeriaiah MS (1960) Karyomorphologic and cytotaxonomic studies in *Aegilops.* Acta Horti Gotob 23: 85–178

Gill BS (1981) Evolutionary relationships based on heterochromatin bands in six species of the Triticinae. J Hered 72:391–394

Gill BS, Kimber G (1974) Giemsa C-banding and the evolution of wheat. Proc Natl Acad Sci USA 71:4086–4090

Greilhuber J, Deumling B, Speta F (1981) Evolutionary aspects of chromosome banding, heterochromatin, satellite DNA and genome size in *Scilla* (Liliaceae). Ber Dtsch Bot Ges 94:249–266

Gustafson JP, Krolow KD (1978) A tentative identification of chromosomes present in tetraploid triticale based on heterochromatic banding patterns. Can J Genet Cytol 20:199–204

Iordansky AB, Zurabishvili TG, Badaev NS (1978) Linear differentiation of cereal chromosomes. 3. Rye, triticale and 'Aurora' variety. Theor Appl Genet 51:281–288

John B (1981) Heterochromatin variation in natural populations. Chromosomes Today 7:128–137

Kihara H (1940) Verwandtschaft der *Aegilops*-Arten im Lichte der Genomanalyse. Ein Überblick. Züchter 12:49–62

Kihara H, Yamashita K (1956) Wheat and its relatives. Wheat Inf Serv 4:16–24

Linde-Laursen I (1978) Giemsa C-banding of barley chromosomes. I. Banding pattern polymorphism. Hereditas 88:55–64

Maan SS, Sasakuma T (1978) Chromosome pairing relationships among the D and M genomes of *Triticum* and *Aegilops* species. Indian Soc Genet Plant Breed 1:322–331

Miller TE (1981) Chromosome pairing of intergeneric amphiploids as a means of assessing genome relationships in the Triticeae. Z Pflanzenzücht 87:69–78

Riley R, Unrau J, Chapman V (1958) Evidence on the origin of the B genome of wheat. J Hered 49:91–98

Schweizer D, Ehrendorfer F (1976) Giemsa banded karyotypes, systematics, and evolution in *Anacyclus* (Asterceae-Anthemideae). Plant Syst Evol 126:107–148

Seal AG (1982) C-banded wheat chromosomes in wheat and triticale. Theor Appl Genet 63:39–47

Sears ER (1941) Chromosome pairing and fertility in hybrids and amphidiploids in the Triticinae. Univ Mo Coll Agric Res Bull 337:1–10

Tanaka M (1955) Chromosome pairing in hybrids between *Aegilops sharonensis* and some species of *Aegilops* and *Triticum.* Wheat Inf Serv 2:7–8

Teoh SB, Hutchinson J (1983) Interspecific variation in C-banded chromosomes of diploid *Aegilops* species. Theor Appl Genet 65:31–40

Vosa CG (1976) Heterochromatic patterns in *Allium.* I. The relationship between the species of the cepa group. Heredity 36:383–392

Vosa CG, Marchi P (1972) Quinacrine fluorescence and Giemsa staining in plants. Nature (London), New Biol 237:191–192