Heterochromatin in American and Japanese Species of *Trillium*

I. Fusion of chromocentres and the distribution of H-segments

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Introduction

In *Trillium* species, certain chromosome regions, the H-segments, show differential contraction during mitosis at low temperatures. These H-segments have been used in several studies as chromosome markers in breeding experiments (e.g. Rutishauser and La Cour 1956), in population studies (e.g. Kurabayashi 1957, 1958) and in X-ray breakage experiments (Darlington and La Cour 1945). The H-patterns that these segments form have been found to characterise the species in several cases (Bailey 1952, 1954, 1958; Kurabayashi, 1958; Darlington and Shaw, 1959). Relatively little study has been made of the H-segment themselves (Wilson and Boothroyd, 1944; Kurabayashi, 1952; Shaw, 1959), but certain observations have revealed apparent differences between American and Japanese species.

Although no comparative studies have been reported, Darlington and Shaw (1959) state that in American species the segments occur in all parts of the chromosomes, are entire and relatively few in number, and rarely completely despiralised. In Japanese species, on the other hand, the segments are proximal, usually occurring as many small segments separated by small euchromatin regions, and very extended. There also appear to be many more alternative patterns in, for example, *T. kamtschaticum* than in *T. erectum*.

Preliminary observations during this investigation confirmed the fact that, as shown in previously published illustrations, *T. kamtschaticum* contains fewer and larger chromocentres in the interphase nuclei than American species with fewer H-segments.

These differences are particularly surprising in view of the close relationship, suggested on taxonomic grounds, between *T. kamtschaticum* and species of the *T. erectum* group, and they may be in part due to differences in the technique. Consequently, a comparative study has been made of the organisation of the chromocentres and of the size and distribution of H-

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segments in American and Japanese species under the same conditions. These observations, which have revealed some of the factors causing variation in the degree of fusion of chromocentres, suggest that H-segments in the two groups of species differ in distribution but in other respects are similar. As described in an accompanying paper (Dyer, 1963 b), a more detailed investigation of their behaviour confirms this, and, while suggesting the causes of variation in the degree of contraction of H-segments, leads to a number of general conclusions concerning their nature and function.

Materials and methods

The species of *Trillium* used, and their sources, are listed in Table 1.

Table 1. The sources and chromosome numbers of the *Trillium* species used. Some individuals of all the American species contained 1 to 9 supernumerary chromosomes in addition to the 10 autosomes

Species	2n	Source	Origin
American			
<i>T. cernuum</i> L.	10	Messrs. Perry, Enfield	N. Carolina
<i>T. chloropetalum</i> (Torr.) How.	10	Edinburgh B. G.	West U.S.A.
<i>T. erectum</i> L.	10	Messrs. Perry, Enfield	N. Carolina
<i>T. grandiflorum</i> (Michx.) Salisb.	10	Messrs. Perry, Enfield	N. Carolina
<i>T. luteum</i> L.	10	Messrs. Perry, Enfield	N. Carolina
<i>T. rivale</i> L.	10	Edinburgh B. G.	West U.S.A.
<i>T. sessile</i> S. Wats.	10	Messrs. Perry, Enfield	N. Carolina
Japanese			
<i>T. kamtschaticum</i> Pall.	10	Prof. Adati, Mie Univ.	Mie Pref.
<i>T. smallii</i> Maxim.	20	G. B. Rawinsky, Farnham	Japan

Low temperature treatment was carried out by placing the whole potted plant in a refrigerator at $1^{\circ}\text{C.} \pm 1^{\circ}\text{C.}$

For studies of chromocentres and chromosomes, the material was stained by the Feulgen method after fixation in a modified Carnoy's fixative (Dyer, 1963 a). Also, in the investigation of the effect of fixation on the appearance of the H-segments, lacto-propionic orcein was used (see Dyer, 1963 a, 1963 c, for method).

For meiosis preparations, orcein squashes were made of anthers dissected out of the large apical bud of the subterranean rhizome at the time appropriate for each species (see Dyer, 1963 d).

Chromosome nomenclature

The five chromosomes in the genome of all *Trillium* species have been labelled according to several different systems by previous authors. The equivalents to the European system used here are given in Table 2.

Table 2

System	Equivalents	Authors
European	A B C D E	Darlington, Rutishauser, Shaw, Dyer
E. American	E B D C A	Wilson, Giles, Bailey, Boothroyd
W. American	I III II IV V	Warmke
Japanese	A C B E D	Matsuura, Haga, Kurabayashi

Section I. The distribution of H-segments - the H-pattern

In a study of 70 plants of *T. erectum*, collected within the same region in N. Carolina, Darlington and Shaw (1959) found only 24 different clones, as indicated by the H-pattern. None of the five chromosome types showed more than three alternatives, and heterozygosity within individuals was only found in Chromosomes C and E, 96% of all pairs being homozygous. This contrasts markedly with the degree of heterogeneity found in most populations of *T. kamtschaticum* (Fukuda and Kozuka, 1958). Consequently, individuals of the same sample of *T. erectum* plants have been re-examined, using a different fixative from that used by Darlington and Shaw, and compared with individuals of two Japanese species, *T. smallii* and *T. kamtschaticum*.

Trillium species are characterised, not only by the H-pattern, but also by the location of nucleolar organising constrictions. As Resende (1940, 1944, 1945) considered that some H-segments are concerned in nucleolar organisation, and Darlington (1941) has suggested that H-segments and typical nucleolar organisers might be incompatible within the same nucleus, the possibility has been investigated that the characteristic distributions of H-segments and nucleolar organisers are in some way related.

As heterozygosity of H-pattern is a marker for the general structural heterozygosity of the chromosomes, the distribution of segments has also been compared with that of another indicator of structural heterozygosity, namely the distribution of bridges associated with acentric fragments at Anaphase I of meiosis.

Observations

H-pattern in T. erectum

In the 16 plants examined (e.g. Fig. 1 a), many pattern types, differing in the distribution of small euchromatin and heterochromatin segments, were found. There may have been other undetected differences in the length of segments in some chromosomes, such as B (short arm), which were classified as having the same pattern. Nevertheless, 11 pattern types were recognisable in Chromosome A, 8 in Chromosome B, 8 in C, 4 in D, and 7 in E (Fig. 1 b). Only 16% of all homologous pairs were apparently homozygous for the H-pattern, and no two plants had the same pattern for all chromosomes (Table 3).

H-pattern in *T. kamtschaticum*

Several plants were examined, and they all showed *H*-patterns included

Fig. 1. a, metaphase chromosomes of an individual of *T. erectum* after 4 days at 1°C. $\times 1,200$. b, the different *H*-patterns seen in 16 individuals of *T. erectum*.

in those described by Fukuda and Kozuka (1958). The *H*-segments were all proximal and similar in appearance to proximal segments in *T. erectum*.

In the individual shown in Fig. 2a, the chromosomes correspond to the types $A_{39}A_{27}$: B_1B_{19} : $C_{13}C_{13}$: D_4D_{11} : E_3E_6 of the Japanese workers.

H-pattern in T. smallii

This species contained less heterochromatin than *T. kamtschaticum* despite being tetraploid. In the single plant examined (Fig. 2b), eight chromosomes had no segments at all. The H-pattern revealed the allopolyploid origin as has been described by the Japanese investigators. As in *T. kamtschaticum*, there were more small intercalated regions of euchromatin in the H-segments than in *T. erectum*.

Table 3. Chromosome composition of individuals of *T. erectum*

Plant No.	Chromosome type					
	A	B	C	D	E	Supernumerary
1	5.6	3.5	2.2	1.2	1.7	
2	5.5	3.4	1.7	1.2	3.4	
3	5.6	1.1	7.7	1.1	4.4	
4	3.3	1.4	1.1	3.3	1.1	
5	6.8	4.5	7.8	2.3	2.5	
6	3.7	2.3	1.2	1.2	3.2	
7	3.3	2.5	1.2	1.2	4.5	
8	5.11	1.3	3.7	2.2	4.7	+1
9	3.5	6.6	4.8	2.4	1.1	
10	3.3	2.2	1.8	1.2	2.4	+9*
11	3.3	4.7	1.7	1.2	4.5	+2
12	1.6	4.4	1.2	1.2	4.6	
13	9.10	7.7	6.8	1.3	4.2	
14	2.2	1.8	1.8	1.2	1.2	
15	3.4	4.4	2.4	1.2	3.3	
16	4.5	3.5	5.5	2.2	2.4	
No. of types	11	8	8	4	7	

* Highest number of supernumerary chromosomes reported in *Trillium*.

H-segments and nucleolar organisers at mitosis

The nucleolar organisers in *Trillium* are terminal, and the satellites extremely small. They are rarely seen, and their distribution is not known for all species. They can be detected more easily after treating excised root-tips for 18 hours in 0.02 M chloral hydrate before fixing, or alternatively by noting the position of nucleoli in late prophase nuclei of endosperms.

Detailed studies were only made on *T. grandiflorum* and *T. rivale*, both of which revealed eight nucleolar organisers in a diploid nucleus. Others were seen in *T. sessile* and *T. cernuum*. These and earlier observations by other authors are summarised in Table 4. The nucleolar organiser on the E chromosome of *T. rivale* was unusual in that it was very long and clearly visible in all preparations, and comprised most of the short arm. In

an endosperm, the short arm of a supernumerary chromosome of *T. grandis*-

Fig. 2. Metaphase chromosomes, after cold-treatment, showing H-segments, and interphase nuclei showing chromocentres, in a) *T. kamtschaticum* and b) *T. smallii*. $\times 1,200$.

florum was associated with a nucleolus, suggesting an origin for the supernumerary as a centric fragment of Chromosome E.

Table 4. The frequency and distribution of ascertained nucleolar organisers on the chromosomes of some species of *Trillium* and the related genus *Paris*

Species	Frequency and distribution of nucleolar organisers												Author
	Total	A		B		C		D		E		f	
		s	l	s	l	s	l	s	l	s	l		
<i>Trillium</i>													
2x													
<i>T. cernuum</i>	1	+											5
<i>T. grandiflorum</i>	4	+					(+)		+	+		+	5
"		+					(+)						3
<i>T. kamtschaticum</i>	3	(+)	(+)							+			7
<i>T. ovatum</i>	2			+ ^h						+			4
"										+			9
<i>T. recurvatum</i>	1					+ ^h							3
<i>T. rivale</i> *	4	+		+				(+)		+ [≠]			5
"										+			8
<i>T. sessile</i>	4							(+)		+			5
"				+ ^h		+				+			3
Total for 7 spp.	19	4	1	3	—	1	2	2	1	5	—		
<i>Paris</i>													
2x													
<i>P. polyphylla</i>	1			(+)									2
<i>P. tetraphylla</i>	1									(+)			6
<i>P. verticillata</i>	1									+ ^x			7
4x													
<i>P. quadrifolia</i>	1									+ ^x			1
6x													
<i>P. japonica</i>	1									(+)			6
Total for 5 spp.	5			1						4			

Key: Symbols

+ Terminal nucleolar organiser.

+^x Interstitial nucleolar organiser.

(+) Nucleolar organiser on arm containing H-segments.

+^h Nucleolar organiser attached to H-segment.+[≠] Long nucleolar organiser visible under all conditions.

* *T. rivale* has not been previously examined for heterochromatin. The one plant examined has very little in the 10 autosomes and none in the single small acrocentric supernumerary chromosome. One small H-segment was situated terminally on one C short arm, one distally in each D short arm and another proximally in one E long arm. None were seen in chromosomes A or B. This H-pattern most closely resembles that of *T. stylosum*.

Authors

1, Darlington, 1941. 2, Darlington and La Cour, 1938. 3, Darlington and La Cour, 1940. 4, Darlington and Shaw, 1959. 5, Dyer, this paper. 6, Haga, 1934. 7, Matsuura, 1938. 8, Warmke, 1937. 9, Warmke and Johansen, 1935.

Although no two species had the same distribution of nucleolar organisers, there is no correlation between this and that of the H-segments revealed after cold-treatment. Satellites have been seen on chromosomes with and without H-segments, individual chromosome arms with and without H-seg-

Fig. 3. *T. grandiflorum* Plant B. Bridges and fragments at Anaphase I. $\times 1,400$. a) B short arm b) A short arm c) B long arm d) C short arm e) E long arm. The constriction in the fragment from C short arm corresponds in size and position to an H-segment. Similar constrictions were sometimes seen on other fragments which contained heterochromatin.

ments, and even attached to H-segments in three cases. Nucleolar organisers are, however, much more frequent on the short arms of chromosomes than on the long arms (15:4 in Table 4), being most common on the shortest, E short arm.

Table 5. Frequency, origin and size of identified acentric fragments in an estimated total of 7,500 P.M.C.s in an individual of *Trillium grandiflorum*

Chromosome	Arm	Length ¹ at anaphase I in μ	Frequency of bridges ²	Length of associated fragments in μ
A	Short	12.0	3	10.0, 11.0, 17.0
"	Long	13.6	1	8.2
B	Short	4.5	2	5.2, 5.6
"	Long	13.5	1	4.8
C	Short	7.5	11	5.6, 7.0, 7.8, 8.4, 9.2, 9.6, 10.3, 11.1, 11.5, 12.3
"	Long	11.1	1	3.3
D	Short	5.1	—	
"	Long	8.1	1	1.1
E	Short	1.5	—	
"	Long	13.5	2	4.0, 19.2
Total		80.4	22	

¹ Mean of 10 measurements.² The origin of 11 other fragments could not be determined.

The lengths were: -3.6, 5.2, 5.6, 6.5, 9.6, 10.5, 11.5, 12.5, 13.7, 14.5, 15.4.

H-segments and anaphase bridges at meiosis

In one individual of *T. grandiflorum*, bridges and fragments occurred at Anaphase I (Fig. 3), indicating the presence of inversion heterozygosity.

An estimated 7,500 of the 20–25,000 Pollen Mother Cells in an anther were scored and bridges were seen in a total of 33 (c. 0.5%). The lengths of these fragments could

Fig. 4. *T. grandiflorum* Plant B. Chromosome map showing the distribution of nucleolar organisers (N), inversion cross-overs (C.O.) and H-segments. The position and relative size of H-segments were determined from cold-treated metaphase chromosomes (see Fig. 5) and the position of cross-overs estimated from the length of identified fragments associated with bridges at Anaphase I (see Fig. 3).

be measured, and in some cases the arm concerned in the bridge could be identified (Table 5). In these cases the approximate position of the middle of the inversion was determined and marked on a chromosome map showing the distribution of nucleolar organisers and H-segments in the same individual (Fig. 4). As the chiasmata were dispersed along the arms, the frequencies of inversion bridges gave an indication of the relative lengths of the inversions.

From this map, it is clear that 8 of the 10 chromosome arms are heterozygous for inversions, 9 or 10 different segments being involved. The inversion in C short arm appears to be a relatively large one. Although there is obviously much more structural heterozygosity than is revealed by the H-segments, it is interesting to note that both of the regions associated with heterozygosity for an H-segment are also heterozygous for an inversion. Thus the large inversion in C short arm is associated with striking heterozygosity for an H-segment which is entirely absent in one homologue, and the heterozygous H-segment in E long arm is close to the only proximal inversion in a long arm.

Bridges have also been seen in individuals of *T. luteum* known to be heterozygous for H-pattern.

Section II. The chromocentres

In several genera, species with H-segments show a variable number of chromocentres, the more or less circular, densely Feulgen positive, bodies lining the nuclear membrane of interphase nuclei, while related species without H-segments show none (Darlington and La Cour, 1938; Dyer 1963a). In

Table 6. The number of chromocentres and the distribution of H-segments in individuals of three species of *Trillium*; *T. grandiflorum* from America, and *T. kamtschaticum* and *T. smallii* from Japan

Species	2n	Position of H-segments	Number of H-segments	Number of chromocentres		
				Mean	Max.	Min.
<i>T. grandiflorum</i>						
Plant B.	10	P, M, D	10	8.1	10	2
<i>T. kamtschaticum</i>	10	P	26	2.2	6	1
<i>T. smallii</i>	20	P	23	8.2	10	5

P=proximal M=median D=distal

American species of *Trillium* the number of chromocentres is closely related to the number of H-segments (Darlington and La Cour, 1945), which has led all previous investigators, with the exception of Resende (1945) and Bailey (1949), to conclude that they represent the same chromosome regions at different stages of the mitotic cycle. The variation in number of chromocentres within an individual is apparently due to a variable degree of fusion. Preliminary observations reveal that the Japanese species *T. kamtschaticum*,

which has many more H-segments than, for example, *T. grandiflorum*, or *T. erectum*, shows fewer, larger chromocentres, presumably due to a much greater degree of fusion. The following observations confirm that the chromocentres and the H-segments are identical chromosome regions, and

Fig. 5. *T. grandiflorum* Plant B. a) Chromosomes at metaphase after cold-treatment showing 10 H-segments; 2 large, 4 medium and 4 small. $\times 1,200$. b) Chromocentres in interphase nuclei from various tissues. i) endosperm; ii) anther wall (small cell); iii) root tip; iv) ovule wall; v) anther wall (large cell); vi) tapetum. $\times 800$. c) Diagram of chromosomes at interphase, showing the relative size of the chromocentres estimated on the assumption that the volume of a spherical chromocentre is proportional to the length of the corresponding H-segment. d) Three interphase root tip nuclei, each showing the maximum number of ten chromocentres. As suggested by Fig. 5c, there are 2 large, 4 medium and 4 small chromocentres. $\times 800$.

indicate the factors which control the fusion of the former.

Observations

In *T. grandiflorum*, the maximum number of chromocentres seen in Table 7. The frequency of chromocentres in cells from different tissues of two plants of *Trillium grandiflorum*

Source of nucleus	Number of chromocentres per nucleus			Number of H-segments at per nucleus	Mean nuclear volume $\times 10^{-4}$ cu. mm.
	Mean	Max.	Min.		
<i>Plant A</i>					
<i>Haploid, $n=5$</i>					
Mononucleate pollen grain	4.88	10	1	c. 5	1.87
Binucleate pollen grain,					
Vegetative cell	4.58	9	3	c. 5	1.31
Generative cell	3.46	7	1	c. 5	0.68
<i>Plant B</i>					
<i>Diploid, $2n=10$</i>					
Root meristem cell	8.1			10	
Ovule wall cell	8.0			10	
Anther wall cell (small)	9.0				
Tapetal cell ("1/2 nucleus")	7.8				
<i>Triploid, $3n=15$</i>					
Endosperm	11.6			15	
<i>Tetraploid?</i>					
Anther wall cell (large)	17.8

Fig. 6. *T. grandiflorum*. a) Mononucleate pollen grain b) Binucleate pollen grain. $\times 1,200$.

root tip nuclei equalled the number of H-segments, which was ten in Plant B (Fig. 5 a, d). Fusion gave rise to as few as two larger chromocentres, the average number being about eight (Table 6). In the absence of fusion, the relative sizes of the ten chromocentres

(Fig 5 d) corresponded closely with those estimated (Fig. 5 c) on the assumption that the volume of an approximately spherical chromocentre was proportional to the length of the H-segment.

The numbers of chromocentres in the resting nuclei of various tissues of two individuals of *T. grandiflorum* were compared (Table 7, Figs. 5, 6). In Plant A, which showed nine H-segments at root-tip mitosis, the nuclei of 50 mono-nucleate pollen grains, and of both nuclei of 50 binucleate pollen grains were examined. The mono-nucleate pollen grains will have received about 5 H-segments at Anaphase II, random assortment at Anaphase I resulting in some variation. Both nuclei in a binucleate pollen grain will have the same number of H-segments as a result of Pollen Grain Mitosis, again about 5, but it was observed that the smaller generative nucleus showed consistently fewer chromocentres. Comparisons of the nuclear volumes were obtained from the dimensions of camera lucida outline drawings at a stage when the nuclei were approximately spherical. In Plant B, chromocentre numbers were recorded in the nuclei of root-tip meristems, interphase nuclei of ovule wall and endosperm, in which the ploidy and number of H-segments was known, and in the large and small anther wall nuclei and in each half of the "double" tapetal nuclei, in which the ploidy could be inferred.

The number of chromocentres was also compared (Table 6) between root-tip nuclei of *T. grandiflorum*, $2n=10$, (Fig. 5biii) in which the 10 H-segments were dispersed along the chromosome arms (Fig. 5a), of *T. kamtschaticum*, $2n=10$, in which the 26 segments were all proximal to the centromeres (Fig. 2a), and of *T. smallii*, $2n=20$, in which the 23 H-segments were also proximal (Fig. 2b).

Conclusions

The H-segments

The heterochromatin of *T. kamtschaticum* does differ from that of *T. erectum* in being entirely proximal, and more frequently interrupted by small euchromatin segments. It has been suggested elsewhere (Dyer, 1963a), from comparisons in this and other genera, that this interruption of H-segments is correlated with the degree of over-lapping of the distributions of chiasmata and H-segments. In *T. kamtschaticum* and other Japanese species, both are concentrated in the proximal regions of the arms, while in the American species both can occur at all positions in the arms. In no species is the distribution of the H-segments correlated with that of the nucleolar organisers.

In other respects, the heterochromatin in *T. kamtschaticum* and *T. erectum* is similar. The extended proximal segments have the same appearance, and the degree of heterogeneity and heterozygosity of the H-patterns in *T. erectum*, which is greater than previously estimated, approaches that of *T. kamtschaticum*. Heterozygosity for H-pattern can in some cases be shown to be associated with heterozygosity for a structural rearrangement, such as an inversion. It is interesting to note that the reassessment of the frequency of heterozygosity of H-patterns has not invalidated the observation

of Darlington and Shaw (1959), on the same sample, that there is an excess of homozygotes over the number expected on the basis of the Hardy-Weinberg equilibrium. Little can be concluded from this because it is not known whether this sample represents a single natural population, but similar analyses, carried out on the data of Fukuda and Kozuka (1958) for 6 populations of *T. kamtschaticum*, show that, in this species also, homozygotes are more frequent than expected.

In a few cases, even individual H-patterns of *T. kamtschaticum* and *T. erectum* are similar, reflecting the relationship between the species long suggested by the taxonomists. Chromosome types A_1 , A_2 , A_4 , A_6 , A_7 , A_8 and E_4 described here appear to correspond to the types A_{52} , A_{53} , A_{43} , A_{44} , A_{42} , A_{70} and D_{12} of Fukuda and Kozuka (1958). Chromosomes A_2 , A_3 , E_4 and E_6 of Darlington and Shaw (1959) resemble A_{21} , A_{61} , D_4 and D_{10} of the Japanese species. It is significant that the eleven chromosome types of *T. kamtschaticum* which correspond to those in the American species are found in the older populations in the south of the species range (Fukuda and Kozuka, 1958). Furthermore, the corresponding chromosome patterns in *T. erectum* occur in plants which also come from the extreme south of the species range (see map, Dyer, 1963 b, Fig. 9). The survival of ancestral chromosome types in both these long-separated species reveals a surprising stability of H-pattern, which is even more surprising in view of the variation that they both show. This slow change in the distribution of segments is, perhaps, explained by the fact that large structural rearrangements of the chromosomes have seldom occurred in this genus, as revealed by the similarity of karyotypes in all *Trillium* species.

A common ancestry for two species with similar amounts of heterochromatin but, on the whole, very different distributions of segments does not support the theory of Darlington and Shaw (1959) that a more distal distribution of H-segments develops as the H-content increases. Even among American species, there is no correlation between H-content and the proportion of segments which are distal, although median and distal segments are restricted to the shorter arms.

The chromocentres

It is clear from the observations on *T. grandiflorum* that, although the chromocentres correspond to the H-segments, not every H-segment will give rise to a separate chromocentre. In most nuclei there is some fusion. While comparisons of nuclei within an individual, which therefore have the same distribution of H-segments, show that the number of chromocentres is closely correlated with the ploidy of the nuclei, a comparison of genetically identical pollen grain nuclei of markedly different size reveals that chromocentre fusion is greater in small nuclei.

It has been suggested elsewhere (Dyer 1963 a) that the degree of fusion

is greater in nuclei with most of the H-segments situated close to each other and to the centromere, than in nuclei where the segments are dispersed. A comparison of the diploid species, *T. kamtschaticum* and *T. grandiflorum*, confirms this. The fact that fusion is less in *T. smallii* than in *T. kamtschaticum*, which has the same number of segments distributed over half as many chromosomes, also supports this idea.

These observations indicate that there are two main factors which determine the number of chromocentres found in a nucleus; the number of segments and their proximity at early interphase. Any factor, such as proximal localisation of H-segments, low chromosome number, or small nuclear volume, which increases the chance of H-segments being adjacent when the centromeres aggregate at telophase, also increases the chance of chromocentre fusion at early interphase. Random distribution of the chromosomes would explain the variation in chromocentre number between otherwise similar nuclei. The striking difference between the chromocentres of *T. grandiflorum*, and other American species, and those of *T. kamtschaticum*, due to the much greater degree of fusion in the latter, is merely a reflection of the difference in the distribution of the H-segments.

Summary

1. Heterochromatin segments, of similar size and position, appear the same, after identical treatments, in American and Japanese species. The segments differ in distribution, being entirely proximal in Japanese species, and proximal, median or distal in American species. They differ also in the amount of intercalated euchromatin. The H-patterns of *T. erectum* and *T. kamtschaticum* are, in a few chromosomes, the same.

2. The distribution of H-segments in a species is not related to that of nucleolar organisers, but heterozygosity for H-pattern does reflect heterozygosity for the adjoining euchromatin as well.

3. The chromocentres of resting nuclei, in the absence of any fusion, correspond with the H-segments in size and number. The fusion of H-segments to form fewer chromocentres increases i) when the nuclear volume is small, ii) when the chromosome number is low, and iii) when the segments are localised near the centromeres. These factors increase the likelihood of segments being adjacent at telophase when the chromocentres are forming.

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