Synaptic responses to concerted genomic evolution in *Lathyrus*. II. Intragenomic effects

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Intragenomic pairing patterns have been examined in pollen mother cells (PMCs) of high chiasma frequency from C_0 autotetraploids, representing seven diploid *Lathyrus* species. Six species were known to display intergenomic correlations between numbers of autonomous synaptic sites and the content of moderately repetitive DNA. Intragenomic variance in chromosome length is unaffected by differences in the size of the genome. Each tetrasomic set of homologues within a nucleus has a similar propensity for multivalent formation and for synaptic exchange. These observations are consistent with the view that each chromosome of a complement is equally affected by genomic changes in the content of moderately repetitive DNA, not least those sequences previously implicated in autonomous chromosomal synapsis.

Keywords: autopolyploidy, genomic evolution, *Lathyrus*, meiosis, multivalents, synapsis.

Introduction

In the previous paper in this series, we reported on studies of pairing behaviour in C_0 autotetraploid *Lathyrus* species and concluded that intergenomic differences in numbers of moderately repetitive sequences are paralleled by different numbers of autonomous synaptic sites (Wallace & Callow, 1993). Here we address the question of how pairing autonomy responds to chromosomal variation within each genome. Pairing autonomy is known to be related to chromosome size in C_0 autotetraploids of *Crepis* (Hamey *et al.*, 1988) and *Schistocerca* (John & Henderson, 1962) but not in those of *Dactylis* (McCollum, 1958), *Festuca* (Simonsen, 1975) or *Lolium* (Simonsen, 1973).

The concerted mode of chromosomal evolution within as well as between genomes of *Lathyrus* (Narayan, 1982) affords an additional test of our earlier conclusions. Our approach is based on indirect evidence because tetrasomic sets of homologues (tetrasomes) could not be identified directly.

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This and the previous paper are dedicated to the memory of our friend and colleague, J. N. Hartshorne.

Materials and methods

Observations

Pairing patterns in 942 metaphase-I pollen mother cells (PMCs), representing 36 C_0 autotetraploid plants of seven species of *Lathyrus*, were analysed for intragenomic variation. The cells were selected from a sample of 2299 PMCs for the presence of at least 28 chiasmata in order to minimize lapses of pachytene pairing due to insufficient chiasma formation (see Wallace & Callow, 1993). Analyses of intergenomic variation are supplied elsewhere, as are details of cytological and horticultural procedures (Wallace & Callow, 1993). Estimates of genomic size are those of Narayan (1982).

Two aspects of synaptic behaviour were recorded, in addition to chiasma frequency: pairing configuration and the minimum number of synaptic exchanges. While the first could be observed directly, the minimum number of synaptic exchanges had to be inferred from metaphase multivalent structure (see Wallace & Callow, 1993).

Statistical design

Although tetrasomes could not be directly identified, indirect evidence of intragenomic variation was provided

by statistical procedures. Heterogeneity in the karyotype was measured as variance in mitotic chromosome length. Thirteen cells were examined at mitotic metaphase, one from *L. cicera* and two from each of six other species. The bias due to measuring both homologues within each diploid complement was counteracted by doubling the within-cell variance. This approach provided an estimate of the karyotypic heterogeneity within each genome. If changes in genome size are *uniformly* distributed throughout the chromosome complement so that *equal* amounts of DNA are added to or subtracted from each chromosome, there should be no change in variance of chromosome size because $V_{x+a} = V_x$ where a is a constant. On the other hand, if the changes are *proportionate* so that each chromosome increases or decreases by the same *relative* amount (e.g. $a = 10$ per cent), then the variance in chromosome length is expected to show a quadratic relation to the size of the genome because $V_{ax} = a^2 V_x$, again with a being constant.

Where each of the seven tetrasomes within a tetraploid nucleus of *Lathyrus* has the same propensity to form pairs of bivalents at pachytene and the same propensity to form quadrivalents, the pairing patterns within cells will conform to a binomial frequency distribution. Thus, given that seven tetrasomes either form pairs of bivalents with probability p or quadrivalents with probability q , then pairing patterns within cells will conform to the expansion of $(p + q)^7$. The proportion of cells, where k tetrasomes form pairs of bivalents and the remaining $7 - k$ tetrasomes form quadrivalents, is given by the general term of the binomial expansion:

$$\frac{7!}{k!(7-k)!} \cdot p^k q^{7-k}.$$

For example, the expected proportion of cells forming four bivalents ($k = 2$) and five quadrivalents under such conditions is $21 p^2 q^5$.

Pairing patterns within cells were tested against such binomial expectations, p and q having been estimated from the average proportions of sets forming pairs of bivalents and quadrivalents respectively and the degrees of freedom in the goodness-of-fit test being reduced accordingly. As in our previous paper (Wallace & Callow, 1993), pairs of univalents were counted as pachytene bivalents, while univalents with trivalents were counted as pachytene quadrivalents.

The minimum number of synaptic exchanges was inferred for each multivalent observed, to give estimates peculiar to particular sets of homologues. Frequencies of minimal synaptic exchange per set of homologues were tested for randomness, using the Poisson distribution.

Results

Genomic diversity in chromosome size

Casual inspection of the karyotypes of the seven *Lathyrus* species examined reveals no apparent relation between morphological heterogeneity and average chromosome size (Fig. 1). The mean chromosome length ($\bar{y}$) showed a highly significant positive relation to the size of the genome (X) ($\hat{y} = 1.85 + 0.29 X$; $t_{[11]} = 3.664$, $P < 0.01$). Despite this obvious scalar relation, variance in chromosome length (V_Y) within genomes remains virtually constant throughout the intergenomic range ($\hat{V}_Y = 1.38 - 0.08 X$; $t_{[11]} = 1.878$, $P > 0.05$). These observations are consistent with the premise that genomic evolution has had a uniform rather than proportionate influence on the constituent chromosomes.

Pairing patterns within tetraploid genomes

Metaphase-I pairing patterns displayed by the 29–391 high chiasma frequency autotetraploid PMCs, representative of each of the seven *Lathyrus* genomes, varied considerably in quadrivalent frequency (Table 1). A typical illustration of such intragenomic variation is provided by *L. odoratus* (Fig. 2). Up to seven quadrivalents were seen in all genomes. Within each tetraploid, the numbers of sets of homologues forming either pairs of bivalents or single quadrivalents agreed closely with binomial expectations (Table 1), which suggests that each set of homologues within a nucleus shows similar pairing behaviour, irrespective of differences in chromosome size.

Synaptic exchange within tetraploid genomes

Minimum numbers of synaptic exchanges were inferred from the metaphase-I pairing configurations of the 203–2,737 tetrasomic sets of homologues examined for each genome (Table 2). Only one of the 6,594 sets in total gave evidence of as many as three synaptic exchanges at this stage. This single example was found in *Lathyrus aphaca*, the species with the highest quadrivalent frequency (Table 2; see Wallace & Callow, 1993). The majority (95 per cent) of the remaining 3,923 quadrivalent configurations required only a single exchange.

Within each species, the minimum incidence of synaptic exchange per set of homologues shows a highly significant deviation from randomness, as predicted by the Poisson distribution. In each case the distribution is rigidly uniform with the variance being approximately half the mean (Table 2). Such uniformity

Fig. 1 Karyotypes of seven *Lathyrus* species. (a) *L. angulatus*, (b) *L. articulatus*, (c) *L. aphaca*, (d) *L. cicera*, (e) *L. sativus*, (f) *L. odoratus*, (g) *L. hirsutus*. Bar = 10 μ m.

indicates that synaptic exchange is evenly rather than randomly distributed between tetrasomes. Evidently within a tetraploid genome, each set of homologues has a similar propensity for synaptic exchange, despite the diversity in chromosome size.

Discussion

Crude measures of chromosome size, such as mitotic length, may be uncorrelated with DNA content. In salamanders of the genus *Plethodon*, for example, a twofold increase in genomic DNA has little effect on chromosome length (Mizuno & MacGregor, 1974) and

must therefore influence chromosome volume or the degree of condensation. Where the relationship between chromosome length and DNA content is more readily apparent, as for example in species of *Lolium*, increases in genome size are dispersed throughout the complements of inbreeding species (Rees & Jones, 1967; Thomas, 1981).

By contrast with the pattern in *Lolium*, inbreeding species of *Lathyrus* have smaller genomes than outbreeders, suggestive of a reduction in the size of the genome during the course of evolution (Rees & Hazarika, 1969). Interspecific reductions in the size of the genome in *Lathyrus* are chiefly traceable to

Table 1 Analyses of pairing patterns within autotetraploids of seven species of *Lathyrus*, based on observations of metaphase-I PMCs each with a minimum of 28 chiasmata. Binomial expectations (*italics*) are based on each of the seven sets of homologues, within a nucleus, forming pairs of bivalents with probability *p*, and quadrivalents (or rarely trivalents plus single univalents) with probability *q*. Pooled classes are bracketed together. The probabilities *p* and *q* have been estimated from average pairing patterns

Species	PMCs	Proportion		2II IV	Pairing pattern								Goodness of fit		
		<i>p</i>	<i>q</i>		7 0	6 1	5 2	4 3	3 4	2 5	1 6	0 7	χ^2	d.f.	<i>P</i>
<i>L. angulatus</i>	62	0.475	0.525	(	1	3 <i>11.63</i>	6	16 <i>15.99</i>	20 <i>17.67</i>	12	3 <i>16.72</i>	1	0.568	2	> 0.70
<i>L. articulatus</i>	94	0.453	0.547	(	0	4 <i>14.75</i>	13	25 <i>22.68</i>	22 <i>27.38</i>	18 <i>19.84</i>	7 <i>9.36</i>	5	2.553	3	> 0.50
<i>L. aphaca</i>	100	0.260	0.740	(	0	0 <i>8.02</i>	2	5	16 <i>18.45</i>	41 <i>31.50</i>	22 <i>29.89</i>	14 <i>12.15</i>	5.679	3	> 0.10
<i>L. cicera</i>	54	0.450	0.550	(	0	3 <i>8.26</i>	6	10 <i>12.89</i>	16 <i>15.76</i>	16 <i>11.56</i>	2 <i>5.53</i>	1	3.589	3	> 0.30
<i>L. sativus</i>	29	0.414	0.586	(	0	0 <i>9.20</i>	1	9	10 <i>8.49</i>	6 <i>11.30</i>	1	2	0.806	1	> 0.30
<i>L. odoratus</i>	212	0.419	0.581	(	0	4 <i>5.15</i>	24 <i>19.41</i>	43 <i>44.85</i>	58 <i>62.19</i>	54 <i>51.74</i>	24 <i>28.66</i>	5	1.803	4	> 0.70
<i>L. hirsutus</i>	391	0.405	0.595	(	1	6 <i>7.89</i>	32 <i>31.67</i>	77 <i>77.56</i>	121 <i>113.94</i>	94 <i>100.44</i>	46 <i>49.19</i>	14 <i>10.32</i>	2.472	5	> 0.70

Fig. 2 Metaphase-I PMCs from C_0 autotetraploid *Lathyrus odoratus*: (a, b) $2_{II} 6_{IV}$, (c) $1_I 4_{II} 1_{III} 4_{IV}$, (d) $4_{II} 5_{IV}$. Bar = 10 μ m.

Table 2 Frequency distributions of minimal synaptic exchange for sets of homologues within autotetraploids of seven species of *Lathyrus*, compared to random expectations based on the Poisson distribution (*italics*). In each species, the incidence of synaptic exchange shows a highly significant deviation from expectation, with the variance (V_r) being substantially less than the mean ($\bar{r}$)

Species	PMCs	Minimal numbers of synaptic exchanges (r)/homologous set				Total sets	$\bar{r}$	V_r	Goodness of fit	
		0	1	2	> 2				$\chi^2_{[2]}$	P
<i>L. angulatus</i>	62	206 <i>254.9</i>	225 <i>135.6</i>	3 <i>36.1</i>	0 <i>7.4</i>	434	0.532	0.263	419.7	<0.01
<i>L. articulatus</i>	94	298 <i>372.1</i>	345 <i>210.4</i>	15 <i>59.5</i>	0 <i>16.0</i>	658	0.570	0.291	150.1	<0.01
<i>L. aphaca</i>	100	182 <i>318.0</i>	485 <i>250.9</i>	32 <i>99.0</i>	1* <i>32.1</i>	700	0.789	0.267	352.1	<0.01
<i>L. cicera</i>	54	170 <i>207.2</i>	189 <i>124.6</i>	19 <i>37.4</i>	0 <i>8.8</i>	378	0.601	0.341	57.8	<0.01
<i>L. sativus</i>	29	84 <i>107.6</i>	109 <i>68.3</i>	10 <i>21.7</i>	0 <i>5.4</i>	203	0.635	0.332	41.1	<0.01
<i>L. odoratus</i>	212	662 <i>791.9</i>	792 <i>497.3</i>	70 <i>156.2</i>	0 <i>38.6</i>	1484	0.628	0.328	297.3	<0.01
<i>L. hirsutus</i>	391	1108 <i>1484.2</i>	1583 <i>908.3</i>	46 <i>277.9</i>	0 <i>66.6</i>	2737	0.612	0.271	856.6	<0.01

*Three synaptic exchanges.

reduced amounts of moderately repetitive DNA (Narayan, 1982). Such reductions are, nevertheless, evenly rather than proportionately dispersed throughout the complement (Narayan & Durrant, 1983). Our own observation that intranuclear variance in mitotic chromosome length remains constant across the genomic range concurs with this conclusion. Thus, in *Lathyrus*, while differences in the size of the genome reflect differing amounts of moderately repetitive DNA, differences in the size of the chromosome mask similarities in the content of moderately repetitive sequences. Our earlier results indicated that a reduction in the size of the genome is accompanied by a loss of autonomous synaptic sites (Wallace & Callow, 1993). The observations reported here suggest that intragenomic differences in the size of the chromosome have not resulted in different numbers of autonomous synaptic sites because each tetrasome within an autotetraploid nucleus has a similar propensity for quadrivalent formation and for synaptic exchange. Evidently, the relation between pairing autonomy and size of genome, reported previously (Wallace & Callow, 1993), is no mere scaling effect because no such relation occurs at the chromosomal level. By contrast, variation in pairing behaviour correlates closely with both patterns of inter and intragenomic variation in the content of moderately repetitive DNA. The present

observations on intragenomic patterns of pairing behaviour therefore support our earlier evidence, derived from partial regression analysis (Wallace & Callow, 1993), that autonomous synaptic sites are most likely to reside in moderately repetitive genomic components.

The independence of multivalent frequency and chromosome size within autotetraploids of *Lathyrus* contrasts markedly with the chromosome-specific pairing observed in autopolyploid *Crepis* (Hamey *et al.*, 1988). In both *C. capillaris* and *C. rubra*, each set of homologues appears to have a characteristic number of autonomous synaptic sites. The pattern of genomic evolution in *Crepis* is evidently less concerted than in *Lathyrus*. Indeed, chromosomal translocations seem to have played an important role in the divergence of species within *Crepis* (Togby, 1943) and within related genera such as *Hypochoeris* (Parker, 1975).

As noted in our previous paper (Wallace & Callow, 1993), we are confident that the relations between genomic composition and pairing autonomy investigated in C_0 autotetraploids are more likely to result from direct mechanical effects than to reflect the intervention of natural selection. Such a situation cannot of course apply to studies of variation in chiasma frequency among the original diploids. On the one hand, the effects of genomic differences are liable to be con-

founded by any concomitant changes in breeding behaviour (Rees & Dale, 1974). On the other, the well known concordance between chromosome size and chiasma frequency (John & Henderson, 1962) is as likely to reflect selection for recombination frequency as any direct mechanical consequence.

Our finding that intergenomic reduction has been accompanied by loss of autonomous synaptic sites contrasts with that of Rees & Durrant (1986), who found no correlation between chiasma frequency and size of genome in *Lathyrus*. Again, while we find no evidence of intragenomic difference in the numbers of autonomous synaptic sites, the mean chiasma frequency in diploid *Lathyrus* increases more or less linearly with chromosome size (Rees & Durrant, 1986; Rees & Narayan, 1988). Such disparate responses to genomic evolution may well be attributable to the degree to which natural selection has interposed between cause and direct effect. In a later paper, we will examine the scale of synaptic responses to genomic evolution, in relation to phenotypic variation and interaction with chiasma frequency.

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