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The odd-even effect in mitotically unstable B chromosomes in grasshoppers

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Abstract. The odd-even effect, by which B chromosomes are more detrimental in odd numbers, has been reported in plants and animals. In grasshoppers, there are only a few reports of this effect and all were referred to as traits related to the formation of aberrant meiotic products (AMPs). Here we review the existing information about B chromosome effects on AMPs, chiasma frequency and the number of active nucleolus organizer regions (NORs) per cell. Polysomy for A chromosomes and B chromosomes are two kinds of chromosome polymorphism frequently found in grasshoppers. In some aspects, e.g. meiotic behaviour and mitotic instability leading to individual mosaicism (in the case of mitotically unstable Bs), polysomic As show similar characteristics to B chromosomes. In fact, polysomy is regarded as one of the main mechanisms for B chromosome origin. Here we review some features of meiotic behaviour in known cases of polysomy and mitotically unstable Bs in grasshoppers, in looking for possible causes for the odd-even effect. In all these traits, the odd-even effect was apparent, although its appearance was not universal in any case, with variation among species or populations within the same species. The equational division and lagging of the extra chromosomes, when univalents, could favour the appearance of abnormal meiotic products, and the formation of bivalents, when there are two or more extra chromosomes, inhibits this process. Therefore, the odd-even effect might be a consequence of the concomitant operation of both aspects of extra chromosome meiotic behaviour. The possibility that the odd-even effect might result from an increase in cell stress generated by odd numbers is suggested.

One of the most intriguing aspects of B chromosome research is the dependence of their effects on whether they are present in odd or even numbers. Darlington and Upcott (1941) found that maize plants with odd numbers of Bs had more chiasmata than did plants with even B numbers. Jones and Rees (1982) explicitly named it the odd-even effect after finding that the between-cell variance in the number of chiasmata in rye plants with odd number of Bs was significantly higher than that observed in plants with even numbers of B chromosomes. A similar odd-even effect has been detected, in a number of plants and animals, for traits such as protein and RNA amounts, dry nuclear mass, exophenotypic characters and fitness related traits (e.g. fertility) (for review, see Jones and Rees, 1982). In general, an odd number of B chromosomes is more detrimental than even numbers.

Mitotically stable B chromosomes show the same number in all cells from the same individual. Mitotically unstable Bs, however, show conspicuous intraindividual variation for the B number due to their mitotic nondisjunction. Therefore, the individuals carrying mitotically unstable Bs are actually mosaic, for which reason, the odd-even effect only makes sense for traits that can be analysed cell by cell (e.g. chiasma frequency or other cytological traits), but not for traits measured in the whole

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Table 1. Analysis of the odd-even effect for chiasma frequency in *Locusta migratoria*

	Odd B number			Even B number		
Sample	Mean	SE	N	Mean	SE	N
Gabias 1983 field	14.42	0.13	122	14.19	0.12	137
Gabias 1983 lab	14.72	0.13	272	14.59	0.12	191
Gabias 1984 field	13.61	0.21	57	14.31	0.24	45
Gabias 1984 lab	13.57	0.12	200	13.69	0.09	289
Padul 1984 field	14.56	0.18	39	14.02	0.12	49
Padul 1984 lab	13.88	0.13	64	13.34	0.09	88

Data taken from Viseras et al. (1988). N = Number of cells analysed.

organism (e.g. fertility, external morphology, etc.). In this paper, we review the available literature in grasshoppers in looking for the odd-even effect in mitotically unstable B chromosomes. In addition, and since polysomy is considered one of the most widely accepted pathways of B chromosome origin (Camacho et al., 2000), we review several cases of male germline polysomy to analyse whether the odd-even effect is, in some way, also manifested for these putative B chromosome ancestors.

Materials and methods

All available literature on unstable B chromosomes and male germ line polysomy in grasshoppers was reviewed in looking for data amenable of analysis for the odd-even effect. Since individuals bearing both types of polymorphisms were mosaic, all traits were analysed at cytological level. These were chiasma frequency and the number of active nucleolus organizer regions NORs in diplotene cells, and the frequency of aberrant meiotic products (AMPs), i.e. macro- and micro-spermatic nuclei and spermatids. B chromosome and polysomy variation was mainly found among testis follicles, with scarce variation within follicles. Therefore macro- and micro-AMPs were scored separately in follicles with different number of extra chromosomes (Bs or A polysomics). A practical way of testing the odd-even effect is to compare the trait between cells with odd and even B numbers.

The statistical tests employed were mixed cross-nested ANOVA for chiasma frequency in *Locusta migratoria*, with "environment" (i.e., caught in the field or bred in the lab) and "odd-even" as independent factors, and "individual male" as a variable nested within "environment", following the linear model:

$$X_{ijkl} = \mu + \tau_i + \theta_j + \tau\theta_{ij} + \Sigma(\theta)_{jk} + \tau\Sigma(\theta)_{ijk} + \varepsilon_{ijkl}$$

where μ is the overall mean, τ_i is the deviation due to the odd-even effect, θ_j is the deviation due to the environment factor (lab vs field), $\tau\theta_{ij}$ is the deviation due to the interaction of the odd-even effect with the environment factor, $\Sigma(\theta)_{jk}$ is the deviation due to the k^{th} individual nested within the j^{th} environment factor, $\tau\Sigma(\theta)_{ijk}$ is the deviation due to the interaction of the i^{th} odd-even effect with the k^{th} individual nested within the j^{th} environment factor, and ε_{ijkl} is the deviation due to the l^{th} replicate (cell) within each odd-even effect-environment factor combination.

In the grasshopper *Dichroplus pratensis*, Bidau (1987) reported data on chiasma frequency and AMPs in three males carrying a mitotically unstable B chromosome. With the data from Table 4 of this paper, we performed a two-way ANOVA with chiasma frequency as the dependent variable, male as a random factor and odd or even B number as a fixed factor.

The data on the number of active NORs per diplotene cell in *L. migratoria* were compared by the Student's t test, and the frequency of macro- and micro-AMPs in seven species (see Table 4) were compared between follicles with odd and even number of extra chromosomes by means of the contingency chi-square test.

Results

Mitotically unstable B chromosomes

The B chromosome in *L. migratoria* is mitotically unstable, with all variation essentially being inter- but not intrafollicular. A first data set on chiasma frequency in Cabrero et al. (1984) showed that mean chiasma frequency in diplotene cells from testis follicles with odd numbers of Bs (16.01, SD = 1.14) was not significantly different from that in follicles with even B numbers (15.93, SD = 0.96) (*t* test: $t = 0.57$, $df = 10$, $P = 0.58$). Later, Viseras et al. (1988) analysed chiasma frequency in males collected at two populations in the years 1983 (Gabias) and 1984 (Gabias and Padul). In all samples, field and laboratory-bred males were analysed. In these males, cells were found with up to six B chromosomes; we compared chiasma frequency between cells with odd (1+3+5) and even (2+4+6) numbers of B chromosomes. Cells with no B chromosomes (which were scarce) were not included in the analysis. Table 1 shows the mean chiasma frequency in the six groups of males in cells with odd and even numbers of B chromosomes. Table 2 shows the results of mixed cross-nested ANOVAs performed on the data in Table 1. It was apparent that individual differences affected chiasma frequency in all populations, an expected result. In addition, environment (lab or field) and odd-even affected chiasma frequency in the Padul population, with cells harbouring odd B numbers showing, on average, about 0.5 more chiasmata than cells with an even number of Bs (see Table 1). In Gabias, however, this effect was not apparent, but there was a significant interaction between odd-even and male in the two years analysed (see Table 2), suggesting that the odd-even effect is dependent on male genotype.

The analysis of chiasma frequency in *Dichroplus pratensis* showed close to significant differences among males ($F = 17.84$, $P = 0.053$), but an odd-even effect was not apparent ($F = 0.03$, $P = 0.86$).

Salcedo et al. (1988) analysed the number of active NORs in diplotene cells from *L. migratoria* male parent and offspring from a laboratory cross performed with a couple from Gabias and another from Padul. The odd-even effect appeared only in the Gabias offspring, with cells bearing an odd number of Bs showing a significantly lower number of active NORs than cells with an even B number (Table 3). The effect, however, was not consistent among populations or generations, although the available sample size in the male parents was actually low (in fact, a single male parent was analysed in each population).

Table 2. Mixed cross-nested ANOVA for chiasma frequency in *Locusta migratoria* (data from Table 1)

(a) Gabias 1983	Type of effect	SS	df	MS	F	*P*
Environment	Fixed	6.18	1	6.18	0.287	0.596788
Male (Environment)	Random	796.16	23	34.62	10.264	**0.000001**
Odd-Even	Fixed	0.66	1	0.66	0.251	0.619581
Environment x Odd-Even	Fixed	0.00	1	0.00	0.000	0.989431
Odd-Even x Male (Environment)	Random	70.78	22	3.22	1.972	**0.005194**
Error		1111.33	681	1.63		
(b) Gabias 1984	Type of effect	SS	df	MS	F	*P*
Environment	Fixed	14.64	1	14.64	0.912	0.348673
Male (Environment)	Random	652.44	24	27.18	6.816	**0.000053**
Odd-Even	Fixed	0.98	1	0.98	0.381	0.541659
Environment x Odd-Even	Fixed	2.95	1	2.95	1.147	0.292449
Odd-Even x Male (Environment)	Random	71.96	21	3.43	2.872	**0.000024**
Error		647.89	543	1.19		
(c) Padul 1984	Type of effect	SS	df	MS	F	*P*
Environment	Fixed	18.22	1	18.22	7.218	**0.018593**
Male (Environment)	Random	34.66	10	3.47	7.231	**0.003581**
Odd-Even	Fixed	9.46	1	9.46	14.821	**0.000405**
Environment x Odd-Even	Fixed	0.24	1	0.24	0.373	0.544800
Odd-Even x Male (Environment)	Random	4.34	9	0.48	0.551	0.835975
Error		214.33	245	0.87		

Environment classified males into field and laboratory grown males. See Materials and methods of analysis for a detailed explanation.

Table 3. Analysis of the odd-even effect for the number of nucleoli in diplotene cells from *Locusta migratoria* males

	Odd B number			Even B number			Student t-test			Variance		F-ratio	
Sample	Mean	SE	N	Mean	SE	N	t	df	*P*	Odd	Even	F	*P*
Gabias parent	6.20	0.57	15	4.00	0.55	5	2.10	18	0.05053	4.89	1.50	3.26	0.26303
Gabias offspring	6.88	0.24	48	8.10	0.23	51	3.62	97	**0.00047**	2.88	2.77	1.04	0.89280
Padul parent	7.31	0.38	13	7.00	0.63	6	0.44	17	0.66838	1.90	2.40	1.26	0.68038
Padul offspring	8.64	0.38	25	8.80	0.20	55	0.41	78	0.68611	3.57	2.27	1.57	0.17018

Data from Salcedo et al. (1988). Those tests which remained significant after the sequential Bonferroni test are marked in bold type.

The formation of aberrant (micro- and macro-) meiotic products (spermatic nuclei and spermatids) has been scored in several grasshopper species with mitotically unstable B chromosomes. Micronuclei and microspermatids are presumably derived from chromosomes lost after irregular meiosis, whereas macronuclei and macrospermatids probably result from restitution nuclei in the presence of chromosome laggards during meiotic anaphases, or else nuclear fusion during spermiogenesis (Nur, 1969).

Data on AMPs are known in eight species of grasshopper, seven of which are shown in Table 4. The trait was first analysed in *Camnula pellucida* by Nur (1969). His findings showed that the frequency of both kinds of AMPs was significantly higher in follicles with an odd (1 or 3) number of a mitotically unstable B chromosome (Fig. 1a and Table 4). In *Locusta migratoria* males carrying a mitotically unstable B chromosome, no macrospermatids and only 0.25% microspermatids were found in follicles with 1B, even though 61.8% of the B univalents divided equationally at the first meiotic metaphase. In *Sphingonotus coerulans*, the frequency of macrospermatids followed a clear odd-even pattern with respect to the number of unstable B chromosomes, since follicles with an odd (1 and 3) number of B chromosomes showed a frequency significantly higher than that in follicles with an even (2 and 4) B number. The frequency of microspermatids, however, did not show such a difference (Fig. 1b and Table 4). In *Cylindrotettix obscurus*, B chromosomes also varied among 1 and 4 but, in this case, microspermatids showed a clear odd-even pattern and macrospermatids failed to show it (Fig. 1c and Table 4). In *Psophus stridulus*, Suja et al. (1989) reported a high frequency of macrospermatids and the absence of microspermatids in males carrying mitotically stable and unstable B chromosomes. This high frequency was especially apparent in individuals carrying the unstable B but, unfortunately, they did not distinguish between follicles with different number of Bs, so we were not able to analyse this case. In *Aiolopus strepens*, microspermatids were also absent, but the frequency of macrospermatids showed a clear odd-even pattern (Fig. 1d and Table 4). In *Dichroplus pratensis*, a highly significant odd-even effect was apparent for both macro- and microspermatids in two different males (Table 4). In *Dichroplus elongatus*, two populations were analysed, Tafi Viejo and Raco, for the frequency of macro- and

Table 4. Frequency of aberrant meiotic products (AMPs) in grasshopper species carrying two kinds of extra chromosomes, i.e. mitotically unstable B chromosomes (B) or extra A chromosomes resulting from polysomy, and comparison between testis follicles with odd and even number of extra chromosomes

Type of extra chromosome	Species	Kind of AMP	% in odd	% in even	chi	*P*	odd>even	% equational	% bivalent	
									Diplotene	Metaphase I
B	*Camnula pellucida*	M	5.30	2.24	125.38	**< 0.0001**	yes	occurs	99	
	Nur, 1969	m	1.35	0.13	95.17	**< 0.0001**	yes			
B	*Locusta migratoria*	M	0					61.8	99.63	97.3
	Cabrero et al., 1984	m	0.25							
B	*Sphingonotus coerulans*	M	12.12	6.35	47.23	**< 0.0001**	yes	occurs		
	Gosálvez et al., 1985	m	0.4	0.29	0.69	0.41				
B	*Cylindrotettix obscurus*	M	0.93	0.72	1.33	0.25		occurs		92.68
	Confalonieri and Bidau, 1986	m	2.95	0.35	84.17	**< 0.0001**	yes			
B	*Aiolopus strepens*	M	3.49	1.61	101.24	**< 0.0001**	yes	no		>90
	Suja et al., 1987	m	0	0						
B	*Dichroplus pratensis*, male no. 2	M	7.83	2.97	267.61	**< 0.0001**	yes	no	98.8	99.1
	Bidau, 1987	m	1.78	0.31	158.39	**< 0.0001**	yes	lagging Bs		
	Dichroplus pratensis, male no. 3	M	17.31	6.01	320.63	**< 0.0001**	yes			
	Bidau, 1987	m	2.49	0.59	73.22	**< 0.0001**	yes			
B	*Dichroplus elongatus* from Tafi Viejo	M	2.25	0.65	62.34	**< 0.0001**	yes			
	Clemente et al., 1994	m	0.94	0.17	36.39	**< 0.0001**	yes			
B	*Dichroplus elongatus* from Raco	M	2.25	2.38	0.25	0.62		lagging Bs	94	
	Clemente et al., 1994; Remis and Vilardi, 1986	m	4.5	1.25	150.94	**< 0.0001**	yes			
Polysomy	*Atractomorpha similis*	m					yes	occurs	100	100
	Peters, 1981									
Polysomy	*Omocestus bolivari*	m	3.34	0.87	10.86	**0.001**	yes		100	100
	Viseras and Camacho, 1984, 1985									
Polysomy	*Chorthippus binotatus*	M	4.71	4.68	0.12	0.73	yes	8.45	96.84	48.44
	Talavera et al., 1990	m	5.66	1.71	43.95	**< 0.0001**				

The occurrence of equational division in anaphase I for the extra chromosomes, when univalents, and bivalent formation, when >1, is also indicated.
M = macrospermatid; m = microspermatid.

microspermatids in males with an unstable B chromosome (Clemente et al., 1994). In Tafi Viejo, the odd-even effect was apparent for both macro- and microspermatids but, in Raco, it was only apparent for the frequency of microspermatids (Table 4).

Male germ-line polysomy

Several cases of germ-line polysomy have been reported in grasshoppers. This kind of chromosome polymorphism is characterised by the presence of extra A chromosomes in the male germ-line and their absence from somatic tissues and females. Intraindividual testis variation for these extra (E) chromosomes is similar to that shown by mitotically unstable B chromosomes, i.e. inter- but not intrafollicular variation. It is thus an appropriate material to compare with unstable B chromosomes.

The first reported case was in the grasshopper *Chorthippus parallelus*. It was exhaustively studied but, unfortunately, none of the papers in the literature (Hewitt and John, 1968, 1970; John and Hewitt, 1969; Westerman, 1969, 1970) contains analyzable information with respect to the odd-even effect. A similar case of polysomy has been found in *Gomphocerus sibiricus* (Gosálvez and López-Fernández, 1981), but no effect was also analysed.

In the case of *Atractomorpha similis*, a clear odd-even effect was apparent from results shown in Fig. 6 of the paper by Peters (Peters, 1981), with follicles bearing odd numbers of E chromosomes showing higher frequency of spermatic micronuclei than follicles bearing even numbers of them. In *Omocestus bolivari*, the frequency of microspermatids followed an odd-even pattern (Fig. 1e and Table 4). In *Chorthippus binotatus*, the frequency of micronuclei showed a clear odd-even pattern, but that of macronuclei failed to show it (Fig. 1f and Table 4).

Discussion

In all traits analysed, the odd-even effect eventually appeared, although its manifestation varied among populations. For instance, it was apparent for chiasma frequency in *L. migratoria* males from Padul but not in those from Gabias, although these latter showed a strong odd-even × male interaction suggesting a significant influence of male genotype on the manifestation of the odd-even effect in this population. The consistency of this result among the two years analysed reinforces this conclusion. The same contradictory results were found in *D. elongatus* for the frequency of macrospermatids, which showed the odd-even effect in Tafi Viejo but not in Raco (see Table 4).

The observation of the odd-even effect for a trait in a species does not seem to imply that it necessarily appears for other traits. In *L. migratoria* from Padul, the odd-even effect was apparent for mean chiasma frequency but not for the number of active NORs. Likewise, in *Dichroplus pratensis*, the odd-

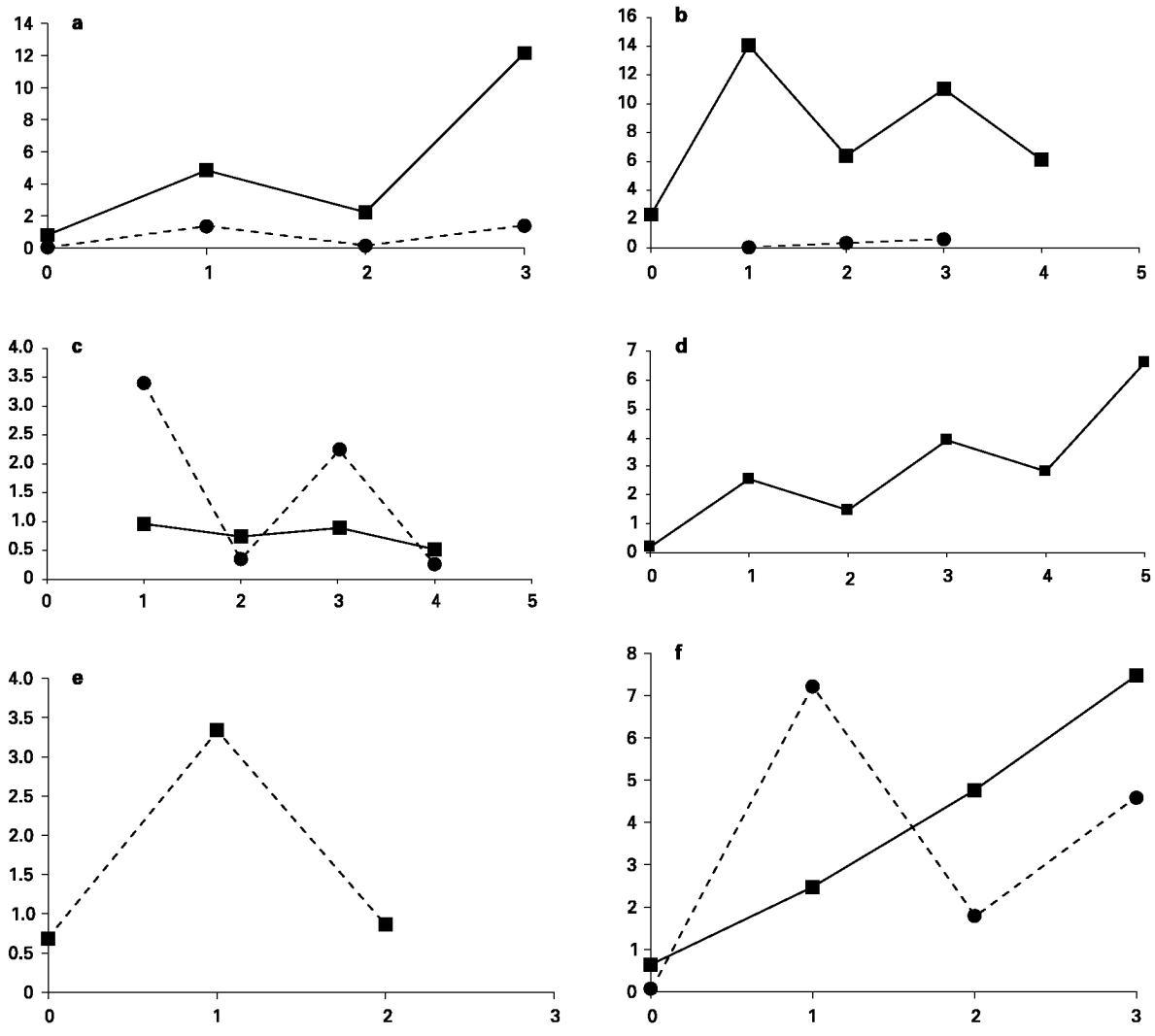

Fig. 1. Frequency (%) of macro- (solid line) and microspermatids (dotted line) (y-axis) in testis follicles with different number of mitotically unstable B chromosomes (x-axis) in (**a**) *Camnula pellucida*, (**b**) *Sphingonotus coerulans*, (**c**) *Cylindrotettix obscurus*, (**d**) *Aiolopus strepens*, and of polysomic A chromosomes in (**e**) *Omocestus bolivari* and (**f**) *Chorthippus binotatus*.

even effect was clearly manifested for AMPs but not for chiasma frequency.

In those cases where the odd-even comparison was significant, the direction of the effect was always consistent with the observation that odd B numbers seem to be more detrimental (Jones and Rees, 1982). For instance, mean chiasma frequency in Padul males of *L. migratoria* was higher in cells with odd B numbers. Bearing in mind that chiasma frequency seems to be a trait sensitive to the presence of parasitic B chromosomes, as predicted by inducible recombination (see Bell and Burt, 1990; Camacho et al., 2003), the odd-even effect seems to indicate that Bs are more perceptible to cells when they are in odd numbers. Likewise, in the case of the number of active NORs in *L. migratoria*, the odd-even effect was manifested by a lower number in cells with odd B numbers, which suggests that these cells might find more difficulty in attending rRNA demands, although information on nucleolus size should be necessary to be confident of this conclusion. Finally, the frequency of AMPs was always higher in the presence of odd B numbers (see Table 4).

No clear explanation is available for the odd-even effect, but in looking for possible causes, we should explore both the trait itself and possible B chromosome behaviour that could cause the odd-even differences. The trait for which more data are available is the formation of AMPs, which showed the odd-even effect in most species reviewed here (see Table 4).

Possible causes for the origin of spermatic macronuclei (and macrospermatids) were first pointed out by Nur (1969): (1) lagging B univalents during meiotic divisions might inhibit cytokinesis to produce restitution nuclei, (2) Bs might promote nuclear or cell fusion and (3) they might simply derive from polyploid spermatogonia. The restitution mechanism would produce only 2C and 4C macrospermatids, whereas cell fusion would yield a continuum of ploidy levels among macrosperma-

tids depending on the number of fused spermatic nuclei. The last event is facilitated by the syncytial nature of testis follicle cysts (Phillips, 1970).

Examples of both kinds of macrospermatid origin seem to exist in the literature. For instance, Gosálvez et al. (1985) measured, by microdensitometry, a sample of spermatids in males of *S. coerulans* carrying unstable B chromosomes and found 1C, 2C and 4C spermatids, as expected from restitution. Consistently, lagging B chromosomes were found in meiotic anaphases. Likewise, Bidau (1986) explained the formation of AMPs in *Metalaptea brevicornis* as a result of effects of lagging Bs on cytokinesis provoking the formation of restitution nuclei (macrospermatids) and micronuclei. In *A. strepens*, however, macrospermatids were found with up to ten centriolar adjuncts, which can only be produced by cell fusion (Suja et al., 1987).

Two aspects of meiotic behaviour of B chromosomes might potentially be associated to the likelihood of macrospermatid formation, depending on B number, and thus might be responsible for the odd-even effect. First, the equational division of B univalents at metaphase I might induce them to lag at anaphase I and thus provoke restitution and the formation of macrospermatids. Second, the formation of B bivalents in spermatocytes with even B number avoids the existence of B univalents that might lag and induce restitution, thus decreasing the likelihood of macrospermatid formation.

In the grasshoppers reviewed here, there seems to be a clear tendency for extra chromosomes, i.e. Bs and polysomic As, to show one or both characteristics favouring the odd-even effect, i.e. equational division of the extra univalents and bivalent formation. As Table 4 shows, one or both effects were found in most species. The odd-even effect, for macrospermatids, was found in *C. pellucida*, *S. coerulans*, *A. strepens*, *D. pratensis* and *D. elongatus*. *L. migratoria* was exceptional since no macrospermatids were found in 1B follicles despite the high frequency of equational division of the B univalents, suggesting that dividing B chromatids do not delay cytokinesis and do not interfere with sperm nuclei individualization within the syncytial testis cysts. Likewise, *C. obscurus* did not show the odd-even pattern for macrospermatids even though the two meiotic characteristics of their Bs were favourable. *D. pratensis* and *A. strepens* might seem to be other exceptions by showing a clear odd-even effect without the equational division of the B but, in *A. strepens*, the available evidence points to macrospermatid origin by cell fusion (Suja et al., 1987), and in *D. pratensis*, Bs were found lagging in about 6% of anaphase II-telophase II cells (Bidau, 1987). As Suja and colleagues pointed out, it is possible that in this species B chromosomes impair the mechanisms that remove the cytoplasmic bridges in the syncytial testis cysts to allow cell and spermatid nucleus fusion. In *C. pellucida* and *D. elongatus*, the odd-even effect for macrospermatids coincides with the occurrence of the two meiotic properties that hypothetically might favour it (see Table 4).

The appearance of microspermatids, or spermatic micronuclei, has been explained by the loss of lagging extra univalents (or chromatids derived from their equational division) (Nur, 1969). Therefore, their frequency should be associated with the same two aspects of extra chromosomes on meiotic behaviour. Table 4 shows that, in most cases where the odd-even pattern was found for microspermatids, the two meiotic properties were met. The clearest cases were *C. pellucida*, *C. obscurus*, *D. elongatus*, *A. similis* and *C. binotatus*. Again, *L. migratoria* was exceptional in showing a very low frequency of microspermatids in spite of a high frequency of B equational division. On the contrary, the absence of microspermatids in *A. strepens* was logical since the Bs were never observed dividing equationally (Suja et al., 1987). In *D. pratensis*, on the contrary, the odd-even effect was present for microspermatids even though the B was never seen dividing equationally (Bidau, 1987).

Out of the 11 cases of the odd-even effect for AMPs reported in Table 4, seven were for microAMPs and four for macroAMPs. This might reflect a possible closer relationship between the odd-even effect for microspermatids and the two mentioned aspects of extra chromosomes meiotic behaviour. Although data are scarce, it seems that polysomic A chromosomes show a high tendency to manifest the odd-even effect for microspermatids, since all three cases tested did (see Table 4). In the three cases, a very high frequency of bivalent formation was found, and in the two species where it was analysed (*A. similis* and *C. binotatus*) the extra univalents sometimes divided equationally. If these extra As were candidates to become B chromosomes, then Bs might have a tendency to show the same meiotic behaviour from their inception. In fact, Table 4 shows a high resemblance in meiotic behaviour of extra As and Bs.

Another interesting point to discuss is the influence of AMP formation in male fertility. In theory, we should expect that macrospermatids would be more influential than microspermatids, since the first imply the loss of whole genome sets whereas the latter perhaps only of the extra chromosomes. But this last assertion might not be always true, since micronuclei can also include A chromosomes, as was shown by Chiavarino et al. (2000), in tapetal cells from maize plants, by FISH with a collection of DNA probes specific for Bs and some As. It is thus conceivable that a certain proportion of the observed microAMPs in the mentioned grasshopper species actually contain lost A chromosomes, with the subsequent aneuploidy generated in some of the apparently normal spermatic products. Anyway, the frequency of macroAMPs found in the cases reviewed in Table 4 (up to 12.12%) tended to be higher than that of microAMPs (up to 5.66%).

The odd-even effects revealed here might result from the stressing effects of B chromosomes. It is known that the incidence of recombination is lowest under optimal conditions and increases as the environment becomes more stressful (Hoffman and Parson, 1991). Recently, Kovalchuk et al. (2003) have provided evidence suggesting the existence of a systemic signal increasing the frequency of homologous recombination in tobacco plants infected with either of two different viruses. The putative molecular signals being responsible for this effect are actually unknown, but the production of the same effect in non-infected tissues from infected plants and the transmission of the effect by grafting free-of-virus leaves from infected plants to healthy non-infected plants, clearly point to their existence. The authors suggest that it might constitute an adaptive response to biotic stress, since this increase in recombination

may provide new specificities in pathogen resistance genes. This meets all expectations of the Red Queen Hypothesis (Van Valen, 1973), which predicts that coevolutionary interactions between parasites and hosts may select for an increase in host sex and recombination resulting in genetically different progeny with an expected lower risk of being infected (Bell, 1982). Such an increase in recombination was named "Inducible Recombination" by Bell and Burt (1990) to explain the increase in chiasma frequency associated with the presence of parasitic B chromosomes. They suggested that parasitized individuals should show greater rates of recombination than unparasitized individuals as a result of selection for genes that increase the rate of recombination only when "some stimuli associated with parasite activity are detected." Strong support for Inducible Recombination came recently from the demonstration that the degree of increase in chiasma frequency depends on the strength of the parasitic B-chromosome attack (Camacho et al., 2002), and Kovalchuk et al. (2003) have shown the existence of a possible "stimuli" for Inducible Recombination. Effects on chiasma frequency might thus be interpreted as a host response to the stress added by parasitic B chromosomes (Bell and Burt, 1990; Camacho et al., 2003), and the odd-even effect for this trait clearly suggests that odd numbers might cause more stress.

At first sight, the existence of a systemic stimulus for B chromosome effects might appear incompatible with the intraindividual odd-even effect observed for mitotically unstable Bs. The stimulus hypothesis would predict that 0B follicles in mosaic males should show B effects even lacking them. This has been tested in two cases. In *Sphingonotus coerulans*, no significant difference was found for the frequency of macrospermatids between 0B males (1.91%) and 0B follicles from B-carrying males (2.32%) (Gosálvez et al., 1985) (contingency $\chi^2 = 0.64$, $P = 0.42$). In *Dichroplus elongatus*, however, a significant increase in the frequency of abnormal spermatids was apparent even in 0B follicles from B-carrying males. For instance, Clemente et al. (1994) reported that, in the Tafi Viejo population, these 0B follicles showed about double frequency of macro- (0.71%) and microspermatids (0.086%) than 0B males (0.3% and 0.047%, respectively). In the Raco population, the frequency of macrospermatids in 0B follicles from B-carrying males was more that ten times higher than that observed in 0B males. Loray et al. (1991) and Clemente et al. (1994) claimed that physiological effects of B chromosomes may explain these increases in testis subunits lacking Bs. Such physiological effects might have much to do with the systemic signal uncovered by Kovalchuk et al. (2003), and the odd-even effect might be the result of additional effects modulated by B presence.

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