B chromosomes and genome size in flowering plants

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Abstract: B chromosomes are extra chromosomes found in some, but not all, individuals within a species, often maintained by giving themselves an advantage in transmission, i.e. they drive. Here we show that the presence of B chromosomes correlates to and varies strongly and positively with total genome size (excluding the Bs and corrected for ploidy) both at a global level and via a comparison of independent taxonomic contrasts. B chromosomes are largely absent from species with small genomes; however, species with large genomes are studied more frequently than species with small genomes and Bs are more likely to be reported in well-studied species. We controlled for intensity of study using logistic regression. This regression analysis also included effects of degree of outbreeding, which is positively associated with Bs and genome size, and chromosome number, which is negatively associated with Bs and genome size, as well as variable ploidy (more than one ploidy level in a species). Genome size, breeding system and chromosome number all contribute independently to the distribution of B chromosomes, while variable ploidy does not have a significant effect. The genome size correlates are consistent with reduced selection against extra DNA in species with large genomes and with increased generation of B sequences from large A genomes.

Key words: B chromosomes, genome size, selfish genetic elements, breeding system, ploidy.

Résumé : Les chromosomes B sont des chromosomes surnuméraires qui peuvent se trouver chez certains, mais pas tous, les individus d'une espèce. Ils sont souvent maintenus grâce à un biais favorable à leur transmission. Les auteurs montrent que la présence de chromosomes B covarie fortement et positivement avec la taille totale du génome (excluant les chromosomes B et corrigé pour la ploïdie) tant globalement que via la comparaison de contrastes taxinomiques indépendants. Les chromosomes B sont pratiquement absents chez les espèces à petit génome. Cependant, les espèces à grand génome sont davantage étudiées que celles à petit génome et il est plus probable que des chromosomes B aient été rapportés chez des espèces qui ont été étudiées davantage. Les auteurs ont tenu compte de ce facteur, l'intensité de la recherche, à l'aide d'une régression logistique. Cette analyse de régression tenait également compte du degré d'allofécondation (un paramètre qui est également associé positivement à la présence de chromosomes B et à la taille du génome), du nombre de chromosomes (un facteur corrélé négativement avec la présence de chromosomes B et à la taille du génome), de même que de la ploïdie variable (plus d'un niveau de ploïdie au sein d'une espèce). La taille du génome, le mode de reproduction et le nombre de chromosomes contribuent indépendamment à la distribution des chromosomes B, tandis qu'un niveau de ploïdie variable n'a pas d'effet significatif. Les corrélations avec la taille du génome suggèrent qu'il existe une moindre pression de sélection à l'encontre d'ADN excédentaire chez les espèces à grand génome et une tendance accrue à la production de séquences B à partir de génomes A de grande taille.

Mots clés : chromosomes B, taille du génome, éléments génétiques égoïstes, système de reproduction, ploïdie.

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Introduction

A major unresolved problem in the study of intragenomic conflict is what controls variation in the frequency of selfish elements across taxonomic units. For some categories of selfish elements we lack all but rudimentary evidence regarding relative frequency. For autosomal drivers, our data points are far too few to permit measures of frequency (evidence being largely limited to four species of *Mus*, one of *Drosophila*, and three of *Neurospora*; Lyttle 1991). We are slightly better off where X–Y drivers are concerned, but can say little more than that X drive has frequently appeared in *Drosophila* (14 times, Jaenicke 2001), stalk-eyed flies (7 species, Lande and Wilkinson 1999), and lemmings (4 species; Bull and Bulmer 1981; Malcolm et al.1986; including sometimes Y drive, Gileva 1987) and that Y drive is known mostly from mosquitoes (Wood and Newton 1991). For small elements such as homing endonucleases, we now have estimates of rates of appearance via horizontal gene movement (which are substantial), as well as rates of subsequent degeneration (Cho et al. 1998; Goddard and Burt 1999; Koufopanou et al. 2002). Transposable elements are nearly

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universal in eukaryotes and substantial evidence is available on the frequency of different categories for a few select species (e.g. DNA transposons relatively more common in *Drosophila* than in humans), but little systematic work has been done reviewing relative frequency across major taxonomic units (Smit 1999; Arkhipova and Meselson 2000). In one striking case, the maize genome appears to have doubled in size since its split with the sorghum lineage about 17 million years ago, largely because of the repeated (and mostly nested) retrotranspositions of elements from more than 10 families in the last 6 million years (SanMiguel and Bennetzen 1998; SanMiguel et al. 1998).

B chromosomes provide special opportunities for studying the frequency of selfish genetic elements across a wide range of species, because they are known from more than 1300 species of plants and almost 500 species of animals, as well as several species of fungi (for excellent recent reviews, see Jones 1995; Camacho et al. 2000; Puertas 2002; the standard text is Jones and Rees 1982). B chromosomes are extranumerary chromosomes found in some individuals within a species, but not in all. By definition then, they are unnecessary for normal development. Much evidence suggests that their usual effect on the phenotype is negative, certainly so at higher numbers of Bs per individual (Jones and Rees 1982), and that they are often maintained by drive, i.e., they give themselves a selfish benefit in replication (evidence reviewed in Jones 1985, 1991).

As emphasized by Jones and Rees (1982), the chief obstacle to our ability to make comparative statements about B frequency is the very different degree to which different evolutionary lineages have been subject to cytogenetic study. Species with well-described karyotypes not known to harbor Bs presumably often lack them, but better studied species are obviously more likely to yield sightings of B chromosomes. No one has presented estimates of B frequency within groups corrected for intensity of chromosomal study. A major goal of the present study is to make up for this deficiency, at least regarding the variables we measure (genome size, chromosome number, and ploidy). That is, we measure "intensity of study effort" and then correct for it.

By concentrating on one well-studied flora, British flowering plants, Burt and Trivers (1998) showed that B chromosomes are largely limited to outbred species, as expected (Bell and Burt 1990). Using the same date set, we show that across 353 species of British flowering plants existence of B chromosomes is much more likely in species with large genomes (A chromosomes, measured as picograms of DNA in 4C cells, where C represents one complete haploid genome). This holds true under independent taxonomic contrasts and when corrected for intensity of study and ploidy. We also control for the degree of outbreeding, which is itself positively associated with genome size across all species (Govindaraju and Cullis 1991; this study), but shows no association in an independent taxonomic units test (this study). A subsidiary finding is that B chromosomes are inversely associated with chromosome number.

Materials and methods

We used the data set compiled by Burt and Trivers (1998) for the presence and absence of B chromosomes in British flowering plants (and also the breeding system). Information on B chromosomes was obtained from the B chromosome atlas in Jones and Rees (1982), which lists all species reported to have Bs. Our data set excludes species reproducing vegetatively or apomictically because, without meiosis, it can be difficult to distinguish Bs from fragmented As. Such species are included when considering the relationship between breeding system and genome size, independently of B status. Data on 4C genome size was collated from published compilations (Bennett and Smith 1976, 1991; Bennett et al. 1982; Bennett and Leitch 1995, 1997; Bennett et al. 1998) and a database on the World Wide Web (Bennett et al. 1998; now located at http://www.rbgkew.org/uk/cval/homepage.html). Data on genome size was available for 12 additional species in Grime et al. (1988) (Grime et al. report only 2C DNA amounts, which we have doubled to give 4C DNA). More than 90% of these studies used Feulgen densitometry to measure picograms of DNA in given cell types. If more than one estimate of genome size exists for a species, the mean value was used. Bennett and colleagues eliminated any estimates that were clearly erroneous and for >75% of species only one genome size is given. Data on genome size was obtained for 226 species, of which 41 (18.1%) are reported to have B chromosomes (complete data set available from the authors).

In many cases, genome size was reported without data on ploidy and (or) number of A chromosomes. Information on ploidy and chromosome number was obtained from Darlington and Wylie (1956), Grime et al. (1988), and *Biological Flora of the British Isles*, published in the *Journal of Ecology* (volumes 42–88, 1952–2000). In cases where there was ambiguity owing to variable or unknown ploidy within a species, that data point was excluded from analyses of genome size when corrected for ploidy. Variable ploidy refers to chromosomes races or cytotypes characterized by ploidy. We also analyzed chromosome number and ploidy, independent of genome size, among species with and without B chromosomes. Chromosome number data was obtained for 364 species in our data set. Owing to intraspecific variation in chromosome number, we performed separate analyses using the smallest and largest values reported. Ploidy is known for 349 of these species, 47 of which have B chromosomes.

To verify that our measure of study intensity, the number of published estimates of genome size, is correlated with intensity of chromosomal study, we performed a computer search for karyotypic studies in subsample of 25 species from our data set. Of the species for which we have information on genome size, we randomly selected 10 species with B chromosomes and 10 without. We also added five additional species with no estimates of genome size (one had Bs). For each species, we simultaneously searched three Ovid databases (MEDLINE, 1966 to present; Wilson Biological and Agricultural Index, 1983 to present; BIOSIS Previews, 1990 to present) for papers containing some form of the word "karyotype" in the title, abstract, or keywords. We then recorded the number of studies found, after checking titles for relevancy and subtracting any duplication among databases. The number of studies of genome size is significantly correlated with the number of karyotype studies found in the search (Spearman rank correlation, $Z = 2.74$, $P < 0.01$, $\rho = 0.56$). The species with the largest number of ge-

nome size studies also had the largest number of karyotype studies, whereas only one of the five species lacking genome size estimates had a karyotype study uncovered by this search. Thus, we feel that the number of studies of genome size does give an indication of the relative intensity of cytogenetic study among species.

To truly quantify the number of karyotypic studies for each species in our dataset would be an enormous task. For example, Darlington and Wylie's *Chromosome Atlas of Flowering Plants* was published in 1956 and lists only the most "recent" references, except in cases of disagreement among authors, yet this compilation has over 2400 references. The computer search described above clearly could not uncover all relevant references, since the oldest database searches back only to 1966, and most of the references that we found were on BIOSIS, which extends back only to 1990. Our data on the number of studies of genome size are therefore much more accurate than any readily available estimate of the number of karyotypic studies, since genome size compilations include nearly all published estimates of genome size until 1998.

Because we found that Bs were more likely to be reported in well-studied species, we controlled for intensity of study using logistic regression. This regression analysis also controlled for correlations with degree of outbreeding, chromosome number, and variable ploidy (where the latter refers to more than one race or cytotype that differ by ploidy). Statistical analyses were performed using StatView (SAS Institute 1999).

In addition to analyses of the entire data set, we controlled for effects of phylogeny, using the method of independent contrasts (Felsenstein 1988; Burt 1989). We based these contrasts on Clapham et al.'s (1962) taxonomy of British plants, updated with more recent taxonomic information (Takhtajan 1997).

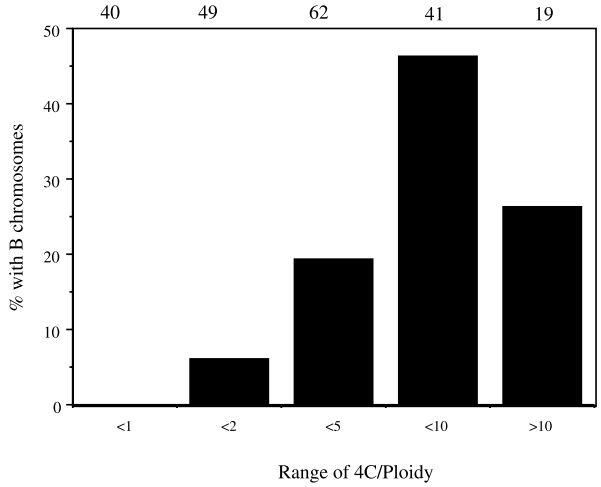
Results

B chromosomes and genome size

The average 4C genome size of 41 species with B chromosomes (mean ± SE = 18.62 ± 1.93 pg) is about 60% larger than that of 185 species without B chromosomes (11.57 ± 1.18 pg), a highly significant difference (Mann–Whitney U test, $Z = 4.93$, $P < 0.0001$). However, genome size is obviously affected by ploidy level, and throughout the rest of this paper we correct genome size for ploidy, using 4C genome size divided by ploidy. The relationship between genome size and B chromosomes remains highly significant when genome size is corrected for ploidy. Mean 4C/ploidy for species with Bs is 6.21 ± 0.58 ($N = 39$), versus 4.03 ± 0.52 ($N = 172$) in species lacking Bs ($Z = 5.41$, $P < 0.0001$). B chromosomes are completely absent from the species with the smallest genomes (Fig. 1).

There were 22 independent taxonomic contrasts (16 genera, 4 tribes, 1 subfamily, and 1 family) with variation in Bs and data for genome size: 15 comparisons were in the predicted direction, 6 in the opposite, and one tie. This is significant with a one-tailed sign test ($P < 0.04$; Table 1). The relationship between reported B chromosome presence and genome size also holds up when controlled for intensity of cytogenetic study (see below).

Fig. 1. Percentage of species reported to have B chromosomes across ranges of genome size (4C/ploidy). Numbers above the figure indicate sample size (number of species).



However, there are differences among taxa in the relationship between B chromosomes and genome size. Our data set is large enough to compare genome size among dicots and monocots separately, and within two families, Compositae and Gramineae. For composites and dicots in general, species with B chromosomes have significantly larger genomes (Table 2), but this relationship is not significant for grasses (Gramineae), and monocots in general show a significant effect in the other direction (Table 2). The negative relationship between genome size and Bs among monocots seems to be largely the result of there being only one monocot with Bs outside of the grasses in our data set, whereas several non-grass monocots have large genomes.

Monocots have, on average, larger genomes than dicots, whether corrected for ploidy (6.79 ± 1.20, $N = 64$ vs. 3.40 ± 0.33, $N = 147$; Mann–Whitney U test $Z = 4.06$, $P < 0.0001$) or not (19.39 ± 2.43, $N = 73$ vs. 9.57 ± 0.87, $N = 160$; $Z = 4.50$, $P < 0.0001$).

Study effort

Because B chromosomes are not present in all individuals or populations of a species, it is possible that differences in the distribution of Bs could result from differences in intensity of study. We used the number of published estimates of genome size of a species as an index of intensity of cytogenetic study. The frequency of species reported to have B chromosomes increases steadily as the number of studies of genome size increases (Fig. 2). Bs are therefore increasingly more likely to be reported as species are increasingly well-studied. Part of this correlation may also result from researchers focusing on species in which Bs are known to occur. The relationship between B chromosomes and intensity of study holds up after analysis by independent contrasts (15 positive, 6 negative, 3 ambivalent, 55 ties; 15 vs. 6, one-tailed sign test, $P = 0.04$; data not shown).

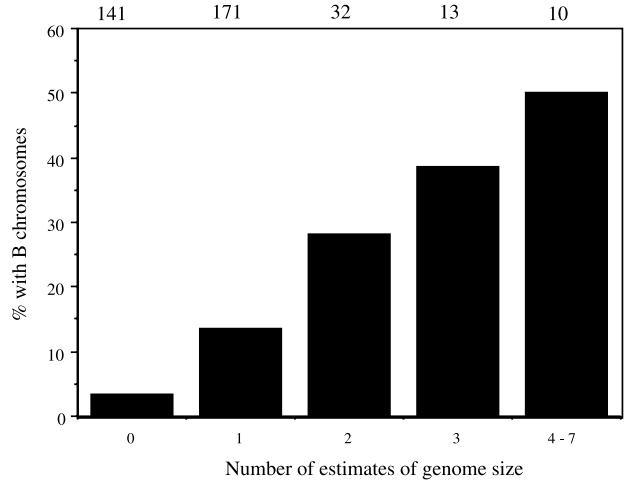
Species with large genomes and (or) few chromosomes are easier to study than species with many small chromosomes, and thus may be preferentially studied. Therefore, the relationships that we found between B status, genome size, and chromosome number (see below) could be confounded by correlations with intensity of study. Genome size

Table 1. Independent contrasts: 4C/ploidy for species with and without B chromosomes.

Taxon	With Bs	*N**	Without Bs	*N*	Difference
Genera					
Agrostis	7.00	1	3.49	2	+3.51
Alopecurus	6.80	1	8.07	2	–1.27
Avenula	10.10	1	11.97	1	–1.87
Bromus	5.74	1	13.61	3	–7.87
Centauria	3.55	1	1.80	1	+1.75
Deschampsia	9.00	1	5.48	1	+3.52
Festuca	6.32	3	3.61	1	+2.71
Holcus	3.63	1	3.03	1	+0.60
Lamium	2.20	1	2.20	1	0.00
Leontodon	5.28	1	2.85	1	+2.43
Luzula	3.60	1	0.18	1	+3.42
Papaver	7.05	1	5.47	2	+1.58
Plantago	2.50	1	1.37	2	+1.13
Ranunculus	13.25	3	6.56	4	+6.69
Rumex	3.30	1	1.94	3	+1.36
Trifolium	1.27	1	1.21	3	+0.06
Tribes					
Anthemideae	6.23	3	8.16	4	–1.93
Cichorieae (minus No. 10)	5.85	2	2.18	5	+2.18
Phleeae (minus No. 2)	3.09	1	3.98	1	–0.89
Poeae (minus Nos. 1, 3, 6, 7, 8)	6.39	9	5.22	8	+1.17
Subfamilies					
Rhinanthoideae	7.90	1	1.62	4	+6.28
Families					
Ranunculaceae (minus No. 14)	9.43	1	21.60	1	–12.17

Note: There are 21 comparisons (22 in total, but 1 tie): 15 positive, 6 negative; one-tailed sign test, $P = 0.04$.
*N, number of species.

Fig. 2. Percentage of species reported to have B chromosomes across ranges of intensity of cytogenetic study (number of published studies of genome size). Numbers above the figure indicate sample size (number of species).



is positively correlated with the number of studies of genome size (Spearman rank correlation, $N = 218$, $Z = 2.73$, $P = 0.0064$, $\rho = 0.19$), indicating that species with large genomes are preferentially studied. However, this relationship does not hold for independent contrasts (11 positive, 18 negative, 3 ambiguous; data not shown), and the genome size of species with B chromosomes varies very little with intensity of study. We control for potential biases owing to intensity of study with logistic regression (see below).

Table 2. B chromosomes and genome size (4C/Ploidy) within taxa.

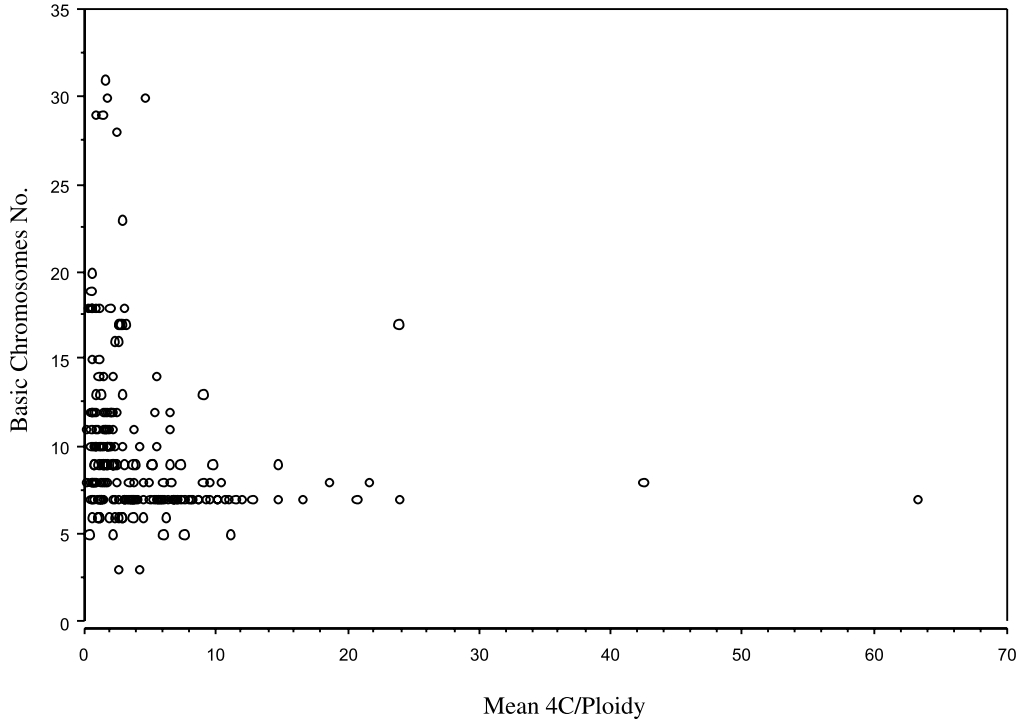
Taxon	Mean	*N*	SE	*Z* value*	Probability
Dicots					
With Bs	6.14	19	0.99	4.15	<0.0001
No Bs	3.00	128	0.33		
Monocots					
With Bs	6.27	20	0.63	2.03	0.043†
No Bs	7.03	44	1.73		
Compositae					
With Bs	5.60	7	0.84	2.46	0.014
No Bs	3.79	19	0.71		
Gramineae					
With Bs	6.41	19	0.65	1.47	0.14
No Bs	5.62	28	0.88		

*Mann–Whitney *U* test.
†Opposite predicted direction.

B chromosomes and ploidy

There is a nearly significant negative effect of ploidy on presence of B chromosomes. Excluding species with more than one reported ploidy level, species with Bs have a mean ploidy of 2.4 ± 0.21 ($N = 22$), whereas species without Bs have a mean of 3.0 ± 0.13 ($N = 204$) (Mann–Whitney *U* test,

Fig. 3. Basic chromosome number (x) versus genome size (4C/ploidy). If more than one value for chromosome number has been reported, the minimum value is used. Spearman rank correlation, $Z = 5.40$, $N = 210$, $P < 0.0001$, $\rho = 0.37$.



$Z = 1.94$, $P = 0.053$). Even if real, the effect is very modest. Excluding species with reports of both diploidy and polyploidy, 18 of 26 species with Bs are diploid (69.2%) compared with 135 of 236 species without Bs (57.2%) ($\chi^2_1 = 0.94$, $P = 0.33$).

Variable ploidy (the presence of more than one published ploidy level for a species) has no effects in the logistic regression (see below), although, considered alone, it strongly predicts B frequency. Species with more than one ploidy are more likely to shelter Bs (20.3% of 123 species) than species with only one ploidy (9.7% of 226 species) ($\chi^2_1 = 6.79$, $P = 0.0092$). However, this result is probably entirely an effect of intensity of study, because alternate ploidy levels and B chromosomes are both more likely to be detected in well-studied species (data not shown).

Genome size and breeding system

There is a strong positive effect of degree of outbreeding on genome size ($P = 0.0013$; see Table 3). However, a comparison of independent taxonomic units fails to reveal any pattern (17 positive, 20 negative, 1 tie, and 3 ambiguous; data not shown). Because both B chromosome presence (Burt and Trivers 1998) and genome size are positively associated with degree of outbreeding, we control for possible confounding effects with logistic regression analysis (see below).

Chromosome number and B chromosomes

B chromosomes are more likely in species with few A chromosomes. Minimum reported chromosome number is significantly higher in 318 species lacking Bs (30.6 ± 0.98) than in 46 species with B chromosomes (18.59 ± 1.29) (Mann–Whitney U test, $Z = 5.66$, $P < 0.0001$). However, independent taxonomic contrasts are not significant (17 positive, 9 negative, 3 ties; two-tailed sign test, $p = 0.168$; data not shown). Variation in maximum chromosome number is in the same direction, but is nonsignificant (40.6 ± 1.45 vs. 36.6 ± 3.27; $Z = 1.34$, $P = 0.18$). Genome size is also inversely related to chromosome number (Vinogradov 2001; Fig. 3), so we include chromosome number in the logistic regression analysis (see below).

Logistic regression analysis

To control for the potentially confounding effects of study intensity on the relationship between B chromosome presence and genome size and between B presence and ploidy variation, we performed multiple logistic regression. This regression analysis also included effects of degree of outbreeding and chromosome number. B status was the dependent variable and log-transformed 4C/ploidy, breeding system (selfing + "mixed" vs. outcrossing), minimum reported chromosome number, presence or absence of multiple reported ploidy levels, and the number of estimates of genome size were the independent variables (whole model log likelihood = –66.91, $r^2 = 0.33$, $\chi^2_5 = 66.53$, $P < 0.0001$). Logistic model coefficients are given in Table 4. Logistic likelihood ratio tests indicate that genome size, degree of outbreeding, and chromosome number all have significant, independent effects on the likelihood of B presence (Table 4), whereas the effects of study intensity and variable ploidy are not statistically significant (Table 4).

Discussion

Study effort

Our first discovery is a methodological one. Amount of effort devoted to research on genetics — study effort — has

Table 3. Effect of breeding system on genome size (4C/ploidy).

Breed	Mean	*N*	SE
Asexual	1.7	6	0.62
Selfing	3.9	31	2.00
Mixed	4.1	107	0.42
Outcrossing	5.2	70	0.77

Note: Kruskal–Wallis test, $H_3 = 15.66$, $P = 0.0013$.

a strong positive influence on the chance that a species will be described as possessing B chromosomes. We used the number of published studies of genome size of a species as an index of study intensity. Since species with large genomes are studied genetically more often (as measured by frequency of published estimates of genome size), study effort alone will generate a positive correlation between genome size and frequency of B chromosomes. This bias may be considerable and suggests that comparative work on the presence or absence of B chromosomes should routinely try to include some correction for study effort. When all data are taken together in a regression analysis, study effort drops out as having a significant effect, but just barely so. We therefore do not pretend that our correction is fully adequate. Something like "amount of cytogenetic study effort" would have been closer to our needs, but more difficult to quantify. Researchers studying chromosomes may be more likely to focus on species with large chromosomes (and thus large genomes) than researchers measuring genome size. Multiple ploidy levels are also more likely to be reported as study effort increases, and multiple ploidy disappears as a factor affecting B presence, when corrections are made for study effort (and the other variables: Table 4).

Genome size and Bs

Our most striking finding is a strong positive association between genome size and presence of B chromosomes, especially when corrected for ploidy, across plant species as a whole and in a comparison of 22 independent taxonomic contrasts. It is noteworthy that this correlation does not hold for monocots, but is very highly significant in the dicots. We have no idea why this is true, though monocots have larger genomes, on average, than dicots (Vinogradov 2001; this study). It may be significant that B chromosomes in grasses typically show drive at the 1st pollen grain mitosis, whereas many Bs in dicots show drive at female meiosis (Jones and Rees 1982). The latter is more likely to show centromeric drive.

The simplest explanation for the association between B chromosomes and genome size is that relaxed selection against large genome size also means weaker selection against Bs (owing to reduced phenotypic costs). Whether larger genomes also mean that individual Bs are relatively smaller (and, therefore, less costly) is unknown without a study of B chromosome size across the same species. The same can be asked for species with few chromosomes: are individual Bs the size of the smallest A chromosome or larger? Or, are they sometimes smaller fragments? For plants in general, fully one half of all B chromosomes are smaller than the smallest As and half of these are microchromosomes (Jones 1995).

Larger genomes may also donate more new B chromosomes. Almost all between-species variation in genome size is accounted for by variation in non-coding DNA, which, in turn, appears to be a major constituent of B chromosomes (Puertas 2002). Thus, it is possible that more A chromosomal DNA means more repetitive DNA is donated to incipient Bs, increasing their chance of propagation.

One possible force generating our correlation is differential rates of DNA excision, a factor recently emphasized for genome size by Petrov (2001). Natural rates of DNA excision appear to be 40 times higher in the small-genomed *Drosophila* than in the relatively large genome of a cricket (Petrov et al. 2000). Imagine that rates of excision against B DNA proceed at the same rate as excision of extraneous A DNA. In large-genomed species, B chromosomes will probably initially be large but, in any case, subject to low rates of gene excision, just as are the As. Bs will endure in large-genomed species. In small-genomed species, B may initially begin small but, in any case, will be subject to high rates of gene excision. They will tend to be reduced more quickly to the smallest size consistent with drive. Thus, species with high rates of excision will have especially tiny B chromosomes, which, in turn, are the least likely to be detected The question then becomes, what determines differences between species in rates of genome loss, a question for which we have no answer.

The only studies co-varying B chromosome frequency and genome size *within* a species were both conducted with maize. Rosato et al. (1998) show that Bs and genome size correlate oppositely with latitude and, thus, with each other. On the other hand, Porter and Rayburn (1990) found no correlation between B chromosomes and genome size, altitude, or number of C bands.

Genome size and breeding system

It is noteworthy that although outbreeding is associated across all species with increased genome size and, separately, with presence of B chromosomes, when using independent taxonomic contrasts, only the association with B chromosomes remains significant (Burt and Trivers 1998). One possibility is that the mostly within-genus contrasts of this test compare species that have deviated in breeding system only recently (between 100 000 and several million years). This is plenty of time to select against B chromosomes, which are dispensable, but genome size of the A chromosomes may be much more difficult to reduce, especially in species like crickets, which have 40-fold lower rates of removal of retrotransposons than *Drosophila* (Petrov et al. 1996, 2000).

Ploidy

Jones and Rees (1982, p. 14) found no evidence for an association between Bs and ploidy level. They compared the proportion of polyploid species with Bs with the frequency of polyploidy in general. We also find no significant relationship between B chromosome presence and whether a species is polyploid or diploid, but there is a nearly significant trend toward species with Bs having, on average, slightly lower ploidy levels than those without Bs ($P = 0.053$).

Table 4. Logistic model coefficients and logistic likelihood ratio tests for genome size, breeding system, chromosome number, study intensity, and variation in ploidy.

				Logistic likelihood ratio tests		
	Coefficient	SE	Partial *r*	χ^2	d.f.	Probability
Intercept	1.79	0.90	–0.10			
Log (4C/ploidy)	1.55	0.54	0.18	9.48	1	0.002
Breeding system	1.67	0.45	0.24	14.83	1	0.0001
Chromosome no.	–0.09	0.03	–0.19	13.11	1	0.0003
No. estimates	0.30	0.21	0.00	2.10	1	0.15
Variable ploidy?	0.36	0.44	0.00	0.68	1	0.41

Note: Breeding system entered as selfing + mixed vs. outcrossing and chromosome number as minimum reported number of chromosomes.

That ploidy may be (slightly) negatively associated with Bs while other sources of genome size are positively associated suggests two things. First, that the act of increasing ploidy does not in itself select for Bs. Second, that it could actually lead to the loss of Bs. Ploidy can be increased in a single individual in one generation. There is a slight chance that this event might lead to the disappearance of a B chromosome in the following manner. Imagine a B that shows drive when univalent but no drive when bivalent (a not uncommon occurrence). Doubling ploidy for an individual with one B will result in progeny with two Bs that pair, may form chiasmata, and show no drive, exactly as expected of A chromosomes. Is it possible that such an entity would permit reactivation of silenced genes or be invaded by functional genes from elsewhere, so as to become a normal, functioning A chromosome? Perhaps only recently split-off B chromosomes would qualify. A related proposal for the way in which a B chromosome may be incorporated into the A genome has recently been made for a haplodiploid species (Araujo et al. 2001).

Chromosome number

J.P.M. Camacho (personal communication) has suggested to us that species with many small chromosomes may have evolved stronger meiotic systems for the correct separation and transmission of chromosomes, thus making selection against B chromosomes easier. This seems to us especially likely if B chromosomes are small relative to As in large-genomed species.

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