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The distribution of B chromosomes across species

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Abstract. In this review we look at the broad picture of how B chromosomes are distributed across a wide range of species. We review recent studies of the factors associated with the presence of Bs across species, and provide new analyses with updated data and additional variables. The major obstacle facing comparative studies of B chromosome distribution is variation among species in the intensity of cytogenetic study. Because Bs are, by definition, not present in all individuals of a species, they may often be overlooked in species that are rarely studied. We give examples of corrections for differences in study effort, and show that after a variety of such corrections, strong correlations remain. Several major biological factors are associated with the presence of B chromosomes. Among flowering plants, Bs are more likely to occur in outcrossing than in inbred species, and their presence is also positively correlated with genome size and negatively with chromosome number. They are no more frequent in polyploids than in diploids, nor in species with multiple ploidies. Among mammals, Bs are more likely to occur in species with karyotypes consisting of mostly acrocentric chromosomes. We find no evidence for an association with chromosome number or genome size in mammals, although the sample for genome size is small. The associations with breeding system and acrocentric chromosomes were both predicted in advance, but those with genome size and chromosome number were discovered empirically and we can offer only tentative explanations for the very strong associations we have uncovered. Our understanding of why B chromosomes are present in some species and absent in others is still in its infancy, and we suggest several potential avenues for future research.

B chromosome research is presently focused on two main areas of investigation, molecular organization and transmission genotypes (for review see Camacho et al., 2000; Puertas, 2002; Jones and Houben, 2003). Interest is centered around the idea of host-parasite interaction between selfish Bs and the host genome, and on the origin and evolution of Bs, especially as analyzed at the molecular level. The earlier phases of work dealt more with the occurrence of Bs in various species, modes of inheritance, effects and ecological and adaptive significance in populations (Jones and Rees, 1982). This extensive phase of research, covering many species and many decades, provided the base of knowledge about B chromosomes, and the platform on which the more recent transmission genetics and molecular studies are now being built.

With rare exceptions, attempts to find an adaptive value for Bs at the level of the individual ran into virtual dead-ends and attention was redirected toward two areas. One was the co-evolution of the host-parasite relationship itself and the other was a description of sequence organization on Bs. We have also reached the point now where most studies involve only a handful of species, and for the rest we are leaving behind many unanswered questions. Despite the vast body of knowledge which we now have on Bs within species, we have as yet hardly touched on the question of what factors determine the distribution of B chromosomes across different species. Why do some species and groups of species carry Bs while others do not? Is there some innate property of a genome, or a breeding system, or a taxonomic group, or a karyotype, for instance, which determines whether a species is likely to carry Bs or not? Here we review what is known about the distribution of Bs across species, with special attention to correcting for variation in the intensity with which groups are studied cytogenetically. In addition to reviewing the available studies—which suggest that major genetic and social variables are associated with the distribution of Bs, we also present new analyses using updated data and incorporating additional variables.

The questions asked here are appropriate for all classes of selfish genetic elements, which are often maintained despite phenotypic costs by transmission at higher than Mendelian frequencies, but Bs are particularly well suited to answer them. Being so easily visible under the microscope, they have been studied for nearly a century and are known for a large number of species (N~2000) across a broad range of taxonomic groups (Jones and Rees, 1982; Camacho et al., 2000).

The major problem confronting any study of the frequency of B chromosomes across species is that, by definition, they are not present in all individuals of a species. Nor are they always present in all populations—nor all tissues within an individual, e.g., root cells, themselves often used for karyotypic work (Chen et al., 1993) or stems and leaves (Wu, 1992). Due to this variability in B presence among populations, individuals, and tissues, and also due in part to differences among taxa in the ease of chromosomal study, we do not know that a species with no reports of Bs truly lacks them. Bs are especially likely to be overlooked if a karyotype is based on a single individual, which was once true of 17,000 plant species (Darlington and Wylie, 1956). Study intensity (and ease of cytogenetic study) likely also contributes to the apparent distribution of Bs across taxa, as Bs are relatively common in grasses (Gramineae = Poaceae), lilies and allied taxa (Lilianae), and grasshoppers (Orthoptera), all of which have been subject to intensive cytogenetic study (Jones and Rees, 1982; Camacho et al., 2000; Camacho, 2004). In other taxa, such as fungi (Covert, 1998), the identification of Bs depends on the use of recently developed techniques, and thus Bs are known in only a small number of species.

Study intensity

There is no simple, single cure for the problem of variation in study intensity. In principle, well studied groups are preferable, if only to improve statistical power. For this reason, Burt and Trivers (1998) chose to analyze B chromosome presence and degree of outcrossing in British flowering plants, a group in which both variables were well studied and there was no reason to expect degree of outcrossing to be associated with amount of cytogenetic work. Since intensity of study also varies within well-studied groups, spurious correlations can occur when the variable of interest co-varies with study effort.

The ideal solution is to statistically correct for variation in study effort. The best measure of study effort would be the total number of karyotypic studies on each species, but such information is often difficult to find and time consuming to compile. To give perhaps the extreme case, in our work on Bs and genome size in 353 species of British flowering plants, it would have been an enormous task to truly quantify the number of karyotypic studies for each species. For example, Darlington and Wylie's *Chromosome Atlas of Flowering Plants* was published in 1956 and typically lists only the most "recent" references, yet this compilation has over 2400 references. The available computer literature databases do not search prior to 1965 and most do not search past 1980. Yet databases listing numbers of karyotypic studies would be very valuable to build, especially for well-studied groups. (A complexity is that the effect of study effort on apparent B frequency may be exaggerated if discovery of Bs in a species causes the number of karytotypic studies on that species to increase.)

An alternative is to measure some other aspect of study effort in genetics that is believed to be correlated with karyotypic study effort (and, then, test this assumption on a sub-sample of the data). The underlying assumption is that some species are well studied genetically and others not,

so degree of study of genome size will correlate positively with intensity of karyotypic study, for example. The former is relatively easy to measure, since online databases of genome size studies exist (Bennett and Leitch, 2003; Gregory, 2001a). A sub-sample of 25 species of British flowering plants shows that number of estimates of genome size does correlate positively with number of cytogenetic studies ($p < 0.01$, Spearman's Rho = 0.56; Trivers et al., 2004).

At the very least, comparative studies must exclude species whose chromosomes have never been counted, since obviously Bs could not be found in such species. This simple correction alone can change the rank order of relative B frequency among plant families (Levin et al., manuscript in preparation).

In many cases the influence of study effort will be unbiased, at least within taxa. For example, Palestis and colleagues (2003) demonstrate that Bs are more frequent in mammals with karyotypes consisting of mostly acrocentric autosomes (see Chromosome shape). There is no reason to suspect that species with mainly acrocentric chromosomes are studied more frequently than those with mainly bi-armed chromosomes, and, indeed, there is no correlation between study effort and the percentage of autosomes that are acrocentric across mammals ($F_{1,944} = 0.427$, $p = 0.513$, $r^2 = 0.0005$). Study effort was indexed by the number of studies listed in an online database of mammalian karyotypes (Institute of Cytology and Genetics, 2000). The effect of study effort on apparent B frequency is enormous. Among species with fewer than three studies cited in the online database (n = 647), 2.5 % have reports of B presence, while 30 % of species with greater than 15 references (n = 27) have Bs. But since there is no apparent bias by percentage of acrocentric As, the fact that many species with few karyotypic studies may be misclassified as non-B species would only decrease the chance that a significant correlation between A chromosome shape and B presence would be found. We have added study effort as a variable in a regression analysis and find highly significant, independent effects of both study effort and A chromosome shape on the presence of B chromosomes (Table 1). The strength of the correlation between acrocentric As and B presence is unchanged by correcting for study effort.

However, there are situations when differences in study effort can introduce bias. Trivers and colleagues (2004) show that study intensity is correlated with the presence of B chromosomes in British flowering plants. The index of study intensity used was the number of studies of genome size on a species, as described above. The chief variable of interest in this study was genome size, with the prediction that Bs would be more frequent in species with large genomes (see Genome size). In this case, study effort led to a bias, because species with large genomes tend to be studied more often than those with small genomes (Trivers et al., 2004). This difference probably results from the greater ease of studying species with large chromosomes (and few chromosomes—genome size and chromosome number are inversely correlated; Vinogradov, 2001; Trivers et al., 2004). Simply finding a correlation between B presence and genome size would be insufficient to demonstrate a relationship, since study effort is positively correlated with both B presence and genome size. It is therefore necessary to control statistically for intensity of study effort using multiple regression. This analysis shows that genome size (as well as breeding system and chromosome number) does make a highly significant contribution to B chromosome presence, independent of study effort (Table 2). Again, correcting for study effort does not affect the strength of the correlation with the variable of interest.

In other cases, biases due to differences in study effort can lead to spurious correlations that disappear when study effort is controlled for, such as an apparent correlation between intraspecific variation in ploidy and B presence (Trivers et al., 2004; Table 2). Increased study

effort not only leads to more discoveries of Bs, but also to more discoveries of chromosomal races and ploidy variation.

Correcting for phylogeny

Another potential confounding factor in studies of B chromosome presence is non-independence of species due to evolutionary relatedness. For example, imagine a case where 10 species have Bs and 10 do not. Of the 10 species with Bs 9 have trait *x*, while only two of those without Bs have trait *x*. This would appear to be evidence of a correlation between trait *x* and B chromosome presence. However, what if the 10 species with Bs were all in one genus and the 10 without were all in another genus? Now there is really no good evidence of an association between Bs and trait *x*. While this example is extreme, it does point out the potential problem of pseudoreplication due to phylogenetic relatedness. We have used the method of independent taxonomic contrasts (Felsenstein, 1988; Burt, 1989) to control for possible phylogenetic biases.

That Bs may be phylogenetically clustered does not necessarily mean that there is no effect of the variable of interest on B frequency. In the hypothetical example above, it is possible that expanding the study to include many genera would reveal a relationship between Bs and trait *x*. Perhaps most genera with high B frequencies also have high frequencies of the trait. In addition, such clustering may suggest factors associated with B presence that had been previously overlooked, if groups with high B frequencies share common characteristics (see Future research). Phylogenetic clustering can also suggest that Bs may survive speciation events, if the Bs are similar in closely related species (Camacho, 2004).

Breeding system

In 1969 Moss stated that Bs in plants are most frequent in outbreeding species, but unfortunately published only an abstract with no data (Moss, 1969). A recent comparative study of 226 species of British flowering plants demonstrates that Bs are, indeed, more likely to be present in outbreeding than in inbreeding species (Burt and Trivers, 1998). This result holds after correcting for study effort and other variables, such as genome size (which is correlated with both degree of outbreeding and B presence), chromosome number, and ploidy (Trivers et al., 2004; see Table 2).

Experimental studies also support the relationship between Bs and outcrossing. Müntzing (1954) inbred the normally outbreeding rye (*Secale cereale*), and B frequency declined. Similarly, Bs experimentally introduced into *S. vavilovii*, which does inbreed, rapidly declined in frequency (Puertas et al., 1987). Empirical evidence based on comparisons of heterozygosity among individuals with and without Bs is mixed, however. Presence of Bs is associated with heterozygosity of RAPD loci in wild roe deer (*Capreolus pygargus*; Tokarskaia et al., 2000), but Bs in rye are associated with homozygosity at isozyme loci (Benito et al., 1992).

An association between Bs and outbreeding is expected on theoretical grounds (Puertas et al., 1987; Bell and Burt, 1990; Shaw and Hewitt, 1990). In inbred or asexual species, natural selection acts among competing lines of descent. Lines without Bs are expected to outcompete those with Bs, if Bs decrease fitness. In outcrossed species Bs can continually infect new lineages and can escape extinction if they drive. Inbreeding also increases the variance in B number

among individuals, which increases the power of natural selection to decrease B frequency. Harmful effects of Bs are typically most prevalent at high numbers of Bs per individual, and inbreeding among "infected" individuals would increase the number of offspring with high numbers of Bs.

Interestingly, Cruz-Pardilla et al. (1989) demonstrate that rye individuals with Bs have higher rates of outbreeding than those without Bs. Since plants with Bs also had decreased fertility, it is possible that this result reflects mortality of zygotes from selfing, due to high B numbers (Shaw and Hewitt, 1990). If so, this result would support the contention that the harmful effects of parasitic Bs are amplified under inbreeding.

It is important to remember that not all Bs are harmful, and some, such as those in *Allium schoenoprasum* appear to be beneficial (Bougourd and Jones, 1997). The relationship between Bs and breeding system is greatly influenced by the direction of the phenotypic effects of the Bs. Burt and Trivers (1998) show in a population genetic model that outcrossing, which favors the spread of harmful Bs, has the opposite effect on beneficial Bs, which do best under inbreeding. Selfing species that do have Bs may be interesting species to study, since they may represent cases where Bs are no longer parasitic and are possibly mutualistic (Burt and Trivers, 1998).

Genome size

While reviewing the literature on genome size, it was noticed that taxa with relatively large genomes tend to have more species with B chromosomes than do taxa with smaller genomes (e.g. monocots vs. dicots, Orthoptera vs. Diptera). As recently noted by Vujosevic and Blagojevic (2004, this issue), the absence of Bs in birds may be related to their uniformly small genome size. Trivers and colleagues (2004) tested the possible association between genome size and Bs within British flowering plants. A strong positive correlation was found, with Bs completely absent from species with very small genomes. This relationship holds among all British flowering plants, both globally and in a test of taxonomically independent contrasts. The relationship is also statistically significant among dicots and, within the dicots, among the Compositae (Asteraceae), but does not reach statistical significance among the grasses (Gramineae). Oddly the correlation is in the opposite direction for monocots as a whole, but the data set included only one monocot with Bs outside of the Gramineae. The global relationship between genome size and Bs remains highly significant when statistically controlled for potentially confounding variables, such as breeding system, chromosome number, ploidy and study effort.

One potential weakness of the Trivers study (Trivers et al., 2004) is that, although British flowering plants are presumably a well-studied group, the presence or absence of B chromosomes was based on data only up to 1982, the B chromosome atlas in Jones and Rees (1982). We have since updated our data set using a database maintained by RNJ though 1994 and by performing computer searches for records of Bs since 1994. We have uncovered only four species that had been misclassified as non-B species. Reclassifying these four species has little effect on the logistic regression analysis (the new analysis is in Table 2), but does cause the effect of genome size on B presence in the grasses to approach significance ($p = 0.076$). Amazingly, reclassifying just four species greatly improves the independent taxonomic contrasts analysis. Previously 15 contrasts were in the predicted direction and 6 were in the opposite direction ($p = 0.04$). These four "new" species add three new contrasts and reverse the direction of one; now 19 are in the predicted direction versus 5 in the opposite direction ($p = 0.003$). This large impact of a small

number of misclassified species points to the effect that differences in study effort can have on statistical power. It is also reassuring that as the data set improves, even marginally, correlations become stronger.

The most obvious possible explanation for the relationship between genome size and B presence is that species with small genomes may be less able to tolerate the effects of Bs. The shape of the relationship between genome size and Bs in British flowering plants supports this explanation, as Bs are absent from species with tiny genomes and B frequency levels off once genome size is relatively large (and may actually decline at very large genome sizes) (Trivers et al., 2004). Another possibility is that large genomes, which contain largely non-coding DNA, may provide a source of DNA for the creation of Bs, which also consist largely of non-coding DNA (Puertas, 2002; Jones and Houben, 2003). One possible sampling artifact cannot be ruled out. Plants with small genomes often have many small A chromosomes, while those with large genomes tend to have a small number of large A chromosomes (Vinogradov, 2001; Trivers et al., 2004), which could increase the relative difficulty of detecting Bs in small-genomed species.

We have also attempted to test for a relationship between genome size and B chromosome presence in mammals, but genome size data is known for only a small number of mammals with Bs, and nearly all of these are rodents. At least among rodents, species with and without Bs have remarkably similar genome sizes (with Bs: mean 2C genome size ± SE = 3.5 ± 0.2 pg, n = 12; without Bs: 3.6 ± 0.1 pg, n = 51; genome size data from Gregory, 2001a). If a relationship between genome size and B presence does exist in mammals or other amniotes it is unlikely to be a strong effect, because there is little variation in genome size among the amniotes (Gregory, 2001a,b). Within our data, angiosperm 2C-values vary from approximately 0.1 to over 60 pg, while rodent 2C-values vary only from approximately 2 to 8.5 pg.

Chromosome number and ploidy

As stated above, there is a negative correlation between genome size and chromosome number in flowering plants (Vinogradov, 2001; Trivers et al., 2004). Since there is a positive correlation between genome size and B presence, it is not surprising to find a negative relationship between chromosome number and B presence. However, this correlation is not simply a side-effect of the relationship with genome size. In a multiple logistic regression of factors affecting B presence in British flowering plants, chromosome number has a highly significant effect, independent of genome size or other variables (Trivers et al., 2004; Table 2). We do not know why this would be true. Camacho (personal communication) suggests that species with many small chromosomes may have evolved more efficient meiotic mechanisms, which could allow removal of Bs to be easier.

Because we found such a strong negative relationship between chromosome number and B chromosome presence in plants, we included chromosome number as a variable in our analysis of Bs in mammals. There is no evidence for an effect of chromosome number on B frequency in mammals (Table 1), and average chromosome number is nearly identical among species with Bs (45.7 ± 1.7, n = 63) and without Bs (43.3 ± 0.4, n = 1112). While this adds to the mystery of the effect of chromosome number in plants, in mammals chromosome shape appears to be the more important variable (see Chromosome shape).

The positive correlation between Bs and genome size in flowering plants leads to a natural prediction: Bs should also be associated with polyploidy. This is not the case. Jones and Rees

(1982, see also Jones, 1995) show that the frequency of polyploidy among species with Bs is similar to the frequency among all flowering plants. Trivers and colleagues (2004) demonstrate that polyploidy clearly has no positive effect on B presence, and may actually have a slight negative effect. Additionally, all positive relationships between Bs and genome size improve after correcting for ploidy. Importantly, these results imply that doubling the number of As does not cause B formation. Trivers and colleagues (2004) suggest that doubling chromosome number may actually remove Bs if it results in bivalency among Bs, which could cause the Bs to act as As. Hypothetically, these bivalent Bs could eventually become part of the A chromosome set (see also Araujo et al., 2001 for a similar suggestion involving haplodiploidy in a wasp). Regardless of whether the initial act of doubling chromosome number directly influences B formation or fate, one would still expect a positive correlation between Bs and ploidy level for the same reasons that Bs are associated positively with genome size. For example, if increased genome size per se allows a species to tolerate B presence, the same should be true for increased ploidy.

Levin (personal communbication) suggests a possible explanation for the lack of a positive correlation between Bs and polyploidy. Polyploids contain less DNA than the sum of their parental genomes, and presumably have lost non-coding DNA (Levin 2002; Leitch and Bennett 2004; Levin and Palestis, unpublished data). Selection against junk DNA may also include selection against Bs, and may also limit the formation of new Bs from A chromosome junk. The lack of a positive association between polyploidy and Bs may also result from differences in breeding system, since polyploids are much more likely than diploids to reproduce via apomixis (Levin, 2002), which would select against Bs (see Breeding system). Another possible, though less likely, explanation is suggested by the fact that average DNA amount per diploid genome is negatively correlated with the prevalence of polyploidy (Grif, 2000). Grif states that this result is due to an upper limit on total DNA content set by the maximum size of the nucleus. If species with small genomes are more likely to become polyploid than are species with large genomes, and species with small genomes rarely harbor Bs, then it is unlikely that polyploid species would contain Bs, at least initially. Additionally, it is possible that polyploid species are close to this upper limit, and thus could not add Bs.

Chromosome shape

Most mammals have either mostly acrocentric A chromosomes (with one long arm) or mostly metacentric or submetacentric A chromosomes (with two arms), while relatively few species have intermediate karyotypes (Pardo-Manuel de Villena and Sapienza, 2001a). Pardo-Manuel de Villena and Sapienza (2001a) suggest that this distribution results from a bias during female meiosis that favors either more centromeres (thus favoring acrocentrics) or fewer (thus favoring metacentrics), depending on whether the egg or polar body side of the spindle is more efficient at capturing centromeres (see also Pardo-Manuel de Villena and Sapienza, 2001b). The direction of this bias switches frequently over evolutionary time, often resulting in closely related species with completely different karyotypes.

We predicted that a meiotic environment favoring increased centromere number should also favor Bs, and that Bs should therefore be more frequent in species with mainly acrocentric autosomes (Palestis et al., 2003). We demonstrate that Bs are more common in species with acrocentric karyotypes among all mammals, among rodents, among non-rodents, and in a comparison of independent taxonomic contrasts. Although A chromosome shape explains only a

small proportion of the variation in B presence ($r^2 = 0.048$), the numerical difference in the average frequency of acrocentrics in species with and without Bs is quite large (approximately 68 % versus 43 % of chromosomes acrocentric, 59 % versus 36 % of chromosome arms on acrocentrics; in statistical comparisons we use arms rather than chromosomes, since one metacentric can be formed by the same number of evolutionary events as two acrocentrics (Palestis et al., 2003). This result holds up after adding a correction for study effort (Table 1). This study demonstrates that comparative research on B chromosomes, when used to test theoretical predictions, can have implications beyond identifying factors associated with B presence. In this case B chromosome research provided independent support for the theory of centromeric drive (Henikoff et al., 2001; Pardo-Manuel de Villena and Sapienza, 2001a,b; Henikoff and Malik, 2002a,b).

The prediction that Bs will be more frequent among species with acrocentric As depends on B chromosome drive occurring during female meiosis. Unfortunately, for most mammals we do not know whether this assumption is true. It probably is true for *Rattus fuscipes* (Thomson, 1984) and *R. rattus* (Yosida, 1978; Stitou et al., 2004, this issue). It is likely that the same relationship between karyotype and B presence will occur in grasshoppers. Hewitt (1976) demonstrates that B drive in *Myrmeleotettix maculatus* is through females, and is also based on a functional asymmetry of the meiotic spindle poles, favoring nonrandom segregation of B chromosomes to the egg pole (Pardo-Manuel de Villena and Sapienza, 2001b). Bidau and Martí (2004, this issue) show an intraspecific correlation between B frequency and acrocentricity in *Dichroplus pratensis*, but it is unknown whether female meiotic drive occurs in this species. Among plant B chromosomes, female meiotic drive does occur in some species (e.g. *Lilium callosum*; Kayano, 1957). However, because of the presence of the gametophyte generation in plants, B drive often occurs during mitosis. For example, among grasses the most common method of B drive is nondisjunction at the first pollen grain mitosis (Jones and Rees, 1982; Jones, 1991). Among such species we expect to see no relationship between A chromosome shape and occurrence of Bs.

If acrocentric As and B presence are both associated with higher centromere number, it is odd that we see no relationship between Bs and chromosome number in mammals (see Chromosome number and ploidy). We would predict Bs to be more frequent with high chromosome number, the opposite direction of the correlation in plants. Chromosome number in mammals does increase as the proportion that are acrocentric increases ($F_{1, 1167} = 441.74$, $p < 0.0001$, $r^2 = 0.27$), but there is a lot of scatter. In fact, the species with the highest chromosome number (*Tympanoctomys barrera*, $2n = 102$) has only one pair of acrocentric chromosomes.

In addition to demonstrating an effect of A chromosome shape on B chromosome presence in mammals, we also found a relationship between A chromosome shape and B chromosome shape (Palestis et al., 2003). Excluding species with multiple B morphologies, Bs in species with acrocentric As are typically acrocentric, while Bs in species with metacentric As are typically metacentric. Camacho (2004) reports a similar relationship in grasshoppers. This relationship suggests recent derivation of Bs from As, but B morphology can clearly evolve quickly leading to many different shapes and sizes of Bs within a species (e.g. *Eyprepocnemis plorans*; López-León et al., 1993).

Future research

Throughout this review it has probably been obvious to the reader that nearly all of the examples of comparative studies of B chromosome presence that we cite come from our own work. There is a good reason for this—very few others have attempted similar studies. We hope that this review and the online publication of R.N. Jones' extensive database on species with Bs, as part of this issue, will stimulate additional research in this area. Several online databases exist for relevant variables (e.g., plant genome size: Bennett and Leitch, 2003; animal genome size: Gregory, 2001a; plant chromosome numbers: Missouri Botanical Garden, 2003; mammalian karyotypes: Institute of Cytology and Genetics, 2000, and supplement to Pardo-Manuel de Villena and Sapienza, 2001a). Here we suggest some potential avenues for future research.

Most of the variables that we have considered are genetic (genome size, several aspects of karyotype), with breeding system being the exception. However, many studies have examined the importance of ecological factors, such as temperature, rainfall and altitude, on B presence when comparing populations within one species (reviewed in Jones and Rees, 1982). It is possible that some environmental variables may also influence B presence across species. (Altitude probably does not, since, while it is negatively correlated with B frequency in many species, the correlation is in the opposite direction in others (Beukeboom, 1994). In most cases authors of studies on ecological variation in B frequency have postulated that clines result from Bs being more common in the optimal habitat of the species, where presumably Bs could be tolerated more easily. However, this explanation cannot be true in all cases, because it would not apply to beneficial Bs and also ignores the effects of history (i.e. site of B origin and subsequent geographic spread) and genetic drift (Beukeboom, 1994).

It is also possible that some ecological factors associated with B presence may act directly on the mechanisms of B transmission, rather than or in addition to acting indirectly via the individual's phenotype (Jones and Rees, 1982). If this is the case, then we would expect to see ecological variables that affect Bs similarly across species. Shaw and Hewitt (1984) demonstrate that transmission of Bs in *M. maculatus* males is reduced at low temperatures, and B frequency is positively correlated with temperature in this species. If the relationship between temperature and B transmission holds true for other organisms (most likely other ectotherms), then we would expect Bs to be more prevalent in species that inhabit warmer climates.

Another relevant line of research is the search for phyletic "hot-spots" for Bs (Levin et al., manuscript in preparation). Among flowering plants, some lineages have unusually high frequencies of particular chromosomal features, such as polyploidy or translocations (Levin, 2002). Analyzing heterogeneity among taxa in B frequency is not as simple as counting the number of species with Bs, because meaningful comparisons require knowing the number of species whose chromosomes have been counted. B frequency can then be expressed as the proportion of characterized species with Bs. Of course, differences in study effort are particularly problematic here. Nonetheless, B hot-spots and cold-spots clearly emerge. For example, 27.2 % of characterized species in Order Commelinales harbor Bs, while there are no reports of B presence in several other orders (Levin et al., manuscript in preparation). Why are some taxa B-rich and others B-poor? Taxonomic patterns can also emerge that suggest correlations between B presence and other variables. For example, very few Bs have been reported in the non-monocot basal angiosperms (Levin et al., manuscript in preparation). The lack of Bs in these ancient lineages may be related to the small ancestral genome size of angiosperms (Leitch et al., 1998; Levin, 2002; Soltis et al., 2003). In addition, Bs may be present in an unusually high percentage

of species, relative to other dicots, in the Loranthaceae, the family with the highest average genome size among the dicots. Comparing B frequency among higher taxa thus provides another method of testing the same correlations we have examined by comparing among species.

We have demonstrated that comparative studies of the factors influencing B presence can be fruitful, despite the problem of differences in study effort among species. Future studies can either identify new variables associated with B presence or can expand the analysis of the variables we have studied. It will be exciting to see if the relationships reviewed here can be applied more generally, both in studies of B chromosomes and in studies of other kinds of selfish genetic elements.

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References

Araujo SMSR, Pampolo SG, Perfectti F, Camacho JPM: Integration of a B chromosome into the A genome of a wasp. Proc R Soc Lond B 268:1127–1131 (2001).

Bell G, Burt A: B-chromosomes: germ-line parasites which induce changes in host recombination. Parasitology 100:S19–S26 (1990).

Benito C, Romera F, Díez M, Figueiras AM: Genic heterozygosity and fitness in rye populations with B chromosomes. Heredity 69:406–411 (1992).

Bennett MD, Leitch IJ. Plant DNA C-values Database (release 2.0, Jan. 2003). http://www.rbgkew.org.uk/cval/homepage.html

Beukeboom LW: Bewildering Bs: an impression of the first B-chromosome conference. Heredity 73:328–336 (1994).

Bidau CJ, Martí DA: B chromosomes and Robertsonian fusions of *Dichroplus pratensis* (Acrididae): intraspecific support for the centromeric drive theory. Cytogenet Genome Res XX:(2004).

Bougourd SM, Jones RN: B chromosomes: a physiological enigma. New Phytol 137:43–54 (1997).

Burt A: Comparative methods using phylogenetically independent contrasts. Oxf Surv Evol Biol 6:33–53 (1989).

Burt A, Trivers R: Selfish DNA and breeding system in flowering plants. Proc R Soc Lond B 265:141–146 (1998).

Camacho JPM: B chromosomes, in Gregory TR (ed): The Evolution of the Genome (Elsevier, Amsterdam, 2004).

Camacho JPM, Sharbel TF, Beukeboom LW: B-chromosome evolution. Phil Trans R Soc Lond B 355:163–178 (2000).

Chen Q, Jahier, J, Cauderson Y. The B chromosome system of Inner Mongolian *Agropyron* Gaertn. I. Distribution, morphology, and cytological behaviour. Carylogia 46:245–260 (1993).

Covert SF: Supernumerary chromosomes in filamentous fungi. Curr Genet 33:311–319(1998).

Cruz-Pardilla M, Vences FJ, Garcia P, Pérez de la Vega M: The effect of B chromosomes on outcrossing rate in a population of rye *Secale cereale* L. Heredity 62: 319–326 (1989).

Darlington CD, Wylie AP: Chromosome Atlas of Flowering Plants (Macmillan, New York, 1956)

Felsenstein J: Phylogenies and quantitative characters. Ann Rev Ecol Syst 19:445–471 (1988).

Gregory TR: Animal Genome Size Database. http://www.genomesize.com (2001a).

Gregory TR: Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol Rev 76:65–101 (2001b).

Grif VG: Some aspects of plant karyology and karyosystematics. Int Rev Cytol 196:131–175 (2000).

Henikoff S, Ahmad K, Malik HS: The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293:1098–1102 (2001).

Henikoff S, Malik HS: Conflict begets complexity: the evolution of centromeres. Curr Opin Genet Devel 12:711–718 (2002a).
Henikoff S, Malik HS: Selfish drivers. Nature 417: 227 (2002b).
Hewitt GM: Meiotic drive for B-chromosomes in the primary oocytes of *Myrmeleotettix maculatus* (Thunb.) (Acrididae: Orthoptera). Chromosoma 56:381–391 (1976).
Institute of Cytology and Genetics. Diploid Numbers of Mammalia. SB RAS, Novosibirsk. http://www.bionet/nsc.ru/chromosomes (2000).
Jones RN: B-chromosome drive. Am Nat 137:430–442 (1991).
Jones RN: B chromosomes in plants. New Phytol 131:411–434 (1995).
Jones RN, Houben A: B chromosomes in plants: escapees from the A chromosome genome? Trends Plant Sci 8:417–423 (2003).
Jones RN, Rees H: B Chromosomes. (Academic Press, London 1982).
Kayano H: Cytogenetics studies in *Lilium callosum*. III. Preferential segregation of a supernumerary chromosome in EMCs. Proc Jpn Acad 33:553–558 (1957).
Leitch IJ, Bennett MD: Genome downsizing in polyploid plants. Biol J Linnean Soc (2004).
Leitch IJ, Chase MW, Bennett MD: Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. Ann Bot 82:85–94 (1998).
Levin DA: The Role of Chromosomal Change in Plant Evolution. (Oxford University Press, 2002).
López-León MD, Cabrero J, Pardo MC, Viseras E, Camacho JPM, Santos JL: Generating high variability of B chromosomes in the grasshopper *Eyprepocnemis plorans*. Heredity 71:352–362 (1993).
Missouri Botanical Garden: Index to Plant Chromosome Numbers (IPCN). http://mobot.mobot.org/W3T/Search/ipcn.html (2003).
Moss JP: B-chromosomes and breeding systems. Chromosomes Today 2:268 (1969).
Müntzing, A. Cytogenetics of accessory chromosomes (B chromosomes). Caryologia S6:282–301 (1954).
Palestis BG, Burt A, Jones RN, Trivers R: B chromosomes are more frequent in mammals with acrocentric karyotypes: support for the theory of centromeric drive. Proc R Soc Lond B 271:S22–S24 (2004).
Pardo-Manuel de Villena F, Sapienza C: Female meiosis drives karyotypic evolution in mammals. Genetics 159:1179–1183 (2001a).
Pardo-Manuel de Villena F, Sapienza C: Nonrandom segregation during meiosis: the unfairness of females. Mammal Genome 12:331–339 (2001b).
Puertas MJ: Nature and evolution of B chromosomes in plants: a non-coding but information-rich part of plant genomes. Cytogenet Genome Res 96:198–205 (2002).
Puertas MJ, Ramirez A, Baeza F: The transmission of B chromosomes in *Secale cereale* and *Secale vavilovii* populations. II. Dynamics of populations. Heredity 58:81–86 (1987).
Shaw MW, Hewitt GM: The effect of temperature on meiotic transmission rates of the B chromosome of *Myrmeleotettix maculatus* (Orthoptera:Acrididae). Heredity 53:259–268 (1984).
Shaw MW, Hewitt GM: B chromosomes, selfish DNA and theoretical models: where next? Oxf Surv Evol Biol 7:197–223 (1990).
Soltis DE, Soltis PS, Bennett MD, Leitch IJ: Evolution of genome size in the angiosperms. Am J Bot 90:1596–1603 (2003).
Stitou S, Zurita F, Díaz de la Guardia R, Jiménez R, Burgos M: Transmission analysis of B chromosomes in *Rattus rattus* from Northern Africa. Cytogenet Genome Res XX (2004).
Thomson RL: B chromosomes of *Rattus fuscipes* II. The transmission of B chromosomes to offspring and population studies: support for the parasitic model. Heredity 52:363–372 (1984).
Tokarskaia ON, Efremova DA, Kan NG, Danilkin AA, Sempere A, Petrosian VG, Semenova SK, Ryskov AP: Variability of multilocus DNK markers in populations of the Siberian (*Capreolus pygargus* Pall.) and European (*C. capreolus* L.) roe deer. Genetika 36:1520–1530 (2000).
Trivers R, Burt A, Palestis BG: B chromosomes and genome size in flowering plants. Genome 47:1–8 (2004).
Vinogradov AE: Mirrored genome size distributions in monocot and dicot plants. Acta Biotheor 49:43–51 (2001).
Vujosevic M, Blagojevic J: B chromosomes in populations of mammals. Cytogenet Genome Res XX (2004).
Wu T: B chromosomes in *Sorghum stipoideum*. Heredity 68:457–463 (1992).
Yosida TH: Some genetic analysis of supernumerary chromosomes in the black rat in laboratory matings. Proc Jpn Acad B 54:440–445 (1978).