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THE ROYAL SOCIETY

B-chromosome evolution

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B chromosomes are extra chromosomes to the standard complement that occur in many organisms. They can originate in a number of ways including derivation from autosomes and sex chromosomes in intra- and interspecies crosses. Their subsequent molecular evolution resembles that of univalent sex chromosomes, which involves gene silencing, heterochromatinization and the accumulation of repetitive DNA and transposons. B-chromosome frequencies in populations result from a balance between their transmission rates and their effects on host fitness. Their long-term evolution is considered to be the outcome of selection on the host genome to eliminate B chromosomes or suppress their effects and on the B chromosome's ability to escape through the generation of new variants. Because B chromosomes interact with the standard chromosomes, they can play an important role in genome evolution and may be useful for studying molecular evolutionary processes.

Keywords: B chromosomes; transposons; evolution; heterochromatin; repetitive DNA; Muller's ratchet

1. INTRODUCTION

Eukaryotic genomes are composed not only of genes found in normal chromosomes (A chromosomes) but also of myriads of selfish genetic elements which do not obey Mendelian laws of inheritance. Notable among these elements are the transposons, segregation distorters, various cytoplasmic factors and B chromosomes. Of these, the latter were really the first selfish genetic elements to be described (Wilson 1907), although their parasitic nature (Östergren 1945; Nur 1966, 1977) and selfishness (Jones 1985; Werren *et al.* 1987) were recognized many years following their initial descriptions.

The B chromosomes, also referred to as supernumerary or accessory chromosomes, are 'additional dispensable chromosomes that are present in some individuals from some populations in some species, which have probably arisen from the A chromosomes but follow their own evolutionary pathway' (J. P. M. Camacho & J. S. Parker, in Beukeboom 1994*a*). In addition, their irregular mitotic and meiotic behaviour allows them to accumulate selfishly in the germline, enabling non-Mendelian inheritance with transmission rates exceeding those of normal chromosomes (0.5). They have been found in all major groups of animals and plants.

B chromosomes have traditionally attracted much interest and various aspects of their biology have been reviewed several times (for an overview, see Jones 1995), of which the most recent comprehensive treatise is the monograph by Jones & Rees (1982). In this review, we discuss current insights into B-chromosome evolution and point out new developments and directions in B-chromosome research that have occurred during the last two decades. A large number of recent studies have revealed new features of B chromosomes and some have shed light on previously unanswered questions. Among them are descriptions of previously unknown mechanisms of B-chromosome inheritance, an extreme example being the paternal sex-ratio (PSR) chromosome of the wasp *Nasonia*, which accumulates through its ability to destroy paternal chromosomes (Werren 1991). Long-term studies on specific B-chromosome systems (e.g. the grasshopper *Eyprepocnemis plorans*; Camacho *et al.* 1997*a*,*b*) have provided evidence for ongoing interactions between B-chromosome morphs and the standard genome at the level of local populations. Indeed, B-chromosome evolution may be viewed as the outcome of continuous conflict between parts of the genome with different interests, i.e. B-chromosome influences may shift back and forth from parasitic to neutral and possibly beneficial effects. Undoubtedly, the most important progress comes from the development and application of new molecular techniques. Data obtained from molecular analyses of several B chromosomes are now available to shed light upon questions of their origin and subsequent chromosomal evolution and sophisticated *in situ* hybridization techniques have additionally contributed to a better understanding of B-chromosome DNA composition and organization.

2. ORIGIN

In recent years, the types of DNA sequences residing on B chromosomes have been analysed extensively in some organisms. The first analyses in the 1970s and 1980s demonstrated that B chromosomes contained DNA that

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Table 1. *Classification of the origins of B chromosomes*

(At the highest classification level, B chromosomess are distinguished based upon their origination from the genome of their actual host species (intraspecies) or from the genome of another species (interspecies or hybrid origin). The second level of classification differentiates between origin from standard A chromosomes (autosomes) and sex chromosomes. For organisms with heteromorphic sex chromosomes, a further distinction can be made between origin from the homogametic or heterogametic sex chromosome. Evidence: 1, similar banding patterns of B chromosomes found among standard complement (including sex chromosomes); 2, (repetitive) DNA sequences of B chromosomes shared with standard complement (including sex chromosomes); 3, (repetitive) DNA sequences of B chromosomes not shared with standard complement, but present in related species; 4, direct observation of origination of chromosomal fragments in interspecific crosses; and 5, phylogenetic analysis of transposable elements shared by B chromosomes and standard complement (intra- and interspecies). See Beukeboom (1994*a*) and Hackstein *et al.* (1996) for details on the techniques used.)

classification			species	evidence	reference
intraspecific	autosomal		*C. capillaris*	2	Jamilena *et al.* (1994, 1995)
			S. cereale		Jones & Flavell (1983), McIntyre *et al.* (1990), Sandery *et al.* (1990), Blunden *et al.* (1993), Cuadrado & Jouve (1994), Houben *et al.* (1996)
			Z. mays	2	Peacock *et al.* (1981), Viotti *et al.* (1985), Alfenito & Birchler (1993), Stark *et al.* (1996)
			C. plumosus	1	Keyl & Hägele (1971)
			D. subsilvestris	2	Gutknecht *et al.* (1995)
			Petauroides volans	2	McQuade *et al.* (1994)
			Reithodontomys megalotis	2	Peppers *et al.* (1997)
			B. dichromosomatica	2	Leach *et al.* (1995), Houben *et al.* (1997*a*)
intraspecific	sex chromosomal	no heteromorphy	*E. plorans*	2	López-León *et al.* (1994)
intraspecific	sex chromosomal	homogametic	no examples		
intraspecific	sex chromosomal	heterogametic	*Glossina* spp.	2	Amos & Dover (1981)
			L. hochstetteri	2	Sharbel *et al.* (1998)
interspecific	autosomal		*Coix*	2,4	Sapre & Deshpande (1987)
			N. vitripennis	3,5	McAllister & Werren (1997)
			P. formosa	4	Schartl *et al.* (1995)
interspecific	sex chromosomal	no heteromorphy	no examples		
interspecific	sex chromosomal	homogametic	no examples		
interspecific	sex chromosomal	heterogametic	no examples		

was similar to that found on the A chromosomes (for a review, see Jones & Rees 1982). Research in the 1990s has involved the isolation, cloning and sequencing of numerous repetitive DNAs located on B chromosomes of various species. Some of these are specific to the B chromosomes while others are shared with the A chromosomes (reviewed in Beukeboom 1994*a*; Hackstein *et al.* 1996).

The traditional view, which is still widely accepted, is that B chromosomes are derived from the A chromosomes (Jones & Rees 1982). From this perspective, we could consider the origin of the B chromosome as a simple by-product of the evolution of the standard karyotype. For example, a B chromosome could derive itself from polysomic A chromosomes, from centric fragments resulting from A-chromosome fusions or from amplification of the paracentromeric region of a fragmented A chromosome. The first clear evidence in favour of the latter hypothesis was obtained by Keyl & Hägele (1971), who demonstrated that the polytene band pattern in the B chromosome of *Chironomus plumosus* was similar to that found near the centromere of chromosome IV.

Recent cytological and molecular studies support the notion that most B chromosomes seem to be derived from the autosomal complement of their current host species, but these studies have also demonstrated other modes of B-chromosome origin (table 1). Intraspecific origin from A chromosomes is the most likely scenario for eight B chromosomes investigated, given the identification of similar repeat DNA sequences on them. For example, all repetitive DNA sequences isolated by micro-dissection from the B chromosome in *Crepis capillaris* are also present in the A chromosomes, although it has not been possible to identify from which autosome the B chromosome originated (Jamilena *et al.* 1994, 1995).

Sex chromosomes have previously been proposed as ancestors of B chromosomes since they may be more easily tolerated in the polysomic state (Hewitt 1973*a*). An example of a sex-chromosome-derived B chromosome is the B_2 chromosome of the grasshopper *E. plorans,* where the arrangement of two DNA sequences (a 180 bp tandem repeat and ribosomal DNA) with respect to the centromere coincide specifically with that of the X chromosome (López-León *et al.* 1994). This suggests that the B chromosome of *E. plorans* has been derived from the paracentromeric region of the X chromosome, with subsequent amplification of the two types of sequences contained there. Another example is the B chromosome of the New Zealand frog *Leiopelma hochstetteri*, which appears to be derived from a univalent (heteromorphic) W sex chromosome based on DNA sequence comparisons (Sharbel *et al.* 1998) and morphological similarities with the univalent W chromosome (Green *et al.* 1993).

The idea that B chromosomes in one species could have originated from the A chromosomes of a closely related species, which was originally proposed by Battaglia (1964), has recently gathered strength (Sapre & Deshpande 1987; McVean 1995; Schartl *et al.* 1995). Sapre & Deshpande (1987) demonstrated the spontaneous origin of B chromosomes in interspecific crosses between *Poecilia aquaticus* and *P. gigantea*. Possible evidence of the origin of B chromosomes by interspecific hybridization was recently found in the gynogenetic fish *Poecilia formosa*, a hybrid species between *P. mexicana* and *P. latipinna*. This unisexual species requires sperm of a sexual parental species to initiate egg development, but paternal chromosomes are eliminated from the developing zygote (Dawley 1989). Laboratory crosses between individuals of *P. formosa* and males of a black strain, both lacking B chromosomes, produced some black-pigmented offspring (frequency = 0.001), most likely the result of paternal pigmentation genes located on B chromosomes which appeared in the offspring because of incomplete elimination of paternal A chromosomes (Schartl *et al.* 1995).

The detection of DNA sequences that are restricted to the B chromosome of one species, but found on the A chromosomes of a closely related species would imply an interspecies origin. Although such a situation was previously reported for a B chromosome in *Brachycome dichromosomatica* (John *et al.* 1991), the repeat was later discovered at very low copy number in the standard genome (Leach *et al.* 1995). The best documented case of hybrid origin is that of the PSR B chromosome of the wasp *Nasonia*. McAllister & Werren (1997) used a phylogenetic analysis of DNA sequences of a retrotransposable element to show that the copies on the PSR were most similar to those of the species from the closely related genus *Trichomalopsis*. Hybridization studies with a linear piece of DNA from the PSR further supported a hybrid origin. Further phylogenetic analyses of sequences that are shared by B chromosomes of different species, such as those reported in the genus *Brachycome* (Houben *et al.* 1997*a*), may prove useful in elucidating the evolutionary history of B chromosomes.

Reproductive mechanisms that are based on chromosome elimination, as in the above-mentioned *Poecilia*, may be especially conducive to the origination of B chromosomes. The frequent occurrence of aneuploidy among sperm-dependent parthenogenetic (= gynogenetic) organisms (Beukeboom & Vrijenhoek 1998) supports this idea. Recently, Sharbel *et al.* (1997) described three different B-chromosome morphs in one population of the sperm-dependent parthenogenetic flatworm *Polycelis nigra*. Because the mechanism of sperm chromosome expulsion is imprecise (Benazzi Lentati 1970; Beukeboom *et al.* 1996), these B chromosomes may have originated from incompletely expelled autosomes. Similarly, the PSR B chromosome of *Nasonia* may have originated as a paternal fragmented chromosome following an escape from sperm chromosome destruction due to cytoplasmic incompatibility between *Nasonia vitripennis* and a species of *Trichomalopsis* (McAllister & Werren 1997). Although there are alternative explanations to cytoplasmic incompatibility, Ryan *et al.* (1985, 1987) showed that fragments of paternal chromosomes sometimes survive in cytoplasmic incompatible crosses.

3. MOLECULAR EVOLUTION

At the time of their origin, B chromosomes would be expected to share sufficient sequence and structural homology with their progenitor chromosomes such that they could synapse and recombine. However, their independent evolution and differentiation through processes analogous to Muller's ratchet require genetic isolation from any such elements within the nucleus (Green 1990; Beukeboom 1994*a*). It follows that newly arisen B chromosomes must have some predisposition to undergoing the relatively rapid structural modification required to induce synapsis failure. Intraspecific B-chromosome origin, which is probably the prevalent mode compared with those associated with hybridization, therefore presents a conundrum. If a neo-B chromosome originates from another chromosome, what initially inhibits synapsis between the two related chromosomes and allows the B chromosome to begin its journey towards independent evolution? Although these initial processes of chromosome evolution remain largely unknown, some indications are provided by data on polysomy in grasshoppers (Peters 1981; Talavera *et al.* 1990). These extra chromosomes, which are restricted to the germline and not inherited, are generated *de novo* each generation from autosomes through non-disjunction. In addition, they are heteropycnotic and do not pair with the original A chromosome. This suggests the presence of some cellular mechanism which can cause rapid heterochromatinization of extra elements (*sensu* genomic imprinting; Thomas 1995) and this could constitute the basis for B-chromosome differentiation (see also Hewitt 1973*a*). In contrast, a chromosome fragment crossing a species boundary will likely be sufficiently different to inhibit ectopic pairing with its new chromosome complement and, thus, such an element would immediately be univalent and prone to evolve as a B chromosome.

Subsequent to synaptic isolation within their respective genomes, elements of both types of origin (i.e. intra- and interspecific) will follow similar paths of molecular evolution and be subject to the same processes which act upon non-pairing chromosomes (Charlesworth 1978). B chromosomes thus converge upon a characteristic degenerate morphology, a reflection of the processes acting upon them rather than their mode of origin (Green 1990). Encouragingly, we are gaining some insights into the molecular evolutionary processes that act upon chromosomes once they have become isolated from the rest of the genome, in particular from studies of sex chromosomes and we discuss how these processes may play a role in B-chromosome evolution.

(a) *DNA repeat sequences*

B chromosomes are typically composed of repeated DNA sequences which vary dynamically in terms of repeat type and copy number (Amos & Dover 1981; Matzke *et al.* 1990; Sandery *et al.* 1990; Eickbush *et al.* 1992; Zeyl & Green 1992; Wilkes *et al.* 1995; Franks *et al.* 1996), a result of unequal crossing over and reduced recombination (Charlesworth *et al.* 1986; Stephan 1987). Repeats may form a significant part of the B-chromosome genome, as has been shown with different repeat families on the PSR (Eickbush *et al.* 1992; McAllister & Werren

997) and, in some cases, repeats may be the exclusive onstituent, as with the pSsP216 repeat unit in the B hromosomes of *Drosophila subsilvestris* (Gutknecht *et al.* 995). McAllister & Werren (1997) have additionally nown that certain repeat sequences isolated from PSR of *N. vitripennis* are also found in the genus *Trichomalopsis*, hus providing evidence that they may be associated with obile genetic elements (see § 3(c)). The typically hetero-hromatic nature of B chromosomes, as revealed by chro-osome C banding, similarly demonstrates the presence f repeat DNA, as constitutive heterochromatin is gener-lly composed of satellite blocks (Bigot *et al.* 1990; Char-esworth *et al.* 1994). In several cases, B chromosomes ontain much larger amounts of repetitive DNA when ompared to the genome from which they originated, hus suggesting massive amplification of repeat motifs ver a relatively short time-scale, e.g. within one genera-ion following a hybrid cross. It has also been suggested hat repeat family amplification may be a mechanism hrough which a chromosome fragment (i.e. a neo-B hromosome) may become stabilized and positively elected for within a nucleus (Reed *et al.* 1994; Leach *et al.* 995).

Repeat sequences have been implicated in lower verte-rate (Nanda *et al.* 1990, 1992, 1993) and plant (Guttman Charlesworth 1998) sex-chromosome evolution and, hus, their influence upon B chromosomes may be analo-ous to the mechanisms leading to the evolution of eteromorphic sex chromosomes. In poeciliid fish, early ex-chromosome differentiation appears to have been nitiated by the accumulation of simple repeat sequences djacent to coding regions for sex determination (Nanda *al.* 1990, 1992, 1993). In the white campion (*Silene utifolia*), a Y-chromosome-linked gene (MROS3) having n active X-chromosome-linked homologue appears to ave been degenerated and silenced by multiple inser-ion–deletion events in addition to the accumulation of ononucleotide repeats (Guttman & Charlesworth 1998). ince the expression of this gene is limited to developing ale flowers, a single active X chromosome copy in XY-hromosome plants is viable, thus alleviating any selec-ion pressure to maintain function in the Y-chromosome omologue (Guttman & Charlesworth 1998). Genes on B hromosomes (assuming they were derived from tran-criptionally active autosomal regions) are similarly nder little or no selection pressure for maintenance of olecular genetic activity and, thus, they could probably ndergo analogous suppressive changes through time. However, such genes still remain to be found on B chro-osomes.

Once in position, repeat sequences may behave as uclear protein targeting signals (Gilson *et al.* 1986; Char-esworth *et al.* 1994; Mitas *et al.* 1995) which can be highly pecific, as evidenced by a protein that binds to hetero-hromatic autosomes but not to heterochromatic B chro-osomes in male mealybugs (Epstein *et al.* 1992). Protein ssociation with such sequences has been suggested as a echanism through which significant conformational hange in chromatid structure is established and efficient airing with homologous regions of a sister element is revented, effectively isolating these regions from recom-ination (Nanda *et al.* 1993). Mammalian X-chromosome nactivation is similarly mediated by chromatin–protein association, as evidenced by the histone protein variant mH2A, which binds to non-heterochromatic regions of the X chromosome and probably causes changes in chromatin structure to induce transcription silencing (Costanzi & Pehrson 1998).

Finally, the accumulation of GACA and GATA repeats has been associated with the qualitative differentiation of cytologically indistinguishable sex chromosomes in the fish *Poecilia velifera* and *Xiphophorus maculatus* (Nanda *et al.* 1993). Southern hybridization of repeat sequence probes to genomic blots of closely related species lacking discern-ible sex chromosomes has demonstrated that many different types of repeat sequences have independently accumulated on the sex chromosomes of this group (Nanda *et al.* 1993). Thus, as a general mechanism through which synapsis between undifferentiated sex chromosomes can be inhibited, the exact repeat motif involved may be less important than the actual accumula-tion of microsatellite DNA itself. The accumulation of repeat sequences with subsequent meiotic isolation through conformational change in DNA structure may thus be the initiators of early heteromorphic sex-chromosome differentiation (Nanda *et al.* 1990, 1993) and these may represent plausible mechanisms through which intraspecific neo-B chromosomes are able to differentiate rapidly from their homologue progenitors.

(b) *Ribosomal DNA*

One form of tandemly repeated DNA which has been frequently described from B chromosomes is rDNA (see Green 1990). These genes, which encode ribosomal RNAs and exist as clusters of repetitive units, are typically visualized as secondary constrictions (nucleolar organizer regions or NORs) on metaphase chromosomes (e.g. through silver staining). Interesting insights into both B-chromosome origin and evolution may be made from rDNA.

It has been suggested that NOR regions are prone to chromosome breakage and this may provide a mechanism through which B chromosomes can be generated. NOR regions typically exhibit different times of expression (see Dai *et al.* 1994; Lin *et al.* 1995) relative to other autosomal genes and species-specific differences in rDNA condensa-tion have been proposed as having led to the formation of a neo-B chromosome in a somatic hybrid between *Solanum brevidens* and *S. tuberosum* (Mitchell McGrath & Helgeson 1998). This process appears to have also acted in the genus *Brachycome* to generate different rDNA-containing B chromosomes (Houben *et al.* 1997*a*).

Chromosome regions containing rDNA show dynamic variation in repeat numbers and this has been attributed to deletions, duplications and unequal sister homologue exchange (Garrido *et al.* 1994; Garrido-Ramos *et al.* 1995). Intrahomologue recombination has additionally led to biased excision of rDNA between the recombining units in *Neurospora* (Butler & Metzenberg 1989). Assuming intrahomologue recombination and excision to be ubiqui-tous processes acting on rDNA clusters, the extent of NOR contraction is clearly limited in terms of organismal viability in autosomal regions but would potentially be under little or no selection on B chromosomes. A B chro-mosome which originates as an autosomal fragment containing an NOR region may lose its rDNA through

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ıtrachromosome recombination and this may partially xplain how B chromosomes degenerate, as variation in ıe number of rDNA repeats may significantly influence hromosome size (Adam 1992; Pukkila & Skrzynia 1993). his scenario does not necessarily exclude the possibility ıat the presence of rDNA on B chromosomes may give ome selective advantage to B-chromosome carriers Beukeboom 1994*a*); it could be that any selective advan- ıge of having an rDNA-containing B chromosome mply decreases through time as rDNA copy numbers re decreased. Such a mechanism leading to the overall oss of rDNA repeat units may explain what have been onsidered anomalous results in repeated studies of NORs ı different B-chromosome systems (Jones *et al.* 1989; Wilkes *et al.* 1995).

(c) *Transposable elements*

It has been proposed that B chromosomes might accu- ıulate DNA from various sources (Beukeboom 1994*a*) xisting as amalgamations of transposable DNA. This as been suggested as a mechanism through which some f the variability in mammalian Y chromosomes has risen, as random insertions of transposable DNA into ifferent regions of the Y chromosome would result in lements differing with respect to DNA composition and ructure (Marshall Graves 1995). Compelling evidence or early Y-chromosome structural modification and llele silencing resulting from transposable element inser- on comes from the TRAM element of the neo-Y hromosome of *Drosophila miranda* (Steinemann & Steine- ıann 1997). Transposable elements also appear to be nvolved in ectopic recombination (Montgomery *et al.* 991), providing a plausible pathway through which equences may be transferred across different homo- ogues.

Theoretically, transposons should accumulate in egions not subject to recombination (Zeyl & Bell 1996) nd this is supported by the transposable elements TRIM nd TRAM the copy number of which on the neo-Y hromosome of male *D. miranda* (which undergoes recom- ination) is comparable to that of the complete female enome (Steinemann & Steinemann 1991, 1992; Steine- ıann *et al.* 1993). Clear evidence of a B chromosome roviding a safe haven for a mobile element comes from ork on the retrotransposon NATE (NAsonia Transpo- ble Element) which has been described from the PSR lement of *N. vitripennis* (McAllister 1995; McAllister & Verren 1997). A retrotransposon has also been invoked in ıe transposition of chloroplast DNA into the repeat lement Bd49 of the B chromosomes of *B. dichromosomatica* Franks *et al.* 1996). Mobile element insertion may thus be esponsible for the generation of structural variability in chromosomes. This mode of differentiation should roceed in a stepwise manner, with a B chromosome rising through the duplication of a major element ollowed by transposable element insertion. A duplicated utosomal region found on a B chromosome could thus apidly lose homology with its parental sequence, the verall result being suppressed recombination between ıem. Such a scenario may be a contributing factor to the ifficulties in elucidating B-chromosome origins.

Finally, any active genes inherited from the original rogenitor elements of the B chromosomes may become silenced either by insertions of transposable elements within the gene or through disruptions in ordered chromatin structure, as has been shown with silenced larval cuticle protein (*Lcp*) genes on the neo-Y chromosome of *D. miranda* (Steinemann *et al.* 1993). B chromosomes which are transcriptionally active and have no apparent phenotypic effects (Green *et al.* 1993) could thus conceivably have had their transcripts nullified through transposon insertion.

(d) *Epigenetic changes in B chromosomes*

Stem–loop structures are good candidates for protein binding sites and have been associated with heterochromatin condensation in the hymenopterans *Diadromus pulchellus* and *Eupelmus muilleti* (Bigot *et al.* 1990). The highly heterochromatic nature of B chromosomes may therefore (in part) be attributed to the presence of such secondary DNA structures. In the PSR chromosome, small palindromic sequences are associated with exchanges between repeats, suggesting that they enhance recombination between repeat units (Reed *et al.* 1994). Support for the potential of B-chromosome DNA to form hairpins *in vivo* also comes from the micro-dissected B chromosomes of the frog *L. hochstetteri* (Sharbel *et al.* 1998).

At the structural level, the single-stranded loop component of a hairpin may be prone to nucleolytic degradation (Mitas *et al.* 1995), a process that has been connected with chromosome breakage (Chen *et al.* 1995). For example, a G/CTT consensus sequence for topoisomerase I is found in certain eukaryote loop structures where single-stranded DNA cleavage occurs. The formation of hairpin structures in B chromosomes may attract DNases (Vogel *et al.* 1990), and expose enough single-stranded DNA to induce single-stranded cleavage and chromosome breakage, predisposing them to chromosomal rearrangements.

Methylation is hypothesized to cause sex-chromosome inactivation (Holliday 1987) and may therefore play a role in B-chromosome evolution. The B chromosome repeat family Bd49 of the Australian daisy (*B. dichromosomatica*) is hypermethylated and, thus, transcriptionally inactive and this is supported by an absence of Bd49 transcripts in leaf RNA extractions (Leach *et al.* 1995). In addition to effects on transcription, non-Mendelian B-chromosome behaviour may also be influenced by methylation. Neves *et al.* (1992) showed that induced demethylation (or blocking of methylation) in rye (*Secale cerale*) causes B chromosomes to undergo mitotic non-disjunction, a known B-chromosome accumulation mechanism.

Finally, chromatin packaging probably influences transcriptional regulation and the relative acetylation of histone molecules causes gene silencing in several organisms (see Houben *et al.* 1997*b*; Costanzi & Pehrson 1998). Houben *et al.* (1997*b*) showed that the B chromosomes of *B. dichromosomatica* are underacetylated relative to the autosomes and that this, in conjunction with late replication of B-chromosome DNA, may cause B chromosomes to become genetically inert. Similarly, autosomal rDNA transcription in *Allium* during different stages of mitosis is blocked by chromosome condensation (González-Fernández *et al.* 1993). Genetic inactivity of B chromosomes may thus not only be considered in terms of non-coding or non-functional DNA, but also from the

erspective of the many protein–DNA complexes which an be physically affected by chromatin structure.

It is becoming increasingly evident that the apparent imilarities between B and sex chromosomes are more han just coincidental and that the molecular evolution of chromosomes may therefore be interpreted in the ontext of sex-chromosome evolution (see Appendix A). nitially, a process must be available which can isolate a ewly formed progenitor B chromosome relatively apidly, such that homologous (or homoeologous) pairing prevented. Such processes may be found in those analo- ous mechanisms which act upon heteromorphic sex hromosomes which have been studied in more depth. ubsequent to their isolation within the nucleus, B chro- osomes would be expected to degenerate, in both struc- ure and DNA sequence composition. This will make the dentification of their progenitors more difficult over time.

4. FREQUENCY

B chromosomes have been described in more than 1300 pecies of plants and almost 500 species of animals (for eviews, see Jones & Rees 1982; Jones & Puertas 1993; ones 1995) and in various species of fungi (Mills & IcCluskey 1990; Miao *et al.* 1991*a*,*b*; Tzeng *et al.* 1992; eiser *et al.* 1996; Leclair *et al.* 1996). These chromosomes ave been described predominantly from certain taxo- omic groups, although the high frequency of B chromo- omes in these taxa probably reflects the intensity and echnical ease with which each group has been studied. It not surprising, therefore, that B chromosomes have requently been reported in the Graminea, Liliacea and Orthoptera, groups which satisfy both these conditions. n fact, the discovery of B chromosomes in fungi was ossible only after the development of a pulse-field gel lectrophoresis technique for karyotyping these organ- sms. Thus, it is likely that many more species, when nalysed with sufficient intensity, will be found to possess chromosomes.

B chromosomes can attain extremely high frequencies n natural populations, depending both on the degree to which a particular species can tolerate these additional lements and on the strength of the B chromosomes' accu- nulation mechanism (if there is one). A stable frequency f B chromosomes is often found for several years in the ame population, which has prompted authors in the past o conclude that polymorphism is in a state of equilibrium nd that the frequency is a result of the action of two pposing forces—the accumulation of the B chromosome which tends to increase the B-chromosome frequency) nd the harmful effects on the fitness of the individuals arrying the B chromosome (which tend to decrease the requency). However, as will be described later, B- hromosome polymorphism may be best interpreted as a ynamic system in which the frequency continually shifts ue to an arms race between the A and B chromosomes.

In addition, interpopulational differences in B chromo- ome frequency depend on selective factors (i.e. the eco- ogical tolerance of B chromosome carriers in terms of the ermissiveness of the environmental conditions for a articular population), historical factors (i.e. the number f generations since B-chromosome origin), transmission actors (related to the differences between populations in the accumulation intensity of the B chromosome) and random factors (i.e. the action of genetic drift in populations of finite size). The four types of factor probably act simultaneously, making it difficult to evaluate the relative importance of any single one, even under intense analysis. Some insight into the relative importance of these factors may be obtained from the distribution of B-chromosome counts among individuals sampled from one population analogous to tests on transposable elements in *Drosophila* (Charlesworth & Lapid 1989; Charlesworth *et al.* 1992*a*,*b*).

The maximum number of B chromosomes that a species is capable of tolerating, measured by the maximum number of B chromosomes found in adult individuals, varies broadly, although it ultimately depends on the relative intensities of the above-mentioned factors. Corn plants have been found with 34 B chromosomes (involving a 155% increase in nuclear DNA content; see Jones & Rees 1982), a situation which is probably tolerable to the plant because of its domestication. In wild plants, such as *Lolium perenne* (Jones & Rees 1982) and *B. dichromosomatica* (Carter 1978), individuals have not been found with more than three B chromosomes, although in *Allium schoenoprasum* plants have been reported carrying up to 20 B chromosomes (Bougourd *et al.* 1995). In the grasshopper *E. plorans* (Camacho *et al.* 1997*b*) and the flatworm *P. nigra* (Beukeboom *et al.* 1996), it is also rare to find individuals with more than three B chromosomes in natural populations, while individuals of the endemic New Zealand frog *L. hochstetteri* can have up to 15 mitotically stable B chromosomes (Green *et al.* 1993).

5. EFFECTS

Most B chromosomes are heterochromatic, promoting the general idea that these elements are genetically inert. Analyses of general transcriptional activity using tritiated uridine have supported this idea (Fox *et al.* 1974; Ishak *et al.* 1991). Nevertheless, some B chromosomes show transcriptional activity, as has been shown in the plumose state in the frog *L. hochstetteri* (Green 1988) or in the polytene state of the mosquito *Simulium juxtacrenobium* (Brockhouse *et al.* 1989). In addition, many B chromosomes have been found to carry ribosomal genes (for reviews, see Green 1990; Beukeboom 1994*a*; Jones 1995), although they are for the most part inactive (Donald *et al.* 1997). Some effects of the B chromosomes appear to be attributable directly to the products of their genes, as is the case with genes controlling resistance to rust in the B chromosomes of *Avena sativa* (Dherawattana & Sadanaga 1973), and the genes conferring resistance to antibiotics in the B chromosomes of the fungus *Nectria haematococca*, thereby favouring its pathogenicity (Miao *et al.* 1991*a*,*b*). These examples indicate that not all B chromosomes are genetically inactive. However, much more information is needed to support the generally accepted opinion that most B chromosomes lack major genes.

There is ample evidence that B chromosomes can affect a multitude of cellular and physiological processes in both plants and animals. The effects are rarely manifest in the external phenotype, although the B chromosomes of *Haplopappus gracilis* influence the colour of the achenes (Jackson & Newmark 1960) and, in corn, plants with B

hromosomes develop striped leaves (Staub 1987). More requently, B chromosomes affect processes or characters ssociated with vigour, fertility and fecundity. Jones & Rees (1982) summarized a broad range of mostly detri- ental effects from the B chromosomes in many species f plants and animals. These negative influences on host tness pointed to the parasitic nature of B chromosomes. Nevertheless, some B chromosomes, when present in low umbers, have beneficial effects upon their carriers and, us, may have a different biological significance discussed in § 7(a)). For example, the B chromosomes of arious species of plants are associated with increased ermination vigour or speed (see table 4.2 of Jones & Rees (1982)).

The influence of B chromosomes may stem either from heir presence or from the activity of genes found on hem. For example, the B chromosomes of the plant *Scilla utumnalis* (Ruíz-Rejón *et al.* 1980; Oliver *et al.* 1982) and *l. schoenoprasum* (Plowman & Bougourd 1994) alter the xpression of A-chromosome genes for an esterase and ndosperm protein, respectively. The presence of B chro- osomes can also influence the expression of NORs on he A chromosomes, as is the case for the grasshopper *E. plorans* (Cabrero *et al.* 1987). As mentioned above, many chromosomes contain ribosomal genes, the activity of which could give the cell higher levels of translation (but ee Donald *et al.* 1997). It would therefore be informative o study the possible effect that B chromosomes possessing ctive NORs might have on growth rates.

It should be emphasized that B-chromosome effects epend on the environmental conditions acting upon a opulation and can be characterized by both spatial and emporal variation. It is therefore risky for the effects etected in one population to be extrapolated over the ntire distribution range of that species. Each case should e analysed thoroughly in many populations and the ffects should be studied under the most natural condi- ons possible.

6. TRANSMISSION

Given that B chromosomes do not always occur in airs and segregate to opposite poles during meiosis (the ehaviour that stabilizes chromosome number in A chro- osomes), they do not conform to a Mendelian system. Non-B univalent chromosomes would be expected to have eiotic transmission rates of 0.5, but this is typically ower as they are unstable in meiosis and/or mitosis. Many B chromosomes register transmission rates clearly reater than 0.5, i.e. they show accumulation, the most mportant property of parasitic B chromosomes. Accumu- ation can take place before, during or after meiosis; ones (1991) exhaustively reviewed the principal cyto- ogical mechanisms that cause this accumulation. Regarding a pre-meiotic mechanism, it suffices to ention B-chromosome accumulation in the locust *ocusta migratoria* derived from their mitotic instability nd the preferential destiny of cells with a high number of chromosomes to become spermatogonia (Nur 1969). Meiotic accumulation has been described from female eiosis in various species of plant and animal and is ased on the inherent asymmetry in the production of nly one ovule from each oogony; the B chromosome migrates preferentially to the secondary oocyte instead of to the first polar body. In the insect *Pseudococcus affinis*, the B chromosomes accumulate during male meiosis by escaping the heterochromatinization and elimination of a chromosomal set characteristic of spermatogenesis (Nur 1962). Post-meiotic accumulation is frequent in plants, where the formation of pollen grains involves two post-meiotic mitotic divisions that give rise to the generative and vegetative nuclei; the non-disjunction of the B chromosome in this mitosis and the preferential migration of the two B chromatids to the generative nucleus are responsible for B-chromosome accumulation.

There is even a case of ameiotic accumulation of a B chromosome in the parasitoid wasp *N. vitripennis* (Werren 1991), where the B chromosome (PSR) present in the spermatozoa causes the condensation and loss of the paternal chromosomes accompanying it, transforming the diploid (female) zygote to a haploid male carrying the B chromosome. Through this mechanism the B-chromosome's transmission rate approaches one and, because it reduces the fitness of its host to zero, this B chromosome is considered one of the most parasitic of all known genetic elements.

In two species, rye (for references, see Jones & Rees 1982) and *L. migratoria* (Pardo *et al.* 1994), B chromosomes accumulate through both sexes, whereas in the grasshopper *Myrmeleotettix maculatus* B chromosomes show drive through females but drag through males (Hewitt 1973*a*,*b*,*c*). However, not all B chromosomes show accumulation, as in the plants *Poa alpina* (Håkansson 1954), *P. trivialis* (Bosemark 1957), *Centaurea scabiosa* (Fröst 1958), *Ranunculus acris* (Fröst 1969), *A. schoenoprasum* (Bougourd & Parker 1979) and *Guizotia scabra* (Hiremath & Murthy 1986). In animals, the most notable case is that of the grasshopper *E. plorans*, in which the three most frequent types of B chromosomes lack accumulation mechanisms (López-León *et al.* 1992*a*). These examples suggest the existence of other models of B-chromosome evolution that differ from the parasitic one, as we shall discuss in § 7.

7. DYNAMICS

In general, B chromosomes could be considered genome symbionts the population dynamics of which depend on two important properties, i.e. their effects on genome fitness and their transmission ratio. Several outcomes are theoretically possible from the interaction of these two properties (table 2). It is clear that, subsequent to their origin, B chromosomes require accumulation mechanisms, otherwise their proliferation may only be explained in terms of beneficial effects on carriers. These are the only ways in which these chromosomes can increase in frequency and establish a polymorphism in a natural population (categories 1, 4 and 7–9). A newly risen B chromosome falling into category 5 would fail to establish a polymorphism, as failure to synapse and irregular meiotic behaviour would preclude its ability to become fixed by genetic drift. As opposed to the A chromosomes and the genes they contain, which normally follow the laws of Mendelian inheritance, the B chromosome is destined to extinction through random forces. Therefore, a near-neutral B chromosome (category 5) is

Table 2. *Possible outcomes of the effect on host genome fitness and B-chromosome transmission rate on the establishment of a B chromosome*

(Δw_G, change in host genome fitness due to B chromosome presence. $k_B - 0.5$ indicates the difference between the B-chromosome transmission ratio (k_B) and the Mendelian one (0.5); therefore + indicates B chromosome accumulation and − indicates B chromosome elimination.)

category	Δw_G	$k_B - 0.5$	net result	evolutionary significance
	−	+	B polymorphism	parasitism
	−	0	B disappears	—
	−	−	B disappears	—
	0	+	B polymorphism[a]	attenuated parasitism
	0	0	B disappears	near-neutral B chromosome
	0	−	B disappears	—
	+	+	B polymorphism[a]	mutualism
	+	0	B polymorphism[a]	mutualism
	+	−	B polymorphism	mutualism

[a] Infinite accumulation is prevented because individuals with high numbers of B chromosomes have reduced fitness.

presumably derived from an attenuated parasitic B chromosome (category 4) that has lost drive or from a mutualistic B chromosome (e.g. category 8) that is no longer beneficial for genome fitness. If the new B chromosome were harmful to the carriers, it would only be able to persist if its propensity to accumulate outweighed any negative effects upon its carriers. This may explain the origin of most of the parasitic B chromosomes known today from a multitude of species (category 1).

(a) *Equilibrium models*

The two most widely accepted models of B-chromosome evolution, the heterotic model (White 1973) and the parasitic (Östergren 1945; Nur 1966, 1977) or selfish model (Jones 1985; Shaw & Hewitt 1990), assume that the frequencies of B chromosomes are in equilibrium in current populations and are used to contrast the antagonistic forces responsible for the equilibrium. The heterotic model assumes a balance between the positive fitness effects of B chromosomes (which show no accumulation) when they occur in low numbers and their negative effects when they occur in high numbers. Typically, it has been applied to category 8, but could equally fit categories 7 and 9 (table 2). The only known B chromosome which has a strong likelihood of being heterotic is that of the chive *A. schoenoprasum*. While this B chromosome does not show accumulation, it has been demonstrated that plants with B chromosomes survive better in natural habitats than those without B chromosomes (i.e. in terms of the development from seed to seedling; Holmes & Bougourd 1989) due to the fact that the B chromosomes boost the germination rate under drought conditions (Plowman & Bougourd 1994).

For the parasitic-selfish model, the equilibrium is the result of B-chromosome accumulation (which increases its frequency) and, typically, of its detrimental effects on the fitness of B-chromosome carriers (which reduce the frequency of the B chromosome). The great majority of B-chromosome systems that have been analysed in detail fall into category 1 (table 2) and are thus compatible with the parasitic model (see Nur 1977; Jones 1985, 1995; Nur & Brett 1985, 1987, 1988; Ruíz-Rejón *et al.* 1987; Shaw & Hewitt 1990).

In most of these studies, B-chromosome frequencies are minimized through their increasing negative effects on host fitness when they increase in number, but other selective pressures may also play a role. A good example is the PSR chromosome of the parasitoid wasp *N. vitripennis*, in which population structure and fertilization proportion affect the spread of the PSR (Beukeboom & Werren 1992; Werren & Beukeboom 1993). The PSR has a transmission rate to sperm of nearly one but causes destruction of the paternal chromosomes, except for itself, shortly after egg fertilization. Owing to haplodiploidy, this results in the conversion of diploid (female) eggs to haploid (male) eggs that carry the PSR. *Nasonia vitripennis* parasitizes fly pupae that occur in temporary patches (e.g. at carcasses) resulting in a demic population structure where flightless males mate locally with their emerging sisters. A theoretical analysis showed that the PSR equilibrium frequency is strongly affected by deme size (the number of founding females) and the fertilization proportion. Population experiments under laboratory conditions confirmed most of the theoretical predictions, i.e. it led to loss of the PSR from populations consisting of small deme sizes and when the fertilization proportion was low. Although these laboratory results have not been repeated under natural conditions, Beukeboom (1994*b*) showed that the PSR causes such minor effects on various traits related to carrier fitness that population structure and fertilization proportion play the major role in determining the frequencies of the B chromosome in natural populations.

(b) *Tolerance to B chromosomes*

It has been a parasitological dogma that a well-adapted parasite should not damage its host, as debilitation and death of the host can cause the death of the resident parasites (Hoeprich 1977; Alexander 1981). However, theoretical (Anderson & May 1982; May & Anderson 1983) and comparative analyses (Ewald 1987) have suggested that this is not necessarily so. For instance, the evolution of parasite virulence (the effect of a parasite on host fitness) may be strongly influenced by the parasite's mode of transmission (Anderson & May 1982; Lipsitch *et al.* 1995), i.e. parasites transmitted horizontally should be more virulent than those transmitted vertically, because the latter have their fitness linked to the fitness of their host and, therefore, harming of the host will reduce parasite fitness. In contrast, horizontally transmitted ones can be more virulent because they may contagiously infect other individuals. Mathematical models predict that parasites that are only vertically transmitted should evolve towards less virulence (Lipsitch *et al.* 1995) and several comparative (Ewald & Schubert 1989; Herre 1993; Clayton & Tompkins 1994) and experimental (Bull *et al.* 1991) studies have shown that the degree of vertical transmission in nature is positively correlated with benignity.

B chromosomes are exclusively vertically transmitted parasites and, hence, fit the expectation of the evolution towards attenuated parasitism, e.g. a change from category 1 to 4 in table 2. This may result from the appearance of less virulent B-chromosome types and/or the evolution of more tolerant host genotypes. As Shaw (1984, p. 93) pointed out, 'alleles on the A chromosome set that reduce the selection operating against animals carrying B's will be selected, as will B-chromosomes that are less damaging to their carrier'. Although many detrimental effects of B chromosomes have been reported (Jones & Rees 1982), it should be borne in mind that the evolution of B-chromosome tolerance depends on the existence of appropriate genetic variation and a high increase in B-chromosome frequency, because selection for B-chromosome tolerance can only take place in B-chromosome-carrying individuals. It is thus conceivable that B-chromosome tolerance has not evolved in all known systems. The inability to detect significant effects of B chromosomes on carrier fitness would be consistent with the evolution of B-chromosome tolerance in a natural population. For instance, the locust *L. migratoria* (Castro *et al.* 1998) harbours attenuated parasitic B chromosomes that do not produce apparent deleterious effects in B-chromosome carriers (category 4). Likewise, the grasshopper *E. plorans* (López-León *et al.* 1992*a*,*b*; Camacho *et al.* 1997*a*,*b*; Martín-Alganza *et al.* 1997) possesses B chromosomes that were originally parasitic but whose drive has subsequently been neutralized by the host genome. These B chromosomes have no apparent effects on carrier fitness. Interestingly, a new parasitic B-chromosome variant (B_{24}) that has recently replaced the neutralized B-chromosome version (B_2) significantly reduced egg fertility (Zurita *et al.* 1998). This suggests that newly arisen parasitic B-chromosome variants are more harmful than older B-chromosome versions. These conclusions are preliminary since the evolution of tolerance to B chromosomes has not received much consideration in the past, mainly because of the difficulty in detecting slight effects. Nevertheless, there are a large number of B-chromosome systems where no significant B-chromosome effects have been detected.

(c) *Suppression of drive*

An absence of accumulation does not necessarily indicate that a B chromosome is heterotic. Parasitic B chromosomes impose a genetic load upon carrier populations and, thus, favour the evolution of any gene variants on the A chromosomes which would tend to reduce this load, either by eliminating B-chromosome accumulation (= B-chromosome resistance genes) or by buffering any detrimental effects (the evolution of B-chromosome tolerance genes; see § 7(b)). The presence of some type of A-chromosome genetic control over B-chromosome accumulation has been demonstrated in *S. cereale* (Müntzing 1954; Romera *et al.* 1991; Jiménez *et al.* 1995), *Festuca pratensis* (Bosemark 1954), *Zea mays* (Carlson 1969; Rosato *et al.* 1996), *Hypochoeris maculata* (Parker *et al.* 1982), *H. maculatus* (Shaw & Hewitt 1985; Shaw *et al.* 1985), *Pseudococcus affinis* (Nur & Brett 1985, 1987, 1988), *Aegilops speltoides* (Cebriá *et al.* 1994) and *E. plorans* (Herrera *et al.* 1996). Such evidence has been extrapolated mostly from variation in transmission rates between individuals, from the success of artificial selection in obtaining lines of high and low transmission rates and from the different results obtained through intra- and interpopulational crosses. Recently, evidence has been provided that B chromosomes in rye possess genes controlling their own transmission (Puertas *et al.* 1998).

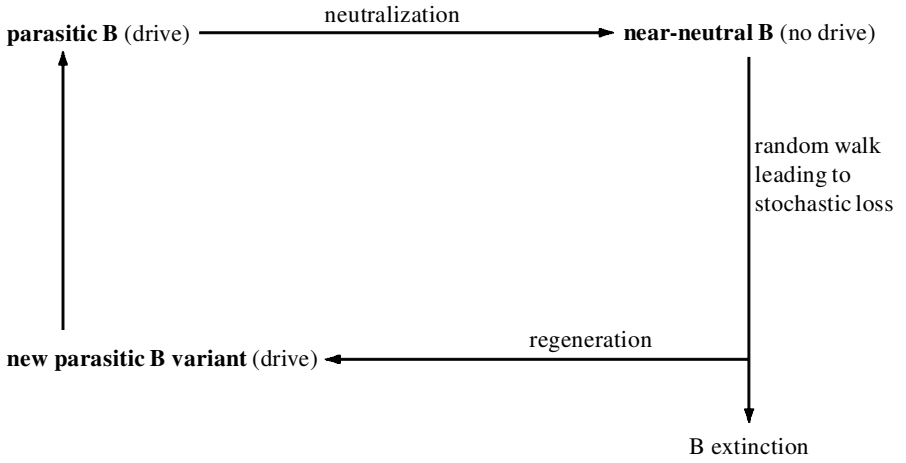
(d) *A non-equilibrium model of long-term evolution*

Parasitic B chromosomes that have lost their accumulation mechanisms are doomed to disappear, unless they become heterotic or recover accumulation at some point during the long process towards random extinction, thereby transforming them into a new type of parasitic B chromosome. According to the magnitude of negative effects exerted by the B chromosomes at the time of losing accumulation, they would disappear rapidly (large effects), slowly (small effects) or very slowly (imperceptible effects) from the population. In this last case, we can consider B chromosomes to be near neutral (category 5), a type of B chromosome that is not found in equilibrium, but that, as we shall see below, constitutes a transitory stage towards disappearance or towards regeneration of the polymorphism.

The only proof of the existence of near-neutral B chromosomes, that is those that have lost accumulation and produced insignificant effects on the fitness of the carriers, is presently provided by the B chromosome of the grasshopper *E. plorans* (see Appendix B). While the transmission ratio of B chromosomes in *E. plorans* is usually close to 0.5 in most individuals (López-León *et al.* 1992*a*), these ratios can vary greatly between individuals in many species with parasitic B chromosomes (Bosemark 1954; Müntzing 1954; Parker *et al.* 1982; Nur & Brett 1985, 1987, 1988; Shaw & Hewitt 1985; Shaw *et al.* 1985; Romera *et al.* 1991; Cebriá *et al.* 1994; Jiménez *et al.* 1995). This could be due to the fact that the suppression of B-chromosome accumulation imposes negative pleiotropic effects of the genes involved, thereby impeding any marked increase in their frequency and preventing complete suppression of B-chromosome accumulation.

The B-chromosome system in *E. plorans* not only illustrates the presence of parasitic chromosomes neutralized by the A-chromosome genome, but goes further to provide evidence of one of the few evolutionary paths remaining for these B chromosomes (apart from disappearing, but this is of minor evolutionary interest); it involves the regeneration of the polymorphism through the appearance of a new parasitic B-chromosome variant that starts the cycle again (figure 1).

Overall, the polymorphism for the B chromosome of *E. plorans* has been regenerated on at least three occasions on the Iberian Peninsula (assuming that B_1 was the ancestral type, given that it is predominant in the majority of the populations analysed): (i) when B_1 was substituted by B_2 in the province of Granada and the eastern part of the province of Málaga, (ii) when B_1 was replaced by B_5 in the zone of Fuengirola (Málaga), and (iii) when B_2 was replaced by B_{24} in Torrox. This polymorphism illustrates that B-chromosome polymorphisms must not be seen necessarily as a system in equilibrium, but rather as a dynamic succession of stages through which the same polymorphism can change from parasitism to near neutrality and then to parasitism again. Thus, the B



igure 1. Arms race making up the long-term evolution of a parasitic B chromosome. The life cycle of the B chromosome begins ith the parasitic stage in which the B chromosome possesses drive (which is the property facilitating its establishment in the opulation) and is harmful for the individuals carrying it. Neutralization consists of the suppression of B-chromosome drive by he evolution of appropriate genes in the A-chromosome genome, paralleled by the evolution of an A-chromosome genome more olerant to B-chromosome effects and/or less harmful B-chromosome variants. The near-neutral B chromosome possesses no drive nd causes minor effects on carriers, at least at low numbers. This leads it to a random walk towards stochastic loss (since -chromosome fixation is impossible because of meiotic irregularity and harmfulness at high B-chromosome numbers) that may e long in large populations. However, if in the course of the random walk the B chromosome mutates to a new variant capable f driving, then the polymorphism regenerates and the cycle starts again.

hromosome's existence can be prolonged by dynamically esisting A-chromosome genome assaults which tend to orce the B chromosome to disappear.

It is not possible at present to determine how many ystems of B chromosomes might be similar to the ystem of *E. plorans*, but a thorough analysis of those that o not show accumulation or that show it in some popu- ations but not in others will almost certainly show imilar cases in the near future. The non-equilibrium odel developed for the *E. plorans* B chromosomes illumi- ates the possible long-term evolution of not only para- itic B chromosomes but also other selfish genetic lements (Johnson 1997).

8. ROLE

The role of B chromosomes in the evolution of ukaryotic genomes appears at first to be somewhat uperfluous. Given that their presence is not needed for urvival or reproduction of the individual, these chromo- omes appear at first to be simply 'genomic junk', a waste roduct of the eukaryotic genome. Nevertheless, given hat B chromosomes cannot originate as junk (that is, as eutral or slightly harmful), but most probably begin by eing selfish (because if they had no accumulation they ould not increase in frequency), it is plausible that, of all he extra chromosomes produced over the evolution of enomes of most organisms, only the selfish ones can be ransformed into B chromosomes.

We have already discussed the neutralization of a arasitic B chromosome, but one might ask whether a eutralized B chromosome could become heterotic. The dea that a parasitic B chromosome could become eterotic was proposed first by Kimura & Kayano (1961) nd has also been defended by Ruíz-Rejón *et al.* (1987). he main problem arising from this possibility involves he fact that the genes of the B chromosomes are generally inactive and can therefore accumulate a large number of mutations. One must then ask how the B chromosomes evolved a beneficial characteristic for the carrier, somehow resisting the effect of Muller's ratchet. There are several possible answers: (i) as we observed above, some genes of the B chromosomes are active, thus conserving some of their functional requirement, suggesting that under certain circumstances these genes may be advantageous to the genome, and (ii) B chromosomes can capture genes from the A-chromosome genome and, thus, become indispensable to the host. This is the case mentioned above for the gene present in the B chromosome of the fungus *N. haematococca*, which makes it resistant to pisatine, an antibiotic produced by its host plant, the pea (Miao *et al.* 1991*a*,*b*).

In the same light, it has recently been proposed that the Y chromosome of *Drosophila* may have evolved from a supernumerary chromosome with the characteristics of a B chromosome (see Hackstein *et al.* (1996) for details of the argument). This idea, which suggests one of the forms in which a B chromosome can ultimately integrate itself into the A-chromosome genome, makes sense only when the B chromosome provides more or less essential functions and achieves regularity in meiosis. For example, in the zebra finch *Taeniopygia guttata*, a supernumerary chromosome has recently been reported which is restricted to the germline such that all males and females carry one (Pigozzi & Solari 1998). The authors have proposed a mechanism by which one copy of the supernumerary is always present in all individuals, i.e. through complete elimination in male meiosis and total preferential segregation to the oocyte in females. If this hypothesis were correct, this could be a stabilized B chromosome transmitted through a single sex (the female), a case reminiscent of the *Nasonia* one. We could imagine the zebra finch situation as a possible solution for many selfish B chromosomes that show strong drive through the

emale and are partly eliminated through the male, e.g. *M. maculatus* (see §6). Their absence from the somatic ne avoids most of their harmful effects on carriers and heir special transmission mechanism assures their egular presence in the germline. In addition, it is onceivable that B chromosomes can integrate themelves into the genome by translocation, a possibility first uggested by White (1973) and supported by the spontaeous translocations that have been recorded between he A and B chromosomes in *E. plorans* (Henriques-Gil *et l.* 1983; Cabrero *et al.* 1987). At first sight, the possibility hat B chromosomes may become transformed into ssential members of the A-chromosome genome (even orming part of some of the chromosomes of the A set) is remote one. However, there are some recent data uggesting this possibility since a population analysis in he Brazilian wasp *Trypoxylon albitarse* has shown that, in ost populations in the Viçosa region, most males have ne B chromosome and most females have two B chromoomes. Since males are haploid and females diploid, it eems that this B chromosome is close to stabilization in hese populations (S. M. S. R. Araujo, personal communiation). Perhaps these B chromosomes could represent only fraction of the total heterochromatin of these species and heir fate could depend on their possible role in the enome. The molecular analysis of this system is an interesting task for future research in the B-chromosome field.

9. PERSPECTIVES

We have discussed a variety of B-chromosome ystems each with its particular transmission dynamics. lthough most B chromosomes show accumulation of ne form or another (Jones 1991), some exceptional B hromosomes transmit at nearly Mendelian rates and, herefore, cannot be categorized as selfish. This is the ase, for example, with the B chromosomes of the chive l. *schoenoprasum* (Bougourd & Parker 1979) and of the rasshopper *E. plorans* (López-León *et al.* 1992*a*), studies hich offer new perspectives on the evolution and iological meaning of these enigmatic chromosomes. We ave seen the discovery of new B-chromosome systems ith previously unknown mechanisms of (accumulaion) transmission and we can expect more in the uture.

Recent molecular studies of B chromosomes have evealed that they do not have a single mode of origin, ut instead can arise in a variety of ways. Although the ource of the B chromosomes can sometimes be traced ith a degree of certainty (e.g. the autosomal complenent, sex chromosomes or a closely related species), it has till been difficult to pinpoint the exact progenitor DNA egion(s). The extent to which this will be possible is nclear at the moment. It will depend critically on intenive molecular study as well as our ability to determine he speed and nature of the molecular processes involved n chromosome evolution. This should also reveal why we annot find any traces of silenced (relict) genes on xisting B chromosomes (other than rDNA).

B chromosomes are being recognized as suitable ystems for studying genome evolution. As single chromoomes that have been freed from the selection pressures hat act on the maintenance of standard chromosomes, they may prove useful in studying processes of molecular degeneration analogous to studies of heterogametic sex chromosomes. We expect that they will become important in understanding chromosomal evolution, including evolution of repetitive DNAs, gene silencing and transposable elements.

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APPENDIX A. REASONS FOR SIMILARITY BETWEEN SEX CHROMOSOMES AND B CHROMOSOMES

B chromosomes are often similar to sex chromosomes in terms of meiotic behaviour, size, morphology and heteropycnocity (Hewitt 1979; Amos & Dover 1981; Jones & Rees 1982; Green 1990), and this resemblance can be interpreted in a number of ways.

(i) In the orthopteran, *Melanoplus femur-rubrum*, B-chromosome similarity to their X chromosomes has been explained in terms of chromosome inactivation, as the heteropycnocity of both elements is likely required for normal meiosis (Nur 1978). As such, the B and sex chromosomes of *M. femur-rubrum* converge upon a common morphotype due to the functional constraints of meiosis (i.e. to prevent pairing of B and X chromosomes with autosomal homologues; Dover & Riley 1972).

(ii) Shared similarity between B and sex chromosomes may also imply real homology, as has been shown by Amos & Dover (1981), who demonstrated that the B chromosomes of the fly *Glossina* have arisen from a duplicated Y chromosome and have subsequently become differentiated from the Y chromosome through the accumulation of tandem repeat DNA or as in the frog *L. hochstetteri*, whose B chromosomes have been derived from the univalent W sex chromosome (Green *et al.* 1993; Sharbel *et al.* 1998).

(iii) B chromosomes and univalent members of heteromorphic sex chromosomes may converge upon a typical degenerate morphotype due to similar molecular evolutionary processes acting upon them (Green 1990).

APPENDIX B. THE B-CHROMOSOME SYSTEM OF *E. PLORANS*

The B-chromosome polymorphism of *E. plorans* is xtremely widely distributed over Mediterranean and outhern Atlantic coastal regions of the Iberian Peninsula Henriques-Gil *et al.* 1984), the north of Africa Henriques-Gil 1984) and Italy (López-Fernández *et al.* 992). Its most important characteristics are as follows.

i) The propensity to mutate, as exemplified by the high number of novel B-chromosome types described to date (more than 40 have been differentiated on the basis of size, morphology and C-banding; Henriques-Gil *et al.* 1984; Henriques-Gil & Arana 1990; López-León *et al.* 1993) and that new types of B chromosome can appear between the offspring of controlled crosses where none of the offspring carried this type of B chromosome (López-León *et al.* 1993). The most widely distributed B chromosome, called B_1, is considered the ancestral B chromosome from which the rest were derived (Henriques-Gil *et al* 1984) by replacement processes (Henriques-Gil & Arana 1990).

ii) The three most frequent types, B_1, B_2 and B_5, lack accumulation (López-León *et al.* 1992*a*) and lack significant effects over several traits related to the fitness of the carriers (López-León *et al.* 1992*b*; Camacho *et al.* 1997*a*,*b*).

iii) B_2 is capable of accumulating in females crossed with males from populations having no B chromosomes, but not when the same females are crossed with males from the same population (Herrera *et al.* 1996), thus suggesting that the B chromosomes originally had an accumulation mechanism which was lost due to the evolution of drive suppressor genes in the A chromosomes, which, logically, are not found in the males of the population that lacks B chromosomes.

In the population of *E. plorans* captured in 1984 near orrox (Málaga), Henriques-Gil & Arana (1990) verified he dominance of a B-chromosome type termed B_{24} (with mean number of B chromosomes of 0.344), which was ifferent from the predominant type in adjacent popula- ons (B_2). This new B chromosome was like B_2, but with duplicated proximal band associated with a greater mount of repetitive 180 bp DNA than possessed by B_2, as ell as a lesser amount of ribosomal DNA (Zurita *et al.* 998). After also finding B_2, although at a very low requency, Henriques-Gil & Arana (1990) proposed that $_2$ was being replaced by B_{24} in this population. In 1992, urita *et al.* (1998) captured specimens in this same loca- ion which showed a B-chromosome frequency of 0.975, normously exceeding that of 1984 and made a series of ontrolled crosses which indicated that B_{24} had a strong endency to accumulate through females, their mean ransmission ratio (0.696) being significantly higher than Mendelian one. However, they did not find any traces f B_2 in the sample analysed from 1992 and it would thus ppear that B_{24}, a new selfish variant showing accumula- ion, has completely replaced B_2 (a neutralized B chro- osome incapable of accumulating) over the last few ears in Torrox. In 1994, a new sample of individuals revealed that the B_{24} frequency had continued to increase, reaching its highest value ever recorded in a natural population of *E. plorans* (1.533) and that B_2 was no longer present. It seems, therefore, that the regeneration of polymorphism had already been completed in this population. If our theory regarding the dynamic evolution of the B-chromosome polymorphism is correct, B_{24} should be neutralized within the next few years. In fact, the first evidence of suppressor genes against B_{24} drive has already appeared: a small proportion of the crosses made by Zurita *et al.* (1998) showed a B_{24} transmission rate close to a Mendelian one and they even found one female with one B chromosome to transmit this chromosome to only 15.2% of her offspring.

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