

A correlation between B chromosome frequency and sex ratio in *Exochomus quadripustulatus*

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Abstract. A survey of natural populations of the British ladybird *Exochomus quadripustulatus* revealed the presence of a single large, acrocentric, supernumerary (B) chromosome in all sites visited. Studies were confined to male meiosis, where more than one B was never found to accompany the six bivalents and neo-XY sex pair. The percentage of males possessing B chromosomes varied from 6.4% to 28.6% in 14 different populations. The sex ratios present in these populations also varied. In some equal numbers of males and females were present, in others there were significant excesses of females. A linear regression was found between the percentage of B chromosomes and the percentages of males and females in those populations. It is suggested that the B chromosomes are not in themselves responsible for the sex ratio differences found for similar differences in sex ratio have been found in related neo-XY species lacking B chromosomes. It is more likely that those factors affecting sex ratio are also responsible for affecting the frequencies of B chromosomes in different populations.

Introduction

Considerable interest has been shown in B chromosomes during the past 20 years. This has included population studies of B chromosomes in plant and animal species, molecular studies of their DNA content, and analyses of their effects in cereals of economic importance. Derived from the normal members of the complement (A chromosomes) by some form of breakage and reunion, they cannot be produced by simple fragmentation as was once proposed, for their relative stability indicates the presence of telomeres as well as centromeres. For many years they were considered to be relatively inert, but an increasing number of phenotypic effects have now been revealed (Rees and Jones 1977). Such effects include the modification of rate of growth and development of a planarian (Melander 1950), increase in the duration of the mitotic cycle in *Lolium* and rye, decrease in fertility with increasing numbers of Bs in rye and decrease in tiller number and straw weight in rye (Müntzing 1963). Changes may also be found in the chiasma frequencies of those cells in which they are found. Thus in the grasshopper *Myrmeleotettix maculatus* (John and Hewitt 1965a, b; Hewitt and John 1967) chiasma frequencies are increased in the presence of Bs. Similar in-

creases have been found in Maize (Ayonoadu and Rees 1968) and *Listera* (Vosa and Barlow 1972). In *Lolium*, Cameron and Rees (1967) found the opposite effect, a reduction. In rye Jones and Rees (1967) found that introduced Bs were without effect on chiasma frequencies, while Zečević and Paušović (1969) found that naturally occurring (wild) Bs did increase chiasma frequencies in rye.

Despite these demonstrations of the effects of B chromosomes on various aspects of the phenotype of their species, it does not necessarily follow that these effects are, in themselves, the cause of any selective advantage which the B-containing individuals may possess. It is equally possible that they may be side effects, quite unrelated to some more important effect, as yet incompletely, or not, understood. The fascination of B chromosomes will no doubt continue for many years to come.

Because satellite DNA sequences may also be variable additions to the chromosome complement in the way that B chromosomes are, a correlation was sought, and claimed to exist, in the grasshopper *Myrmeleotettix* by Gibson and Hewitt (1972). A re-analysis of the DNA of this species by Dover and Henderson (1976), however, revealed that this was not the case, and that the B chromosomes contained essentially the same type of DNA as the rest of the chromosomes. This observation highlights one of the most inexplicable aspects of the evolution of B chromosomes. Although B chromosomes are sometimes small, they are also often quite large, involving large portions of the chromosomes from which they were derived. The problem which requires explanation is how, during the origin of a B, can a fairly large chromosome segment become inactivated more or less immediately, to give rise to a reproductively normal individual, when aneuploidy for that chromosome, or for that chromosome segment (e.g., by translocation) would almost certainly give rise to an abnormal individual? The more one studies different aspects of B chromosome structure, function and phenotypic effects, the more one is also likely to learn of the subtleties of interchromosomal effects and gene regulation in general.

In the ladybird *Exochomus quadripustulatus* a fairly large B chromosome is present in British populations. Its frequency varies at different sites, as does the sex ratio. Although no phenotypic effects of the B have yet been identified, an interesting and unique correlation has been discovered between the frequency of B chromosomes and the sex ratio of each population.

Materials and methods

The ladybird *E. quadripustulatus* is a small black beetle with four red spots on its elytra. It belongs to the Chilocorinae, a sub-family of the Coccinellidae. It is arboreal, found mainly, in this country, on Douglas Fir and Pine, where it feeds on Woolly Aphids (Adelges), Coccids and other small creatures. In other countries it has adapted to other food on other trees and may be found in orchards on the Ukraine, citrus groves in Turkey and on Eucalyptus trees in Australia.

In a population survey of the British Isles carried out from 1984–1986, over 30 populations were studied in the southern half of England. These have been listed and described in a recent publication (Henderson and Albrecht 1988) and need not be re-listed here. *Exochomus* has only one generation per year. Overwintered adults mate in the spring, larvae pupate in the summer and new adults emerge in July and August. Meiosis begins in the pupal testis and is completed within a week or two of emergence. Testes were vivisected in insect saline and either fixed in 1/3 acetic/alcohol and stored in a refrigerator or squashed directly into propionic orcein.

Results

Testis development

There are two testes in ladybirds which may either be widely separated laterally within the abdomen or close together in the middle. Each testis is normally composed of some half a dozen or so spherical follicles, united to a single duct. Within each follicle the organisation and behaviour is usually more or less identical. At one end a cluster of gonial cells gives rise by mitosis to spermatocytes. These undergo meiosis to form spermatids, and clusters of relatively small mature sperm accumulate at the duct end of the follicle. Mitosis and meiosis are often both continuous after emergence from the pupa and throughout adult life, varying in intensity if the insect hibernates or emerges in spring.

In *Exochomus* and other Chilocorids a new pattern of behaviour has evolved. In larvae and young pupae testis organisation is similar to that described above, with two widely separated testes, each consisting of some half a dozen spherical follicles. Mitosis and meiosis both take place within these spherical follicles and even some spermatid formation occurs. Prior to sperm formation, however, in the mid-pupal period, the previously free follicles come close together and elongate. The result of this intimate association is a structure similar to an orange, with each follicle resembling the segments of the orange. Shortly after this stage is reached the *external* membranes of the follicle walls break down and a large, visibly single sphere is formed. This elongates as sperm formation begins and the contents undergo some reorganisation. Although not visible in squash preparations, the *internal* membranes separating the fused follicles do not undergo complete breakdown. Thin membranes separating the internal regions contributed by each of the separate follicles can be seen in thin sections. The comparison with the segments of an orange within an orange skin is most appropriate.

When adults emerge from the pupa their testes are at a slightly elongated, or rugby ball shaped, stage of develop-

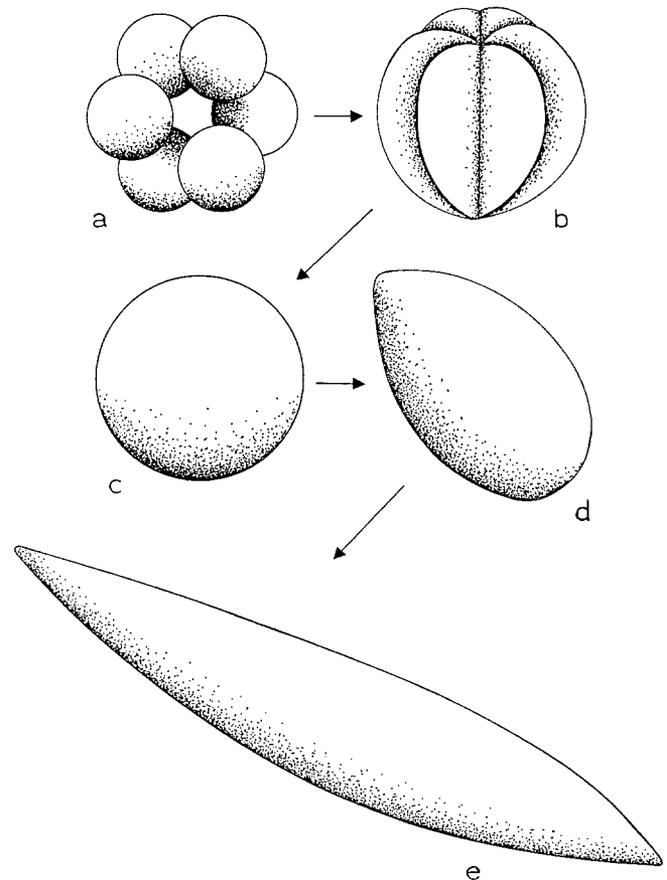


Fig. 1. Changes in testis structure and morphology during pupal and adult development of *Exochomus quadripustulatus*. Free follicles first fuse and the resulting sphere elongates to form a single elongated tubule

ment. Only a limited amount of mitosis and meiosis continues into adult life. This lasts for only a week or two in *E. quadripustulatus*. As sperm elongation occurs, testis elongation occurs also, and the small, oval structure present in newly emerged adults rapidly elongates to a long, thin, cucumber shape. The sperm bundles come to extend more or less from one end of the elongated testis to the other. These morphological changes are summarised in Figure 1.

Cytological observations

The complement. Chromosome studies are not easy in population samples of *Exochomus* because meiosis is of relatively short duration and is easily missed. As indicated above, it begins in the pupal testis and is completed within the first few weeks of adult life in a series of short bursts, alternating with periods of little or no division. The result is that, in heterogeneous population samples of young adults of indeterminate age, only a limited number will be found to possess metaphase Is and many will be found to be unscorable. This makes widespread population sampling, of the type widely practised in grasshoppers, for example, extremely difficult, for one has a narrow window of only a few weeks at the end of July and beginning of August in which to make collections. Thus, although many individuals were collected from 32 populations (Henderson and Albrecht 1988) reasonable numbers of metaphases giving

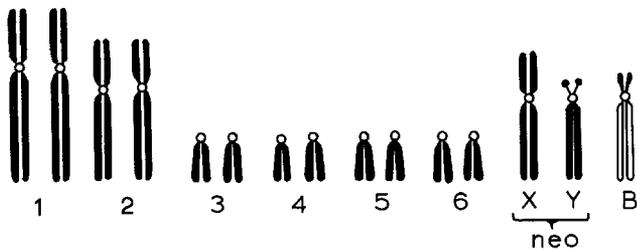


Fig. 2. Karyotype of *Exochomus quadripustulatus*. Four pairs of small telocentrics accompany the two pairs of large metacentrics. The acrocentric neo-Y chromosome has satellited short arms. The large B chromosome is approximately the same size as the neo-Y, but has much larger short arms

accurate estimates of B chromosome frequency could only be obtained from 14 of these populations in the limited time available.

Female mitosis and meiosis do not take place in the ovaries of newly emerged females during July and August when collections are most easy. They appear to take place the following spring, prior to mating, at times when the weather is poor, populations more dispersed, and collections much more difficult. For these reasons, this initial study concentrated on male meiosis which, for the above reasons, proved difficult enough to sample in most populations.

The chromosome number in this species was first listed by Smith (1957, 1960). He examined specimens derived from material introduced into California for Biological Control purposes. The complement appears to be the same as that in Britain. It consists of six autosomal bivalents and a neo-XY, sex pair (Figs. 2, 4a). Of the autosomes, two are large metacentrics and the remainder small telocentrics. The neo-X is metacentric, and the neo-Y is acrocentric, with satellited short arms. Smith (1960) also listed the presence of supernumerary chromosomes in these Californian specimens, but gave no details. No observations of British specimens had been undertaken before this work was begun and hence it was not known whether Bs would also be found in British populations.

The B chromosomes. In all populations where samples were large enough to detect them, B chromosomes were found. The 14 populations involved are listed in Table 1, together with their B frequencies. It will be seen that the frequencies varied from 6.4% in Old Warden (Bedfordshire) to 28.6% in Brooks Green (Sussex). The low percentage at Old Warden is unlikely to be due to sampling error as some 47 individuals were scored.

In all cases only a single B was found at male meiosis. It was relatively large, with visible coiling, and acrocentric (Figs. 3, 4b, d). It often lay off the plate at metaphase I and lagged at anaphase I (Fig. 5). It divided at telophase I and the single B chromatid present at metaphase II was usually situated off the metaphase plate, closer to one pole (Fig. 6a, b). Accurate chiasma frequency scores were not possible with the small numbers of cells found at metaphase I and diplotene, but the Bs did appear to increase slightly the chiasma frequencies of the cells in which they were found.

In two of the most southern populations, Brooks Green and Eartham Wood, single individuals were found to possess a very small additional chromosome, in some cells of

Table 1. B chromosome frequencies and sex ratios in 14 populations of *Exochomus*

Location	- B	+ B	Totals	B (%)	Female (%)	Male (%)
Brooks Green	10	4	14	28.6	66.7	33.3
Swaffham	5	2	7	28.6	70.6	29.4
Eartham Wood	13	4	17	23.5	71.7	28.3
Hereford	17	5	22	22.7	68.3	31.7
Quantocks	9	2	11	18.2	64.8	35.2
Lowestoft	5	1	6	16.6	62.5	37.5
Grimes Graves	5	1	6	16.6	57.7	42.3
Sandy	42	8	50	16.0	50.1	49.9
Peterborough	47	7	54	12.9	50.0	50.0
Santon Downham	34	5	39	12.8	53.6	46.4
Lakenheath	40	4	44	9.1	52.4	47.6
Kings Forest	20	2	22	9.1	47.7	52.3
Sandringham	23	2	25	8.0	58.4	41.6
Old Warden	44	3	47	6.4	60.7	39.3
Totals	314	50	364			
Means				13.7	59.7	40.3

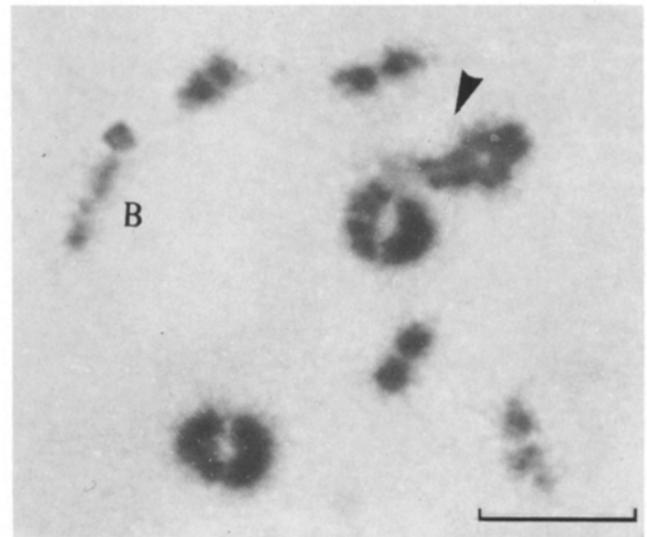


Fig. 3. Diakinesis in *Exochomus*. Two large ring bivalents and four small rod bivalents accompany the neo-XY bivalent (arrowed) and a large B chromosome. The terminal segment of the neo-X, representing the ancestral X, is diffuse and weakly staining. The short arm of the B forms a heterochromatic blob. Bar represents 10 μ m

their testes (Fig. 4c). Its size was similar to that of the short arm of the acrocentric large B. It is not possible to be sure, from such small samples, whether there is a consistent polymorphism for a second, small B in these populations at a low frequency, or whether spontaneous fragmentation, or misdivision, of the large B had occurred.

Sex ratios. In the previous publication on this species (Henderson and Albrecht 1988), the sex ratios of 32 populations of *Exochomus* were found to vary from a conventional 1:1 and to differ from site to site. In Table 1 the sex ratios of the 14 populations with recorded B frequencies are provided for comparison. If one plots the frequency of B chromosomes against the population sex ratio, a scattered relationship is found, possibly reflecting sampling error in B chromosome frequency, but a significant regression is evi-

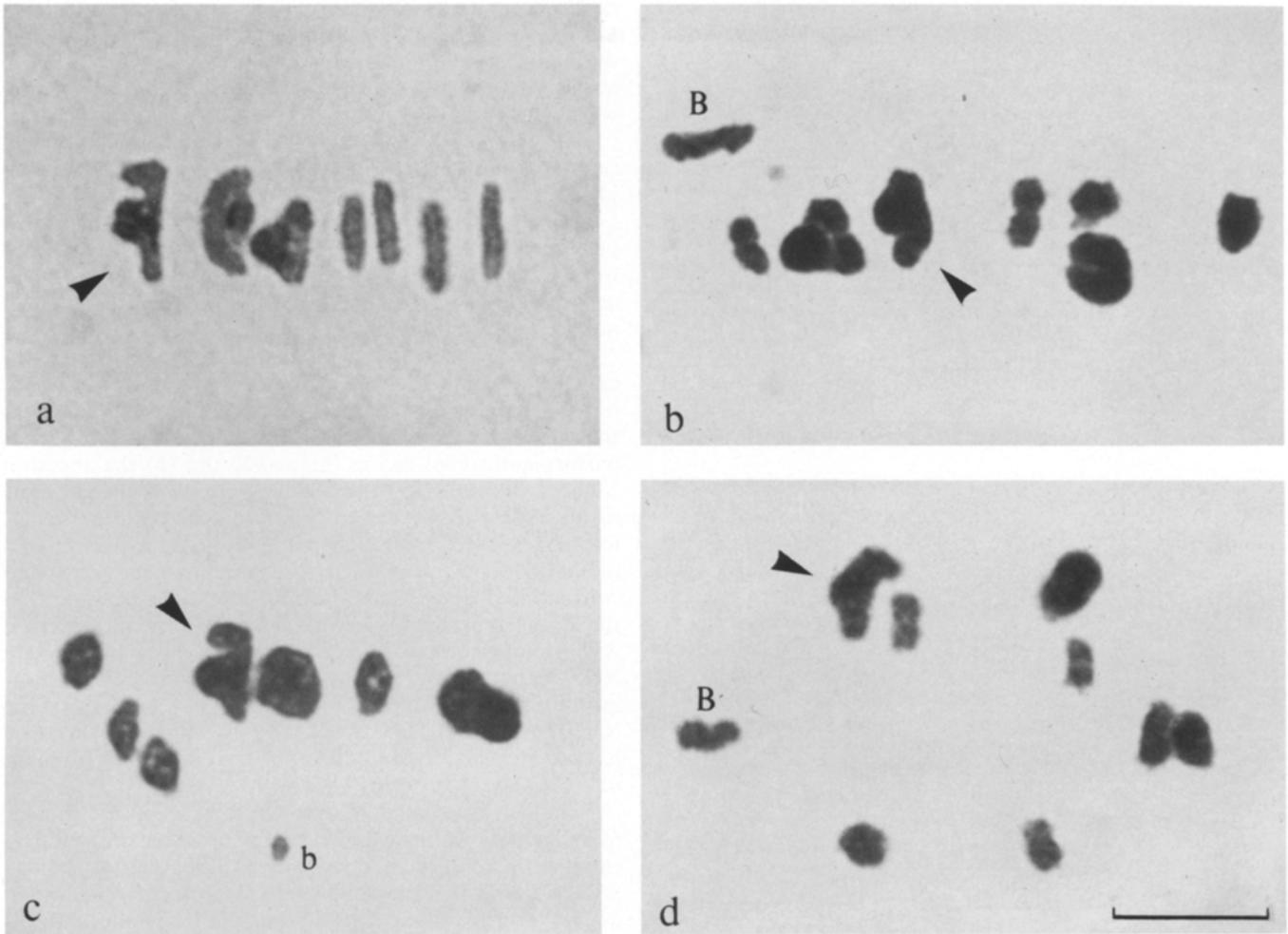


Fig. 4a-d. The chromosome complement at metaphase I in *Exochomus*. The neo-XY sex bivalent is *arrowed*. **a** The basic complement, **b** individuals with large B, **c** individual with small B (?), **d** individual with large B. Bar represents 10 μ m

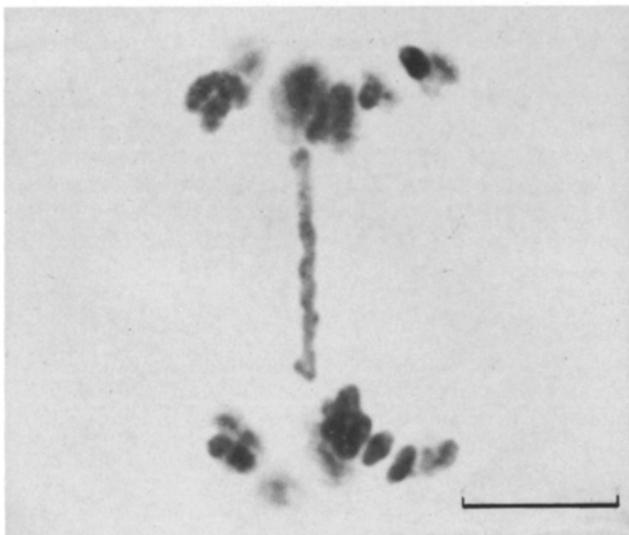


Fig. 5. Lightly squashed anaphase I in *Exochomus* showing the lagging B chromosome separating between the two polar groups. The size of the acrocentric short arms is clearly visible. Bar represents 10 μ m

dent (Fig. 7). Variations in sex ratio are not, in themselves, of widespread occurrence, of course, and this is probably the first recorded correlation between B chromosome frequency and sex ratio.

Discussion

The demonstration that B chromosomes are found throughout *Exochomus* populations in this country as well as in introduced Californian populations, shows that polymorphism for B chromosomes is possibly a world-wide feature for this species. Indeed, I have also confirmed that the same large B chromosome was present in a population sample collected from Brittany, France, by J.S.M. Albrecht, though the number of scoreable individuals was not large enough to provide an estimate of its frequency. If the B should prove to be the same in the Californian populations, it would indicate that the polymorphism originated a long time ago. It would also appear to be of some selective advantage for it was found in 14 of the populations sampled in Britain. On the other hand, the fact that only one B was ever found at male meiosis, strongly suggests that the possession of more than one is lethal in the male. It would

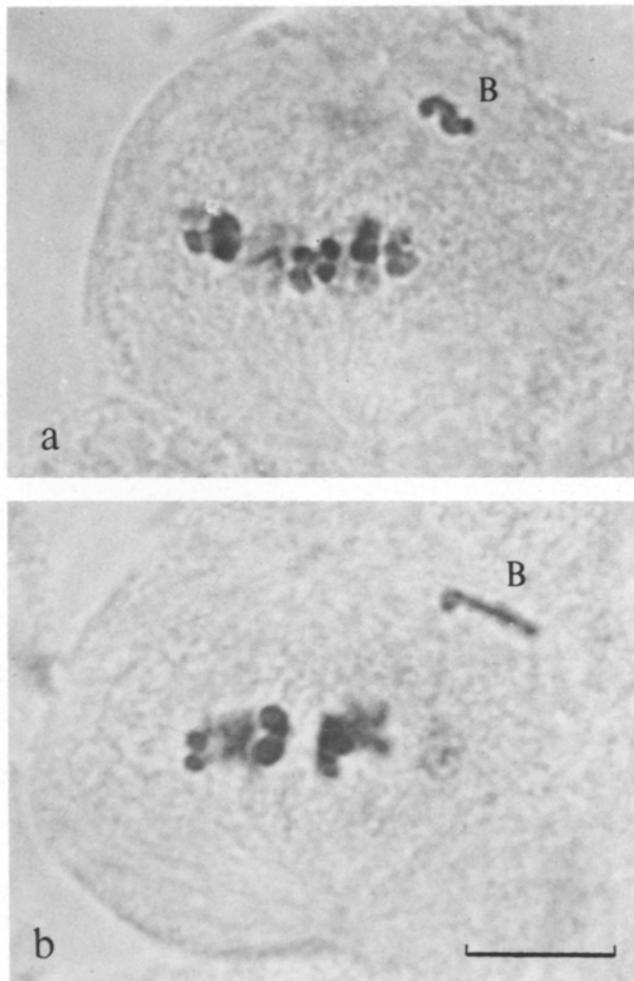


Fig. 6a, b. Unsquashed metaphase II in *Exochomus* showing the orientation of the single B chromatid off the metaphase plate. In **a** chromatid coiling is evident. In **b** the short arm is well shown, and some neocentric activity is suggested by the straightness of the strand. (Unstained, phase contrast). Bar represents 10 μ m

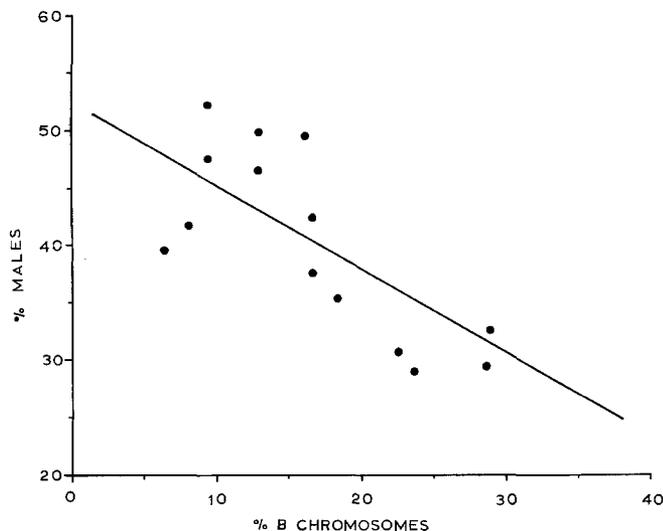


Fig. 7. Regression of B chromosome frequency and sex ratio in 14 population samples of *Exochomus quadripustulatus*

be of interest to know what numbers are present in females, but this would be technically difficult to determine, far more difficult than a comparable study in grasshoppers, for example, and must await a more detailed study directed towards this end in the future.

It might look at first glance as if the correlation observed between sex ratio and B chromosome frequency implied a causal relationship – the Bs being responsible for, or influencing, the sex ratio differences found. This might be possible, of course, if the B chromosome was derived from a sex chromosome with incomplete inactivation of some of its genes. However, in my opinion this is definitely not the case for the following reasons.

In this species, *E. quadripustulatus*, a neo-XY sex mechanism is present. The neo-Y chromosome was, prior to the translocation, a conventional autosome. Its two main roles should be the orientation and segregation from (a) its partner autosomal loci in the neo-X and (b) the ancestral X chromosome arm of the neo-X. Were the B chromosome to be derived from the neo-Y, without inactivation, its effects on sex determination might be expected to be less important than the characteristic (usually lethal) effects of conventional autosomal trisomy. It is quite possible that the B chromosome is derived from the original ancestral X chromosome arm. Indeed, in several grasshoppers with XO males, including *Myrmeleotettix maculatus*, the B chromosomes present appear to have been derived from the X chromosomes (Hewitt 1973, 1979) but in no instance have these been found to affect the sex ratios in the individuals in which they occur.

On the other hand, during the course of the present investigations we have found comparable sex ratio differences in a number of other ladybird species which also possess neo-XY sex chromosome mechanisms, but which do not possess any B chromosomes (Henderson and Albrecht 1988). They have also recently been found in a neo-XY weevil lacking B chromosomes (Holloway 1985). The sex ratio differences themselves are therefore more likely to involve the neo-XY sex mechanism, together, possibly, with elements of the type involved in hybrid dysgenesis (Henderson and Albrecht 1988).

The likelihood that the factors involved in affecting the sex ratios themselves are more likely to be responsible for affecting the frequency of B chromosomes than the reverse, is of greater interest than the simple functional correlation involved in the alternative possibility. It raises a number of questions including the possibility that elements of the type involved in hybrid dysgenesis may be involved in affecting both the viability of members of a neo-XY sex chromosome system and also the survival of B chromosomes which might, ancestrally, have been derived from one of them.

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