SINGLE LOCUS INHERITANCE IN THE TETRAPLOID TREEFROG HYLA VERSICOLOR, WITH AN ANALYSIS OF EXPECTED PROGENY RATIOS IN TETRAPLOID ORGANISMS

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ABSTRACT

The recently evolved autotetraploid frog, Hyla versicolor, was examined electrophoretically for evidence of genomic restructuring leading to diploidization. Loci were tested against the progeny ratios expected if inheritance was disomic versus tetrasomic. Two loci (Mpi and Sod-2) appeared to be inherited tetrasomically, one (Mdh-2) appeared to be inherited disomically, and one (Tpi) appeared to be inherited disomically in one family and tetrasomically in another family, when tested conventionally against 1:2:1 and 1:4:1 segregation ratios. The minimum number of progeny required for this type of analysis for codominant alleles is shown to be 92. Progeny resulting from double reduction were observed, and the occurrence of a null allele class at Mpi was noted. A reexamination of expected progeny ratios in tetraploid organisms reveals that tetrasomic inheritance patterns cannot be predicted without adequate knowledge of the amount of crossing-over, the proportion of tetravalents versus random bivalents that are formed, and the ratio and types of centromere segregation (alternate and adjacent) that occur from tetravalents in the species being studied. However, disomic inheritance can be unambiguously confirmed only by the production of all heteroallelic gametes from homobivalent, symmetrically heterozygous individuals. In addition, a method is described for estimating gene-centromere distances using the ratio of progeny genotypes in certain crosses in tetraploid species.

INTRODUCTION

The ultimate source of all variation that leads to evolutionary change is alteration of the DNA. A species' potential for evolution is therefore limited to some extent by the size of its genome. The most drastic method of increasing the genome size is to duplicate it one or more times (autopolyploidization). A single genomic duplication results in an autotetraploid organism with four identical copies of each chromosome which may associate as tetravalents or random bivalents during meiosis. However, for the 'extra' DNA to be useful for evolution, it is necessary for the chromosomes to collect sufficient structural changes that they only pair in two specific bivalent sets (structural diploidization). This is achieved instantly in allo-tetraploids, which form by the union of two non-homologous genomes. At this point one locus can carry out the normal functioning of the gene, while the other is free to evolve, since the two homeologous pairs will not be exchanging genetic material through crossing-over. Allendorf, Utter and May (1975) demonstrated that, while the gene dosages in tetraploids can be determined electrophoretically, it is not possible to confirm a tetrasomic mode of inheritance of these loci using population data. Rather, it is necessary to use inheritance data to determine gametic segregation patterns.

Species that have undergone genomic duplication relatively recently are of particular interest, because analysis of their genomes allows us to observe the rate at which rediploidization

occurs. One such species is the North American tetraploid gray tree frog Hyla versicolor (4N=48), which is suspected to have diverged from the diploid H. chrysoscelis (2N=24) approximately 375,000 - 4 million years ago (Ralin, Romano and Kilpatrick 1983; but see Maxson, Pepper and Maxson 1977 and Ralin 1978 for additional discussion). Extensive comparisons of their morphology (Ralin and Rogers 1979), ecology (Ralin 1968, 1981), physiology (Kamel, Marsden and Pough 1985; Ralin 1981), and cytology (Bachmann and Bogart 1975; Toivonen et al. 1983) have revealed essentially no differences between the two species except in the trill rate of their mating call (Gerhardt 1974; Johnson 1966; Ralin 1977); however, this important difference serves to isolate the diploid and tetraploid reproductively, making them functionally distinct species.

An examination of genome restructuring in H. versicolor was started by Danzmann and Bogart (Danzmann 1982; Danzmann and Bogart 1982, 1983). They studied the inheritance of four loci (Aat-1, Ldh-1, Mdh-1, Sod-1) and found both disomic and tetrasomic modes of inheritance. In addition, several of their crosses yielded data that were intermediate between disomic and tetrasomic modes of inheritance. To provide additional evidence on the status of genome restructuring in this species, we re-examined several of these loci, plus 5 others, in a series of laboratory crosses. We also re-examined the conventional gamete ratios expected in a tetraploid organism, in the light of recent cytological information on the behavior of chromosomes in tetraploids.

MATERIALS AND METHODS

Laboratory crosses

Male H. versicolor were collected in 1983 and 1984 from two locations in the northeast (near Ithaca, New York and Norfolk, Virginia) and two locations in the midwest (Columbia, Missouri and Bastrop, Texas). Females were collected locally in Ithaca and Auburn, New York so that they could be used for crosses in the laboratory immediately after collecting. Males were identified in regions where they are sympatric with H. chrysoscelis by their call. Species identification was confirmed electrophoretically by noting gene dosages; that is, a heterozygous locus in a diploid will produce allelic products only in a 1:1 ratio, whereas a tetraploid can be asymmetrically heterozygous (AAAA' or AA'A'A'), producing allelic products in a 3:1 ratio. These two cases are readily distinguishable by band intensities on a gel.

Artificial breeding methods were used as described in Rugh (1965) and Nace et al. (1974). All females were collected in amplexus early in the night, thus ensuring the presence of large numbers of eggs in the oviduct. Females were manually stripped of eggs within a few hours of capture and were immediately frozen at -80^{o}C. In a few cases, when stripping did not produce sufficient eggs, the females were killed and the eggs dissected

from the oviduct. Sperm were obtained by dissecting both testes from a male and macerating them in 2-4 ml of Amphibian Ringer's solution; the male carcass was then frozen. After sperm motility was observed under a microscope, the sperm solution was pipetted over the eggs to achieve fertilization. By stripping eggs into two or more clumps, it was possible to use a single female in more than one cross. Likewise, some males were used for more than one cross in the same night. Parental origins of the crosses analyzed are given in Table 1.

Eggs were maintained in Amphibian Ringer's solution until hatching. Tadpoles were raised in aged tap water to prevent chlorine poisoning, and fed boiled lettuce supplemented with blocks of Purina rabbit chow mixed with agar (as described by Nace et al. 1974).

Electrophoresis

Samples of muscle, liver, and heart were removed from the parents just prior to electrophoresis. Electrophoretic techniques, staining protocols, interpretation of banding patterns, and genic nomenclature followed May, Wright and Stoneking (1979) and Harris and Hopkinson (1976). Seven buffer systems were used: C (CT of May, Wright and Stoneking 1979), R (Ridgway, Sherburne and Lewis 1970), 4 (continuous tris-citrate type 4 of Selander et al. 1971), A (AZH from May, Wright and Stoneking 1979), S-9 (tris-maleate type 9 of Selander et al. 1971), 4-disc (a modified discontinuous system using 4 buffer for the gel and Markert and Faulhaber's (1965) electrode buffer), and

C5.4 (a modified C buffer with its pH lowered to 5.4 with HCl). The parents were screened for electrophoretic variation for 19 enzymes using the buffer systems and tissues in which the enzymes were best resolved (Table 2). Tadpoles were sacrificed by freezing to -80^{o}C just after their back legs began to develop. The intestines were removed and the body was homogenized with two drops of extraction buffer (0.05 M Tris/HCl at pH 7.1) using a 8mm glass rod in a 12x75mm test tube. Each family of tadpoles was selected for analysis based on parental genotypes, and was analyzed only for those loci for which the parental genotype crosses were appropriate for studying single locus segregation.

For enzymes encoded by more than one locus, the locus with products closest to the origin was labelled 1. Alleles at a given locus were designated according to the relative mobilities of their protein products, with the most common homomeric allele designated as 1. Successive alleles were numbered, based on the mobility of their protein product, from least anodal to most anodal. Occasionally a new allele was noted later in the study, and was given the next highest number, regardless of the mobility of its homomeric protein product, to avoid renumbering the alleles. The mobilities of the protein products, relative to that of the most common allele, are given in Table 2. The genotype of an individual at a particular locus is given by the locus name, its number (hyphenated), and the alleles listed in parentheses. For example, an individual symmetrically heterozygous for alleles 1 and 2 at the faster of the two Mdh loci would be designated Mdh-2(1122). Genotypes were scored on

the assumption that each individual possessed four gene doses at each locus. The presence of an individual with three or less gene doses would have suggested the existence of a null allele or the presence of a diploid or triploid in the cross. Such an individual would be detectable by aberrant progeny ratios in some, but not all, cross combinations.

Analysis

The mode of inheritance of loci was determined by comparing the ratio of 11, 12, and 22 gametes produced by symmetrically heterozygous individuals with the ratios expected from disomic inheritance (1:2:1) and conventionally tested tetrasomic inheritance (1:4:1). The expected progeny genotype ratios are given in Table 3 for the two cross combinations that can be used to distinguish disomic from tetrasomic inheritance: a symmetrical heterozygote mated with a homozygote or with an asymmetrical heterozygote. We used a modification of the Likelihood Ratio Test, described in Appendix 1, to determine the minimum number of progeny needed to distinguish between these two models with 95% confidence; this minimum number is 92.

All crosses in which one allele was present in a single dose were analyzed for evidence of double reduction, or chromatid non-disjunction (Burnham 1962). This phenomenon results from tetravalent pairing with a cross-over between a locus and the centromere, followed by adjacent segregation. In the tetravalent illustrated in Figure 1, segregation of chromosome a with c and b with d will result in two 11 gametes (i.e., gametes with two

copies of allele 1 from chromosomes a and c) and either two heteroallelic (12) gametes or two double reduction gametes (11 and 22) after meiosis II in the cell containing chromosomes b and d. Products of double reduction are expected in low frequencies at loci on chromosomes that form tetravalents during meiosis and are sufficiently distal to the centromere to make cross-over events likely to occur between the locus and the centromere. Double reduction is detectable in any cross in which an allele is present in a single dose in the parents; the double reduction progeny will have two doses of the allele. For instance, in a 1112 x 1111 cross, double reduction is detectable by the presence of 1122 progeny. Note that double reduction gametes may also be formed by a symmetrical heterozygote, but cannot be differentiated from gametes formed in the absence of crossing-over. Thus double reduction progeny in an asymmetrical heterozygote provide unequivocal evidence of tetrasomic segregation and crossing-over. It is interesting to note that two closely linked loci will both be doubly reduced in the same gamete if a cross-over occurs between the most proximal locus and the centromere. This provides a method for immediate detection of linkage.

All loci were examined for evidence of meiotic drive, which is the unequal distribution of alleles into the gametes. To check this, each asymmetrically heterozygous x homozygous cross was tested for the expected 1:1 offspring ratio of homozygotes to

asymmetrical heterozygotes using the chi-square test (Sokal and Rohlf 1981). Lack of fit could also indicate differential survival of offspring.

RESULTS

Fourteen polymorphic and 13 monomorphic loci were adequately resolved with the buffer systems used in this study (Table 2). The 14 polymorphic loci that were resolved are discussed below.

Enzymes

Four gene doses were observed for all polymorphic systems. Aspartate amino-transferase (called glutamic oxaloacetic transaminase by Danzmann and Bogart) and peptidase with leucyl-alanine were both clearly resolved as polymorphic loci in the adults, but could not be reliably scored in the tadpoles. In addition, glycerol-3-phosphate dehydrogenase and an unidentified NAD-dependent dehydrogenase were observed in the parents but did not appear to be present in the offspring. In future studies the tadpoles could be raised to metamorphosis before sacrificing in order to score these enzymes in the progeny.

Aspartate aminotransferase: AAT was encoded by two loci. Aat-1 activity appeared anodally close to the origin. In all cases the bands were too tightly clustered to distinguish phenotypes consistently.

Isocitrate dehydrogenase: IDH was encoded in adult H. versicolor by two loci with anodally-migrating gene products; in the tadpoles the locus with slower migrating products (Idh-1) had much less enzymatic activity than in the adults, which resulted in unscorable phenotypes for this locus. Products of two alleles were observed at Idh-2, and their bands conformed to ratios expected for a dimeric enzyme. In two families (E841, H842) an apparent fast variant at Idh-1 was noted in the tadpoles. However, the band intensity ratios did not conform to those expected for a dimer, and the 'variants' appeared in much lower frequencies than would be expected given the parental genotypes. It was therefore presumed that these bands were due to an artifact of unknown origin.

Lactate dehydrogenase: LDH is a tetramer encoded in vertebrates by two loci, the product of an ancient gene duplication (Ohno, Wolf and Atkin 1968). In H. versicolor, the products of one locus migrated cathodally and the other anodally, both lying close to the origin. The locus coding for the anodal form was arbitrarily designated Ldh-1. Band intensities, where they were clearly resolved, conformed to ratios expected for a tetramer encoded by four gene doses. A minimum of three alleles was noted for Ldh-1, but the bands in all cases were tightly clustered near the origin such that it was not possible to identify them consistently. Ldh-2, encoded by a minimum of 3 alleles, was equally difficult to interpret, and could only be reliably scored in one family (G841). Ldh-2 in this study corresponded to Ldh-1 in Danzmann (1982).

Malate dehydrogenase: MDH was encoded by two loci whose activity appeared anodally; the locus with slower migrating products (Mdh-1) was monomorphic in all of the animals examined. The banding patterns of Mdh-2 conformed to a ratio for a dimeric enzyme with three alleles. Mdh-2 in this study corresponded with Mdh-1 in Danzmann (1982) and Danzmann and Bogart (1982).

Peptidase with leucyl-alanine: PEP-LA was encoded in the parents by a single locus with two alleles whose protein products migrated anodally. Banding patterns appeared to conform to ratios expected for a monomeric enzyme.

Superoxide dismutase: SOD was encoded by two loci whose products migrated anodally. Banding patterns for both loci conformed to ratios expected for a dimeric enzyme. Two different allele products at Sod-1 were clearly resolved on tadpole gels stained for LDH; however, Sod-1 activity was low in the parents, resulting in unscorable phenotypes. Six alleles were noted at Sod-2, which corresponded to Sod-1 in Danzmann (1982).

The following four loci have not previously been described in H. versicolor:

Adenylate kinase: AK was encoded by one locus with anodally-migrating gene products; the band ratios corresponded to a monomeric enzyme encoded by three alleles. Band intensities varied with time of incubation of the gel, and Ak progeny ratios were aberrant in all families. This enzyme must be studied in more detail in order to understand its behavior.

Mannosephosphate isomerase: MPI was encoded by one locus with anodally-migrating gene products, and behaved as a monomer. Four alleles were noted in the adults, plus a null allele class.
Phosphogluconate dehydrogenase: PGD was encoded by two loci with anodally-migrating gene products. Banding patterns conformed to ratios expected for a dimeric enzyme. Pgd-2, whose products migrated faster than Pgd-1, was monomorphic for all individuals sampled. Products of the two alleles at Pgd-1 appeared close to the origin and could only be reliably scored in two families (D843 and H842), as the slower, rare allele was frequently obscured by its proximity to the origin.
Triosephosphate isomerase: TPI was encoded by one locus with anodally-migrating gene products; the band ratios corresponded to a dimer encoded by three alleles.

Single locus segregation:

Apparent evidence of meiotic drive or differential mortality due to genotype was seen in three crosses: Tpi in cross B842, Pgd-1 in cross D843, and Mpi and Sod-2 in cross H842 (Table 4). Because Sod-2 and Mpi showed normal segregation in other crosses, it seems likely that the aberrant ratios for these loci are due to the small sample size in cross H842. Pgd-1 was not scored in any other cross; therefore this single aberrant cross may be indicative of an unusual segregation pattern at this locus or low viability of the homozygous phenotype. Note that 34 tests for normal segregation were performed at the .05 confidence level; if segregation is normal in each cross for each locus, then 1.7 is

the expected number of tests (out of 34) incorrectly identified as significant, and the probability that four or more tests will be incorrectly identified as significant is 8.8%.

Double reduction was seen in two crosses. In cross F841 for Sod-2 (1112 x 1366), one out of 40 progeny had the genotype 1226, indicating double reduction in the female. In cross A844 for Mpi (1111 x 124Ø), out of 112 progeny at least one had the genotype 1122, and at least two had the genotype 1144, indicating double reduction in the male. The presence of the null allele in this cross made it difficult to distinguish dosages; e.g., the 112Ø phenotype could not always be differentiated from 1112 or 1122 on these gels. Thus the number of double reduction progeny is a minimum estimate, including only those individuals whose genotypes we could accurately identify. The low frequency of double reduction at Sod-2 in cross F841 would suggest that either the chromosomes that carry this locus do not consistently form multivalents, adjacent segregation between them is rare, there is little crossing-over between the locus and the centromere, or a combination of these three events occurs. Despite the fact that several crosses were diagnostic for double reduction at Sod-2 (i.e., a homozygote x an asymmetrical heterozygote), double reduction progeny only appeared in the one cross.

Of the crosses that were diagnostic for single locus segregation, that is, a symmetrical heterozygote crossed with a homozygote or an asymmetrical heterozygote, only four (A844, B841, B842, and D843) had the required number of progeny (92) to test for inheritance mode (Table 5; see Appendix 1). Of these

crosses, three indicated a conventional tetrasomic mode of inheritance for Mpi, one indicated tetrasomic inheritance for Sod-2, and one indicated tetrasomic inheritance for Tpi. Of the crosses with insufficient progeny, one indicated tetrasomic inheritance for Mpi and two fit either a tetrasomic or a disomic mode of inheritance for this locus; one indicated tetrasomic inheritance and two indicated either mode of inheritance for Sod-2; one indicated disomic inheritance for Tpi, and one indicated either mode of inheritance for Mdh-2. The ambiguity of the results from families with low numbers of progeny confirms the need to have a minimum N=92.

DISCUSSION

What is the status of the H. versicolor genome with respect to diploidization? The formation of multivalents in meiosis (Bogart and Wasserman 1972) indicates that minimal structural differentiation of the chromosomes has taken place. The inheritance data of Danzmann and Bogart (1982, 1983) and Danzmann (1982) suggest that the Aat-1 (Got-1), Ldh-1, Sod-1, and Mdh-1 loci apparently segregate both disomically and tetrasomically among individuals within a population, when the observed frequencies of progeny genotypes are used to test between 1:2:1 (disomic) and 1:4:1 (tetrasomic) ratios of gametes. Our data, analyzed in the same way and including additional loci, support this conclusion. However, it must be kept in mind that only five

of Danzmann and Bogart's 13 homozygote x symmetrical heterozygote crosses and four of our 11 homozygote x symmetrical heterozygote crosses had sufficient progeny ($\geq$ 92) to test adequately between these two ratios. In our study, these four crosses indicated only tetrasomic inheritance for Mpi, Sod-2 (Danzmann and Bogart's Sod-1), and Tpi. In Danzmann and Bogart's work, one of these crosses indicated disomic inheritance for Ldh-1, two indicated disomic inheritance for Mdh-1, and two indicated tetrasomic inheritance for Mdh-1.

In light of these data, we re-examined the conventional 1:4:1 gamete ratio expected for tetrasomic inheritance in a symmetrical heterozygote with two alleles. This ratio was first described by Muller (1914), who considered random segregation of chromosomes but did not include crossing-over. Haldane (1930), taking crossing-over into account, estimated the expected gamete ratios if all eight copies of the locus were randomly distributed into gametes. The resulting ratio, which he called random chromatid segregation, is 3:8:3. For this model he assumed incorrectly that sister chromatids are disassociated prior to meiosis. However, the same ratio would be achieved after multiple gene-centromere cross-overs between all eight chromatids, which would effectively randomize the locations of each allele with respect to their centromeres. This phenomenon could only occur if the chromosomes associated in a bundle, i.e., with all four homologous arms in contact with each other. Since there is no cytological evidence that this type of chromosome association

occurs, the theoretical 3:8:3 gamete ratio is an unrealistic expectation.

In H. versicolor and the tetraploid-derivative salmonids, multivalent associations of chromosomes in meiosis are often seen as ring tetravalents (or the functionally equivalent rod tetravalents with two acrocentric and two metacentric chromosomes), indicating pairing of the type shown in Figure 1 (Wright et al. 1983, Bogart and Wasserman 1972). The ring is formed by the terminalization of chiasmata in diakinesis (Mather 1936). The word 'tetravalent' will be used to refer to this type of association in the following discussion. The association of chromosomes as tetravalents during meiosis and the types of centromere segregation that can occur from a tetravalent (Burnham 1962; see Fig. 1) restrict the possible allelic pairs that can be formed. Burnham (1962) calculated the gamete ratio expected if tetravalents are always formed, a single cross-over occurs between each homologous chromosome pair (between gene and centromere), and all three types of centromere segregation take place with equal frequency. This ratio, referred to as "maximum equational segregation", is 2:5:2. We have extended the calculation of expected gamete ratios by taking into account all possible combinations of cross-over frequency, types of centromere segregation, and types of chromosome association in meiosis. In a tetraploid organism, the chromosomes may associate in meiosis in one of four ways: (1) preferential bivalents, where the same two homologous chromosomes always pair together, (2) random bivalents, (3) tetravalents, or (4) 'bundles' (discussed

above). Univalents and other multivalents are also occasionally seen, but for simplicity are not included in this discussion. Preferential bivalents will always produce a 0:1:0 ratio (with homobivalents) or 1:2:1 ratio (with heterobivalents) of 11, 12, and 22 gametes, regardless of the frequency of crossing-over, and random bivalents will always produce a 1:4:1 ratio. However, the expected ratio of gametes produced when tetravalents are consistently formed will vary with the frequency of crossing-over and the types of centromere segregation that occur. The expected gamete genotypic ratios are summarized in Table 6 for all combinations of these events in a tetravalent. A detailed explanation of how these ratios were derived is given in Appendix 2. It should be kept in mind that these ratios represent theoretical extremes, and that actual distributions of gamete types will be expected to fall between these limits. It is immediately apparent from Table 6 that a 1:4:1 ratio is only expected in the absence of adjacent 1 (for locus X) or adjacent 2 (for locus Y) segregation, or when no crossing-over occurs. If all three types of centromere segregation occur in equal frequency, then the expected ratio could be 1:4:1, 2:5:2, or 11:32:11, depending upon the frequency of crossing-over between the gene and the centromere.

Theoretically, alternate 2 segregation is unlikely to occur, since it involves non-disjunction of homologous chromosomes. In the tetraploid-derivative salmonids this would result in unbalanced gametes, which have never been observed (Wright et al. 1983). It is possible that this type of segregation does, in

fact, occur, but results in non-viable gametes. In H. versicolor, which has not undergone as much structural differentiation of homeologous chromosomes, there may be no restriction to the survival of gametes formed by adjacent 2 segregation.

Typical studies of disomic versus tetrasomic inheritance test only between a 1:2:1 and a 1:4:1 ratio (e.g. in potatoes, Martinez-Zapater and Oliver 1984, Quiros and McHale 1985; in alfalfa, Quiros 1982); the latter two studies did not even take gene dosage into account. The sample sizes necessary for these analyses are rarely achieved in such studies. The conclusion to be drawn from our calculations is that, in order to test for inheritance mode in organisms of tetraploid origin, one must (1) have an adequate sample size to distinguish between the ratios being tested, (2) know the percentage of tetravalents, random bivalents, and preferential bivalents formed by the chromosomes carrying the locus being examined, (3) know the types and relative frequencies of centromere segregation that occur in these tetravalents, and (4) know the frequency of crossing-over between the gene and the centromere. These are difficult criteria to meet. For instance, the presence of double reduction progeny is evidence that at least one of the two types of adjacent segregation has occurred; to know which type requires knowing on which chromosome arm the locus is carried. In Figure 1, locus X will yield double reduction progeny only with adjacent 1 segregation, while locus Y will yield double reduction progeny only with adjacent 2 segregation. If we consider a worst-case

analysis, in which all types of segregation take place, then one must have sufficient progeny to be able to distinguish a 1:2:1 ratio from the closest tetrasomic ratio, which is 2:5:2. The minimum N required for this test, using the method outlined in Appendix 1, is 872. (The equally close tetrasomic ratio 5:8:5 requires the same minimum N of 872.)

Mather (1936) developed a parameter, alpha, which takes into account the frequencies of tetravalent formation and crossing-over, and can be used to calculate expected gamete ratios for any combination of these events. However, one cannot use alpha to derive expected ratios unless these frequencies are known, and the frequencies cannot be known without examining progeny ratios.

There is one definitive method of detecting disomic inheritance. If some loci are indeed segregating disomically, then we would expect some symmetrically heterozygous individuals to be homobivalent (i.e., the homologous chromosome pairs are homozygous for different alleles) and others to be heterobivalent (i.e., both homologous chromosome pairs are heterozygous). The latter will produce a 1:2:1 ratio of gametes; the former will produce only heteroallelic gametes (see Table 3). At the onset of structural diploidization the two alleles will be in equal frequency at both loci and approximately one third of the symmetrically heterozygous individuals will be homobivalent. As the frequency of the alleles changes at the two loci, this percentage of homobivalent individuals will increase until only homobivalents occur at fixation (functional diploidization). The three families that apparently showed disomic inheritance in this

study (E84l for Mdh-2 (Danzmann and Bogart's Mdh-1), and E84l and F84l for Sod-2) all produced a ratio of progeny genotypes close to 1:2:1. Among Danzmann and Bogart's crosses, however, the male in cross 79-21 produced only heteroallelic gametes for Ldh-1; both parents in cross 79-14 produced only heteroallelic gametes for Sod-1; the symmetrically heterozygous male used in crosses 80-13 and 80-26 produced only 2 homoallelic gametes out of 29 in the first cross and 13 out of 79 in the second for Sod-1; the same male produced 8 homoallelic gametes out of 80 in cross 80-26 and 5 out of 30 in cross 80-31 for Mdh-1; the male in cross 79-17 produced 2 homoallelic gametes out of 21 for Got-1 (Aat-1) and the female in crosses 79-20 and 79-21 produced 5 homoallelic gametes out of lll and 1 out of 12, respectively, for the same locus. None of these individuals was used in other crosses. This is quite convincing evidence that chromosomes do pair as specific bivalents at least some of the time. The symmetrically heteroallelic gametes produced by these individuals must, however, have resulted from either occasional mispairing of the chromosomes with their homeologs, or tetravalent associations. This could also explain the crosses showing progeny ratios intermediate between those expected for disomic and tetrasomic inheritance.

It is important to note the distinction between random chromatid segregation, which is the result of multiple (i.e. 0-infinity), randomizing cross-overs within a chromosome 'bundle' association resulting in a 3:8:3 gamete ratio, and maximum equational segregation, which is single gene-centromere cross-

overs in a tetravalent resulting in a 2:5:2 ratio. The latter represents the theoretical maximum number of double reduction progeny in a tetraploid. These ratios are frequently confused: Danzmann (1982) and Danzmann and Bogart (1982, 1983) refer to the 2:5:2 ratio as the "segregation ratios expected after completely random chromatid crossing-over"; Redei's text (1982, p. 249) defines maximum equational segregation as the situation when "genes segregate independently from the centromere"; and Strickberger's text (1968, p. 456) lists the 2:5:2 ratio under the ambiguous title "complete chromatid segregation" . Similarly, the 3:8:3 ratio is used by some texts (Suzuki et al. 1976 p. 289, King 1965 p. 172-176) as the expected gamete ratio if crossing-over takes place, without consideration of the consequences of tetravalent formation (as defined here). Clearly, these are simplistic (if not inaccurate) treatments of a subject that is highly complex.

The presence of a null allele class at Mpi is an interesting parallel to evolution in the tetraploid-derivative salmonid and catostomid fishes. Species in both of these families have lost approximately half of their original duplicate gene expression (Allendorf, Utter and May 1975, Ferris and Whitt 1978). Duplicate gene expression is lost by the loss of DNA or by the fixation of an allele that codes for a non-functional protein or no protein at all (Stoneking, May and Wright 1981). These null alleles are presumably less deleterious in a tetraploid than a diploid population due to the 'buffering' effect of having four copies of each gene. Loss of duplicate gene expression (i.e.,

fixation of a null allele at one locus) can only occur subsequent to the establishment of disomic inheritance; however, it is clear from our data and those of Danzmann and Bogart, who postulated a null allele at the Aat-1 locus (Danzmann & Bogart 1982), that diploidization is not a prerequisite for the establishment of null alleles in a tetraploid population. Apparently H. versicolor is just beginning the process of genomic restructuring and gene silencing. Null alleles caused by loss of DNA can provide the preliminary structural differentiation that leads to preferential bivalent pairing.

An interesting point emerges from the ratios in Table 6. Consider a species in which crossing-over is unrestricted, and all modes of centromere segregation take place. If a locus is close to the centromere, so that virtually no recombination takes place between the locus and the centromere, then disomic and tetrasomic inheritance will give progeny ratios of 1:2:1 and 1:4:1, respectively. However, if there are at least 50 map units between the locus and the centromere, so that cross-over events between the locus and the centromere are likely to occur during each meiotic division, and tetravalents are consistently formed in meiosis, then the ratio of progeny for a tetrasomic locus will be 11:32:11 (see Appendix 2 for the derivation of this ratio). This difference in progeny ratios dependent upon the distance of the locus from the centromere permits us to estimate gene-centromere distances in 1111 x 1122 crosses for these loci. For this purpose it is clearer to express progeny genotypes as a proportion of the total progeny, rather than as a ratio of three

genotypes. Thus, if the locus is close to the centromere, the expected proportion of asymmetrically heterozygous progeny (1112) is 4/6 (= .667); if there are at least 50 map units between the locus and the centromere, the expected proportion will be 32/54 (= .593). An observed ratio will lie between these two values; it will vary directly with the distance of the locus from the centromere.

In summary, we suggest that the only conclusive evidence of disomic inheritance in H. versicolor comes from crosses containing homobivalent individuals who produce mostly heteroallelic gametes. All of these individuals, with the exception of two crosses with very small sample sizes (79-14, N=12 and 79-21, N=13), also produced homoallelic gametes; this indicates that even chromosomes which primarily pair as specific bivalents have some residual random bivalent or tetravalent pairing. The presence of double reduction progeny in our study is conclusive evidence that tetravalents, crossing-over, and tetrasomic inheritance do occur in H. versicolor. More loci must be examined in a larger number of families with adequate numbers of progeny, using parents from different geographic areas. Gene-centromere mapping data would enable us to test our hypothetical gamete ratios, which are dependent upon the location of loci along chromosome arms. In addition, linkage data are needed to determine whether there is differential diploidization within and among chromosomes. Studies of meiosis using chromosome banding will reveal whether tetravalents are consistently formed between the same chromosome types in all individuals. This work should

be extended to other tetraploid amphibians, especially those suspected of having undergone genome duplication earlier than H. versicolor, so we can begin to estimate the time needed for genome restructuring events to take place. It is important to determine the types of chromosome associations that occur, and whether there are differences in this respect between plant and animal tetraploids. Claims of disomic or tetrasomic inheritance in tetraploid organisms in general should be treated with caution unless appropriate cytological data are available and adequate sample sizes are used, or until crosses producing all asymmetrically heterozygous offspring are seen.

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Appendix 1

Statistical procedure used to determine minimum sample size necessary to distinguish between progeny ratios of 1:2:1 and 1:4:1 in a cross of a symmetrical heterozygote with a homozygote

Let the null hypothesis be H_0: the progeny ratio is 1:2:1. Let the alternative hypothesis be H_1: the progeny ratio is 1:4:1. We require

$$\begin{aligned} P[\text{reject } H_0 \mid H_0] &= \alpha = .05 \quad , \\ P[\text{accept } H_0 \mid H_1] &= 1 - \beta = .05 \quad . \end{aligned} \tag{A1}$$

The Neyman-Pearson Lemma (Mood, Graybill and Boes 1974, p. 411) states that, with a simple H_0 and a simple H_1, the most powerful test of specified size α is the Likelihood Ratio Test of the form: reject H_0 if $\lambda \leq k^*$, accept H_0 if $\lambda > k^*$, where k^* is a calculated constant and

$$\begin{aligned} L_0 &= (1/4)^x(2/4)^y(1/4)^z \quad , \\ L_1 &= (1/6)^x(4/6)^y(1/6)^z \quad , \\ \lambda &= L_0/L_1 = (6/4)^{x+z}(12/16)^y = (3/2)^n(1/2)^y \quad . \end{aligned} \tag{A2}$$

Here x, y, and z denote the observed frequencies of homozygous, asymmetrically heterozygous, and symmetrically heterozygous progeny, respectively, and $n = x + y + z$.

We need to find suitable k^* and n that make both error probabilities in (A1), α and $1-\beta$, equal to .05. The probabilities and hypotheses in (A1) can be re-expressed as

$$P[\lambda \leq k^* \mid H_0] = \alpha = .05 \quad , \tag{A3}$$

$$P[\lambda > k^* \mid H_1] = 1 - \beta = .05 \quad ; \tag{A4}$$

H_0: (x,y,z) ~ multinomial(n;1/4,1/2,1/4), so y ~ binomial(n,1/2) ;

H_1: (x,y,z) ~ multinomial(n;1/6,4/6,1/6), so y ~ binomial(n,2/3) .

The event $\lambda = (3/2)^n(1/2)^y \leq k^*$ in (A3) can be expressed equivalently as

$$y \geq k = \{n\ell n(2/3) + \ell n(k^*)\}/\ell n(1/2) \quad .$$

The binomial(n,1/2) distribution of y under H_0 is well approximated by a normal distribution with mean $\mu = n/2$ and variance $\sigma^2 = n/4$ (Mood, Graybill and Boes 1974, p.120). Thus equation (A3) holds when $k \approx \mu + 1.645\sigma = n/2 + 1.645(n/4)^{\frac{1}{2}}$, where 1.645 is the .95 quantile (which corresponds to $\alpha = .05$) of the standard normal distribution.

Similarly, the event $\lambda > k^*$ in (A4) can be expressed as $y < k$. The binomial(n,2/3) distribution of y under H_1 is well approximated by a normal distribution with $\mu = 2n/3$ and $\sigma^2 = 2n/9$. Thus (A4) holds when $k \approx 2n/3 - 1.645(2n/9)^{\frac{1}{2}}$. Solving the equation

$$k = n/2 + 1.645(n/4)^{\frac{1}{2}} = 2n/3 - 1.645(2n/9)^{\frac{1}{2}}$$

for n gives n = 91.93. Thus the minimum number of progeny needed to distinguish between the progeny ratios 1:2:1 and 1:4:1 with error probabilities of at most 5% under both hypotheses is n = 92. Solving for the corresponding value of k gives k = 53.89, so with n = 92 we accept H_0 if $y \leq 53$ and reject H_0 (accepting H_1) if $y \geq 54$.

The same process can be used to determine the minimum number of progeny needed to distinguish between the expected progeny ratio for disomic inheritance (1:2:1) and the ratio for tetrasomic inheritance, a:b:a. For notational convenience, define c = 2a + b. The likelihood L_0 of (A2), which corresponds to H_0, remains unchanged, while L_1 of (A2) is replaced under the more general H_1 of ratio a:b:a by

$$L_1 = (a/c)^x(b/c)^y(a/c)^z \quad .$$

Under H_1, y has a binomial(n,b/c) distribution, which is well approximated by a normal distribution with $\mu = bn/c$ and $\sigma^2 = b(c-b)n/c^2 = 2abn/c^2$.

For the case of $b > 2a$, or equivalently $b/c > .5$, equations (A3) and (A4) hold when

$$k = n/2 + 1.645(n/4)^{\frac{1}{2}} = bn/c - 1.645(2abn/c^2)^{\frac{1}{2}}$$

which leads to

$$n = (1.645[.5 + (2ab)^{\frac{1}{2}}/c]/[b/c - .5])^2 \quad . \qquad \text{(A5)}$$

Applying these results when a:b:a equals 2:5:2, the tetrasomic ratio with $b > 2a$ that is closest to the disomic ratio 1:2:1, results in $n = 871.34$. Thus the minimum number of progeny needed to distinguish between the ratios 1:2:1 and 2:5:2 with error probabilities of at most 5% under both hypotheses is $n = 872$. The corresponding value of k is $k = 460.29$, so with $n = 872$ we accept H_0 if $y \leq 460$ and reject H_0 (accepting H_1) if $y \geq 461$.

For the case of $b < 2a$, or equivalently $b/c < .5$, equations (A3) and (A4) hold when

$$k = n/2 - 1.645(n/4)^{\frac{1}{2}} = bn/c + 1.645(2abn/c^2)^{\frac{1}{2}} \quad ,$$

which leads again to (A5) for the minimum number of progeny needed.

Appendix 2

Derivation of expected gamete ratios in a symmetrically heterozygous tetraploid individual

In a tetraploid organism, the chromosomes may associate in meiosis in one of three ways: (1) preferential bivalents, (2) random bivalents, or (3) tetravalents. (1) Preferential bivalents will occur when the chromosomes have accumulated sufficient structural mutations that two chromosomes are more likely to pair with each other than with the other two. Since alleles cannot be exchanged between the two chromosome pairs, inheritance is disomic. The gametes from a symmetrically heterozygous individual (1122) will be in a 1:2:1 ratio (11:12:22) or all heteroallelic (12), depending upon whether the bivalents are heteroallelic or homoallelic, respectively. We propose the use of the terms heterobivalent and homobivalent to refer to these two arrangements. Note that crossing-over between the gene and the centromere will not change the gamete ratio if inheritance is disomic. Disomic inheritance can most easily be detected by the presence of homobivalent individuals who produce only heteroallelic gametes.

(2) If random bivalents are formed (i.e., any two of the four chromosomes may pair with each other), inheritance will be tetrasomic. The expected gamete ratio will be 1:4:1, regardless of the frequency of crossing-over. However, if the chromosomes are metacentric and are sufficiently similar to form random bivalents, they will probably also associate as tetravalents (see

Fig. 1). Acrocentric chromosomes in autotetraploids will form random bivalents.

(3) If the chromosomes associate in a tetravalent during meiosis, then the prediction of expected gamete ratios is complicated by gene-centromere crossing-over and the three types of centromere segregation that can occur (Burnham 1962; see Figure 1). In the following discussion, we consider segregation of the X locus. If no cross-overs take place between the gene and the centromere, then a 1:4:1 ratio of 11:12:22 gametes is expected, regardless of the types and ratios of centromere segregation (Muller 1914; see Table 6). If a single cross-over always occurs in each chromosome pair (i.e., four cross-overs in each tetravalent formed from four metacentric chromosomes), as has been shown in salmonids (Thorgaard et al. 1983), and all three types of centromere segregation occur in equal ratios, then the predicted gamete ratio is 2:5:2 (Burnham 1962). Note that this will only be the case if the cross-over occurs between the gene in question and the centromere; thus this ratio will only be found for loci distal to the centromere. If all three segregation types occur in equal frequency in conjunction with randomizing cross-overs (i.e., all frequencies of crossing-over between zero and infinite can occur, thus randomizing the location of the alleles with respect to their centromeres), then the predicted gamete ratio is, rather startlingly, 11:32:11. [Note that this is very close to the 3:8:3 ratio predicted by Haldane in 1930. Haldane achieved his ratio by assuming completely random chromatid segregation. That is, a

symmetrically heterozygous individual has four chromatid copies of each allele; all possible pairwise combinations of these alleles give a 6:16:6 ratio of 11, 12, and 22 gametes. This is correct in theory; however, the association of chromosomes in a tetravalent inhibits the randomness of chromatid pairing.] In order to derive the 11:32:11 ratio, we must consider an essentially infinite population of pre-meiotic cells each containing a symmetrically heterozygous tetravalent, each of which may undergo any one of three types of centromere segregation. Each tetravalent may have any one of three chromosome arrangements: chromosomes with like alleles may be juxtaposed, opposite one another, or tangential to one another (see Fig. 2). In the opposite arrangement, crossing-over will not result in an altered arrangement of alleles; adjacent 1 segregation will produce a 1:1 ratio of 11 and 22 gametes, while the other two types of segregation will produce only 12 gametes. If the chromosomes are arranged juxtaposed or tangential to each other, adjacent 1 segregation will produce a 1:4:1 ratio of gametes (i.e., the two meiosis I products will both contain two chromatids carrying a 1 allele and two chromatids carrying a 2 allele randomized with respect to their centromeres by multiple cross-overs). Either adjacent 2 or alternate segregation will produce a 1:2:1 ratio of gametes, as follows: after crossing-over, each chromatid pair (= chromosome) has probabilities 1/6, 4/6, and 1/6 of being 11, 12, and 22, respectively. Two such chromosomes will segregate together, and in meiosis II will separate into two chromatids per gamete; the gamete ratios can be

calculated by making a Punnet square (Table 7). By equally weighting and then totalling the gamete ratios produced by the three types of segregation and the three types of tetravalent configurations (Table 8), the ratio 11:32:11 is found.

The preceding discussion is in reference to locus X in Figure 1. For estimating gamete ratios for a locus in the Y position, the ratios resulting from adjacent 1 and adjacent 2 segregation will be reversed.

Table 1. Artificial crosses made with Hyla versicolor, with origin of parents.

Cross Number	Origin of parents and date of collection: Female	Male
A844[a]	Ithaca, NY 6/84	Bastrop, TX 4/83
B841	Ithaca, NY 6/84	Ithaca, NY 6/84
B842	Ithaca, NY 6/84	Columbia, MO 5/83
D843	Aurora, NY 6/84	Bastrop, TX 4/83
E841	Ithaca, NY 6/84	Bastrop, TX 6/84
F841	Ithaca, NY 6/84	Bastrop, TX 4/83
F842	Ithaca, NY 6/84	Richmond, VA 5/84
G841	Ithaca, NY 6/84	Ithaca, NY 6/84
G842	Ithaca, NY 6/84	Richmond, VA 5/84
H841	Ithaca, NY 7/84	Bastrop, TX 6/84
H842	Ithaca, NY 7/84	Columbia, MO 5/83

[a]The letter refers to the female in the cross, and the number refers to the male. Thus B841 and B842 used the same female, while F842 and G842 used the same male.

Table 2. Loci examined in *H. versicolor*, with tissues and buffer systems on which they were best resolved.

Enzyme	Locus	E.C. no.	Alleles resolved	Buffer System	Parent tissue used[a]
Aconitase	*Ac*	4.2.1.3	mono	R	L
Adenosine deaminase	*Ada*	3.5.4.4	mono	R	L
Adenylate kinase	*Ak*	2.7.4.3	*3* (308) *2* (200) *1* (100)	C	M
Aspartate aminotransferase	*Aat*-1	2.6.1.1	*1* (100) *2* (750)	A,C	M
	Aat-2	2.6.1.1	mono	A,C	M
Creatine kinase	*Ck*	2.7.3.2	mono	4	H
Diaphorase	*Dia*	1.6.4.3	mono	R	M
Galactosaminidase	*Gam*	3.2.1.23	mono	R	M

Galactose-1-phosphate uridylyl transferase	Galt-2	2.7.7.10	mono	4	M
Glutathione reductase	Gr-1,2	1.6.4.2	mono	C	M
Glycerol-3-phosphate dehydrogenase (NAD^+)	G3p	1.1.1.8	1 (fast) 2 (slow)	C	M
Isocitrate dehydrogenase	Idh-1	1.1.1.42	1 (100) 2 (81)	4	H
	Idh-2	1.1.1.42	1 (100) 2 (97)	4	H
Lactate dehydrogenase	Ldh-2	1.1.1.27	3 (400) 2 (200) 1 (100)	R (C, C5.4)	M
Malate dehydrogenase	Mdh-1	1.1.1.37	mono	C	H,L
	Mdh-2	1.1.1.37	2 (129) 1 (100) 3 (86)	C	H,L

Mannosephosphate isomerase	Mpi	5.3.1.8	1 (100) 2 (94) 3 (89) 4 (79) Ø	R	M
a-D-Mannosidase	a-Man	3.2.1.24	mono	M	L
NAD-dependent dehydrogenase	Ndh	1.1.1.-	1 (100) 2 (111)	4disc	L
Peptidase with leucyl-alanine	Pep-la	3.4.11-13	1 (83) 2 (100)	C	H
Phosphoglucomutase	Pgm	2.7.5.1	mono	C,4	M
Phosphogluconate dehydrogenase	Pgd-1	1.1.1.43	1(fast) 2(slow)	C	M
	Pgd-2	1.1.1.43	mono	C	M
Superoxide dismutase	Sod-1	1.15.1.1	1 (130) 2 (100)	C	M

	Sod-2	1.15.1.1	5 (173) 3 (166) 2 (142) 4 (118) 6 (107) 1 (100)	4disc	L
Triosephosphate isomerase	Tpi	5.3.1.1	3 (137) 2 (116) 1 (100)	M (R)	L

See methods section for description of buffer systems used. When different buffer systems were used for tadpoles and adults, the buffer used for the tadpoles is listed in parentheses. Relative mobilities of alleles are given in parentheses. Mono = monomorphic locus.

[a]H = heart, L = liver, M = muscle.

Table 3: Expected progeny genotypes at a single locus with two alleles under either disomic or tetrasomic inheritance (excluding double reduction events).

Parental genotype		Possible gametes		Progeny	Expected ratios[a]		
parent 1	parent 2	parent 1	parent 2	Genotypes	DI	DII	TI
1111	1122	11	11	1111		1	1
			12	1112	1	2	4
			22	1122		1	1
1112	1122	11	11	1111		1	1
		12	12	1112	1	3	5
			22	1122	1	3	5
				1222		1	1

[a]DI = disomic inheritance with homozygous bivalents (homobivalents)
DII = disomic inheritance with heterozygous bivalents (heterobivalents)
TI = tetrasomic inheritance (conventional ratio)

Table 4. Tests for normal segregation of chromosomes in Hyla versicolor by testing for random segregation of single alleles.

Cross	Locus	Parental genotypes Female	Male	Allele	Progeny genotype ratio 111	111X	11XX[a]	X^2	p-value[b]
A844	Mpi	1111	124Ø	2	49	60	1	1.11	-
	"	"	"	4	54	55	2	.01	-
	Idh-2	1111	1112	2	65	47		2.89	-
	Sod-2	1111	1112	2	51	61		.89	-
	Tpi	1113	1111	3	59	53		.32	-
B841	Tpi	1113	1111	3	61	59		.03	-
B842	Idh-2	1111	1112	2	66	52		1.66	-
	Tpi	1113	1112	2	74	44		7.63	.006
	"	"	"	3	60	58		.03	-
	Sod-2	1111	1114	4	61	59		.03	-
D843	Sod-1	1111	1112	2	53	49		.16	-
	Sod-2	1111	1134	3	77	66		.85	-
	"	"	"	4	76	67		.57	-
	Mdh-2	1111	1112	2	68	75		.34	-
	Pgd-1	1112	1111	2	56	85		5.96	.015
E841	Mdh-2	1111	1123	2	22	24		.09	-
	"	"	"	3	28	18		2.17	-
F841	Mpi	1111	1112	2	20	19		.03	-
	Sod-2	1112	1366	2	18	19	1	.03	-
	"	"	"	3	16	21		.68	-

	Mdh-2	1111 1112	2	20	19	.03	-
F842	Sod-2	1112 1113	2	76	70	.25	-
	"	" "	3	76	70	.25	-
G841	Ldh-2	1111 1112	2	36	35	.01	-
G842	Tpi	1113 1111	3	16	24	1.60	-
	Sod-2	1113 1111	3	24	16	1.60	-
H841	Tpi	1113 1112	2	22	24	.09	-
	"	" "	3	19	27	1.39	-
	Sod-2	1112 1555	2	21	25	.35	-
	Mdh-2	1111 1112	2	27	19	1.39	-
H842	Tpi	1113 1122	3	23	28	.49	-
	Mpi	1111 1112	2	18	34	4.92	.027
	Idh-2	1111 1112	2	24	28	.31	-
	Sod-2	1112 1166	2	18	34	4.92	.027

Expected progeny genotypes are a 1:1 ratio of homozygotes to asymmetrical heterozygotes. Alleles shown are those being tested for random segregation in crosses where more than one allele is present in a single dose. The allele being tested is represented by an 'X' in the progeny genotypes.

[a] denotes products of double reduction (see text).

[b] denotes X^2 value significant below the .05 level. All tests have 1 d.f.

Table 5. Observed and expected segregation of single locus allozymes in artificial crosses of H. versicolor.

Cross	Locus	Parental genotypes Female	Male	N	Observed progeny genotypes [expected tetrasomic ratios (1:4:1)] {expected disomic ratios (1:2:1)} 1111	111X	11XX	1XXX	X^2(d.f.)	p-value
A844	Mpi	1111	1240	109	16	71	22			
					[18.17	72.66	18.17]		.99 (2)	.610
					{27.25	**54.5**	**27.25}**		**10.7 (2)**	**<.005****
B841	Mpi	1122	1111	120	23	84	13			
					[20	80	20]		3.1 (2)	.212
					{30	**60**	**30}**		**20.9 (2)**	**<.001****
B842	Mpi	1122	1112	120	8	61	43	8		
					[10	50	50	10]	4.2 (3)	.241
					{15	**45**	**45**	**15}**	**12.3 (3)**	**.006****
E841	Mpi	1112	1122	46	2	14	26	4		
					[3.8	19.2	19.2	3.8]	4.7 (3)	.195
					{5.75	**17.25**	**17.25**	**5.75}**	**8.0 (3)**	**.046***

G842	Mpi	1122	1111	40	7	26	7		
					[6.6	26.8	6.6]	.07 (2)	.966
					{10	**20**	**10}**	**3.6 (2)**	**.165**
H841	Mpi	1111	1122	46	11	33	2		
					[7.7	30.6	7.7]	5.8 (2)	.055
					{11.5	**23**	**11.5}**	**12.2 (2)**	**.002****
D843	Sod-2	1111	1134	143	30	93	20		
					[23.83	95.34	23.83]	2.3 (2)	.317
					{35.75	**71.5**	**35.75}**	**13.7 (2)**	**.001****
E841	Sod-2	1111	1144	46	9	23	14		
					[7.7	30.6	7.7]	5.7 (2)	.058
					{11.5	**23**	**11.5}**	**1.1 (2)**	**.577**
F841	Sod-2	1112	1366	37	7	21	9		
					[6.16	24.66	6.16]	2.0 (2)	.368
					{9.25	**18.5**	**9.25}**	**.89 (2)**	**.641**
H842	Sod-2	1112	1166	52	11	37	4		
					[8.7	34.6	8.7]	3.3 (2)	.192
					{13	**26**	**13}**	**11.2 (2)**	**.004****

D843	Tpi	1133	1111	**140**	26	96	18		
					[23.3	93.4	23.3]	1.6 (2)	.449
					{35	**70**	**35}**	**20.2 (2)**	**<.001****
H842	Tpi	1113	1122	52	19	25	8		
					[8.7	34.6	8.7]	14.9 (2)	<.001**
					{13	**26**	**13}**	**4.7 (2)**	**.095**
E841	Mdh-2	1111	1123	46	14	22	10		
					[7.7	30.6	7.7]	8.3 (2)	.016*
					{11.5	**23**	**11.5}**	**.78 (2)**	**.677**

Progeny genotypes are given in a generalized form: homozygous, assymetrically heterozygous, and symmetrically heterozygous for any two alleles. Thus a 1123 individual can be treated as a symmetrical heterozygote by scoring the 2 and 3 alleles as 'X'. P-values at or below the .05 and .01 level are indicated by * and **, respectively.

Table 6. Summary of all expected ratios of 11, 12, and 22 gametes in a symmetrically heterozygous individual (1122) in which tetravalents are consistently formed in meiosis.

centromere segregation type	no cross-overs	one cross-over	randomizing cross-overs
adjacent 1 adjacent 2 alternate	1:4:1	2:5:2	11:32:11
only adj. 1	1:4:1	1:1:1	5:8:5
only adj. 2	1:4:1	1:4:1	1:4:1
only alt.	1:4:1	1:4:1	1:4:1

Table 7. Punnett square showing the gametes that would be produced from alternate or adjacent 1 segregation from a tetravalent after randomizing crossing-over.

	(1 : 4 : 1)			
	11	**12**	**22**	TOTALS:
(1) **11** :	**11** (1)	**11,12** (2:2)	**12** (1)	**11** **12** **22** (9 : 18 : 9)
(4) **12** :	**11,12** (2:2)	**11,12,22** (4:8:4)	**12,22** (2:2)	= (1:2:1)
(1) **22**	**12** (1)	**12,22** (2:2)	**22** (1)	

Further explanation of this table is given in the text and in Figure 1. The post-cross-over chromosome pairs that enter meiosis II are given outside the box. Predicted ratios of genotypes are given in parentheses; genotypes are given in boldface. Note that the genotype ratios above and to the left of the box may have any values without changing the final gamete ratio, so long as these genotype ratios are in the form m:n:m above the box and m':n':m' to the left of the box, where m, n, m', and n' are arbitrary positive numbers.

Table 8. Determination of the gamete ratios expected from a symmetrical heterozygote with randomizing crossing-overs and all types of centromere segregation from a tetravalent.

tetravalent configuration	centromere segregation type: adj. 1	adj. 2	alt.
opposite	**11,22** (1:1)	**12** (all)	**12** (all)
juxtaposed	**11,12,22** (1:4:1)	**11,12,22** (1:2:1)	**11,12,22** (1:2:1)
tangential	**11,12,22** (1:4:1)	**11,12,22** (1:2:1)	**11,12,22** (1:2:1)

The possible pairing configurations in the tetravalent are shown for each row (see Figure 2).

Figure 1. Type of cross-over event in a tetravalent which may lead to double reduction gametes at the X locus following adjacent 1 segregation (a with c and b with d). See text for further explanation.

adjacent 1 segregation - a with c, b with d

adjacent 2 segregation - a with b, c with d

alternate segregation - a with d, b with c

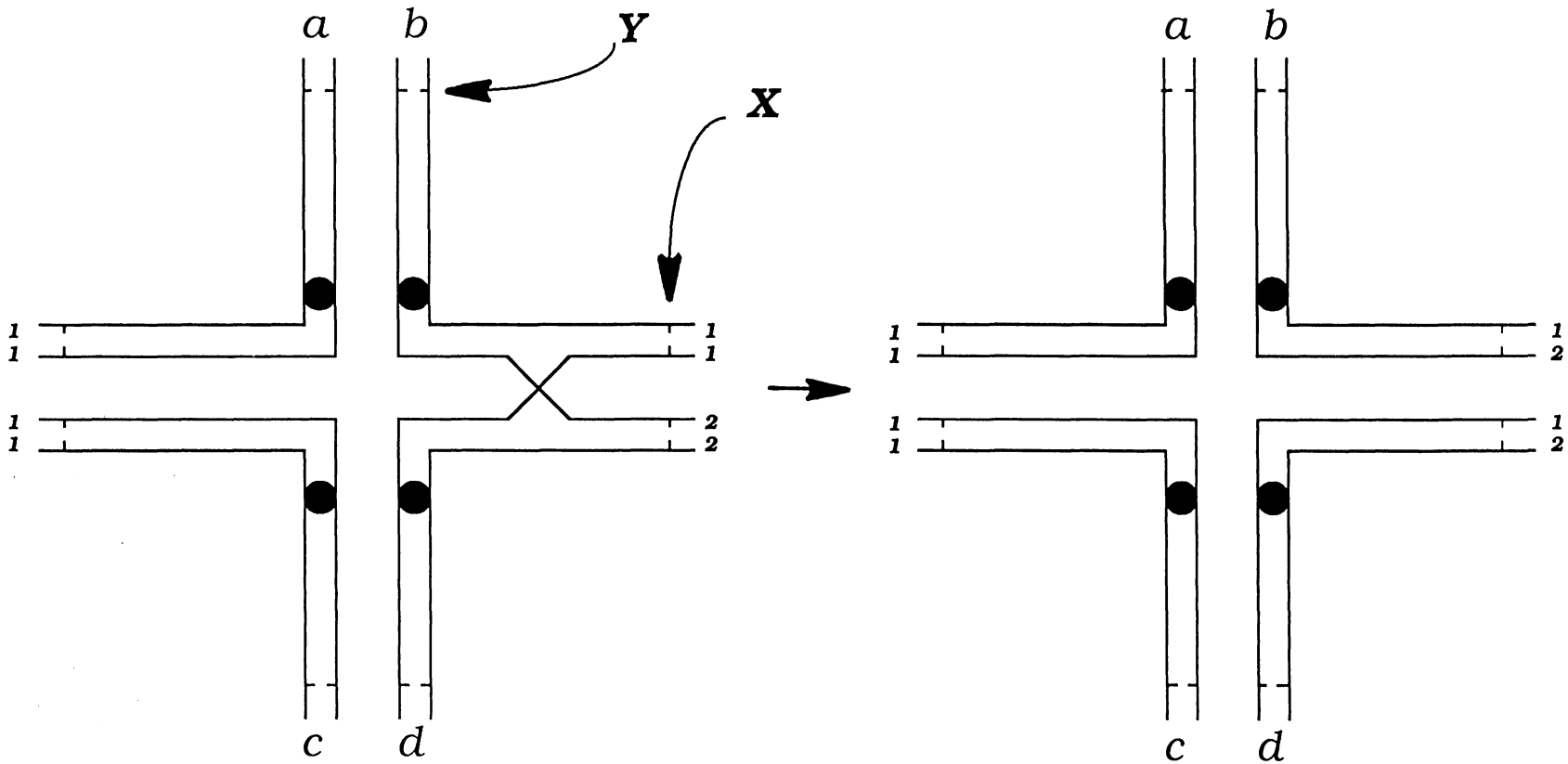
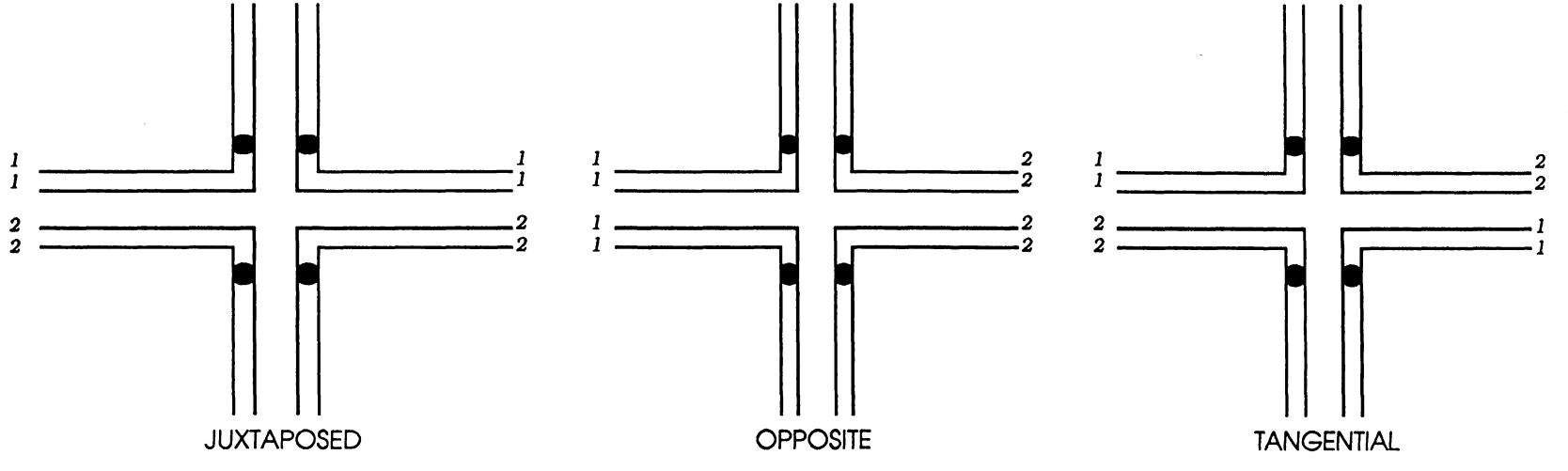

a
b
Y
X
1
1
1
1
1
1
2
2
c
d
a
b
1
1
1
2
1
1
1
2
c
d

Figure 2. Possible configurations of alleles in a tetravalent from a symmetrically heterozygous individual.


1
1
1
1
2
2
2
2
JUXTAPOSED
1
1
2
2
1
1
2
2
OPPOSITE
1
1
2
2
2
2
1
1
TANGENTIAL