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Diploid-polyploid cryptic species pairs: a possible clue to evolution by polyploidization in anuran amphibians[1]

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Abstract

Three of the seven currently recognized bisexual polyploid anuran species have morphologically very similar diploid "cryptic species" counterparts. The North American diploid-tetraploid cryptic species pair *Hyla chrysoscelis* and *H. versicolor* is analyzed and compared with diploid and tetraploid populations of the South American nominal species *Odontophrynus americanus*. It is postulated that, through an intermediate triploid stage, tetraploidy could arise independently in diploid species by way of suppressed maturation in oöcytes. Tetraploid populations arising sympatrically from diploid species may maintain their integrity bio-acoustically. Polyploidization in the Anura is an important evolutionary mechanism and may prove to be quite wide spread when species are carefully examined on a population level.

Speciation by polyploidy is fairly common in plants (STEBBINS, 1950, 1966) but relatively rare among animals. WHITE (1954) and DOBZHANSKY (1951, 1970) have maintained that polyploidy cannot be sustained in naturally occurring biparental populations of animals. On the basis of DNA content studies of various vertebrates, OHNO (1967) has the belief that various degrees of polyploidization must have occurred in the evolution of vertebrates.

Among reptiles, polyploidy has invariably been associated with parthenogenetic reproduction (PENNOCK, 1965; WRIGHT and LOWE, 1968; HALL, 1970; MASLIN, 1971). Similarly, gynogenetic all-female triploid races are

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known for the salamanders *Ambystoma platineum* and *A. tremblayi* (UZZELL, 1964; UZZELL and GOLDBLATT, 1967). Despite objections which have been raised against the possibility of bisexual polyploidy in vertebrate species, seven polyploid, biparental anuran species have been well documented. Polyploid species which have been described from South America include: *Odontophrynus americanus* (4n) (M. L. BEÇAK et al., 1966; BOGART, 1967), *Ceratophrys ornata* (8n) (BOGART, 1967; BARRIO and RINALDI DE CHIERI, 1970b), *C. dorsata* (8n) (M. L. BEÇAK et al., 1967), *Pleurodema bibroni* (4n) and *P. kriegi* (4n) (BARRIO and RINALDI DE CHIERI, 1970a), and *Phyllomedusa burmeisteri* (4n) (M. L. BEÇAK et al., 1970). Recently, WASSERMAN (1970a) found that the North American common tree toad, *Hyla versicolor*, is also a polyploid (4n), sexually reproducing species. *H. chrysoscelis* is apparently identical morphologically to *H. versicolor*, and both range widely over eastern North America. On the basis of acoustical analysis and hybridization experiments, JOHNSON (1959, 1963) and RALIN (1968) proved that *H. versicolor* and *H. chrysoscelis* are separate species. BLAIR (1965) considers this species pair to be an example of speciation resulting from climatic events of the Pleistocene.

The purpose of this paper will be to compare and analyze the chromosomes of the closely related hylid species *H. versicolor* and *H. chrysoscelis* and compare these results with an apparently parallel instance of diploidy-tetraploidy in the South American species *Odontophrynus americanus*.

Materials and methods

Adults and larvae of *Hyla versicolor* and *H. chrysocelis* were obtained from several localities in Texas, South Carolina, New Jersey, Louisiana, Mississippi, Indiana, and Illinois. Tadpoles were also obtained from artificial crosses using the techniques outlined by RUGH (1948) and JOHNSON (1959). Calls in breeding congregations were recorded with a Uher 4000 report L tape recorder and analyzed with a Kay Electric Co. Sona-Graph 6061A and a Tektronix type 502A oscilloscope. Chromosomes were obtained from squash preparations of corneal epithelium, tadpole tail tips, and testis. The technique used was essentially the same as outlined by BOGART (1968). Chromosomes were measured at a final magnification of 45,000× with a "map-measurer", with the original negatives enlarged in a rear projection device. Idiograms were constructed from the averaged measurements of six cells from each species. The total length of all chromosomes from a cell was assigned a value of 100%, and each individual chromosome was expressed as a percentage of the total complement length in the idiogram. The scale was multiplied by 10 to produce units of normalized length. Living sperm cells and dried blood cells were photographed under phase contrast.

Results and discussion

Hyla versicolor *and* H. chrysoscelis

JOHNSON (1966) and RALIN (1968), in their description of *H. chrysoscelis*, were able to distinguish this species only on the basis of bio-acoustical analysis and genetic compatibility experiments. JOHNSON indicated that further studies should reveal differences not requiring sound spectrograms. It is now known (WASSERMAN, 1970a) that *H. versicolor* is a tetraploid species having 48 chromosomes, whereas *H. chrysoscelis* has 24 chromosomes (2n). These two cryptic species may be considered a diploid-tetraploid pair. Chromosomes of both species are shown in fig. 1a, b. Photomicrographs of blood and sperm cells of the two species are compared in fig. 2. In each case the cells of *H. versicolor* are larger than those of *H. chrysoscelis*. The genetic incompatibility between these two cryptic species (JOHNSON, 1959, 1963) is understood more easily in the light of the polyploid discovery. Hybrids resulting from reciprocal crosses between *H. versicolor* and *H. chrysoscelis* are triploids, having complements of 36 chromosomes (fig. 3). The high degree of offspring mortality in backcrosses between hybrids and individuals of either parent species, as found by JOHNSON (1963), must be due to chromosome imbalance in the gametes of the hybrids.

Portions of the ranges of two species have been delineated acoustically by BLAIR (1965), JOHNSON (1966), RALIN (1968), and ZWEIFEL (1970). In this study, when it was possible, tentative identifications on the basis of trill rate were made in the field as the animals were being collected. This was essential where the two species occurred in sympatry. In every case cytological examination confirmed identifications made in the field; i.e., slow-trilling animals proved to be tetraploid, and fast-trilling individuals were diploid. Idiograms were constructed for a population of *H. versicolor* from Bastrop, Texas, and *H. chrysoscelis* from near Elgin, Texas (fig. 4). These idiograms were constructed from the averaged normalized chromosome lengths and centromeric ratios presented in table I. From table I it is evident that the average chromosome lengths and centromeric ratios of *H. versicolor* are included within the range of lengths and ratios of *H. chrysoscelis*. Indeed, the averaged chromosome measurements of every population sampled did not differ markedly from the average presented for either species in table I. It was not possible, therefore, to distinguish any differences in chromosome lengths or centromeric ratios between any of the populations of *H. versicolor* and *H. chrysoscelis*. We believe that any chromosomal mutations which have taken place since these two species were reproductively isolated are not of the

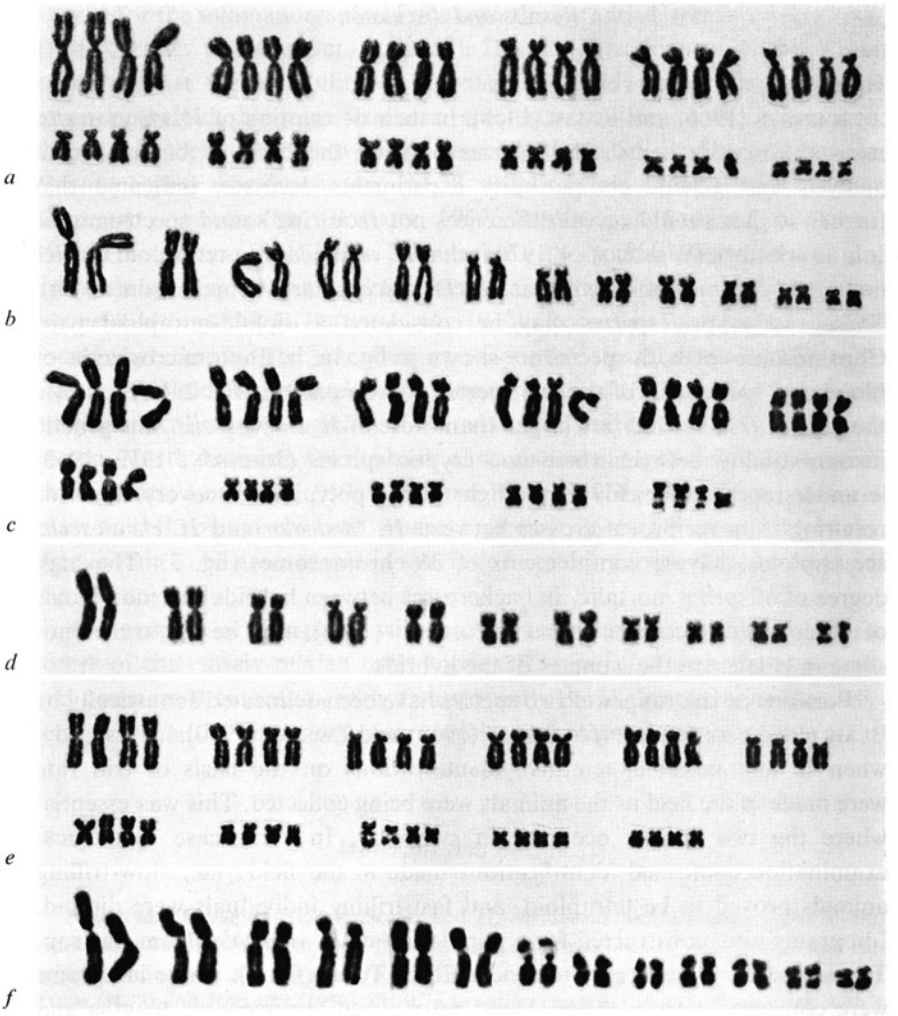

Fig. 1. Karyotypes. *a. Hyla versicolor* male, Bastrop, Texas. *b. H. chrysoscelis* male, Elgin, Texas. *c. Odontophrynus americanus* male, Tucumán, Argentina. *d. O. americanus*? male, Córdoba, Argentina. *e. Pleurodema kriegi* male, Pampa de Achala, Córdoba Prov., Argentina. *f. Ceratophrys ornata*? juvenile, Córdoba Prov., Argentina.

kind or magnitude that could be detected using our present method of analysis.

During the course of this study, four distinct secondary constrictions were found to occur in the karyotypes of some individuals of *H. versicolor*

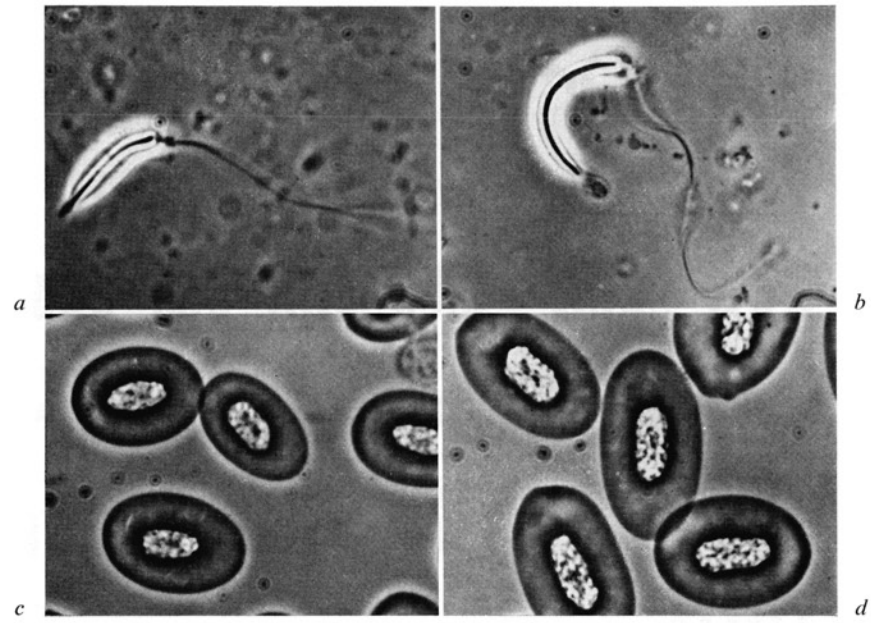

Fig. 2. a. Sperm cell of *Hyla chrysoscelis*. *b.* Sperm cell of *H. versicolor*. *c.* Blood cells of *H. chrysoscelis*. *d.* Blood cells of *H. versicolor*.

and *H. chrysoscelis*. A submedian secondary constriction on the long arm of chromosome 1 (fig. 5a) was present in two *H. chrysoscelis* individuals from Elgin, Texas, and one *H. chrysoscelis* from Tillman, South Carolina. A subtelocentric secondary constriction on the long arm of chromosome 4 which reproduced a satellite (fig. 5b) was found in most of the sampled individuals of *H. versicolor* from Alpine, New Jersey, and in one *H. versicolor* from Bastrop, Texas. A subtelocentric secondary constriction on the short arm of chromosome 4 (fig. 5c) was the most commonly found secondary constriction and was present in karyotypes of *H. versicolor* from Alpine, New Jersey, and Bastrop, Texas, and of *H. chrysoscelis* from Elgin, Texas, Griffy, Indiana, and Bastrop, Texas. A subtelocentric secondary constriction on the long arm of chromosome 3 (fig. 5d) was found in karyotypes of *H. versicolor* from Alpine, New Jersey, and Bastrop, Texas.

Unlike many hylid species (BOGART, unpublished), *H. versicolor* and *H. chrysoscelis* karyotypes do not always demonstrate the presence of secondary constrictions. Thus, it is difficult to draw any conclusions concerning the presence or absence of certain secondary constrictions until many

Fig. 3. Tadpole karyotypes. *a. Hyla chrysoscelis* ♀ × *H. versicolor* ♂ triploid hybrid. *b. H. versicolor* ♀ × *H. chrysoscelis* ♂ triploid hybrid. *c.* Hexaploid *H. versicolor.*

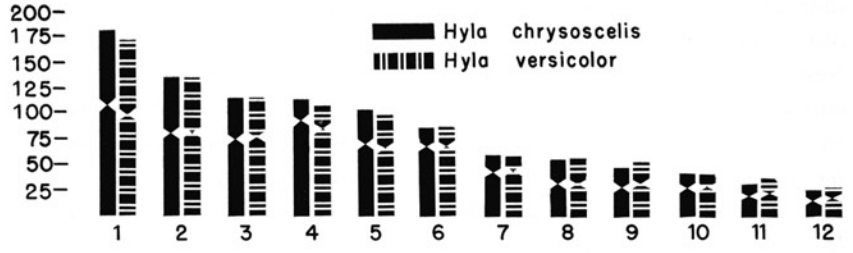

Fig. 4. Comparison of the idiograms of *Hyla chrysoscelis* and *H. versicolor;* constructed from the data in table I.

Table I. Analyses of haploid chromosome complements of *Hyla versicolor* and *Hyla chrysoscelis*.

Chromosome number	Normalized haploid chromosome length		Centromeric ratio	
	Mean	Range	Mean	Range
H. versicolor				
1	171	165–180	1.4	1.4
2	134	132–135	1.6	1.6–1.8
3	116	114–120	2.0	1.8–2.0
4	108	106–113	3.6	3.5–3.9
5	101	94–107	1.9	1.8–2.3
6	87	84–91	4.0	3.4–4.3
7	60	54–63	2.7	2.2–3.4
8	58	55–60	1.4	1.2–1.6
9	54	51–57	1.6	1.4–1.9
10	42	41–44	1.9	1.5–2.2
11	38	37–39	1.7	1.3–2.0
12	30	28–32	1.8	1.6–2.1
H. chrysoscelis				
1	182	170–196	1.5	1.4–1.6
2	135	126–138	1.5	1.3–1.6
3	115	103–129	1.9	1.8–2.1
4	114	100–125	4.0	3.3–4.6
5	103	90–112	2.2	1.9–2.6
6	87	83–94	4.4	2.7–5.6
7	60	54–63	2.6	1.1–3.8
8	56	53–61	1.2	1.0–1.4
9	48	36–55	1.6	1.5–1.8
10	43	38–48	2.1	1.6–2.4
11	32	27–39	2.0	1.8–2.5
12	29	24–34	1.4	1.1–1.5

more individuals and populations are sampled. Some secondary constrictions seem to be present in both species, and both satellites associated with chromosome set 4 (fig. 5b, c) may be present on different chromosomes in the same set in *H. versicolor*.

SAEZ and BRUM (1959) were the first to describe meiotic multivalent association of the chromosomes in *Ceratophrys ornata* and *Odontophrynus americanus*. Later, M. L. BEÇAK et al. (1966) confirmed multivalents in the

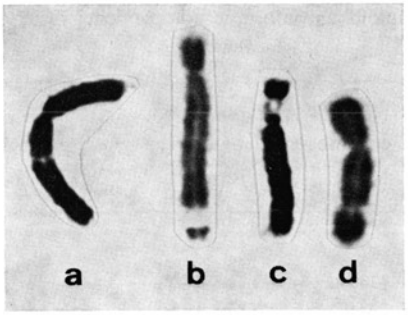

Fig. 5. Secondary constrictions found in individuals of *Hyla versicolor* and *H. chrysoscelis.*

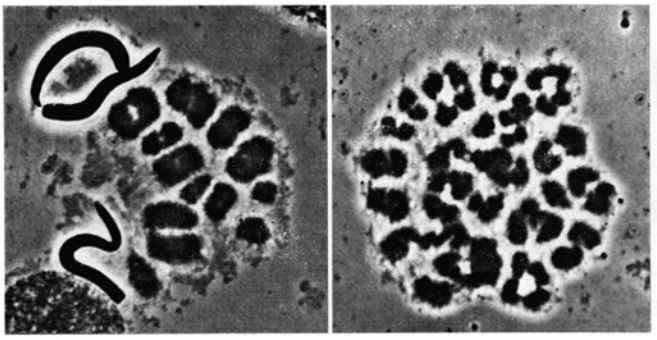

Fig. 6. Testis squashes. *a.* Metaphase I in *H. chrysoscelis. b.* Metaphase I in *H. versicolor.*

tetraploid *O. americanus* and presented multivalents for the octoploid *Ceratophrys dorsata* (M. L. BEÇAK et al., 1967). Multivalent associations in meiosis have been found also in *C. ornata* (BARRIO and RINALDI DE CHIERI, 1970b) and *Phyllomedusa burmeisteri* (M. L. BEÇAK et al., 1970); however, neither of the tetraploid *Pleurodema* species demonstrates multivalent association (BARRIO and RINALDI DE CHIERI, 1970a). Squashes of *H. versicolor* testicular material revealed multivalents among the meiotic metaphase chromosomes. In normal meiosis, homologous chromosomes pair during synapsis, forming bivalents recognized from diakinesis through metaphase as 12 single ring structures (see fig. 6a for *H. chrysoscelis*), and during anaphase, the 12 homologs migrate to opposite poles. In *H. versicolor*, however, there is a union of four "homologous" chromosomes to form

quadrivalents rather than bivalents (fig. 6b). This union is maintained through metaphase I. During anaphase, each quadrivalent separates into two bivalents, and so 24 "homologs" migrate to opposite poles.

Odontophrynus americanus

Tetraploid *O. americanus* have been described from populations in Campos de Jordão, São Paulo, Brazil (M. L. BEÇAK et al., 1966), and Tucumán, Argentina (BOGART, 1967) (fig. 1c). *O. americanus* specimens from Córdoba, Argentina, proved to have only 22 chromosomes (fig. 1d) rather than the 44-chromosome complement described as a species characteristic by SAVAGE and CEI (1965). *Hyla versicolor* and *H. chrysoscelis* were distinguished originally by sound-spectrum analysis, and, disregarding cell size, they were considered indistinguishable morphologically. Thus, it is interesting to examine the sound spectrum of the diploid and tetraploid populations of *O. americanus*. Eleven calls from five diploid individuals and 14 calls from four tetraploid individuals were analyzed and compared. At 20°C, the diploid population was found to have an average pulse rate of 127.5 pulses per second (pps) and a dominant frequency of 1175 cycles per second (cps) (fig. 7a). At 25°C, the tetraploid population was found to have an average pulse rate of 94 pps and a dominant frequency of 875 cps (fig. 7b). Two diploid individuals were recorded at 17°C. At this lower temperature the average pulse rate was 104.5 pps with a dominant frequency of 1000 cps. The pulse rate and the dominant frequency appear to vary directly with temperature. This relationship, with respect to the pulse rate, holds in *O. americanus* as it has been shown to do in *H. versicolor* and *H. chrysoscelis* (RALIN, 1968; ZWEIFEL, 1970). The temperatures at which the diploid and tetraploid *O. americanus* were recorded in this study were not the same, but the differences demonstrated between these populations would be even greater at the same temperature.

Possible mechanisms for the formation of polyploids

In the case of *Hyla versicolor* and *H. chrysoscelis*, separation into two species was warranted on the basis of hybridization experiments and bioacoustical analysis performed prior to the discovery of chromosomal differences. The diploid and tetraploid *O. americanus* appear to be South American counterparts of *H. versicolor* and *H. chrysoscelis*. The similarity is striking. In both instances the tetraploid is apparently morphologically indistinguishable from the diploid, quadrivalents are formed during meiosis in the tetraploid, and the tetraploid's call has a reduced pulse rate. Even

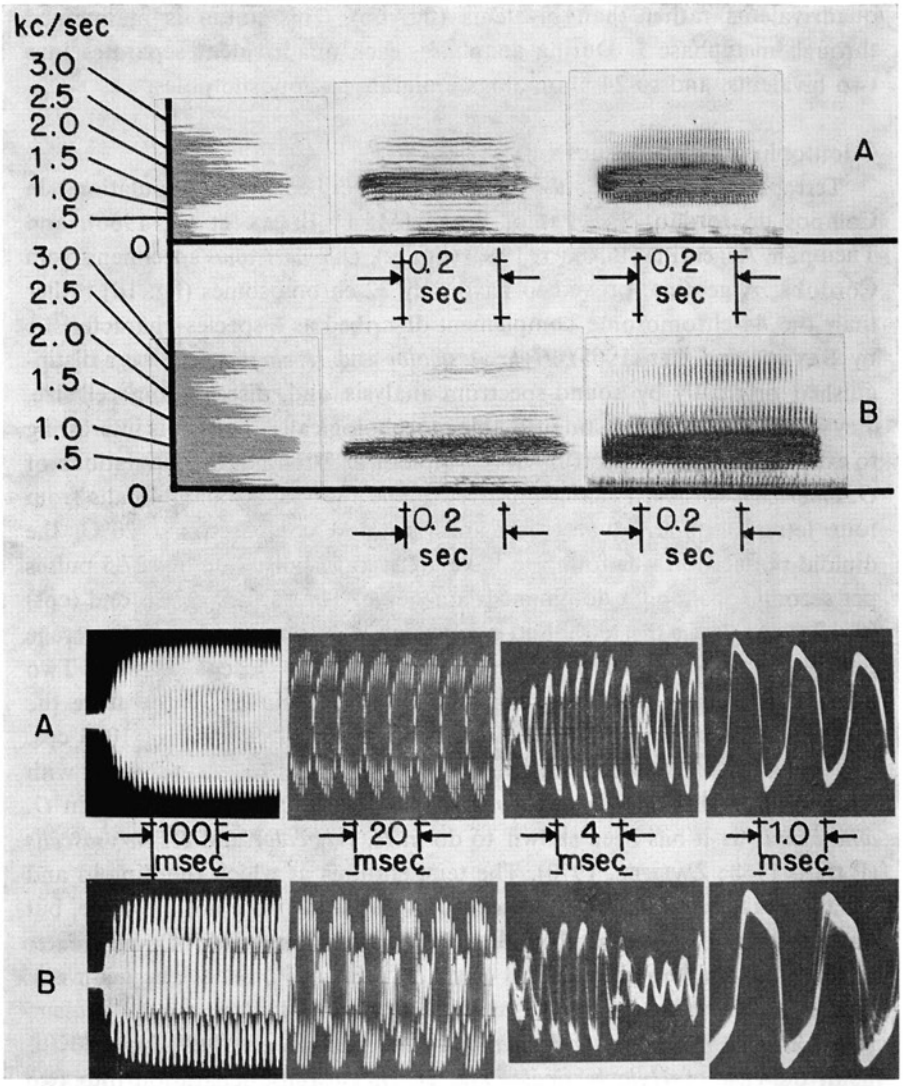

Fig. 7. Sonagrams and oscilloscope tracings of diploid (*A*) and tetraploid (*B*) *Odontophrynus americanus* individuals.

though the diploid and tetraploid *O. americanus* have yet to be hybridized, W. BEÇAK et al. (1968) found that viable triploid hybrids resulted from the cross of a tetraploid *O. americanus* ♀ and an *O. cultripes* ♂. Development in the reciprocal cross did not progress past gastrulation. Since these two species are

considered to be the most distantly related species in the genus (SAVAGE and CEI, 1965), the failure in the reciprocal cross might be expected owing to the genetic divergence between the two species. If the diploid-tetraploid *O. americanus* populations are parallel to the more thoroughly studied situation with *H. versicolor* and *H. chrysoscelis*, *O. americanus* will undoubtedly be established as another diploid-polyploid cryptic species pair. Similarly, diploid populations of the octoploid species *Ceratophrys ornata* have been discovered by BARRIO and RINALDI DE CHIERI (1970b) (see also fig. 1f). This species should be divisible into a diploid-octoploid cryptic species pair when more specimens have been studied.

Specication by polyploidization must now be considered, not as a single isolated event, but as an important evolutionary mechanism in the Anura. Five genera are represented, and the seven known cases of anuran polyploidy represent at least five independent occurrences. BARRIO and RINALDI DE CHIERI (1970a) suggest that the two tetraploid *Pleurodema* species were derived from a common polyploid ancestor, and none of the diploid *Pleurodema* species is very similar morphologically to the tetraploid species. *Ceratophrys dorsata* and *C. ornata* may have undergone speciation after the establishment of polyploidy, since *C. dorsata* apparently lacks a diploid-polyploid pair of populations. However, few populations of *C. dorsata* have been examined cytologically or bio-acoustically.

In plants, the formation of quadrivalents is often an indication of an allotetraploid (SWANSON, 1957), and DOBZHANSKY (1951) states that more and more forms once regarded as autopolyploids are being shown by more careful studies to have been of hybrid origin. Since *H. versicolor*, the tetraploid *O. americanus*, and the octoploid *C. ornata* all have morphologically very similar diploid counterparts, it might be assumed that each of the polyploids arose directly from corresponding diploid populations (i.e., that they are autopolyploids). We cannot, however, reject the possibility that the formation of polyploidy was caused or facilitated by secondary contact of genetically isolated and differentiated populations (allopolyploids). Distinguishing between allopolyploids and autopolyploids will be very difficult at the present time. For example, *H. versicolor* appears to divide *H. chrysoscelis* in some parts of its range. Populations of *H. chrysoscelis* on each side of a *H. versicolor* barrier could be dissimilar genetically, and it would be difficult to determine whether initial speciation of *H. versicolor* evolved either (1) by autopolyploidy from a diploid parent population of *H. chrysoscelis* or (2) by juxtaposition of formerly isolated and divergent populations, between which hybridization occurred in the zone of secondary contact.

The latter would be an instance of allotetraploid speciation. In either event, the intrusion of the tetraploid *H. versicolor* serves presently as a genetic barrier isolating two populations of *H. chrysoscelis*. This does constitute an effective barrier, since where the two species occur sympatrically, intermediate call types or triploids do not occur.

The discovery that corresponding chromosomes of the diploid and tetraploid forms of *O. americanus* are the same size (fig. 1), together with the DNA determinations for the latter species and for the diploid *O. cultripes* (W. BEÇAK et al., 1967), suggests that earlier views of the evolution of polyploidy by chromatid autonomy (BOGART, 1967) are now untenable. Triploid and pentaploid embryos are often found among tadpoles in artificially produced experimental and control crosses of *Bufo*, *Hyla*, *Odontophrynus*, and *Scaphiopus* (WASSERMAN, 1970b; BOGART, 1972 and unpublished). Polyploid amphibians produced under experimental conditions and even spontaneous occurrences have been well documented (see reviews by FANKHAUSER, 1945, and MOORE, 1955). In every case, triploids are the most commonly found class and tetraploids are very rare. Triploid and pentaploid embryos are thought to be produced as a result of suppressed maturation divisions in oöcytes (TCHOU-SU, 1936; MOORE, 1955; BOGART, 1972). If all the polar bodies were retained from the first maturation division, tetraploid eggs would result, and after fertilization, the embryo would be pentaploid. More commonly, the polar body in the second maturation division may be maintained, producing a diploid egg, which, when fertilized, could give rise to a triploid zygote. Gynogenetic diploid and tetraploid embryos could be produced if there were no fertilization, but such events must be very rare.

In *Bufo*, triploids may be either male or female (BOGART, 1972). One experimentally produced triploid male *B. regularis* ($3n = 30$) was crossed with a normal 20-chromosome female from another population of *B. regularis*, and the resulting offspring had various chromosome numbers ranging from 20 to 33 (BOGART, 1972). It is evident that the chromosomes of the triploid male were assorted randomly in the sperm cells.

Temperature shock has been used to induce triploidy by inhibiting the second maturation division (KAWAMURA, 1952; MOORE, 1955). Fertilized eggs of *Hyla versicolor* were subjected to a cold shock treatment following RUGH (1948). Of 28 tadpoles which were sampled, 2 were found to have 72 chromosomes (fig. 3). These hexploids must have resulted from an unreduced egg (48) and a normal *H. versicolor* sperm (24). The same female was used in an artificial cross with a male *H. chrysoscelis*. One tadpole sampled had 60 chromosomes, which probably resulted from the combina-

tion of another unreduced egg (48) and a normal *H. chrysoscelis* sperm (12). Twenty-three other tadpoles had the normal expected triploid complement of 36 chromosomes. All of the control tadpoles sampled had 48 chromosomes. Even though the two hexaploids discovered were in the cold shock experiment, 26 normal tadpoles sampled were subjected to the same treatment, and the one unreduced egg which was probably responsible for the pentaploid *H. versicolor* × *H. chrysoscelis* was not subjected to cold treatment. This suggests that some of the eggs of this female were normally unreduced.

Because it is the most commonly found polyploid class in amphibians, triploidy conceivably could have been important in the production of the various tetraploid species. There are at least three possible methods by which tetraploids could be produced through an intermediate triploid stage:

1. Triploid males or females producing gametes that contain a random assortment of chromosomes and a chance meeting of some of these gametes with normal or abnormal gametes, producing genetically correct, viable tetraploids.

2. Triploid males or females producing haploid and diploid gametes. Chance meeting of two diploid gametes, each from a triploid individual, or the union of a diploid sperm with an unreduced egg would produce the tetraploid.

3. Triploid males or females producing unreduced gametes that combine with normal haploid gametes to produce the tetraploid.

The little evidence which has been gathered suggests that the first two possibilities are not as likely as the third. The triploid male *B. regularis* (above) produced sperm cells that contained a varied assortment of chromosomes and gave rise to short-lived tadpoles. The backcrosses which were performed by JOHNSON (1963) gave results similar to those obtained with the triploid *B. regularis*. Even though the chromosomal evidence was not available in JOHNSON's study, the inviability of the tadpoles suggests that the chromosomes were not balanced. Triploid females have not been tested, since it is very difficult to raise and develop females to the condition of egg deposition under laboratory conditions. We do know (BOGART, 1972, and unpublished), however, that unreduced eggs occur frequently in diploid anuran females and even in the tetraploid *H. versicolor*. It is possible, therefore, that unreduced eggs would occur in triploid females. If a triploid female produced a fairly large percentage of unreduced eggs, then the number of tetraploids produced by one female possibly could be large enough to initiate a population of tetraploid individuals. This, then, would increase the likelihood of tetraploids breeding with each other in the succeeding genera-

tions. The first two possibilities, involving chance meetings of certain gametes, would be more likely to produce very rare tetraploid individuals.

Since unreduced eggs appear to be fairly common, it is possible that unreduced sperm cells also exist. At present, there is little or no evidence for formation of unreduced sperm cells (BOGART, 1972). The possibility also exists that misdivision in the embryo could produce a tetraploid individual, but, again, this mechanism would produce only rare tetraploid individuals, and there is no available evidence for this mechanism.

The major objection to polyploid animal species seems to be focused on imbalance in the sex determination of polyploids; therefore, it is important to discuss the possible mechanisms of overcoming this problem. Distinct heteromorphic sex chromosomes have not been demonstrated satisfactorily for any anuran (SETO, 1964; MIKAMO and WITSCHI, 1966). OHNO (1967) believes that polyploidy may be permitted when the Z and W, or the X and Y, are in such a primitive state of evolution that the W, or the Y, is still equivalent genetically to the Z, or the X. We believe that a partial solution to the problem of sexual imbalance lies in the formation of multivalents in meiosis. It is possible that complete random assortment of homologs does not take place when there is multivalent association. If the sex-determining parent's homologs carrying the Y, or the W, were always to migrate to the same pole and the homologs carrying the X, or the Z, migrated to the opposite pole, then the gametes would always be homozygous for sex determination. Intersexes would occur only if the gametes were heterozygous for sex determination. This particular system could have been selected for rapidly even if it did not occur coincidentally with the formation of the polyploid. Multivalent association would definitely facilitate this mechanism.

Unlike plants, only a few species of polyploid amphibians have been discovered, and most of these have closely related diploid ancestors. No families or genera of polyploid amphibians have been found, and it must be assumed that polyploidy has not been a major factor in the evolutionary history of amphibians. This raises a question as to the selective advantage of the polyploid in relation to the diploid ancestor. From hybridization experiments with *Bufo* (BLAIR, 1972), it is evident that polyploids are definitely more vigorous and attain more advanced stages in hybrid combinations than the diploid hybrids if the parental species were distantly related. Polyploids discovered in control crosses, however, are usually less vigorous than the normal tadpoles. Perhaps the answer lies in the greater possible genetic variability of the polyploid compared to its diploid ancestor. In a

tetraploid, the number of linkage groups doubles. Four alleles may be present in the homologous set of chromosomes instead of the normal pair. Mutations of some of the alleles may be maintained and propagated more easily in the tetraploid, where the mutant alleles are balanced by more normal alleles. A large number of variant alleles could provide variation and plasticity to a polyploid species, thus enabling the species to exploit a larger range of environmental conditions than would be possible in the potentially less variable diploid species. The diploids may be more successful in more stable regions or when conditions are stable for a long period of time.

If the polyploid species were able to maintain its integrity for a long enough time, rearrangements of genetic material coupled with the dissociation of meiotic multivalents could bring about the diploidization of the polyploid, resulting in a diploid species possessing twice as many chromosomes as its closest relatives. This might explain the absence of complete homology in the mitotic chromosome set and of multivalents in the two tetraploid *Pleurodema* species. Indeed, it may be argued successfully that these two species are, in reality, true diploid species and that the tetraploids which have diploid counterparts are more recently derived forms.

Initially, a polyploid species must arise sympatrically from a diploid ancestral population. In order to facilitate the continuance and subsequent spread of the tetraploid population, some mechanism would be required so that the diploid and tetraploid species would not interbreed. For many years anuran mating calls have been recognized as important isolating mechanisms (BOGERT, 1960). FRISHKOPF and GOLDSTEIN (1963) demonstrated that the auditory nerve in the bullfrog is tuned very selectively to hear only certain parameters of its particular call. The derived calls of the tetraploid *O. americanus* and *H. versicolor* have both independently reduced the pulse rate from those of their diploid ancestors. It is tempting to speculate that the reduction in pulse rate is related to polyploidy. Assuming selective tuning, the call emitted by a tetraploid male may be heard only by other tetraploid individuals of the same species. Thus, in one generation, there possibly could be formed a bisexual, reproductively isolated, polyploid species complete with a premating isolating mechanism living in sympatry with its diploid counterpart.

Considering the inadequate study of many anuran species, it is not surprising that more diploid-polyploid species pairs have not been encountered. In 1960, BLAIR documented the existence of fast- and slow-calling "races" of *Hyla eximia* in Mexico. It is very possible that these "races" represent yet another diploid-polyploid cryptic species pair.

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