Evolution of Globin Expression in the Genus
Xenopus (Anura: Pipidae)

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Comparison of electrophoretic globin phenotypes of 18 different Xenopus taxa reveals four different basic types of banding patterns. Each type includes species that also are similar in their morphological, cytogenetical, and biochemical characteristics. Three of these patterns reflect distinct evolutionary lines, while the fourth may be interpreted as the intersection of two of these lines. The composition of the basic pattern of the highly polyploid species is consistent with an allopolyploid origin of most of these species. The number of distinct globin polypeptides—four in the only extant diploid species, X. tropicalis, and five or more in most of the tetraploid species, including X. laevis—suggests that primordial globin genes had undergone duplication either before or after the tetraploidization event. Finally, the individual globin phenotypes are excellent molecular markers that are of great help in identifying the various species but not the subspecies.

Introduction

Genome duplications have been major diversification events in the evolution of the genus Xenopus. Karyological studies have shown that the oldest extant species of the genus, X. tropicalis, has conserved a diploid chromosome number (2n) of 20 (Tymowska 1973), whereas all other species possess diploid chromosome numbers that represent either four, eight, or 12 haploid sets of nine or 10 (Tymowska and Fischberg 1982). Because the DNA content also increases with the increase in the chromosome number (Thiebaud and Fischberg 1977), it is safe to assume that these species developed by polyploidization.

Comparative studies of albumins and vitellogenins and of their coding genes in X. tropicalis and X. laevis (2n = 36) provided serious indications of a tetraploid origin of X. laevis, dating back to some 30 Myr ago (Bisbee et al. 1977; Wahli et al. 1979; Westley et al. 1981; Jaggi et al. 1982; Westley and Weber 1982). Hentschel et al. (1979) reported the existence of six electrophoretically distinct adult globin polypeptides in X. laevis. Subsequently, it was shown that these globins are encoded by three α-like and at least two β-like globin genes (Kay et al. 1980; Patient et al. 1982; Hosbach et al. 1983). Unlike the organization of mammalian globin genes, four of these are localized in two gene clusters, each composed of one α- and one β-like sequence (Jeffreys et al. 1980). A similar linkage arrangement of single adult α- and β-globin genes in X. tropicalis represents one of the most convincing genetic indications of a tetraploid origin for X. laevis (Jeffreys et al. 1980).

1. Key words: globin expression, Xenopus, polyploidization, allopolyploidy, evolution.

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The genome duplications that gave rise to the tetraploid species were followed by further polyploidization steps. The more recently duplicated genes of the resulting highly polyploid *Xenopus* species are expected to have diverged much less than those of *X. laevis*. We suppose, therefore, that electrophoretic globin patterns of these species should include two sets of similar globin polypeptides if they have had an autopolyploid origin. On the other hand, allopolyploid species may reveal patterns that are very similar to those of their ancestors. The more these ancestors had diverged prior to hybridization, the greater the chance would be to recognize them in the patterns of an allopolyploid species.

We have compared the electrophoretic globin phenotypes of 18 species and subspecies of the genus *Xenopus*. The results presented here show that globins are excellent molecular markers. They allow a more detailed analysis of the relationship of these taxa than do the intact hemoglobins (Bürki et al. 1984). The different globin patterns observed are variations of four basic types, and each of these is common to closely related species. The composition of the two basic patterns of the highly polyploid species indicates an allopolyploid origin for most of them.

**Material and Methods**

**Animals**

The origin of the 18 *Xenopus* species and subspecies, their chromosome numbers, and the number of animals analyzed are given in table 1.

**Globin Preparation**

Blood was drawn into 3.2% sodium citrate from the femoral vein of adult animals by a capillary pipette. Erythrocytes were washed three times and lysed by repeated freezing and thawing in at least six volumes of 50% cold amphibian Ringer solution. Partial purification and determination of the concentration of hemoglobin were done as described by Moss and Ingram (1968). The hemoglobin solutions were then diluted to 10 mg/ml with distilled water. The hemoglobin was dissociated by the addition of nine volumes of 7 M urea/5% acetic acid and stored at -70 °C.

**Electrophoresis**

Globins were compared by electrophoresis on slab gels containing 15% acrylamide, 6 M urea, 6 mM Triton-X 100, and 5% acetic acid. Gels were pre-electrophoresed and scavenged as described by Zweidler (1978). Samples containing 15 μg of globin were layered directly on the gel surface, and electrophoresis was performed at room temperature for 16 h with 4 mA/cm gel surface using 5% acetic acid as running electrolyte. Gels were fixed for 1 h in 0.7 M trichloracetic acid/0.185 M sulfosalicylic acid, stained for 30 min at 60 °C in 0.1% Coomassie Blue G-250 (Serva) in 25% ethanol/8% acetic acid, and destained in the solvent.

**Results**

The globins of 18 species and subspecies of the genus *Xenopus*, resolved on polyacrylamide gels, are illustrated in figure 1. The banding patterns of all species and subspecies are composed of multiple globin bands, ranging from four (T) to 11 (W). In comparing individual patterns, we have divided the pherograms into three parts (A–C). The boundary between A and B was chosen to separate α- and β-globins of *X. laevis* (see Patient et al. 1982). Part B separates slow- and fast-
Table 1
Origin, Diploid Chromosome Number (2n), and Sample Size (n) of Each of the Analyzed Species and Subspecies of the genus *Xenopus*

<table>
<thead>
<tr>
<th>Species and Subspecies</th>
<th>Origin (n)</th>
<th>2n</th>
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<tbody>
<tr>
<td>X. tropicalis (Gray)</td>
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<tr>
<td>X. epitropicalis Fischberg et al. (1982)</td>
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<tr>
<td>X. species nova VIb</td>
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<td>X. muelleri (Peters)</td>
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<tr>
<td>X. borealis Parker</td>
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<td></td>
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<tr>
<td>X. clivii Peracca</td>
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<tr>
<td>X. fraseri Bouleneger</td>
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<td>X. species nova III</td>
<td></td>
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<tr>
<td>X. laevis</td>
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<tr>
<td>X. l. laevis (Daudin)</td>
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<tr>
<td>X. l. subspecies nova I b</td>
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<tr>
<td>X. l. sudanensis Perret</td>
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<td>X. l. peteri Bocage</td>
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<tr>
<td>X. l. victorianus Ahl</td>
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<tr>
<td>X. l. bunyoniensis Loveridge</td>
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<td></td>
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<tr>
<td>X. vestitus Laurent</td>
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<tr>
<td>X. wittei Tinsley et al. (1977)</td>
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<tr>
<td>X. amieti Kobel et al. (1980)</td>
<td></td>
<td></td>
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<tr>
<td>X. ruwenzoriensis Fischberg and Kobel (1978)</td>
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</tbody>
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|                  |            |    |
| Adiopodoumé, Ivory Coast (4) | 20*     |
| Sierra Leone (2)           | 20*     |
| Kinshasa, Zaire (3)        | 40*     |
| Ghana (3)                  | 36*     |
| Ifakara, Malawi (3)        | 36*     |
| Marsabit, Kenya (3)        | 36*     |
| Samburu Range, Kenya (1)   | 36*     |
| Kiambu, Kenya (1)          | 36*     |
| Nairobi, Kenya (3)         | 36*     |
| Ethiopia (3)               | 36*     |
| Yaoundé, Cameroon (3)      | 36*     |
| Foulassi, Cameroon (2)     | 36*     |
| Ethiopia (3)               | 36*     |
| Fish Hoek, Cape, South Africa (4) | 36* |
| Malawi (3)                 | 36*     |
| Galim, Cameroon (2)        | 36*     |
| Zimbabwe (2)               | 36*     |
| Mbuye, Rwanda (2)          | 36*     |
| Lake Luhonda, Rwanda (2)   | 36*     |
| Lake Mutanda, Uganda (4)   | 72*     |
| Lake Bunyoni, Uganda (4)   | 72*     |
| Galim, Cameroon (4)        | 72*     |
| Semiliki Valley, Uganda (4) | 108*    |


b Recognized as new taxa by M.F.

* Determined by J.T. and M.F., unpublished.

migrating bands of *X. tropicalis*, while the upper boundary of C was chosen to exclude minor bands that are assumed to represent nonglobin erythrocyte proteins.

*Xenopus tropicalis* (T; 2n = 20) and *X. epitropicalis* (E; 2n = 40) have similar globin patterns, with one major and one minor band in each of parts A and C. In one individual of *X. tropicalis* we observed two weak C bands (not shown) that may represent allelic products.

Both *X. muelleri* (M; 2n = 36) and *X. species nova VI* (SPN VI; 2n = 36) have one major and two minor A and B bands. However, the mobilities of the major and most of the minor globins are different between the two taxa. In contrast to *X. tropicalis*, these two species lack C bands.

*Xenopus borealis* (2n = 36) from Marsabit and Samburu Range (B[M]) populations have one major and two minor A bands (the most cathodal minor band lies so near the major band that it is not visible in fig. 1) and one major and one minor B band. All individuals from Nairobi and Kiambu (B[N]) differ from B[M]) by an additional minor B band. The presence of this additional band is always accompanied by a weakening of the anodal minor A band. These two bands may, therefore, represent allelic variants. The major A band of *X. borealis* is identical to that of *X. muelleri*, while the major B band comigrates with that of *X. sp. n. VI*. All *X. borealis* also lack C bands.
**Fig. 1.**—Electrophoretic globin patterns of 18 *Xenopus* species and subspecies: *X. tropicalis* (T); *X. epitropicalis* (E); *X. sp. n. VI* (SPN VI); *X. muelleri* (M); *X. borealis*, Marsabit and Samburu Range populations (B[M]) and Kiambu and Nairobi populations (B[N]); *X. clivii* (C); *X. fraseri* (F); *Xenopus sp. n. III* (SPN III); *X. l. laevis* (LL); *X. l. subsp. n. I* (LSSPN I); *X. l. sudanensis* (LS); *X. l. petersi* (LP); *X. l. victorianus* (LV); *X. l. bunyoniensis* (LB); *X. vestitus* (V); *X. wittet* (W); *X. amieti* (A); and *X. ruwenziensis* (R).

*Xenopus clivii* (C; 2n = 36) has one major and two minor bands in each of the regions A and B. Both major bands are different in mobility from those of the species mentioned above, but, *X. clivii*, *X. muelleri*, *X. sp. n. VI*, and *X. borealis* have the absence of major C bands in common.

*Xenopus fraseri* (F; 2n = 36) has two major and one minor A bands and two major C bands. The anodal major C band is probably composed of a major and a comigrating minor band. The patterns of *X. fraseri* and *X. tropicalis* are very similar, since both lack B bands. However, migration of all A and C bands differs slightly in the two species.

*Xenopus species nova III* (SPN III; 2n = 36) shows two major and two minor A and B bands. The cathodal major A band comigrates with one of those of *X. fraseri*, while the three anodal A bands are similar to the A bands of *X. clivii*. *Xenopus sp. n. III* further resembles *X. clivii* in the comigration of one of their B bands and in the lack of major globins in part C.

All the subspecies of *X laevis* (LL, LSSPN I, LS, LP, LV, LB; all 2n = 36) have one major and two minor A and B bands. The patterns of all six subspecies are identical, except for the cathodal minor A band, which in *X. laevis laevis* (LL) and in *X. l. subspesies nova I* (LSSPN I) is slightly different from that of the other subspecies. The two major globin bands of the *X. laevis* subspecies have slightly different mobilities from the major bands of the species mentioned above.

Except for *X. fraseri*, all species and subspecies with 2n = 36 are characterized by the absence of major C bands.
Xenopus vestitus (V; 2n = 72) shows two major and two minor A bands, one weak B band, and a major and a minor C band. Some of the A bands comigrate with those of the X. laevis subspecies, while the pattern as a whole, except for the B band, resembles that of X. fraseri.

Xenopus wittei (W; 2n = 72) has the most complex pattern of the whole genus. It is composed of two major and three minor A bands, one major and one minor B band and two major and two minor C bands. The two major C bands were observed only in the pattern illustrated in figure 1. All other individuals presented only the anodal major component and were most probably homozygous for this polypeptide. In both its A bands and the presence of C bands, the pattern of X. wittei resembles that of X. vestitus and X. fraseri, while one B band comigrates with the major B band of X. muelleri (M).

Xenopus amieti (A; 2n = 72) shows two major and two minor A and C bands. This pattern resembles that of X. fraseri in the lack of B bands.

Xenopus ruwenzoriensis (R; 2n = 108) has one major and four minor A bands and five minor C bands. Most of these bands comigrate with those of either X. fraseri or X. amieti in such a way that the pattern of X. ruwenzoriensis appears to be a superposition of the X. fraseri and X. amieti patterns (fig. 2).

The globin patterns of the 18 species and subspecies of the genus Xenopus are variations of four basic patterns. Pattern I, which is common to X. tropicalis and X. epitropicalis, is composed of globin polypeptides with low (A) and high (C)

![Diagram](image)

Fig. 2.—Comparison of the globin pattern of Xenopus ruwenzoriensis (R) with patterns of mixed globins (A + R) from X. amieti (A) and X. ruwenzoriensis (R) and of mixed globins (A + F) from X. amieti (A) and X. fraseri (F). Fifteen µg of globin was separated in each lane; and mixed samples contained equal amounts of the species globins.
detergent affinities. Pattern II is common to *X. fraseri*, *X. amieti*, and *X. ruwenzoriensis*. It has the same composition as pattern I, but the major C band of pattern I migrates more slowly than those of pattern II when the detergent concentration in the gel is reduced (not shown). Pattern III is composed of globin chains with low (A) and medium (B) detergent affinities. It is shared by all species and subspecies with 2n = 36 except *X. fraseri*. Pattern IV includes all three types of globin polypeptides (A, B, and C). It is common to *X. vestitus* and *X. wittei*.

**Discussion**

The present results demonstrate that each *Xenopus* species can be identified by its globin pattern. In a parallel study we have compared the electrophoretic hemoglobin (Hb) patterns in the genus *Xenopus* (Bürki et al. 1984). The diversity of the globins corresponds to the observed differences in the Hb's of the different *Xenopus* species. Yet, the electrophoretic resolution of globin polypeptides, according to their different affinities to the nonionic detergent Triton-X 100 (Zweidler and Cohen 1972), permits a more detailed analysis of the relationship between the different *Xenopus* taxa. Our results show that four basic globin patterns can be recognized in the genus *Xenopus*.

Pattern I is shared by the diploid species *X. tropicalis* and the probably allotetraploid *X. epitropicalis*. Cytogenetic similarities indicate that these two species are more closely related to each other than to any of the other species in this study (Tymowska and Fischberg 1982). The occurrence of the same number of globin bands in both species suggests that diploidization of the globin genes in *X. epitropicalis* has been achieved and that in both species four globin genes are expressed. Jeffreys et al. (1980) reported the existence of single α- and β-globin genes in *X. tropicalis*, but they admitted that highly diverged additional genes may exist.

Pattern II is expressed in the tetraploid *X. fraseri*, the octoploid *X. amieti*, and the dodecaploid *X. ruwenzoriensis*. These three species are morphologically similar (Kobel et al. 1980). To our surprise we have found no similarities in their Hb patterns (Bürki et al. 1984), and differences can also be observed between their globin patterns. The lack of globin B bands in these three species, however, indicates that they are more closely related to each other than to any of the other species in this study. The resemblance of patterns I and II, although it might be partially coincidental, suggests that *X. fraseri* arose by tetraploidization of *X. tropicalis*-like ancestors some 30 Myr ago (Bisbee et al. 1977). However, it is interesting to note that *X. fraseri* and *X. tropicalis* differ considerably in their morphological characters. According to the supposition that polyploid *Xenopus* species arose by allopolyploidy (see Tymowska and Fischberg 1982), we suggest that *X. amieti* emerged by hybridization of *X. fraseri*-like ancestors. Much more recently, hybridization of tetraploid and octoploid ancestors might have given rise to the dodecaploid *X. ruwenzoriensis*. The globin pattern of *X. ruwenzoriensis* is, in fact, almost identical to an artificial mixture of both *X. fraseri* and *X. amieti* globins (fig. 2).

Pattern III is common to all species and subspecies with 2n = 36 except *X. fraseri*. A close relationship between individual taxa of this group can be recognized for *X. muelleri* and *X. borealis*. two species closely related with respect to their biochemical, cytological, and morphological characters (see Bürki et al. 1984). *Xenopus sp. n.* VI is a hitherto unknown taxon (M.F., unpublished results). The three individuals analyzed clearly differ in most of their globins from the other
tetraploid species. Partial similarities can be detected between \textit{X. clivii} and \textit{Xenopus sp. n.} III. This finding is in contrast to the differences observed in their Hb (Bürki et al. 1984) and sperm-specific histone patterns (Mann et al. 1982). Yet, in this case the differences in the Hb patterns certainly hide some similarities in the corresponding globins. The most evident similarities among taxa sharing pattern III can be seen in the almost identical globin phenotypes of the different subspecies of \textit{X. laevis}, confining their close relationship (Müller 1977). The comparison of the basic globin patterns II and III reveals that although \textit{X. fraseri} and the species with pattern III share the same chromosome number, they probably belong to at least two distinct evolutionary lines, i.e., the genome duplication that gave rise to the tetraploid species with 36 chromosomes seems to have occurred more than once during speciation in the genus \textit{Xenopus}.

The six \textit{A} and \textit{B} bands of \textit{X. l. laevis} have recently been identified as three \(\alpha\)-and three \(\beta\)-globin chains, respectively (Patient et al. 1982). Four of these globins are encoded by two unlinked \(\alpha\)-\(\beta\)-gene clusters (Jeffreys et al. 1980), with the two remaining \(\alpha\)- and \(\beta\)-genes of unknown linkage (Patient et al. 1982). Additional genes could have arisen by duplication of a primordial locus in some of the diploid \textit{Xenopus} ancestors. This mechanism could also account for the existence of four globin chains in \textit{X. tropicalis}. It is unlikely that this gene duplication occurred after the tetraploidization events that gave rise to species with 36 chromosomes, since they all have more than four globin bands. This would require either a monophyletic origin of these species or several independent gene duplications.

\textit{Pattern IV} is shared by the two octoploid species \textit{X. vestitus} and \textit{X. wittei}, which are closely related cytogenetically (Kobel and Müller 1977). Their similar globin patterns confirm this close relationship and suggest that both species originated by hybridization of an ancestor of the species with pattern III (presence of \textit{B} bands) and ancestors of the \textit{X. fraseri} line (presence of \textit{C} bands; \textit{A} bands resemble those of \textit{X. fraseri} and \textit{X. amieti}). \textit{Xenopus vestitus} has a relatively small number of globin bands compared to \textit{X. wittei}. This observation suggests that more globin genes have been silenced in \textit{X. vestitus} than in \textit{X. wittei}.

Our study shows that the elucidation of the evolution of globin expression in the species of \textit{Xenopus} may contribute to a better understanding of the evolution of this genus. For this reason it would be interesting to characterize and compare different globin polypeptides of species such as \textit{X. tropicalis} and \textit{X. fraseri}, the ancestors of which seem to have occupied key positions in the evolution of this genus.

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\textbf{LITERATURE CITED}


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