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Sequences of Cμ and the V$_H$1 Family in LG7, a Clonable Strain of *Xenopus*, Homozygous for the Immunoglobulin Loci

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Twenty-eight heavy-chain variable (V$_H$1) region genes and the immunoglobulin (IgM) heavy-chain constant region of an isogenic *Xenopus* hybrid, *X. laevis*/*X. gilli*, LG7, have been sequenced. The LG7 clone represents the first *Xenopus* hybrid that is homozygous for the IgH locus. The V$_H$1 family was specifically investigated because V$_H$1 genes are used by the antibodies produced during the *Xenopus* antidinitrophenol (DNP) response, These V$_H$1 germ-line sequences establish a so-called "dictionary" that is available for studying somatic hypermutational mechanisms in immunized frogs.

KEYWORDS: Isogenic *Xenopus*, immunoglobulin, genes.

INTRODUCTION

The gynogenetic development of diploid eggs produced by *X. laevis*/*X. gilli* hybrids (LG) leads to the production of clones of heterozygous isogenic females (Kobel and Du Pasquier, 1975). The development of the different hybrids and the ease with which large numbers of them can be produced has been a major advantage in the studies of various aspects of the *Xenopus* immune system, particularly antibody diversity (Wabl and Du Pasquier, 1976; Du Pasquier and Wabl, 1978). As useful as they are, these clonable animals are still heterozygous and therefore genetically complex. It would considerably simplify immunogenetic studies if some LG hybrid clones could be made homozygous at the major histocompatibility complex (MHC) or immunoglobulin (Ig) loci. We report here such a clone, LG7, that is homozygous at the Ig heavy-chain (H) locus. In addition, we report most of its V$_H$1 germ-line sequences as well as the sequence of its IgM (μ) heavy-chain gene. This V$_H$ family is involved in the antidinitrophenol (DNP) response, one of the best studied *Xenopus* antibody responses (Brandt et al., 1980; Schwager et al., 1988). Thus, these germ-line sequences establish a "dictionary" necessary for future studies on somatic generation of the antibody repertoire in this species.

RESULTS

LG7 Is Homozygous for the Ig Loci

LG7 frogs first attracted our interest because they yielded fewer restriction fragments than other LG hybrids (Schwager et al., 1988). Whether this was due to homozygosity or to a Ig H chromosome loss was not known. To determine the cause of LG7's unique restriction fragment length polymorphism (RFLP) pattern, we decided to first use a variety of techniques. The previous RFLP analyses were confirmed by testing different digests of LG genomic DNAs with probes from all 11 V$_H$ families, light-chain probes for Vρ and Cρ (kindly provided by our colleague J. Schwager; Schwager et al., 1991) and Cμ probes. The individual patterns could be grouped into four RFLP classes, identical for each probe (LG3,15; LG17,5,46; LG14,6; and LG7). LG7 consistently gave half as many restriction fragments as the other LG7 hybrids and Fig. 1 shows examples of Southern blot hybridized with

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TABLE 1
Absorbance at 405 nm[a]

Mab specificity	Outbred *laevis*	LG15	LG7
14G1 anti-*laevis* IgM			
undiluted	0.38	0.13	0
1/5	0.46	0.17	0
1/50	0.29	0.09	0
10A9 anti-*Xenopus* IgM			
undilted	0.65	0.35	0.33
1/5	0.86	0.31	0.27
1/50	0.80	0.33	0.32
409B8 anti-*Xenopus* IgL			
undiluted	0.11	0.14	0.10
1/5	0.13	0.15	0.14
1/50	0.14	0.15	0.13
11D5 anti-*Xenopus* IgY			
undiluted	0.10	0.15	0.12
1/5	0.01	0.03	0.01
1/50	0.04	0.04	0.02

[a] All assays were performed in duplicate and included enzyme-linked antibody controls and substrate controls. Values are results from one data set after 2 hr incubation. No color development occurred in the LG7 wells even after 18 hr; abosrbance ≤0.08.

various V_H probes. Moreover, every band in LG7 that hybridized to a V_H probe could also be found in LG15; thus, LG15 seems to contain an LG7-like haplotype.

To determine whether there are differences in expressed Ig haplotypes, we analyzed the serum from LG7, LG15, and outbred frogs in enzyme-linked immunosorbent assays (ELISA) with different anti-*Xenopus* Ig monoclonal antibodies (Mab). LG7 was always negative with Mab 14G1, a monoclonal that detects specifically *laevis* μ Ig (Du Pasquier et al., 1985), indicating that only *gilli* μ-type Ig is produced. All three frogs routinely tested positive with Mabs for *Xenopus* IgY (Mab 11D5), *Xenopus* light chain (Mab 409B8), and Lg μ (Mab 10A9; Table 1). Additional evidence for LG7 possessing only the *gilli* Ig haplotype was also found by screening LG7 genomic libraries with a Cμ probe. Only *gilli* μ constant

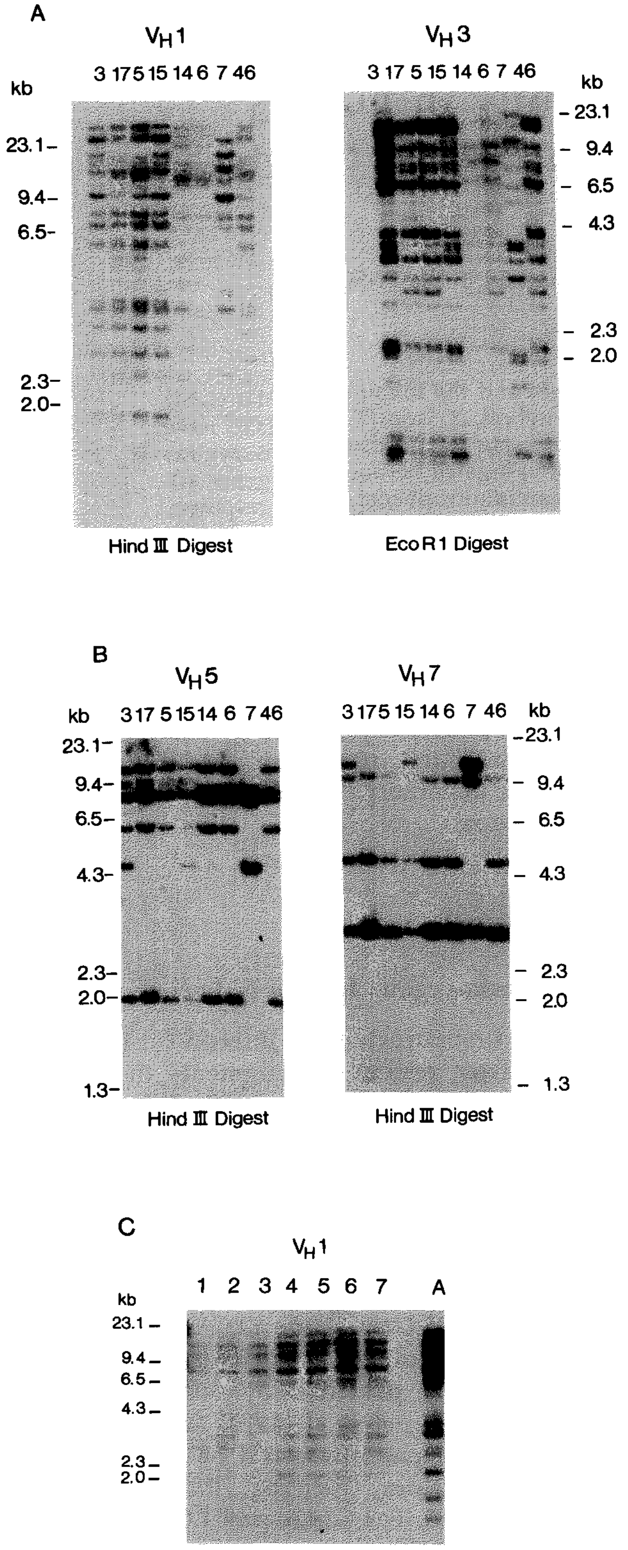

FIGURE 1. Genomic Southern blot analyses of erythrocyte DNA from LG frogs. DNA was digested to completion with either Hind III or EcoRI and electrophoresed on 0.7% agarose gels and Southern blotted. The filters were hybridized with probes for (A) V_HI, V_HIII, (B) V_HV, V_HVII, and washed under stringent conditions. Numbers correspond to LG nomenclature (Kobel and Du Pasquier, 1977). (C) Southern blot analysis of LG7 small-egg progeny. Genomic DNA from seven individual LG7 small-egg tadpoles (numbered 1–7) and an adult LG7 (labeled A) were digested with EcoR1 and hybridized with a V_H probe. Markers (in kb) are indicated in the margins.

regions could be sequenced from two different LG7 genomic libraries. Even though *laevis* and *gilli* $C\mu$ cross-hybridize and share long stretches of sequence identity, the introns are of different sizes, with characteristic repeats and thus are easy to identify (Du Pasquier, unpublished).

Although the foregoing data did not exclude loss of a chromosome or deletion of one of the Ig loci, additional experiments do so. Chromosome spreads of phytohemagglutinin-stimulated LG7 spleen cells contained 36 chromosomes, the number expected of LG hybrids. Moreover, when Southern blots were titrated with $C\mu$ and $C\rho$ probes, the signal intensity of LG7 genomic DNA was equal to that of LG15 and LG14, that is, LG7 contains the same number of copies of $C\mu$ and $C\rho$ as LG15 and LG14 (data not shown).

LG hybrids produce two types of eggs, small eggs that can be either normal haploids or aneuploids, and large genetically identical diploid eggs that are used to propagate the species. If LG7 was heterozygous, segregation of IgH (or any other gene family) polymorphism should be detected in progeny. However, the segregation cannot be followed in the large (2n) eggs because they are always identical. Instead, the small eggs (1n) that contain a segregated set of chromosomes must be analyzed. We compared in Southern blots digests of genomic haploid LG7 embryo DNA with adult LG7 DNA. The RFLP pattern of every haploid embryo tested was identical with the adult parent LG7 pattern when tested with different V_H probes and with a $C\mu$ probe (Fig. 1C). Thus, LG7 is homozygous at the IgH locus. This and the fact that the LG7 pattern of Ig H-chain genes is identical to half of the pattern of LG15 Ig genes suggests that LG7 is homozygous for at least the *gilli* Ig H-chain gene set present in LG15.

LG7 Is Derived from the Gynogenetic Development of an LG15 Small Egg

Small eggs rarely develop beyond hatching unless the second polar body is not extruded so that diploidy is restored and a viable embryo is created. This is a rare occurrence. When small egg embryos do survive, their genetic loci will still be heterozygous if there is a crossover between a locus and the centromere. Thus, heterozygosity is a function of gene position; heterozygosity increases with increasing distance from the centromere (Nace et al., 1970; Kobel and Du Pasquier, 1977).

We believe that the LG7 hybrid developed from an accidentally selected viable diploid LG15 small egg (see Fig. 2). Because all LG hybrids are tested by skin graft as a routine control, this

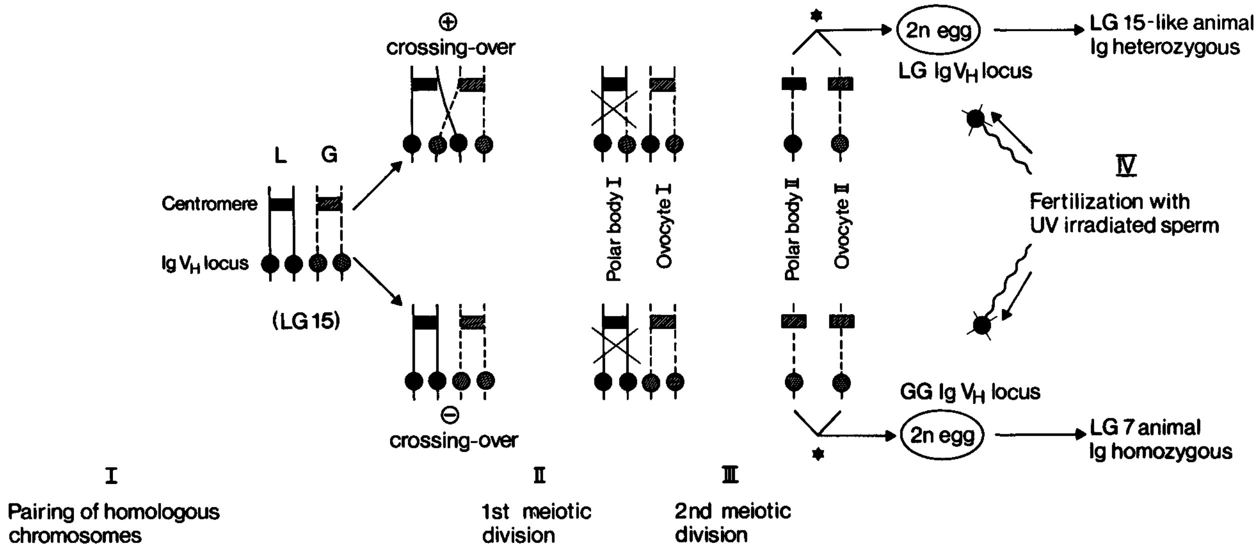

FIGURE 2. Scheme depicting LG7 origins. Pairing of *laevis* (solid lines) and *gilli* (hatched lines) Ig H-chain containing chromosomes in meiosis. Upper pathway generates an LG15-like animal; the lower pathway generates the homozygous LG7 animal. The asterisks (*) represent the point where the prevention of the second polar body extrusion occurs and a viable diploid egg is produced.

animal was easily isolated. Later, it was found to produce both big and small eggs, thus making it clonable.

Genomic V_H1 Gene Sequences

Three genomic libraries representing altogether 1.7×10^6 recombinants phage (three to four genome equivalents) were made from LG7 red cell genomic DNA and screened unamplified with a *Xenopus* family V_H1 cDNA probe at high stringency. A total of twenty eight different V_H1 elements were isolated and sequenced, and the alignments of their encoded amino acids are shown in Fig. 3. All exhibit a typical V_H gene structure (Kabat et al., 1991) and all contain specific features characteristic of *Xenopus* V_H1 genes. The complementarity-determining regions (CDRs) show limited variability and many different genes contain identical CDR1s and very similar if not identical CDR2s. The CDR2s exhibit the most sequence divergence which is restricted to their amino-terminal (NH_2-) portions (Schwager et al., 1988, 1989). Six sets of the genomic V_H1s contain identical CDR1s and three sets contain identical CDR2s (Table 2). The CDR1s are fifteen nucleotides in length and can differ by as much as 60%. Only the nucleotides at positions 100–103, encoding MET 34, never vary. Genomic g44A was the only gene that contained a CDR1 identical to one published previously from a *Xenopus laevis* genome (p LL1.4; Schwager et al., 1989). The largest sequence difference found in the V_H1 CDR1s is a change of nine nucleotides (g46C vs. g345A). LG7 V_H1 CDR2s vary in length from 48–54 nucleotides (16–18 amino acids) and can differ by as much as 43% (g2A vs. g345A). Overall nucleotide sequence identities range from 82–96%, and as expected, the framework (FR) regions are the most highly conserved. The V_H1 nucleotide sequences beginning with the initiation codon (ATG) of the split leader and

TABLE 2
V_H1 Genomics That Share CDRs

CDR1s	CDR2s
g7C, g15	g7B, g7C
g4A, g10A	g342A, g342B
g35, g2A, g13	g352, g2B
g343, g10B	
g342A, g342B	
g22, g349B	

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                                           CDR1                    CDR2
                                          |   |              |                 |
            ----+----1----+----2----+----3----+----4----+----5----+----6----+----7----+----8----+----9----+----0---
LG7G345A    DVQLAQSEPVVIKPGGSHKLSCTASGFTFSSTWMHWVRQASGKGLQWLCQI-HPDGSSNTYYADSVKGRFTISRDNNNSK*YL*MNNLQTEDTAVYYCA----  M97004
LG7G7B      ''''D'''''''''''''''''''''''''T'''S''''SP'N''''VSR'-Y--DA''IN''''''''''V'K''''N'L''Q''''''''''''''TT---  M94853
LG7G7C      '''''''D''''''''''''''''''''''T'''Y'''''P'N''''VSR'-Y--DA''IN''''''''''V'K''''N'L''Q''''''''P''''''T---  M97007
LG7G15      '''''''D''''''''''''''''''''''T'''Y'''''P'N''''VSR'-Y--DA''IN''''''''''V'K''''N'L''Q''''''''P''''''T---  M96997
LG7G4A      ''''''''S'''''''''''''''''''''''''Y'''''P''''''VSI'-TS''''-'NS''''''''''''''''K'LF'Q''''''''A'''''TT---  M97006
LG7G710A    ''''''''S'''''''''''''''''''''''''Y'''''P''''''''H'-''''''-A''''''''''''''''K'N'L''Q''''''''''M''''R---  M94839
LG7G349A    ''''''''S''''''''''''''''''''''N''N'''''P''''''VSY'-SN'''G-'''PS'F''''''''''''N'L''Q''''''''''''''SSVTQ  M94846
LG7G341     ''''V'''''''''''''''''''''''''''''N'''''P''''''VSR'-SSGSG'''''''''''''''''''''N'L''Q'''''''''''''''TK--  M94843
LG7G13      '''''''''''''''''''''''''''''''Y''D'''''P''''''VSA'-IG''''-'''''''''''''''''''N'L''Q'''''''''''''''REG-  M96996
LG7G2A      ''''''''S''''''''''''''''''''''Y''D'''''P''''''VSY'SY'N'TT-'H'''''''''''''''''N'L''Q'''''''''''''''R---  M97000
LG7G35      '''''''''''''''''''''''''''''''Y''D'''''P''''''VSD'-NY''A-E'''''''''''''''''''N'L''Q'''''''''''''''RD--  M94848
LG7G346B    ''''D'''S''''''''''''''''''''''YG'S'''''P''''''VSY'-SSG'GT-I''''''''''''''''''N'LF'Q'''''''''''''''R---  M94845
LG7G10B     ''''D''''''''''''''''''''''''''YG'G'''''P''''''VSA'-SS''G'-'''''''''''''''''''N'L''Q''''''''''''''TR---  M94840
LG7G343     ''''D'''S''''''''''''''''''''''YG'G'''''P''''''VSA'-SSN'G'-'V'''''''''''''''''N'L''Q'''''''E'''''''R---  M94844
LG7G4B      ''''D'''S''''''''''''''''''''''YS'''I'''P'R''''VSA'-SNG'T--'''''''''''''''''''N'L''Q'''''''''G'''''RER-  M94851
LG7G44A     E'''''''S'''''''''''''''''''''DH''S'''''P''''''VSN'-KY''A-E'N'''''''''''''''''N'L''Q'''''''''''''''R---  M94850
LG7G5       ''''''''''''''''''''''''''''''DY''N'I'''P''''''VSA'-SNG''--'''''''''E'---------------------------------  M94852
LG7G349C    ''''D'''S''''L''''''''''''''''DY''S'I'''P'''''RVFY'-RH''GT-'N''''''''''''''SK'N'L''Q''''H'''''''''VR---  M97005
LG7G17B     ''''D''''''''''''''''''V''''''DH''D'''''P'''''''GE'-Y'N'GTTK'-''''''''''''''''N'L''Q'T'''''H'''''''S---  M96998
LG7G342B    ''''''''''''''''''''''''''''''DN''N'''''P''''''VSV'-SSS'GT-I''T'''''''''''''''N'L''Q''''''''''M''''G---  M97002
LG7G342A    ''''''''''''''''''''''''''''''DN''N'''''P''''''VSV'-SSS'GT-I''T'''''''''''''''N'L''Q''''''''''M''''G---  M97003
LG7G22      ''''D''''''''''''''''''''''''TDY''''''''P''''''''H'-'SG'T--'''''''''''''''''S'N'L''Q'''''''''G'''''RERE  M96999
LG7G46C     'L'''''V''''''''''''''''''''''DYY'N'F'''P''''''''''-Y''''T-''''''''''''F''''''N'F''Q'''''''''''''''T---  M97199
LG7G2B      ''''''''S''''''''''''''''''''IGH''''''''P''R''''SD'-NY'''TTR'-''''''''''''''''N'L''QI'''''''''''''TRY--  M94842
LG7G21      'I''D'''''''''''''''''''''''''GYY'''I'''P''''''VSR'-SS''G'-'V'''''''''''''''''N'L''Q''''''''A''''''RD--  M94841
LG7G352     'M''D'''S'''''''''''''''''''''DY''S'''''P'''''''SD'-NY'''TTR'-''''''''''''''''N'L''Q''''''''M'''''VRY--  M94849
LG7G349B    '''''''''''''L''''''''''''''''DY''''''''P'''''''GE'-''N'GT-'N'''''''''''''''''N'L''E'T'''''''''''''T---  M94847
LG7G331     ''''D'''S'''NQ''''''''''''''''GYY'N'''''P''''''VYY'-KG''''-'''''''''''''''''''N'L''Q'''''''''''''''RE--  M97001
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FIGURE 3. Alignments of encoded animo acids of V_H1 genomic genes (in one letter code) beginning with residue 1. The V_H1 genes in bold type were found expressed in a cDNA library made from immunized LG7 frogs. Sequence identities are indicated by (') and gaps (-) are introduced to maximize homology. The asterisks (*) indicate stop codons. The CDR boundaries are marked and are according to Kabat et al. (1991). Genes g345A and g342A are pseudogenes and gene g5 is truncated due to only being partially sequenced. Master sequence was chosen for length. GenBank accession numbers are at the right margin.

continuing to the conserved nonamer of the recombination signal sequence (RSS) are aligned in Fig. 4. The conserved octamer ATGCAAAT and sequences analogous to a TATA box that are found 5' of all V_H genes (Parslow et al., 1984) and act as the Ig H-chain promoter (Wirth et al., 1987) were identified in twenty five of the V_H1 genes. Three of the genes, g349A, g349C, and g343, were cloned using a restriction site just 3' of their leader sequences and so their promoters were not identified. Genes g349A and g343 are both viable V_H genes because they are found expressed in an LG7 cDNA library (Wilson et al., 1992). Very little sequencing was performed 5' of the leader sequences and consequently no second possible promoter regions, as have been previously identified in V_H1 genes (Schwager et al., 1989), were found. The LG7 V_H1 leader regions range from sixteen to nineteen amino acids in length and several genes (e.g., g7B, g7C, g2A, g35) contain almost identical leader sequences. Gene g44A has the longest leader intervening intron of 108 nucleotides (Fig. 4A). The V_H1 genes varied in overall length from 98–101 amino acids. The heptamers of the RSS are identical in all but one of the V_H1 genes. Two nucleotide substitutions in g22 change its heptamer to $\text{CACA}\underline{\text{C}}\text{T}\underline{\text{A}}$. The nonamer sequences are much more variable and only six genes contain the concensus $^{G}/_{A}$CAAAAACA previously identified as the V_H1 nonamer (Schwager et al., 1989). The RSS 23-bp spacers vary by a wide range. Six genes contain identical 23-bp spacers, whereas others differ by more than 50% (g345A vs. g352).

Many of the genomic V_H1s were isolated more than once from both the same library and from the different libraries. Eight of the recombinant phage contained more than one V_H1 gene (e.g., g349A, B, C) and at least four recombinant phage (from seven chosen at random) tested positive for members of additional V_H families by hybridization with family-specific oligonucleotide probes (Haire et al., 1990). Three of the recombinant phage contained V_H II hybridizing fragments and one different recombinant phage contained a V_H VIII hybridizing fragment (data not shown). These results in part agree with recent reports that show evidence for interpersion of *Xenopus* V_H families (Schwager et al., 1989; Haire et al., 1991).

Two of the twenty eight genomic genes are clearly pseudogenes. Gene g345A contains two stop codons in its FR3 region, beginning at nucleotide positions 238 and 247, and gene g242A contains a stop codon in its leader sequence. Genes g342A and 342B are located on the same recombinant phage and differ by four bases. Both genes contain an extra different base in their leader intron sequences and, more important, g342A contains two extra bases in its leader, one of which is responsible for generating the stop codon (TAA) directly after the initiation codon ATG. The close sequence similarities and their close association in the DNA suggest that these two genes are the result of a recent gene-duplication event. Genes g7B and g7C probably also represent another example of recent gene duplication; they are found on the same recombinant phage and differ by thirteen bases in their coding regions. Genes g15 and g7C only differ by one base in their CDR2 regions, and are identical everywhere else from 100 bp 5' of their octamer to 80 bp 3' of their RSS sequences (data not shown). Recombinant phage 15 and 7 are different by RFLP analysis, which suggests that these two sequences represent two genes. However, this evidence, even considered with the low (practically nonexistent) frequency of sequencing error (see Materials and Methods) of our reactions is inconclusive. Both genes g7B and g7C were repeatedly isolated from all libraries, whereas g15 was found only once. Also the EcoR1 fragment containing g15 is approximately equal in size to the EcoR1 fragment containing g7C, and so the difference in the two may well be the result of a sequencing artifact.

IgM Heavy-Chain Region

The four exons ($C\mu1$ through $C\mu4$) encoding the *gilli* $C\mu$ chain of LG7 are shown in Fig. 5. The coding region encompasses 4163 bp from the first amino acid of $c\mu1$ to the polyadenylation signal. The four exons and their splice sites were identified by comparison with the published *laevis* μ heavy-chain cDNA sequence (Schwager et al., 1988) and with LG7 μ cDNA sequences (Wilson et al., 1992). The splice sites obey the traditional rule encountered so far for all Ig genes, the splice junction occurs between the first and second base of the joining amino acid (Brüggerman, 1987). In two places, the exon boundaries of the *gilli* μ gene differ from the boundaries predicted from the *laevis* μ cDNA for the $C\mu2$ exon. The LG7 $C\mu2$

exon is two amino acids shorter, ending with a cystein making the Cμ3 exon two amino acids longer at its amino-terminal end.

The deduced amino-acid sequence of the *gilli μ* gene differs at thirty six positions from the published *laevis μ* cDNA sequence (Schwager et al., 1988). The percent homology of the 452 residues is 79.6% (see Fig. 5); at the DNA level, the homology is 96% (1308/1359 bp identical). This polymorphism, relatively higher at the amino-acid level, is not likely to represent the true allelic polymorphism of *Xenopus* Ig allotypes because *X. laevis* and *X. gilli* are different species even though they can produce fertile offspring. In addition, these substantial differences could explain why species-specific antiheavy-chain

A

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          ----+----1----+----2----+----3----+----4----+----5----+----6----+----7----+----8----+----9----+----0----+----1----+----2
LG7G345A  -ATGAAGTTGTTTCTTGTTATGTT-AAT------GACACTTTTATCAGGTAA----------------TAAAAATAAGGAATAATACAATTACAATTATCACATAACATTTTATTTTTAA
LG7G7B    -''''''''T'''''''''''T''-'T'------'T''''A''G''''''''----------------G''TGG'CTTT'TGG'''TT'GA''TCCA''A'TC'C'T'ACA'T''A''''
LG7G7C    -''''''''''A'''''''''T''-'T'------'T''''A''G''''''''----------------G''TGT'CTTT'TGG'ATATTAG'ACTCC'AT'ATCTCATAACAT'''A'T'
LG7G15    -''''''''''A'''''''''T''-'T'------'T''''A''G''''''''----------------G''TGT'CTTT'TGG'ATATTAG'ACTCC'AT'ATCTCATAACAT'''A'T'
LG7G4A    -'''''''CA''G''''''T'C''-'''TTTCTT''TGTG'''''''''''G----------------G''TGT'CTAATTAT''TG'''A'A'GAAT'ACA''C'T'A'A'TC''''T'
LG710A    -''''''A'T'''T'''''G'TA'-'''------'T'''''''G'''''''G----------------G''TGT'TTAA'''T''TG'''ACT'GAAT'A'A''''T'A'''TAAAACT'
LG7G349A  -'''''''CA'''A'C''''GTAA-T'ACACT--'T''TA''''''''''''------------AACTGTC'G'C'TATT'AT''TA'TAATGCT'GTAAGAT'G'TG''A'TAAAACTG
LG7G341   -'''''''''C''''''''TGT''-'''ATTGCTCT''GG''''''''''''----------------G''TGT'T''''GAT'CTG'''A'TT'AAT'ATTCCTCA'GC'ATCAC'''T
LG7G13    -''''''''TC''''''''''C''-'''ATTACTCT''GG''''''''''''----------------G''TGT'C''''GAT'CTG'''A'TT'AAT'ATTCCTCTTGC'ATCAC'''T
LG7G2A    -'''''''CA''GT'''''''C''-'''GTTCCT'T'GTG'''''''''''G----------------G''T'T'C'''TGAT''CAG''ACTT'CAT'TCTCA'GACA'CAC'''AAC'
LG7G35    -'''''''CA''A''''''T'C''-'''ATTCCT'T'GTG'''''''''''G----------------G''TGT'G'''TGAT''CAG''ACTT'CAT'TCTCA'GACA'CAC'''AAT'
LG7G346B  -''''''''''''''C'''''T''-'T'------'T'G''G'GT''''''''----------------'''TTT''TTT'TGG'GTATTAG'ATTCC'ATCATCTCGTAACG''''''T'
LG7G10B   -'''''''CA''G''''''T'C''-'''GTTCCT'T''TGG'''''''''''----------------G''T'TCCTAATGAT''TG'''A'T'GAAT''TA''C'A'AC''T'''''''
LG7G343   -''''''''''''''CA''''T''-'T'------'T'''''''GT'''''''----------------C''TGT'CTTAT'G'G''TT'GA''TCCA''A'TC''TA'CA''T'A''A''
LG7G4B    -''''''''A'''''''''T'T''-'''------'C''''''''''''''''---------------TATGTTC'GTAAGGAGTA'T'GAC'ATCAC'''TTAT'''T''A'TAAAAACT
LG7G44A   -'''G''''''''''G'''''T''-'''ATTACT'GG''A''''''''''''GGATGTTATATATATA''T'T'''TAT'TAT'''T'TA''T'TAATAG'AT'CCA'ACA''A'A''T'
LG7G5     -''''''''''''''A'''TGT''-'T'ATTCCTCT''GG''''''''''''----------------G''TGT'CT'''GAT'CTGG''A'ATCCAT''CTCA'GGTA'CAC''A'A'T
LG7G349C  -''''''''TA''''''''GGTG'-T''ATTCCT'TT'TG''''''''''''----------------G''TGT'C''''GAT''TGT''ACT'CA'T''C''AC'T''CAATG'AA''C
LG7G17B   -'''''''''''G''''''T'CC'-'''ATCCCT'T'''AGC''''''''''---------------TAC'GTTG''''TNAT'''GTTCAGG'GA''CTGG''GCT'AAA'TACA'C'C
LG7G342B  -'''''''CA''G''''C'T''''-'''ATCCCT'T''TGC''''''''''G---------------GG''GTTGT'AAG'''T'GA'TAATAT'A'TC''AT''TA'''''TG''AA''
LG7G342A  ATGT''''CA''G''''C'T''''G'''ATCCCT'T''TGC''''''''''G---------------GG''GTTGT'AAG'''T'GA'TA''T''''CCA'TTAT'AT''''G''AAA''
LG7G22    -'''''''''''G'T''''T'CC'-'''ATCCC'GT''TGGTT'''''''''----------------''C'GTA'CA'GTATTATA'T'C'GG'GATA'TG'ATG'TAAAAT'ACA'G'
LG7G46C   -''''''''AA'''''''''''''-'''------'''''G''''''''''''----------------'''''TA'G'A'TA'TACA'T'ACA'T'ATCACATA'CAT'''AT''''A'C
LG7G2B    -'''''''CA''GT'''''''CC'-'''ATCCCT'T''TG''''''''''''----------------''C'GTGTTTATGAG'''TCTGATA'TAA'ATTGC'G'AGACA'CAC'''T'
LG7G21    -''''''''''''''A'''TGT''-'T'GTTCCTCT''GG''''''''''''----------------G''T'T'C''''GAT'CTGG''A'TTC'AT''CTCA'G'TA'CAC''A'A'T
LG7G352   -''''''''''''''''''TAT''-'C'ATTCC'GTTATGG'''''''''''----------------G''TGT'T''''GATG''T'TAACATT'CCCA'G''AT''A'G''A'AAACG
LG7G349B  -''''''''''''''A'''T'T''-'''------''''''''''''G'''''----------------G''TGT''TTT'T'G'GTA'TAG'AT'C'TAA'T''GTTA''CA'AAC'ATC
LG7G331   -''''''''''''''''''T'T''-'T'GTTACT'G''TG'''''''''''G----------------G''TGT'CCAC'CAT''TG'''A'TAGCAT'ATAT'T'T''AA'T''A''''
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          ----+----1----+----2----+----3----+----4----+---
LG7G345A  ACAATCGTTATGCCTCTTCTTTTTCC---------AGGAGGTCACTGT
LG7G7B    'A'''ATCAT'ATACTG'A'C'CAT'TTTTCC---'''G'''''''''
LG7G7C    'A''ATA'C''TATA''GTA'C'CATCTTTTCC--'''G'''''''''
LG7G15    'A''ATA'C''TATA''GTA'C'CATCTTTTCC--'''G'''''''''
LG7G4A    'A''A'A'C''TATGTC''''C''TTCC-------'''''T'''''''
LG710A    T'TT'ATG'C'TTT'TCC-----------------'''GT''''''''
LG7G349A  CATT'AT''C'CT''T'AT''C'CT'TTTGTCCT-''''T''''''''
LG7G341   'AT'AAC'GG'TTTATG''AA'CTATTTC------''''T''''''''
LG7G13    'AT'AGCGNG'TTTATG''GA'CTATTTC------'''''''''''''
LG7G2A    'TTGCTC''TAT'T'T'C'----------------'''''''''''''
LG7G35    'ATGCTC''TAT'T'T'C'----------------'''''T'''''''
LG7G346B  TT''AAAAATAC'A'TA'G'C''ATGTTTTCC---'''''T'''''''
LG7G10B   CT'TCGT'ATGT'TGA'CTA''C------------'''''T'''''''
LG7G343   'A'''AC'ATAATG''''A'G'''TTCC-------'''T'T'''''''
LG7G4B    'TC''T''CTCA''T'''C----------------'''''T'''''''
LG7G44A   TA''AAAAA'AAAA''A'TA'G'CT''TCTGTTCC'''G'T'''T'''
LG7G5     'A'C'GT''T'TATGTC''A'C'ATTTC-------'''''''''''''
LG7G349C  GGTC'TTA'G'CT'ATC'T'CC-------------'''''T'''''''
LG7G17B   TTTTATTAAC'TT''T'ATC''AA'----------'''G'T'''''''
LG7G342B  ''T'''A''''TTG'ACT'''C-------------'''''T'T'''''
LG7G342A  CT'TCAT'ATGTTGCA'CT'''C------------'''''T'T'''''
LG7G22    'TTT'GTAA'CTTTAT''A'C''AAAT--------'''T'T'''''''
LG7G46C   TATCATTA'G'CT''TC'T''CC------------'''''''''''''
LG7G2B    TA''CT'''T'TATCA''C----------------'''T'T''G''''
LG7G21    G'AC'GT''TCAT'TC'AA'C'A'TTC--------'''G'T'''''C'
LG7G352   GTT''TA'GTCT'A'A''T''CC------------''''T''''''''
LG7G349B  'TGTCTCA'CGTTT'TC------------------'''''T'''''''
LG7G331   'A'C'GA'C''ATT'TCC-----------------'''T---''''''
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FIGURE 4. Alignment of genomic V_H1 genes. (A) Leader sequences beginning at the initiation codon ATG and continuing to the beginning of the V_H region (residue 1). Sequence identities are indicated by (') and gaps (-) are introduced only to conserve length. The stop codon TAA in g342A is underlined. (B) V_H1 regions begin at residue 1 and continue through the RSS nonamer. The number of nucleotides from the beginning of the first base of residue 1 is shown above the scale. Gaps are introduced to maximize homology. FR and CDR boundaries are according to Kabat et al. (1991). The stops in g345A are underlined. Master sequence was chosen for length.

B

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                                                                                                   |   CDR1      |             120
          ----+----1----+----2----+----3----+----4----+----5----+----6----+----7----+----8----+----9----+----0----+----1----+----2
LG7G345A  GATGTGCAACTTGCCCAGTCAGAGCCAGTGGTGATAAAGCCGGGAGGGTCTCACAAACTGTCCTGCACAGCCTCTGGCTTCACATTCAGTAGCACATGGATGCACTGGGTTAGACAGGCT
LG7G7B    ''''''''''''''AT'''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''C'''C''''''AG'''''''''''''''T''
LG7G7C    ''''''''''''''''T''''''''C''''''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''C'''C''''''T''''''''''''''''''''
LG7G15    ''''''''''''''''T''''''''C''''''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''C'''C''''''T''''''''''''''''''''
LG7G4A    '''''A''''''''''''''''''T'''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''C''''''T'''''''''''''''''''''A
LG710A    ''''''''''''''''''''''''T'''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''C''''''T''''''''''''''''''''
LG7G349A  '''''A''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''AC''''''A''''''''''''''''''''
LG7G341   '''''A''''''''T''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''C''''''A''''''''''''''''''''
LG7G13    '''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''TAC''''''G''''''''''''''''''''
LG7G2A    '''''A''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''TAC''''''G''''''''''''''''''''
LG7G35    '''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''TAC''''''G''''''''''''''''''''
LG7G346B  '''''A''''''''A''''''''''T''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''TATG'''''AG''''''''''''''''''''''
LG7G10B   '''''A''''''''A''''''''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''TATG'''''GG''''''''''''''''''''''
LG7G343   '''''A''''''''A''''''''''T''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''TATG'''''GG''''''''''''''''''''''
LG7G4B    '''''A''''''''A''''''''''T'''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''TTAC'CT'''''''''A'''''''''''
LG7G44A   ''A''''''''''''''''''''''T''''''''''''''''C''''''''''''''''''''''''''''''''''''''''''''''''''GATCAC''''''AG''''''''''''''''''''''
LG7G5     '''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''GA'TAC''''''A'''''A'''''''''''
LG7G349C  '''''''''''''A''''''''''T''''''''''''''''T''''''''''''''''''A''''''''''''''''''''''''''''''GA'TAC''''''AG''''A'''''''''''
LG7G17B   '''''A''''''''AT''''''''''''''''''''''''''''''''''''''''''''''''''''''T'''''''''''''''''''GATCAC''''''G''''''''''''''''''''
LG7G342B  '''''A'''''''''''''''T'A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''GAT'AC''''''A''''''''''''''''''''
LG7G342A  '''''A'''''''''''''''T'A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''GAT'AC''''''A''''''''''''''''''''
LG7G22    '''''A''''''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''C'GATTAC''''''''''''''''''''''''''''
LG7G46C   '''T''''''''''''''''''T'T''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''GATTAT'AT'''A'''''T'''''''''''
LG7G2B    '''''A''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''T'G'TCAC''''''''''''''''''''''''''''
LG7G21    ''CA'A'''''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''G'TTAT'AT'''''''''A'''''''''''
LG7G352   ''CA''''''''A''''''''''T''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''''''''''GATTAC''''''AG''''''''''''''''''''''
LG7G349B  '''''A''''''''''''''''''''''''''''''''''T'''''''''''''''''''''''''''''''''''''''''''''''''GATTAC''''''''''''''''''''''''''''
LG7G331   '''''A''''''''A''''''''''T'''''''''''''C'A'''''''''''''''''''''''''''''''''''''''''''''''''G'TTAT'AT'''A''''''''''''''''''''

                                     |                        CDR2                        |                                    240
          ----+----1----+----2----+----3----+----4----+----5----+----6----+----7----+----8----+----9----+----0----+----1----+----2
LG7G345A  TCTGGGAAAGGATTACAGTGGTTATGTCAAATTCACCCTGATGGGAGTAGTAACACATACTATGCTGATTCAGTCAAAGGAAGATTCACCATCTCCAGAGACAATAATAACAGCAAGTAA
LG7G7B    C'''''''T'''''''''''G'G'C''GT''AT'TGA''CA------'''''''T'A''''''''''''''''T''''''''''''''''''''G''''''A''''''''''''''''''''A'''''T'
LG7G7C    C'''''''T'''''G'''''''G'G'C''GT''AT'TGA''CA------'''''''T'A''''''''''''''''T''''''''''''''''''''G''''''A''''''''''''''''''''A'''''T'
LG7G15    C'''''''T'''''G'''''''G'G'C''GT''AT'TGA''CA------''C''''T'A''''''''''''''''T''''''''''''''''''''G''''''A''''''''''''''''''''A'''''T'
LG7G4A    C'''''''''''''''''''''G'G'C'ATT'''CAC'AGC''''''''''''---''''A'''C'''''''''G''T''''''''''''''''''''''''''''''''''''''''''''''AG''''T'
LG710A    C''''''''''''''''''''''''''''C''''''''''''''''''''''---'G''''''''''''''''''''T''''''''''''''''''''''''''''''''''''G''''A'''''T'
LG7G349A  C''''''''''''''''A''''G'G'C'T'T'''AGTAA''''''A'''G'---''''''''''CC''AGC'''T'T''''''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G341   C'''''''''''''''''''''G'G'C''GT'''AG'AGC'G'A'TG'G''''''''''''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G13    C'''''''''''''''''''''G'G'C'GCT'''ATAGG''''''''''''''---''''''''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G2A    C'''''''''''''''''''''G'''C'T'T'''AGTTACCCAAATG'A'C''CA'''C'T''''''''C'''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G35    C'''''''''''''''''''''G'''C'G'T'''A''TAC''C'''GC'G---'G''''''''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G346B  C'''''''''''''''''''''G'G'C'T'T'''AG'AGC'G''''G'''C---''T''''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G10B   C'''''''''''''''''''''G'G'C'GCT'''AG'AGC''''''G''''---''''''''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G343   C'''''''''''''''''''''G'G'C'GCT'''AG'AGCA'''''G''''---''''GT''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''A'T'
LG7G4B    C''''''G'''''''''A''''G'G'C'GCG'''AG'AA''GA''------''C''''''''''''''''''''''''T'''''''''''''''''''''''''''''''''C''''A'''''T'
LG7G44A   C'''''''''''''''''''''G'''C'A'T'''A''ATA''C'''GC'G---'G'''A''''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G5     C'''''''''''''''''''''G'G'C'GCG'''AG'AA''G'''T''------''''''''''''''''''''''''T''''''GA''''-------------------------------------
LG7G349C  C''''''''''''''''''C''G'G'T'T'T''AAGG'A''''''AG'''C---''''A''''''''''''''''''''T'''''''''''''''''''''''''''''TC'''A''''A'''''T'
LG7G17B   C'''''''''''''''''''''''G''G''''T'''''A'''''G'''C---''''A'G''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G342B  C'''''''''''''G'''''''G'''C'GTT'''AG'AG'AGC'''G'''C---''T'''''''A'''''''G''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G342A  C'''''''''''''G'''''''G'''C'GTT'''AG'AG'AGC'''G'''C---''T'''''''A'''''''G''T''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G22    C'''''''''''''''''''''''''''''C''''''T'''GA''------''C''''''''''''''''''''''''T''''''''''''''''''''''''''''''G''''''A'''''T'
LG7G46C   C''''''''''''''''''''''''''''''''T''''''''''''''''C---''''''''''''''''''''''''T''''''''''''''''T''''T''''''''''''''''''''A'''''TC
LG7G2B    C''''''''C''''''''''''''''C'G'T'''A''TA'''''''''''''CA'---''CG''''''''''''''''T''G''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G21    C'''''''''''''''''''''G'G'C''GT''AAG'AGC''C'''G''''---''''GT''''''''''''''''T'''''''''''T''''''''''''''''''''''''''C''''A'''''T'
LG7G352   C'''''''''''''''''''''''''C'G'T'''A''TA'''''''''''''CA'---''CG''''''''''''''''T''''''''''''''T''''''''''''''''''''''''''''''A'''''T'
LG7G349B  C'''''''''''''''''''''''G''G''''''''''''''A'''''G'''C---''''A''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''''A'''''T'
LG7G331   C'''''''''''''''''''''G'G'A'T'T'''A''AGG'''''''''''''''---''''''''''''''''''''''''T''''''''''''''''''''''''''''''''''''''''''A'''''T'

                                                                               |7-mer|                       | 9-mer | 335
          ----+----1----+----2----+----3----+----4----+----5----+----6----+----7----+----8----+----9----+----0----+---
LG7G345A  TATCTGTAAATGAACAATCTACAAACTGAAGACACTGCCGTGTATTACTGTGCTGG-------------CACAGTGAGAAAGGATCCCAGAGCAGTGCAGCAAAAACA
LG7G7B    ''''''C'''''''''''''''''''''''''''''''''''T''''''''''A''ACAGA----------''''''''C''''TAG''T''''''''''''''''''''''''''AC
LG7G7C    ''''''C'''''''''''''''''''''''''''''''''C''''T'''''''''''''''''''''ACAGA----------''''''''C''''TAG''T''''''''''''''''''A''''''AC
LG7G15    ''''''C'''''''''''''''''''''''''''''''''C''''T'''''''''''''''''''''ACAGA----------''''''''C''''TAG''T''''''''''''''''''A''''''AC
LG7G4A    'T''''C'''''''''''''''''''''''''''TG'''''''''''''''''''''A''ACAAG----------''''''''GC''G''G''ATTT'TG'CCT'TAAGGT'GT'
LG710A    ''''''C'''''''''''''''''''''''''''''''''''''A''''''''''''''A'ATA----------''''''''C''''TAG''T''''''''''''''''''''''''''''
LG7G349A  ''''''C'''''''''''''''''''''''''''''''''''''''''''''''''''T''TCTGTGACACAGTGA''''''''C'C''C'''TG'A'''''A'TGA'''''''C
LG7G341   ''''''C''''''''''''''''''''C'''''''''''''''''''''''''''''''''ACGAAG---------''''''''C''''TA'''A''''''''''''''''''''''''T
LG7G13    ''''''C''''''''''''''''''''''''''''''''''''''''''''''''''''A'AGAGGA-------''''''''C'''''A'''A''''''''''''''T''''''''T
LG7G2A    ''''''C''''''''''''''''''''''''''''''''''''''''''''''''''''A'ACA----------''''''''T''''''''A'A'''''''''''''''''''''CAC
LG7G35    ''''''C''''''''''''''''''''''''''''''''T''''''''''''''''''''A'AGACA--------''''''''C'''''A'''A''T'TG''''''''''''''''''
LG7G346B  'T''''C''''''''''''''''''''''''''''G''A''''''''''''''''''''A'AGA----------''''''''C'''''''A'A'''''''''AGTGCAAC''''A'
LG7G10B   ''''''C''''''''''''''''''''''''''''''''''''''''''''''''''A''A'AGA----------''''''''C'''A''A'A''''''''''''''A''''''''''
LG7G343   ''''''C''''''''''''T''''''''A'''''A'''''T''''''''''''''''''G'A'AGA----------''''''''C''''TAG''T'''''''''''''A''T'''''
LG7G4B    '''''CC''''''''''''''''''''''''''''''''G''''''''''''''''''''A'AGAGAGA------''''''''C''''AC''''T'''T'''''TAA''''T'''
LG7G44A   ''''''C''''''''''''''''''''''''''''''''''''''''''''''''''''A'ACA----------''''''''C'''''A'''A''''''''''''''AA''''''''
LG7G5     ------------------------------------------------------------------------------------------------------------
LG7G349C  ''''''C''''''''''''T''''C''''''''''''''''''''''''''''''''''T'A'AGACA--------''''''''C''''C'ATGACA'AA'C'C'CATTGCTCTT
LG7G17B   ''''''C''''''C''''''''''''''''C'''''''T''''''''''''''''''''A'CA-----------''''''''C'G'A''GAATGAG'TTC'C'GA'''T''CT
LG7G342B  ''''''C''''''''''''''''''''''''''''''''A''''''''''''''''''''AGA----------''''''''C''A''A'A''''''''''''''''''''''''T'
LG7G342A  ''''''C''''''''''''''''''''''''''''''''A''''''''''''''''''''AGA----------''''''''C''A''A'A''''''''''''''''''''''''T'
LG7G22    ''''''C''''''''''''''''''''''''''''''G''''''''''''''''''''''A'AGAGAGAGAGAG-''''C'A'C'''''A'A''''''''''''''''''''T'G'
LG7G46C   ''''''C''''''''''''''''''''''''''''''''T''''''''''''''''''''ACATG----------''''''''C''''TAGC'T''''''''GTGCAGC''''AC
LG7G2B    ''''''C''''A''''''''''''''C''''''''''''T''''''''''''''A''A'ATACA--------''''''''C'''''A''''''''''TCA'AT''''''T'''
LG7G21    ''''''C''''''''''''''''''''''''''G''''''''''''''''''''''''''A'AGATG--------''''''''C''''TAG''T''''''''''''''''''''''''T
LG7G352   ''''''C''''''''''''''''''''''''''''TG''T''''''''T''C'T'A'ATATA--------''''''''A'''AAGCAATG'CAA'AACTCCA'TTCG'T
LG7G349B  '''''CG''''''C''''''''''''''''''''''''''A''''''''''''''''''''ACAGA----------''''''''C''''TAG''A''''''''''''''''''''''''T
LG7G331   ''C'''C''''''''''''''''''''''''''''G''A''''''''''''''''''''A'AGAGA--------''''''''C'''''''A'A''''''''''''''AC''''''G
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FIGURE 4

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   1  GTAATAAGAATGTCAAAAACCCACAGAGAGCCTGTGAAGATTTGTTCTTTGCACTAACATCAAAACTAACCTTAACTCTTCCACAAACCTGGTTCCAGTGAAATTCC   107

                                                                                                      Ala Thr Ser
 108  TACATTTCATCTAACCACCTTGTAATCATTTACCATCTCTAATCCTAACAACAAATACTCATTCCTTCCCCCTTCTCTTTTGCTTGTATTCTCAG  CT ACT TCA   210

      Lys Ser                 10                  15                  20                  25                  30
      Asn Pro Pro Ser Leu Phe Pro Leu Ile Ser Cys Gly Glu Ser Met Asp Pro Val Thr Ile Gly Cys Leu Ala Lys Asp Phe
 211  AAC CCC CCA TCC CTT TTT CCA CTC ATT TCT TGT GGG GAG TCT ATG GAC CCA GTC ACC ATT GGT TGT TTG GCC AAA GAT TTC   291

                      35          Thr     40                  45                  50                  55
      Leu Pro Glu Thr Ile Ser Phe Ser Trp Gly Asp Lys Asn Asn Ala Ser Tyr Ser Thr Gly Leu Lys Ser Tyr Lys Pro Val
 292  CTC CCT GAA ACT ATT AGC TTC AGC TGG GGA GAT AAG AAC AAT GCC AGT TAC TCC ACT GGC CTC AAG AGC TAT AAA CCT GTG   372

              60                  65                  70                  75              Asn Ile     Gln
      Met Gln Ser Ser Gly Thr Tyr Ser Ala Ser Ser Gln Val Asn Val Ala Ser Ala Val Trp Asp Lys Ser Glu Pro Phe Tyr
 373  ATG CAA TCT TCC GGC ACC TAC TCA GCC AGC TCC CAA GTC AAC GTT GCC TCT GCA GTC TGG GAC AAA AGT GAA CCA TTT TAC   453

      85                  90  Asp Thr Ile     95          Leu     100 Asp Pro
      Cys Asn Ala Lys His Leu Glu Ile Thr Lys Ser Val Glu Val Lys Lys Gly Thr V
 454  TGC AAT GCC AAA CAC CTG GAG ATC ACT AAG AGT GTG GAG GTC AAG AAA GGT ACA G GTAACCTTATTTTGAACTTTAAATTCACATAGA   541

 542  AATATATTATCTTGCTCTCTATATTTATTGCCATGAAACATATTTACTTATTGATAAACAGCTCCAAGAATCAACACCACTGATTCAATACAAGTAAGATTATTACT   648

 649  CAGTGATTCTGTCGGAACCACTTTATATTGAGCACAAATAATAAATATAATTTCTTATAGAAGTAATAAAAACATAATTTCTTATATACCTATAATATATATATTTT   755

 756  TTTAATAGAGATGCTGCAAGTGGAATGCATAACTGCATACATATCAAGCAGCCATGCATGAAATATGTGTATTCAATAATTGTCCTCTTTTAAAGGGGTGATATGGC   862

 863  AATCCTAATGAGAACTATACTTTGGTATGCATCTAATACAATAGGCTGAACGCACAAACCCCTACCAAACGATCATATTTCTCTAGGTATTAGTAAACAACTAAAAT   969

 970  GAAAACTTCAGGTGATTAAAATTAATGCTAAATAATTAATGTGTGTTGTTAGTAGTTTCTAGAAGTTAAAATGTTTAATTTCCACATAAGGCTTGTGTCACAATTAA  1076

1077  GGTTATTAGCAGCAACTTTACAACTGTCTGCAAATACAGGTATGGATCTGTTCTTGGAATCTTGGGTTTTTCTGTTTTTTCATTATTTGGAGAACCATGACTTTAGA  1183

1184  TACTAAAATATGTGAACATAAGAAAAAAACTGGGTATGTCATCAGAATGGTATCTCAAATTACAGGGTAGTGTCTAGTTATTACATAGAAAAAAGCAAACCCATAGC  1290

1291  AAGAGAAGAAATGCTTGTTCAAATAGGACTCTATGGGAAATGACAAAAGGTAATTTAAAGGTGAACTACCCCTTTAATTCTTTCCAATTGGCATAATAATCTACTTG  1397

1398  CTTTAAAGAAAACAAAGGAACCTTTTTGGAGCCAGTTTCAAGTAGCAGCTACTTTCCCTGTGTGACATAAGCCTGACTGTGTAAATTATCCTCTTTGCATTCATAAA  1504

1505  TTAAAACTAATAAAACTAATAGAAGTTACATGTAGTACACAACAGAATGTGAACACTAGAAATGATATTTCCTTCCAATATTTTTAGAAAAATCTACTGGTTTATAT  1611

1612  ACAAATGTGACAAAAAACACTGAACTCTTTTTATTATCGGTATTCAGTATACACTGAGGGACAGATTTATTATGTGGTGTTAAAAATGGTGGAATAATACACCACAC  1718

1719  GTCTCCAGCATTGCCGTATAAGAATGGTGTTATTTTTTTTACGCACTTTTTCTTTGACCAACTACTGGCAGAACTTACTTATAAAGGTCTGAATCGATGTGAAATAG  1825

1826  CATTCACATGGCATAAAATAAAACACACCTTGATAAATTCGCCATTTTTACATGGCTTTCTACACCGTTTTTTTTGCCAAATTTCGTTTTACACAGCATAACTATGC  1932

1933  CCCTGAATGTACATATTGCATACAAAAATACAACTGAAGAATGATACATAAAATGAATAGGCCCTGCCCAAAAGAGTTTACGGAGTGACCAGCTTGTAGAGTTGACA  2039

2040  TAAATTATTTTACCCTTTTTTTGTTTATTTGTATCAACACTCACTTTTCTTTAATTAAAGGAGTAACACATCATTAGCTTGGAAGTATACCGGAAATGCGTCTCTGT  2146

                                                              Lys Pro                 110                 115
                                                           al Asn Lys Val Glu Lys Pro Val Val Ser Ile His Pro Pro
2147  TTATCTTATAGGATCGCCTAATCCTATTCTTTTCTATCCCTTTTATCAG    TG AAT AAA GTT GAA AAA CCA GTT GTG TCT ATT CAC CCT CCA   2236

                  120                 125                 130                 135     Thr     Thr His
      Ser Lys Asp Ala Leu Ala Leu Asn Glu Ser Leu Phe Ile Val Cys Leu Ala Thr Asn Phe Asn Pro Lys Asn Ile Val Ile
2237  TCC AAG GAT GCT CTT GCC TTG AAT GAA AGC CTC TTT ATC GTA TGT CTT GCA ACG AAT TTT AAT CCC AAA AAT ATA GTA ATT   2317

          145                 150     Thr     155                 160                 165     Arg             170
      Lys Trp Leu Lys Asn Gly Asn Gln Thr Lys Glu Gly Val Arg Val Glu Glu Pro Val Glu Asp Lys Lys Gly Gly Tyr Glu
2318  AAA TGG CTA AAG AAT GGG AAC CAG ACA AAA GAA GGT GTG AGA GTT GAA GAA CCT GTT GAA GAC AAA AAG GGA GGA TAT GAG   2398

                          175                 180                 185                 190                 195 Glu
      Thr Thr Ser Tyr Leu Ser Ile Thr Arg Lys Glu Trp Asp Leu Asp Thr Leu Tyr Ser Cys Val Val Glu His Ala Gly Ser
2399  ACA ACA TCC TAT CTC TCC ATA ACT AGA AAG GAA TGG GAT TTG GAT ACT TTG TAC TCA TGT GTA GTT GAA CAT GCA GGA TCG   2479

      Ala     200                 205                 210
      Gly Ser Leu Gln Glu Lys Asn Met Ser Lys Ser Leu Met Cys A
2480  GGT TCC TTA CAA GAG AAG AAT ATG AGC AAA TCA CTA ATG TGT G GTAAGTTCTTGTGATGTGATGCTTATTGTTCTACAGATAAGGATCAGTT   2571

2572  TTACTTTAAGAAATTAATTTATGTATAAGTGTTAATACTGTGGGTCATATTTGCTTCAATGCATGTTGGTACATATATAGTTACATATAGTCTTTATCTGATTATGA   2678

2679  CCAATAAAAGCCAAAATATACCAATTTTGAAAGGTATTTTTTGCCTGTAAACAGTGCTAGGAACCGGCACTCACCTCTCCTTAGTACTAATAGCCAGGTATATATAT   2785

2786  ATGAGAGAGATGTTCAGGGTATTTTTTGATGTCATAGTGTTATGCACTACTATTTAAAGGGTTGTGGTCCCTGTGAATTTCAGGTCAGTGAATGTGAAACAGCATCT   2892

2893  CGTTTTCTATTAAAACTGTCTTATGGGGCTAAACATGAAGTGATGATCGGTTATTTTGGTTGAACAAAGCCAAGTAGACTGGCATGTTTGCACAATGTAAGGGTGGC   2999

3000  TTAATAAATGTTTTGTTATTGGAGGGAATTTAACATAAATAATGTATAATTTGCAGTATGGTATCTCACTATTTTTTTTTCCTTTTTTTTCCTTTTTTTTTTCCTTT   3106
```

FIGURE 5

Mabs (like 14G1, see the foregoing) can be produced.

Only 650 bp beyond Cμ4 were sequenced and no transmembrane exons were found in that stretch. The segments encoding the *Xenopus* transmembrane region have been published previously (Du Pasquier and Schwager, 1989) and a complete map of the *Xenopus* IgH locus will be published elsewhere (Du Pasquier, in preparation). The *gilli μ* locus is also characterized by a long intron between Cμ1 and Cμ2 (1618 bp). This intron contains sequences similar to some enhancer motifs described for mammalian Igs (Staudt and Lenardo, 1991). An octamer and sequences analogous to *μ*e4 and *μ*e5 are identified in Fig. 5.

```
                          Pro 215                 220                 225                 230                 235
                   sp Thr His Ile Thr Pro Thr Ser Ile Gln Val Ile Thr Ile Pro Pro Ser Leu Glu Ser Ile Phe Glu Lys
3107  TTCCTCAG     AC ACC CAT ATA ACA CCC ACT AGT ATC CAA GTA ATC ACT ATT CCA CCA TCA TTA GAG AGC ATT TTT GAG AAA    3185

                      240                 245                 250                 255             Val 260
      Lys Ser Ala Thr Leu Thr Cys Leu Val Ser Asn Met Ala Asn Ser Glu Asp Leu Arg Ser Ile Ser Trp Phe Lys Lys Ser
3186  AAA TCT GCC ACA CTT ACC TGC CTG GTC AGT AAT ATG GCT AAC TCT GAG GAT TTG AGA TCA ATA TCC TGG TTT AAA AAA TCT    3266

              265                 270                 275                 280 Tyr         Phe 285
      Gly Thr Gln Glu Ile Pro Leu Lys Thr Glu Leu Gly Asp Ala Ile Tyr Asn Asp Asn Arg Thr Tyr Ser Val Lys Gly Thr
3267  GGT ACT CAA GAG ATA CCA TTG AAA ACA GAA CTG GGA GAT GCA ATC TAT AAC GAT AAC CGC ACC TAT TCT GTA AAA GGA ACC    3347

      290                 Glu                 300                 305                 310             Met 315
      Thr Thr Val Cys Ala Asp Glu Trp Asn Asn Asp Lys Phe Val Cys Lys Val Glu His Thr Glu Leu Ala Ser Val Lys Glu
3348  ACC ACT GTC TGC GCT GAT GAA TGG AAT AAC GAC AAG TTT GTC TGC AAA GTG GAA CAC ACA GAG CTG GCT TCA GTG AAG GAG    3428

          Leu Pro Leu
      Val Phe Leu Phe Lys Glu Lys G
3429  GTC TTT CTC TTT AAA GAA AAA G GTAAGGCCATCTACCTGTACTGCACAGTACTAATCAGACAGAACAACTATAATAAAATAGCCATTATACTTGATACC    3527

3528  AATATAGCACACATTTTTCATGTGCAGAGGTTGTTATTCATCCACTGATCTGATAGCTATTATTAATATACAATTAATAAATTTCTATTAGGGTCTGTTTTAAGCAC    3634

3635  CAAAGACCAACATTCTGTAGAAAGTATAATTTTCTACAATAGAGTAGATCATCAACCTGTAAAATAAATTGTGACATCCATTCGAGTTGTATGTTAAGGGTGTTTTT    3741

                                                                                                     325
                                                                                                  ly Glu Tyr Asn Thr
3742  ACTGATATCTGTAGAGGAAGCATTTGTGTGAGTAATCCCTTCAAAAACTTTGAGATTTGTAACTCCTAATTTTCTATTTTAAAACAG  GA GAA TAC AAT ACA    3842

          330         Phe Ser 335                 340                 345                 350                 355
      Pro Ser Val Tyr Val Phe Pro Pro Pro Leu Glu Glu Leu Ser Lys Arg Glu Thr Ala Thr Leu Thr Cys Leu Val Lys Gly
3843  CCA TCT GTT TAT GTT TTC CCA CCA CCT CTT GAG GAA TTG TCT AAG AGA GAA ACT GCC ACC TTG ACA TGC TTG GTT AAA GGG    3923

                      360                 365         Lys     370                 375                 380
      Phe Ser Pro Ser Glu Ile Phe Val Lys Trp Leu His Asn Asn Glu Ala Val Pro Lys Gln Asn Tyr Ile Asn Thr Ser Ile
3924  TTC AGC CCC TCT GAA ATA TTT GTA AAA TGG CTT CAC AAC AAT GAG GCG GTT CCA AAA CAA AAT TAC ATA AAT ACC AGC ATC    4004

              385     Leu         390                 395                 400     Asn         405 Ile
      Asn Asp Glu Leu Tyr Pro Lys Gly Gln Lys Ser Gly Lys Phe Phe Leu Tyr Ser Leu His Thr Ile Asp Phe Lys Asp Trp
4005  AAT GAC GAG CTT TAT CCC AAA GGA CAG AAG AGT GGA AAG TTC TTT CTG TAC AGT CTT CAC ACC ATA GAC TTT AAA GAC TGG    4085

      410                 415                 420                 425                 430                 435
      Asp Ala Gly Asp Ser Phe Ser Cys Val Val Gly His Glu Ser Leu Pro Leu Gln Leu Thr Gln Arg Ser Ile Asp Lys Ser
4086  GAT GCT GGT GAT AGT TTT TCC TGT GTG GTT GGC CAT GAG TCA TTA CCA CTT CAG CTG ACC CAA AGG AGC ATT GAC AAG TCT    4166

                  440                 445                 450
      Ser Gly Lys Pro Thr Asn Val Asn Val Ser Leu Val Leu Ser Asp Thr Cys  *  *
4167  TCT GGT AAA CCT ACT AAC GTG AAT GTG TCC CTC GTC TTG TCT GAT ACC TGT TAGTGATCATCTCCAAAGACCCTATAACTCCATCTCATG    4256

4257  TTCTTCCCTATATGGTGTAATGGACAAGGCGGAGGGAGAAGTGTTGACTTTATGTTTGTCTGTCTGTTCTTGTACATTTTTTGTCTGCAAAATTAAAAGTAGAAATA  4363

4364  AAAATCAATTTATACAATGTTTGTTTGTATGCATGCAATTTGGGAAAGAAGTGGTGATTTGGAAAATTGAAATACTTCACCACTGGTTTGTAGCCTGCATTAGGGAT  4470

4471  TTGAATTCCAGATACACCTACACCTTTTCATAAAACCCACAGCCATTAGCTTCAGTAGGAATCCAAAAGCTCAGAGCTGCATAGGGTAAGAACTTAACAAAGGTCAC    4577

4578  ATTACTACTTGATTAGTTTCATTTATAAAGAAATTACATATGACTAGGAAAGGGAATGCTTACTAGCCAAGATGTCCAAATAAACATCATTCAGTTAATTTGAAAGT    4684

4685  GTTTCATGTGCTTTCTATGTTATAGGCAATGTGTGTTCAACTTAGAACATATGAGACAAAACAAACTGTTATGGGCATATTTTTCAAAGTGTGAAATTACTCCCACT    4791

4792  TTCAATTCTTTTCTACTGGTTTTTGTTGTACACTACAAG                                                                          4830
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FIGURE 5. Nucleotide and inferred amino-acid sequences of the LG7 IgM heavy-chain gene. The inferred amino acids are indicated above the nucleotides. Only the amino acids of *laevis μ* (from the cDNA sequence; Schwager et al., 1988) that differ from *gilli μ* are shown. These residues are shown above the LG7 *gilli*-encoded amino acids Sequence motifs in the CH1 to CH2 intron that are similar to Ig-enhancer seuqencers are underlined. These include a SITE E, E-boxes (*μ*e5, *μ*e3, *μ*e4), and a *μ*B motif. A sequence identical to the KBF-A motif in the kappa-chain enhancer is underlined and overlined. The octamer sequence is double underlined. Ig-enhancer sequences are from Staudt and Lenardo (1991). Polyadenylation sites are in bold. GenBank accession number of *gilli μ* is M97008.

DISCUSSION

In this study, we examined a clone of isogenic *Xenopus*, the *X. laevis/X. gilli* hybrid LG7, which probably developed accidentally from a LG15 small egg (Kobel and Du Pasquier, 1986) with a recombined genome. Because this clone is homozygous at the heavy-chain locus, its genetic simplicity makes it the best available strain for comparing germ line with cDNA Ig sequences. These comparisons are necessary to detect the presence of somatic mutations, an issue that has remained unanswered in the lower vertebrates for the past 18 years, ever since the major differences in antibody repertoires were first reported between amphibians and mammals.

In addition to establishing a dictionary of V_H1 sequences from LG7, we have sequenced the μ-chain gene. This will be useful for future studies with conventional heterozygous LG hybrids in order to identify origins of μ cDNAs.

All of the twenty eight V_H1 genes sequenced exhibited the typical vertebrate V_H-gene structure (Kabat et al., 1991) and sequence identities ranged from 82–96%, thus easily fulfilling the criteria of V_H-gene family membership (Brodeur and Riblet, 1984). In addition, all of the V_H1s contain specific features and conserved nucleotides previously described for *Xenopus* V_H1 genes (Schwager et al., 1989). Many shared CDR1s and CDR2s are similar even though by overall comparison they show the most sequence divergence. Most of this variability is in the first eight to ten amino acids of the CDR2s. The LG7 V_H1 sequences further reinforce, by allowing for greater comparisons, the hypothesis that the low heterogeneity of the *Xenopus* V_H pool (at least for this family) may be the result of recent expansion events (Schwager et al., 1989; Du Pasquier and Schwager, 1991).

We are aware that the screening process may have missed genes and that calculating V_H family size by Southern blotting can be inaccurate because restriction fragments may contain more than one gene or may comigrate in the gel. The size of the LG7 V_H1 family was originally estimated to contain at a maximum thirty genes and Southern blot analyses with frequent six or even four base cutters are consistent with this estimate (data not shown). Because studies in outbred *laevis* and other LG hybrids place the V_H1 family size at approximately sixty in heterozygous individuals (Schwager et al., 1989; Haire et al., 1990), we believe that very few LG7 genes are missing. Indeed, when the twenty eight germ-line genes sequenced in this study were used to analyze LG7 somatic mutants (Wilson et al., 1992), only four out of fifty five cDNAs could not be unambiguously assigned to one of these germ-line V_Hs.

The LG7 *gilli* Cμ segment conforms to the organizational pattern seen in all other vertebrate Cμ loci. There are four exons that encode the four protein domains of the secreted μ heavy chain and a detailed analysis of the inferred amino acid identities between *Xenopus* μ chain and those of other vertebrates can be found in Schwager et al. (1988). One unusual feature of the *gilli* μ gene is the presence of enhancer motifs in the Cμ1 to Cμ2 intron. Whether these sequences have any enhancer function remains to be investigated. Enhancer like sequences are also found 5' of the putative switch region of the *Xenopus laevis* IgM gene (Du Pasquier, unpublished), a location analogus to the site of the mammalian IgH-chain enhancer. The Cμ1, Cμ2 intron length, however, is not unusual. Introns of similar lengths are found between Cμ1 and Cμ2 of the channel catfish, *Ictalurus punctatus*, and between Cμ2 and Cμ 3 of the horned shark, *Heterodontus francisci* (Wilson et al., 1990; Kokubu et al., 1988).

The LG7 hybrids are the first *Xenopus* clones to be homozygous at their IgH locus and preliminary evidence indicates that they may well be homozygous for the ρ light-chain locus. Except for the MHC locus, which is heterozygous (Bernard et al., 1979), homozygosity at other LG7 genetic loci has yet to be investigated. In the future, it may well prove to be worthwhile to create other clonable homozygous LG hybrids by using pressure or cold-temperature shock to increase the frequency with which the second meiotic division does not occur. This technique was previously exploited to study histocompatibility antigens (Kobel and Du Pasquier, 1977).

MATERIALS AND METHODS

Genomic DNA Preparation and Library Construction

High-molecular-weight DNA was prepared from *Xenopus* LG erythrocytes lysed in TES buffer (10 mM Tris, pH 8.0, 10 mM EDTA, 400 mM

NaCl, 0.2% SDS), containing 100 μg/ml proteinase K. LG7 small-egg progeny DNA was prepared in an identical manner except that tissues from 3-day-old tadpoles were homogenized in TES buffer. DNA was extracted as previously described (Kiefer, 1990).

LG7 genomic libraries were made from DNA partially digested with Sau3A or Mbo1 and size fractionated by sucrose gradient centrifugation (Davis et al., 1986). Fragments of approximately 20 kb were packaged into EMBL3 vectors (Stratagene) following manufacturers' recommended protocol. The libraries contained approximately 3.6×10^5 (Sau3A) and 5.6×10^5 (Sau3A) and 8×10^5 (Mbo1) recombinants. All libraries were screened unamplified with V_H1 or $C\mu$ probes, as previously described (Wilson et al., 1986). Identical V_H1 genes were isolated from all three libraries. The $C\mu$ genes were isolated and sequenced from the library Sau3A 3.

Southern Blot Analysis

Genomic DNA (5–10 μg) was digested to completion with restriction enzymes, electrophoresed on 0.7% agarose gels, transferred to nitrocellulose or nytran filters (Schleicher and Schuell) and hybridized (Schwager et al., 1991). All probes were labeled by the method of Feinberg and Vogelstein (1983). After overnight hybridization, the Southern blots were washed at high stringency (65°C, 0.1×SSC, 0.1% SDS). X-ray film exposure was at −80°C. The DNA probes for V_H1, $V\rho$, $C\rho$ were kindly provided by our colleague, J. Schwager.

Subcloning and Sequencing

Fragments containing V_H1 genes were subcloned into Bluescript plasmid vectors (Stratagene) and sequenced on both strands by primer extension using the dideoxynucleotide triphosphate chain termination reaction method (Sanger et al., 1977). Synthetic oligonucleotides were prepared by H. R. Kiefer's laboratory (Basel Institute for Immunology). The error frequency of the modified T7 polymerase (USB) used in our sequencing reactions must be very low because two independent genomic $C\mu$ clones were sequenced (4830 bp each) with no base differences being found.

Computer Analysis

DNA sequences were aligned in pairs with a program based on the algorithm of Needleman and Wuntsch (1970). Each sequence was aligned to a "master" and their pairwise alignments were used as input for a multiple alignment program based on a heuristic algorithm. All computer programs were written by Charles Steinberg (Basel Institute for Immunology).

Enzyme-Linked Immunoabsorbent Assay and Chromosome Analysis

Serum was obtained from LG7, LG15, and outbred *laevis* frogs (two each) and diluted 1/10 in amphibian PBS (standard PBS diluted 1.3 fold). Microtitre plate wells were coated with 50 μl of diluted frog serum overnight at 4°C. After washing the plates, assays were performed using the β-Galactosidase Hybridoma Screening Kit (BRL) following the manufacturer's recommended procedure. The primary antibodies were Mabs 14G1, 10A9, 409B8, and 11D5 (Du Pasquier et al., 1985).

Chromosome spreads were prepared using a procedure previously described (Du Pasquier et al., 1985).

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REFERENCES

Bernard C.C.A., Bordmann G., Blomberg B., and Du Pasquier L. (1979). Immunogenetic studies on the cell-mediated cytotoxicity in the clawed toad *Xenopus laevis*. Immunogenetics **9:** 443–459.

Brandt D.C., Griessen M., Du Pasquier L., and Jaton J.C. (1980). Antibody diversity in amphibians: Evidence for the inheritance of idiotypic specificities in isogenic *Xenopus*. Eur. J. Immunol. **10:** 731–736.

Brodeur P.H., and Riblet R. (1984). The immunoglobulin heavy chain variable region (Igh-V) locus in the mouse. I. One hundred Igh-V genes comprise seven families of homologous genes. Eur. J. Immunol. **14:** 922–930.

Brüggerman M. (1987). Genes encoding the immunoglobulin constant regions. *In:* Molecular genetics of immunoglobulin, Calabi F., and Neuberger M.S., Eds. (Amsterdam: Elsevier Science Publishers), pp. 51–79.

Davis L.G., Dibner M.D. and Battey J.F. (1986). Basic methods in molecular biology (New York: Elsevier), pp. 171–174.

Du Pasquier L., Flajnik M.F., Guiet C., and Hsu E. (1985). Methods used to study the immune system of *Xenopus* (Amphibian, Anura). *In:* Immunological methods III, Lefkovits I., and Pernis B., Eds. (London: Academic Press), pp. 425–465.

Du Pasquier L., and Schwager J. (1989). Evolutions of the immune system. *In:* Progress in immunology VII, Melchers F., Ed. (New York: Springer Verlag), pp. 1246–1255.

Du Pasquier L., and Schwager J. (1991). Immunoglobulin genes and B cell development in amphibians. *In:* Mechanisms of lymphocyte activation and immune regulation III, Gupta S., Ed. (New York: Plenum Press), pp. 1–9.

Du Pasquier L., and Wabl M.R. (1978). Antibody diversity in amphibians: Inheritance of isoelectric focusing antibody patterns in isogenic frogs. Eur. J. Immunol. **8:** 428–433.

Feinberg A.P., and Vogelstein B. (1983). A technique for radiolabelling DNA restriction fragments to high specific activity. Anal. Biochem. **132:** 6–13.

Haire R.N., Amemiya C.T., Suzuki D., and Litman G.W. (1990). Eleven distinct V_H gene families and additional patterns of sequence variations suggest a high degree of immunoglobulin gene complexity in lower vertebrates, *Xenopus laevis.* J. Exp. Med. **171:** 1721–1737.

Haire R.N., Ohta Y., Litman R.T., Amemiya C.T., and Litman G.W. (1991). The genomic organization of immunoglobulin V_H genes in *Xenopus laevis* shows evidence for interspersion of families. Nucleic Acids Res. **19:** 3061–3066.

Kabat E.A., Wu T.T., Reid-Miller M., Perry H.M., and Gottesman K.S. (1991). Sequences of proteins of immunological interest (Bethesda, MD: National Institutes of Health).

Kiefer H.R. (1990). United States patent no. 4,946,952.

Kobel H.R., and Du Pasquier L. (1975). Production of large clones of histocompatible, fully identical clawed toads *(Xenopus).* Immunogenetics **2:** 87–91.

Kobel H.R., and Du Pasquier L. (1977). Strains and species of Xenopus for immunological research. *In:* Developmental immunobiology, Solòmon J.B., and Horton J.D., Eds. (Amsterdam: North Holland-Elsevier), pp. 299–306.

Kobel H.R., and Du Pasquier L. (1986). Genetics of polyploid *Xenopus.* Trends Genet. **2:** 310–315.

Kokubu F., Hinds K., Litman R., Shamblott M.J., and Litman G.W. (1988). Complete structure and organization of immunoglobulin heavy chain constant region genes in a phylogenetically primitive vertebrate. EMBO J. **7:** 1979–1988.

Nace G.W., Richards C.M., and Asher J.H., Jr. (1970). Pathenogenesis and genetic variability. I. Linkage and inbreeding estimations in the frog, *Rana pipiens.* Genetics **66:** 349–368.

Needleman S.B., and Wunsch C.D. (1970). A general method applicable to the search for similarities in the amino acid sequence of two proteins. J. Mol. Biol. **48:** 443–453.

Parslow T.G., Blair D.L., Murphy W.J., and Granner D.K. (1984). Structure of the 5' ends of immunoglobulin genes: A novel conserved sequence. Proc. Natl. Acad. Sci. USA **81:** 2650–2654.

Sanger F., Nicklen S., and Coulson A.R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

Schwager J., Bürckert N., Courtet M., and Du Pasquier L. (1989). Genetic basis of the antibody repertoire in *Xenopus:* Analysis of the V_H diversity. EMBO J. **8:** 2489–3001.

Schwager J., Bürckert N., Schwager M., and Wilson M. (1991). Evolution of immunoglobulin light chain genes: Analysis of *Xenopus* IgL isotypes and their contribution to antibody diversity. EMBO J. **10:** 505–511.

Schwager J., Mikoryak C.A., and Steiner L.A. (1988). Amino acid sequence of heavy chain from *Xenopus laevis* IgM deduced from cDNA sequence: Implications for evolution of immunoglobulin domains. Proc. Natl. Acad. Sci. USA **85:** 2245–2249.

Staudt L.M., and Lenardo M.J. (1991). Immunoglobulin gene transcription. *In:* Annual review of immunology, Paul W.E., Fathman C.G., and Germain R., Eds. (Palo Alto: Annual Reviews Inc.), pp. 373–398.

Wabl M.R., and Du Pasquier L. (1976). Antibody patterns in genetically identical frogs. Nature **264:** 642–644.

Wirth T., Standt L., and Baltimore D. (1987). An octamer oligonucleotide upstream of a TATA protif is sufficient for lymploid-specific promoter activity. Nature **329:** 174–178.

Wilson M., Middleton D., Alford C., Sullivan J.Y., Litman G.W., and Warr G.W. (1986). Putative immunoglobulin V_H genes of the goldfish, *Carassius auratus,* detected by heterologous cross-hybridization with a murine V_H probe. Vet. Immunol. Immunopath. **12:** 21–28.

Wilson M., Hsu E., Marcuz A., Courtet M., Du Pasquier L., and Steinberg C.M. (1992). What limits affinity maturation of antibodies in *Xenopus*-the rate of somatic mutation or the ability to select mutants? EMBO J. **12:** in press.

Wilson M.R., Marcuz A., van Ginkel F., Miller N.W., Clem L.W., and Warr G.W. (1990). The immunoglobulin μ heavy chain constant region gene of the channel catfish, *Ictalurus punctatus:* An unusual mRNA splice pattern produces the membrane form of the molecule. Nucleic Acids Res. **18:** 5227–5233.