Conserved Vertebrate Chromosome Segments in the Large Salamander Genome

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ABSTRACT

Urodele amphibians (salamanders) are important models for embryological, physiological, and natural history research and are also a biomedically important group because they are the only vertebrates capable of regenerating entire organ systems. To enhance the utility of salamanders for biomedical research and for understanding genome evolution, genetic linkage analysis was used to identify chromosome segments that are homologous between ambystomatid salamanders and distantly related vertebrate model organisms. A total of 347 loci (AFLPs, RAPDs, and protein-coding loci) were mapped using an interspecific meiotic mapping panel (*Ambystoma mexicanum* and *A. tigrinum tigrinum*; family Ambystomatidae). Genome size in Ambystoma was estimated to be 7291 cM, the largest linkage map estimate reported for any organism. However, the relatively large size of the salamander genome did not hinder efforts to map and identify conserved syntenies from a small sample of 24 protein-coding loci. Chromosomal segments that are conserved between fishes and mammals are also conserved in these salamanders. Thus, comparative gene mapping appears to be an efficient strategy for identifying orthologous loci between ambystomatid salamanders and genomically well-characterized vertebrate model organisms.

COMPARATIVE gene mapping among vertebrate species is providing insight into the relative importance and timing of gene duplications, gene losses, gene order rearrangement, and chromosomal exchanges during vertebrate genome evolution (Aparicio *et al.* 1997; Ehrlich *et al.* 1997; O'Brien *et al.* 1997; Force *et al.* 1999; Groenen *et al.* 2000; Naruse *et al.* 2000; Postlethwait *et al.* 2000; Woods *et al.* 2000). Nevertheless, relatively little progress has been made in extending genome analysis to representative species of all the major vertebrate groups. Although chicken is becoming a model for one "reptilian" lineage (Groenen *et al.* 2000), no amphibian genome has been characterized extensively. This is somewhat surprising given the historical importance of amphibians as model organisms and the pivotal phylogenetic position of this lineage for understanding evolutionary transitions from fishes to amniotes.

For more than a century, urodele amphibians (salamanders) have been used as models for embryology, physiology, and natural history research (*e.g.*, Roy *et al.* 2000; Voss and Shaffer 2000). Moreover, salamanders are the only adult vertebrates that can regenerate organ systems completely and are thus a potentially valuable group for understanding mechanisms of tissue repair and repatterning, as well as for designing clinical therapies to treat human trauma, amputation, and disease (reviewed by Dinsmore 1996). To enhance the utility of salamanders for biomedical, genomic, and other research, we initiated a project to develop a comparative gene map that will allow identification of orthologous loci between salamanders and other vertebrates. We chose Mexican axolotl (*Ambystoma mexicanum*) and tiger salamander (*A. tigrinum tigrinum*) because these species are arguably the most important given their long histories as research models and their continued use in contemporary research programs. Although strikingly different in morphology and life history, it is possible to cross the axolotl and tiger salamander (as well as other closely and distantly related species; Voss and Shaffer 1996) to create mapping panels for genetic linkage analysis. In previous work, axolotl/tiger salamander hybrids were backcrossed to axolotl to generate map cross families herein referred to as the AxTg mapping panel. This mapping panel already has been used to map a major quantitative trait locus (QTL) for metamorphic failure (Voss and Shaffer 1997) and to test candidate genes for metamorphic failure and a mutation affecting pigment cell morphogenesis (Parichy *et al.* 1999; Voss *et al.* 2000).

Although previous studies utilized the AxTg mapping panel for genetic linkage analyses (Voss and Shaffer 1997; Parichy *et al.* 1999; Voss *et al.* 2000), there was no investigation of chromosomal segment homologies to other vertebrates. The likelihood of identifying homologous chromosomal segments seems reasonable given that conserved syntenies are being established between fishes and mammals (Naruse *et al.* 2000; Post-

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TABLE 1

Primers used to PCR amplify *Eco*RI (E)/*Mse*I (M) and *Not*I (N)/*Hpa*II (HP) restriction fragments

*Eco*RI primers		*Mse*I primers	
E^+0 = GACTGCGTACCAA TTC		M^+0 = GATGAGTCCTGAG TAA	
E^+0-ACGA	E^+0-ACGC	M^+0-CACA	M^+0-CTAG
E^+0-ACGG	E^+0-ACGT	M^+0-CACG	M^+0-CTAC
		M^+0-CACC	M^+0-CTAT
		M^+0-CACT	
*Not*I primers		*Hpa*II primers	
N^+0 = GACTGCGTATTGG CCGC		HP^+0 = GGCGAATCCTAA GCGG	
N^+0-AAG	N^+0-ATT	HP^+0-ATA	HP^+0-AAC
N^+0-AGG	N^+0-AAT	HP^+0-TAC	HP^+0-CAC
N^+0-ACA	N^+0-TGC	HP^+0-CAC	

The $^+0$ primers for each restriction fragment type are shown followed by more selective primers with plus three or plus four nucleotide extensions.

LETHWAITE *et al.* 2000). However, one potentially confounding factor is the relatively large size of salamander genomes when compared to other vertebrates; although ambystomatid salamanders have relatively few chromosomes ($N = 14$), physical genome size is estimated to be an order of magnitude larger than human (STRAUS 1971). Here we report that Ambystoma also has a correspondingly large genetic linkage map, in fact the largest known in any organism. Despite this large genome and genetic map, we show that protein-coding loci can be mapped efficiently in this system and conserved gene orders can be identified between salamanders and distantly related vertebrates with much smaller genomes. Thus, some regions of chromosomal synteny have been maintained between these amphibians and other vertebrate lineages, suggesting that genome cross-referencing between salamanders, humans, and other model vertebrates ultimately will provide new insights into genome evolution and new tools for biomedical research.

MATERIALS AND METHODS

Salamander crosses, DNA markers, and polymorphism detection: A mapping panel (AxTg) was created by crossing two male F_1 *A. mexicanum*/*A. tigrinum tigrinum* hybrids to a single *A. mexicanum* female (Voss 1995). DNA was isolated from 44 backcross offspring and typed for 314 amplified fragment length polymorphisms (AFLPs), nine randomly amplified polymorphic DNA markers (RAPDs), and 24 protein-coding loci. A total of 262 AFLPs were identified previously (Voss and SHAFFER 1997). Additional AFLP marker loci were scored using primer sets for *Eco*RI/*Mse*I and *Not*I/*Hpa*II restriction fragments (Tables 1 and 2; see Vos *et al.* 1995 and Voss and SHAFFER 1997 for a description of AFLP methods). RAPD primers are listed in Table 3; the RAPD protocol was described previously (Voss 1993). Table 4 lists protein-coding loci that were mapped, as well as PCR primers and conditions. Protein-coding loci were amplified using 150 ng DNA, 75 ng each primer, 1.5 mM $MgCl_2$, 0.3 units Taq, and 32 cycles of a three-step profile (45 sec denature, 60 sec anneal, and 30–60 sec extend). Protein-coding loci were verified by sequencing PCR products from the grandparental *A. mexicanum* and *A. t. tigrinum* individuals from which the mapping panel was derived. Nucleotide sequence alignments between *A. mexicanum* and *A. t. tigrinum* protein-coding loci revealed polymorphisms that were scored using restriction digestion, single-strand conformation polymorphism (SSCP), allele-specific amplification (presence/absence of PCR product), or length variation. SSCP methods followed Voss *et al.* (2000). Length variants were resolved using 1–4% agarose gels.

Genetic linkage analysis and estimation of genome size: Linkage analysis was performed using Map Manager QTXb07 (MEER *et al.* 2000). To heuristically search for syntenic groups, loci were grouped simultaneously into linkage groups using a search/linkage criteria of $P = 0.001$. The method suggested by HULBERT *et al.* (1988) was used to estimate total genome size:

$$\text{Genome size (cM)} = \frac{(\text{no. of markers})(\text{no. of markers} - 1)(\text{cM distance at LOD} = 3.0).}{(\text{no. of marker pairs at LOD} \geq 3.0)}$$

Orthology and establishment of conserved synteny: Preliminary orthologies for Ambystoma protein-coding loci were established using BLASTX searches (ALTSCHUL *et al.* 1997) and the nonredundant protein sequence database (Evalue $\leq 10^{-5}$; http://www.ncbi.nlm.nih.gov/blast/blast.cgi). Conserved syntenies were identified by comparing ambystomatid linkage groups with two or more protein-coding loci to human, mouse, and zebrafish genome maps. Genome maps from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and the Human Genome Project at University of California, Santa Cruz (http://genome.ucsc.edu/index.html) were used to locate positions of presumptive orthologues in human, and the Mouse Genome Database (http://www.informatics.jax.org) and ZFIN (http://zfish.uoregon.edu/) were used to locate presumptive orthologues in mouse and zebrafish.

RESULTS

DNA markers segregated according to Mendelian expectation: As a preliminary step toward building a genetic linkage map for these salamander species, we asked whether molecular markers segregated according to Mendelian expectations. We thus compared the proportions of homozygous and heterozygous genotypes for each of the AFLP and RAPD markers as well as protein-coding loci scored in the AxTg mapping panel. This analysis revealed that none of the protein-coding loci and only 5% of the anonymous molecular marker loci (AFLPs and RAPDs) exhibited segregation patterns that deviated significantly from an expected 1:1 ratio of homozygous and heterozygous genotypes. Thus, the vast majority of loci segregated in a Mendelian manner between these closely related species.

A partial genetic linkage map was constructed: We used the 347 markers scored for segregation in the AxTg mapping panel to generate a framework genetic linkage map in which a total of 56 linkage groups were identified

TABLE 2

Primer pairs used to generate AFLP marker loci

Marker prefix ID	Primer pair	Marker prefix ID	Primer pair
A1	E-ACGG/M-CACA	A26	N-ATT/H-AAC
A2	E-ACGG/M-CACG	A27	E-ACGC/M-CTAC
A3	E-ACGG/M-CACT	A28	E-ACGA/M-CACT
A4	E-ACGA/M-CACC	A29	E-ACGT/ M-CACT
A5	E-ACGG/M-CACC	A30	E-ACGT/M-CACA
A6	E-ACGA/M-CACA	A31	E-ACGT/M-CACC
A7	N-AAG/H-CAC	A32	E-ACGT/M-CACG
A8	E-ACGA/H-CACG	A33	E-ACGC/M-CACA
A9	N-AAG/H-TAC	A34	E-ACGC/M-CACC
A10	N-AAG/H-AAC	A36	E-ACGC/M-CACT
A11	N-AAG/H-ATA	A37	N-AAG/H-CAT
A14	N-AGG/H-CAC	A39	N-ACA/H-CAT
A15	N-AGG/H-TAC	A40	N-ATT/H-CAT
A16	N-ACA/H-AAC	A50	E-ACGG/M-CTAG
A17	N-ACA/H-CAC	A52	E-ACGG/M-CTAT
A18	N-ACA/H-TAC	A53	E-ACGG/M-CTAC
A19	N-ACA/H-ATA	A54	N-TGC/H-CAT
A20	N-AAT/H-ATA	A55	N-TGC/H-TAC
A21	N-AAT/H-TAC	A56	N-TGC/H-ATA
A24	N-ATT/H-ATA	A57	N-TGC/H-CAC
A25	N-ATT/H-TAC		

Primers are described in Table 1.

(Figure 1; updated versions of this map also can be found via the world wide web at http://lamar.colostate.edu/~srvoss/SGP/). The majority of these linkage groups ($N = 31$) consisted of four or fewer linked DNA markers (range, 0–61.7 cM). Nevertheless, several large linkage groups comprising as many as 21 loci were identified, 10 of which were >100 cM (range, 101.1–295.1 cM). A total of 48 loci were found to be unlinked. The entire group of linked markers provided 3475 cM of genome coverage. Given that ambystomatid salamanders have a haploid chromosome number of 14, the observation of many small linkage groups suggests that additional markers will be needed to comprehensively map all chromosomal regions. To estimate the genome size of Ambystoma from these partial linkage data, we determined the total number of marker pairs that were linked at LOD > 3.0 and then applied the method of Hulbert *et al.* (1988). A total of 494 significantly linked marker pairs yielded an estimated genome size of 7291 cM.

Protein-coding loci were mapped efficiently using the AxTg panel, revealing conserved syntenies with other vertebrates: The generation of a partial genetic linkage map, comprising principally anonymous markers, nevertheless allowed us to ask whether protein loci can be mapped easily using the AxTg mapping panel and whether regions of conserved synteny can be identified in salamanders relative to other vertebrates. To these ends, we targeted 26 protein-coding loci for mapping, which represented a diversity of developmental and physiological protein products. Several of these loci are known to be genetically and physically linked in other vertebrate species. Of the 26 loci targeted, 24 (92%) exhibited nucleotide polymorphism between *A. mexicanum* and *A. t. tigrinum* (the exceptions were *POMC* and *TOP1*). Table 5 shows map positions for these 24 protein-coding loci relative to presumptive orthologues from human, mouse, and zebrafish. Despite the large genetic and physical size of the salamander genome, we identified five conserved syntenies between salamander and other distantly related vertebrate species. Four of the conserved syntenies include loci of *HOX* gene complexes. A conserved synteny containing *HOXA* loci was identified by mapping *HOXA4* and *HOXA9* to LG 43. A second conserved synteny was identified by mapping *HOXB13* and three additional loci (*THRA*, *COL1A1*, and *DLX3*) to LG 23. All four of these loci are located on human chromosome 17 (Hsa 17), and combinations of these genes are syntenic in zebrafish and mouse. A third conserved synteny was identified by mapping *HOXC10* and *WNT1* to LG 7. On Hsa 12, *HOXC* complex genes are syntenic with *WNT1*. A fourth conserved synteny was

TABLE 3

Primers used to generate RAPD marker loci

UBC134	AACACACGAG
UBC144	AGAGGGTTCT
UBC274	GTTCCCGAGT
UBC549	CCGGCTTATG
UBC608	GAGCCCGAAA
UBC722	CCTCTCCCTC

TABLE 4

Primers used to amplify protein-coding loci

Locus	Reference/GenBank accession	Primers	Product size (bp)	Detection method
Proopiomelanocortin (*POMC*)	D. Parichy (unpublished data)	F-CCGAAGGAACAGCACGGGGGCCAGT R-TCTTGCCTTTCCATGGCGTCTCCCTCCT	156	NP
Topoisomerase 1 (*TOP1*)	S. Voss (unpublished data)	F-CTTGCTYTGAGAGCWGGCAATGAAAAGG R-TTCTCMACAGGSACYTTGTTRTAG	183	NP
Distaless-homeobox3 (*DLX3*)	U59480	F-GGCGAGGCGCACCTCTCCAACTGGTGA R-AGGCTCCCACCTTCTGAGTTGGGAAGG	184	RD *Dpn*II
Collagen 1a (*COL1A1*)	S. Voss (unpublished data)	F-CACCGAAGCCTCCCAAAACATCAC R-GAGCCCTTCCATCTTAGTCGT	1150	RD *Eae*I
Red cone visual pigment (*RCP*)	AF038947	F-TCCCATACGCAGAAAACCA R-TACCGCAAGGAAATGGGAAGCAGT	327	SSCP
Ultraviolet cone visual pigment (*UVCP*)	AF050653	F-GAAGCGAGGTCTGCAAGGAGGATG R-TACCGCAAGGAAATGGGAAGCAGT	301	RD *Rsa*I
Rod-specific transducin (*GNAT1*)	AF050653	F-CCCATGCTGCGGAAGAGA R-TTAGAGGGCGAAAAAGTGTCATC	486	RD *Dra*I
Cone-specific transducin (*GNAT2*)	AF050654	F-ATGCACAACCCCGATACTACCAAC R-AGTCGGGCCCTTTAAATGAATACG	432	ASA
Fork head protein g1b (*FOXG1B*)	U43547	F-GATGGACTGCTAATCTTTTGAGACCC R-AACAAGTGGGGCAAACAACCAACCAGC	269	RD *Mnl*I
Chondroitin sulfate proteoglycan 2 (*CSPG2*)	Parichy *et al.* (1999)	F-GACAGCAGTGGAGACAGATGAAGAAC R-TCTACAGCGACGGGAGATGACG	708	RD *Rsa*I
Homeo box a4 (*HOXA4*)	D. Gardiner (unpublished data)	F-CTGCAGCACTGGCAGGTCCTGCTG R-TGGCGAGCGCATCTTGGTGTTGG	1190	LV
Homeo box a9 (*HOXA9*)	U20941	F-AGGCACTACGGCATCAAGCC R-ACTCCTTAGGCCGGTCCTTGT	430	SSCP
Homeo box b13 (*HOXB13*)	AF298184	F-GTCTCCTTTTGCTTGATTTCCG R-TCACCAAAATCACAGAACACCTGC	376	RD *Nsi*I
Homeo box c10 (*HOXC10*)	AF298185	F-TGCCCATACAGCGAGCTTACAGACAT R-GCACACGAGACCCACAACAACAAAA	413	RD *Pst*I
Homeo box d8 (*HOXD8*)	AF031246	F-CGGACTGTAAATCGTCCAGTGGTCAC R-GCTGGGAACTTGTCTTTGTTGTTCTC	738	RD *Hpa*II
Homeo box d11 (*HOXD11*)	AF031481	F-ACATCATCTTCGGGACTGTAACAAGG R-AACCAGGTCCCCTTAGTTCTATTCAGG	699	RD *Sau96I*
Glyceraldehyde-3-phosphate dehydrogenase (*GAPD*)	J-X. Ma (unpublished data)	F-AACGCCTCCTGCACCACCAACT R-CTCTGCCATCGCGCCACATTT	336	RD *Dpn*II
Kit ligand (*KITLG*)	AF119044	F-ACCTCCCAAGTGACTACAGTATATC R-ACCTCCCACTCAAACAGCTTC	263	SSCP
Platelet-derived growth factor receptor α (*PDGFRA*)	D. M. Parichy (unpublished data)	F-CGGGTCATTGAGTCCATCAGCC R-CAGTGGGTTTTAACATTTTCACAG	1000	RD/SSCP *Rsa*I
Rhodopsin (*RHO*)	U36574	F-CCAAGAGTTCTGCCATCTACAATCCAG R-CGCAGGAGAAACCTGGCTGGAAGACAC	1200	ASA

(*continued*)

TABLE 4

(Continued)

Locus	Reference/GenBank accession	Primers	Product size (bp)	Detection method
Wingless-type MMTV integration site family, member 1 (*WNT1*)	AF001958	F-CAGCGCCAAATCACACCTCA R-TCCATAAACGCTGCCAAAATAAAG	400	*Hin*cII
Sodium bicarbonate cotransporter (*SLC4A4*)	AF001958	F-TCCGCTTGCAGCAGTCTCCTTGCTCTC R-TAACGGCCTGATTGATGACCAGCGAAG	223	SSCP
Thyroid hormone receptor α (*THRA*)	Voss *et al.* (2000)	F-TCCTGACAGTGAGACGCTG R-TAGGGCCACTTCGGTGTCATC	150	SSCP
Thyroid hormone receptor β (*THRB*)	Voss *et al.* (2000)	F-CCCGGAAAGCAAAACCTT R-TATCATCCAGGTTAAATGAC	136	RD *Xcm*I
Aldehyde oxidase (*AOX1*)	K. Mason (unpublished data)	F-TACAAGGACACGAATAGCGAGACA R-CCACTGAGAACTTTAGGGGTATGA	205	RD *Dde*I
v-kit (*KIT*)	K. Mason (unpublished data)	F-TCCGTGTGGGAATCCAGTCACT R-AGATGGCATATCTGGGACATATTC	950	LV

Polymorphisms were revealed by restriction digestion (RD), length variation (LV), single-strand conformation polymorphism (SSCP), or allele-specific amplification (ASA). No polymorphism (NP) was detected for two loci.

identified by mapping *HOXD8*, *HOXD11*, and *AOX1* to LG 9. On Hsa 2, *HOXD* complex genes are syntenic with *AOX1*. Finally, a fifth conserved synteny was identified by mapping *PDGFRA-KIT* to LG 12.

Several syntenies unique to axolotl also were observed: *KITLG-UVCP*, *FOXG1B-CSPG2*, and *GAPD-d* (*d* = white color mutant; Parichy *et al.* 1999). *UVCP* has not been identified in mammalian models and it has yet to be mapped in reptiles and fishes. The linkage of *FOXG1B* and *CSPG2* in Ambystoma is not observed in human, as presumptive orthologues of these genes are found on Hsa 14 and Hsa 5, respectively. Linkage between *Gapd-d* presumptively localizes the white color mutant to a conserved, vertebrate chromosomal segment containing loci that are syntenic with *GAPD*.

DISCUSSION

This study represents the first attempt to examine amphibian genome structure by determining the order and location of hundreds of molecular markers. In turn, the partial genetic linkage map we constructed has implications for the feasibility of genetic mapping in this research model organism, the size and structure of ambystomatid genomes, and genome evolution in vertebrates.

Genetic linkage analysis in Ambystoma: Our crossing design using the two interfertile species, Mexican axolotl and tiger salamander (*A. mexicanum* and *A. t. tigrinum*), allowed genetic mapping to be accomplished in a highly efficient manner. Nucleotide variation was found for all but two protein-coding loci between these species and all polymorphic loci were subsequently mapped. Clearly, considerable molecular differences have accrued between these two species since their estimated time of divergence some 10–15 mya (B. Shaffer, personal communication). However, these differences do not greatly influence the segregation of molecular marker genotypes in hybrid crosses, and, more generally, do not greatly affect the viability and fertility of hybrid offspring (Voss 1995). The extent to which fertile hybrid crosses can be made among salamanders is somewhat unusual for vertebrate species (Voss and Shaffer 1996). One possible explanation is a conservative rate of chromosomal evolution for ambystomatid salamanders as compared to other vertebrates. For example, giemsa banding patterns of *A. mexicanum* and *A. t. tigrinum* are identical for 4 of the 14 chromosomes, and there is no gross evidence for translocations or inversions among the remainder (Cuny and Malacinski 1985). If chromosomal evolution has occurred at a relatively lower rate in Ambystoma, it may be a particularly useful model for reconstructing the ancestral tetrapod condition that subsequently gave rise to the human genome. Indeed, chromosome number in Ambystoma approximates that expected of the common ancestor of fish and amniotes (Postlethwait *et al.* 2000).

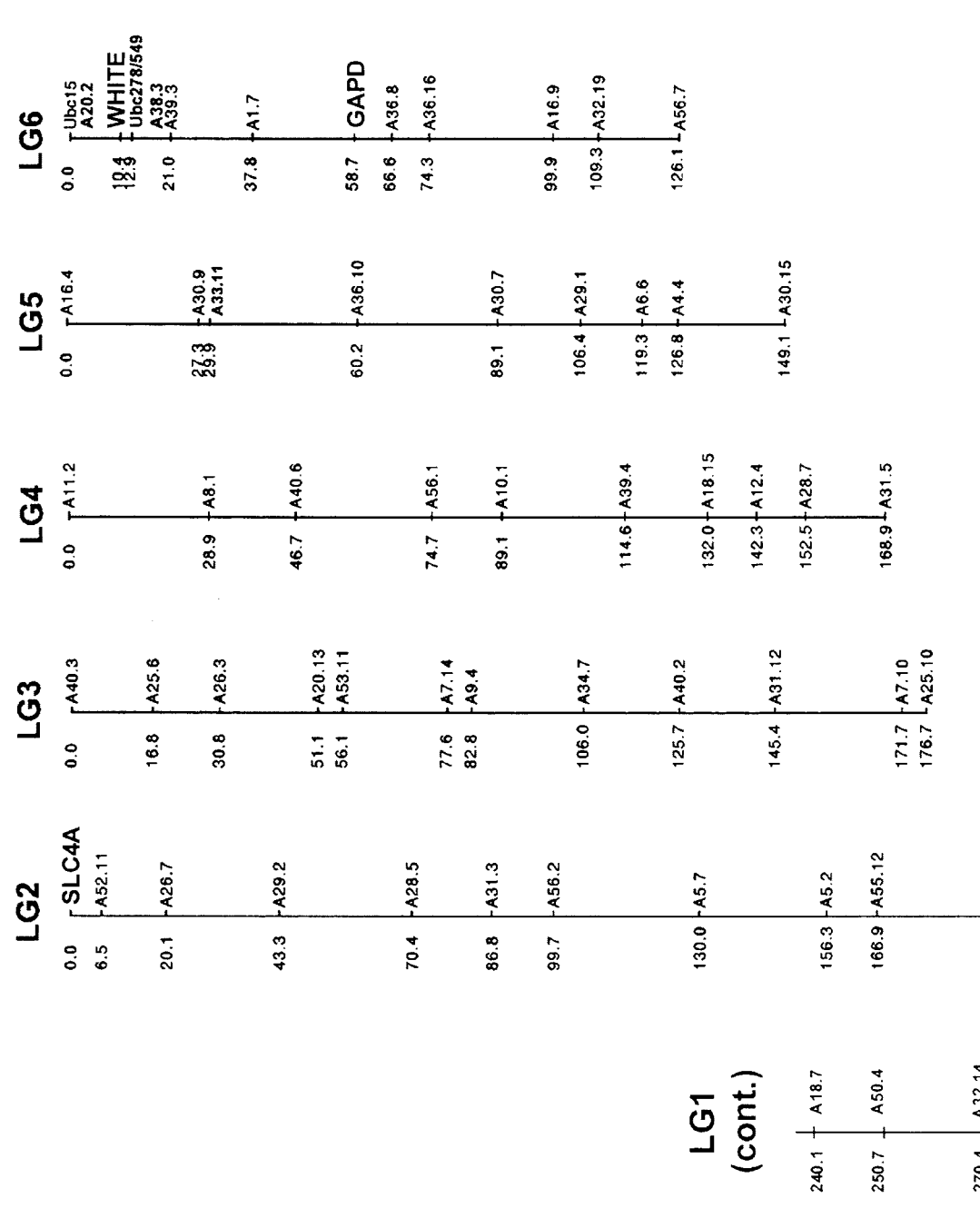

FIGURE 1.—Partial genetic linkage map for Ambystoma using the AxTg panel. For each linkage group, distance (centimorgans) is shown to the left; DNA markers are shown to the right.

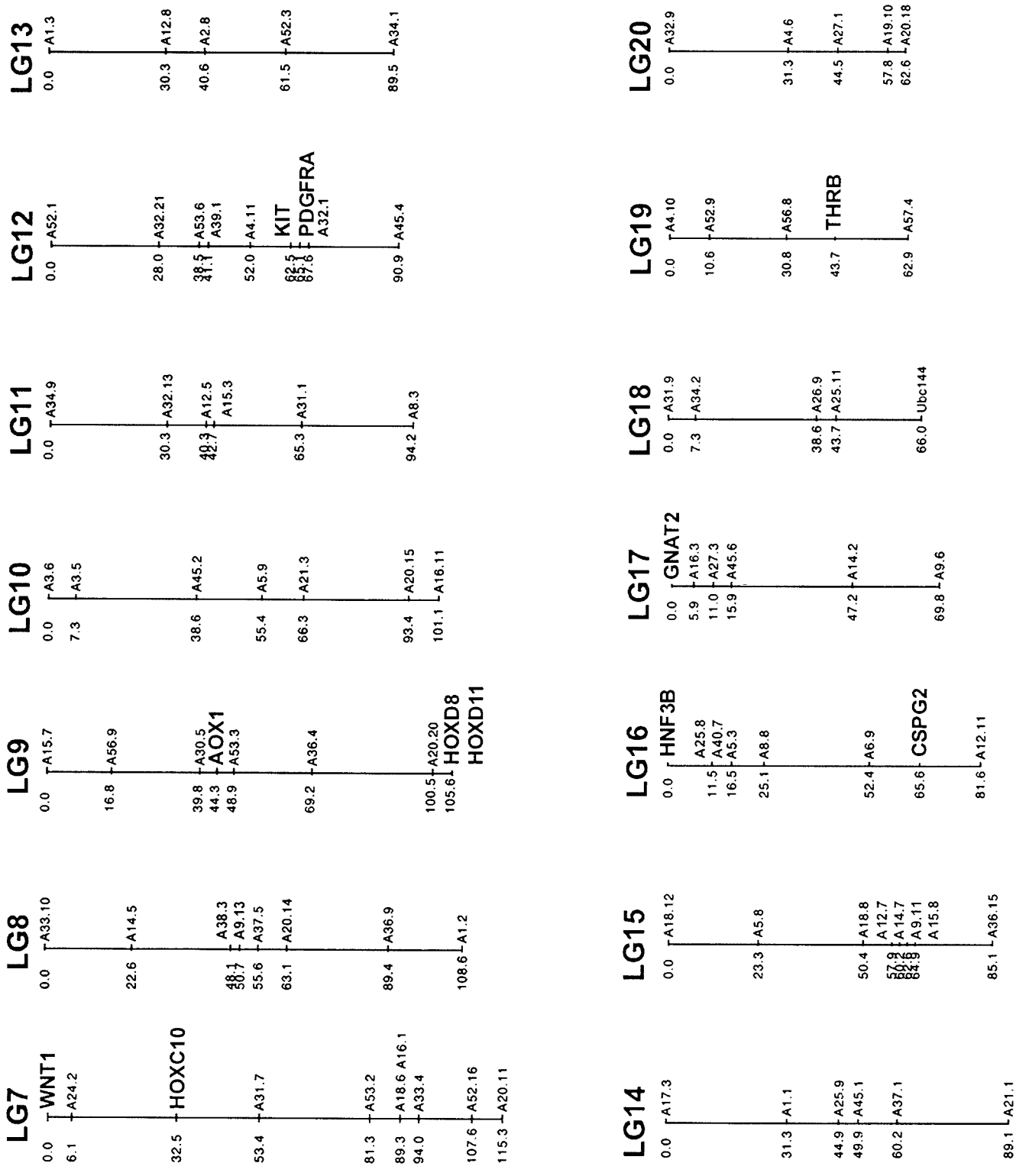

FIGURE 1.—*Continued.*

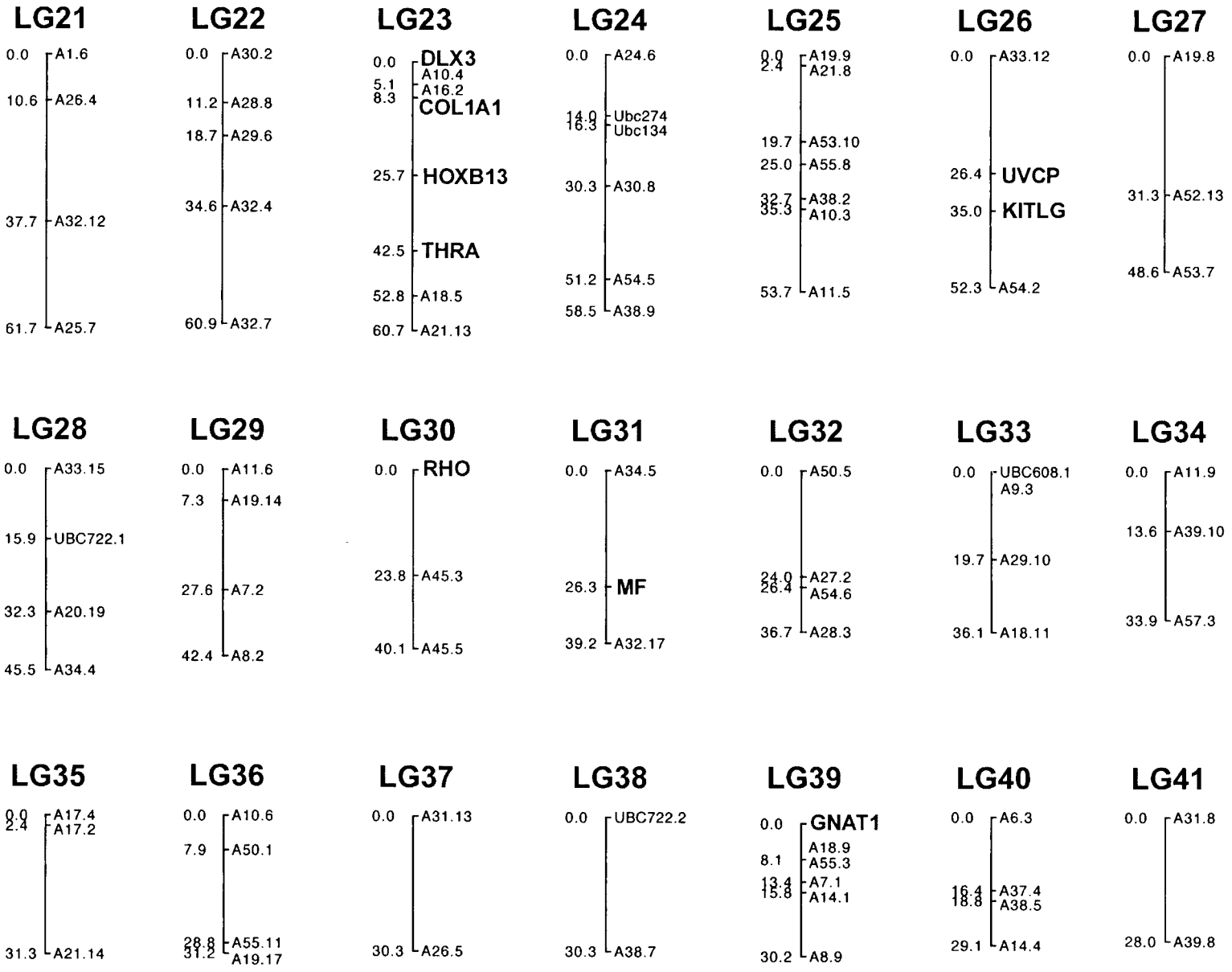

FIGURE 1.—*Continued.*

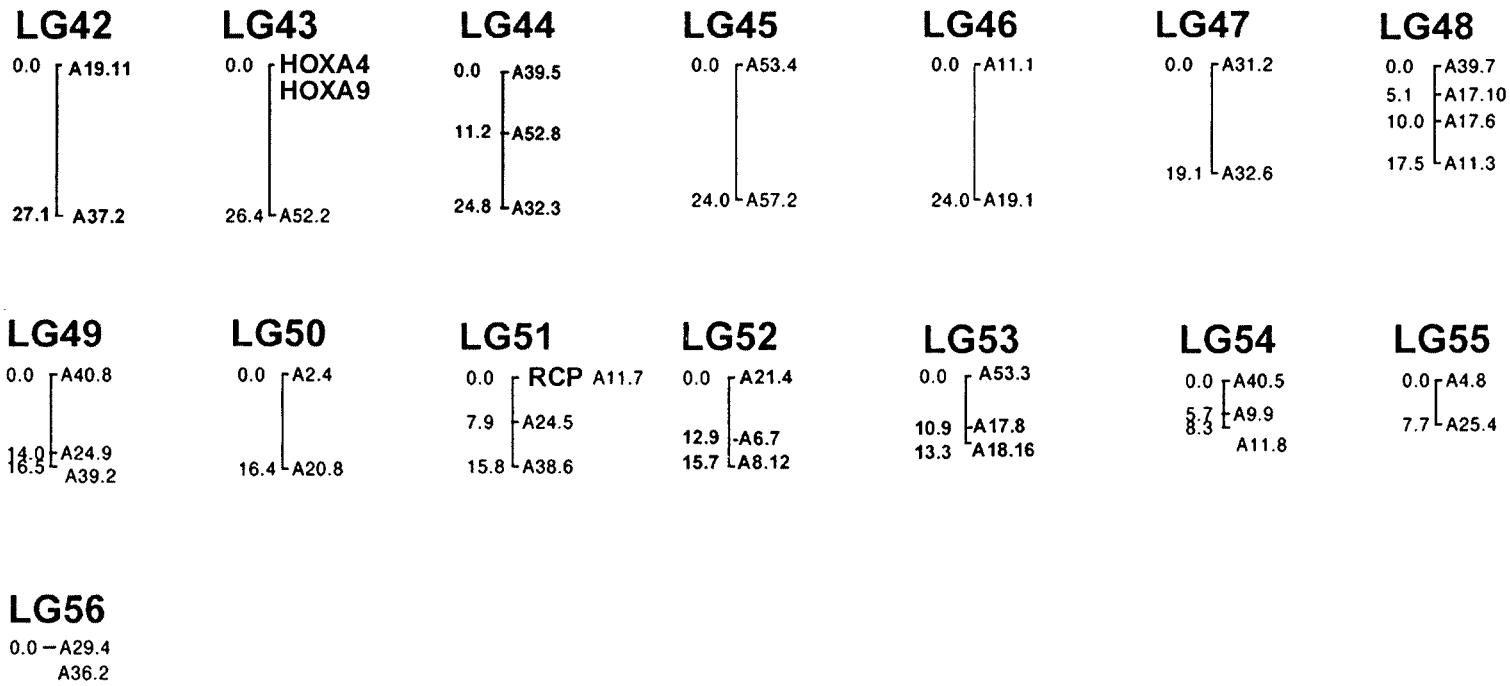

FIGURE 1.—*Continued.*

TABLE 5

Comparison of linkage assignments for protein-coding loci mapped in Ambystoma

Locus	Ambystoma	Human	Mouse	Zebrafish
SLC4A4	2	4q21	UK	UK
KIT	4	4q11-q12	5	20
PDGFRA	4	4q11-q13	5	20
GAPD	6	12p13	6	16
HOXC10	7	12q13	15	11,23[a]
WNT1	7	12q13	15	23
AOX1	9	2q33	1	UK
HOXD8	9	2q31-q37	2	9[a]
HOXD11	9	2q31-q37	2	9[a]
FOXG1B	16	14q12	12	UK
GNAT2	17	1p13	3	UK
THRB	19	3p24.3	UK	UK
DLX3	23	17q21.3-q22	UK	12
COL1A1	23	17q21.3-q22	11	3[b]
HOXB13	23	17q21-q22	11	3,12[a]
THRA	23	17q11.2	11	3
UVCP	26	UK	UK	UK
KITLG	26	12q22	10	UK
RHO	30	3q21-q24	6	8
CSPG2	38	5q12-q14	13	UK
GNAT1	39	3p21	9	UK
HOXA4	43	7p15-p14	6	16[a]
HOXA9	43	7p15-p14	6	16[a]
RCP	51	Xq28	X	UK

UK, unknown.

[a] This particular *Hox* gene has not been mapped in zebrafish. The number refers to the presumptive linkage group(s) containing the corresponding Hox gene member.

[b] A duplicate ortholog of this gene is found on LG12.

Estimate of the ambystomatid genetic map: Our genetic linkage map estimate of 7291 cM is the largest reported for any organism. This is not unexpected because salamanders are known to have physically large genomes (Morescalchi 1975), and genetic and physical map sizes often are correlated (Sybenga 1996). The *C* value of *A. tigrinum* is estimated at 32×10^9 bp (Straus 1971), an order of magnitude larger than most vertebrates but only moderate in size when compared to other salamanders (Graur and Li 2000). Nevertheless, it is possible that our estimate is biased upward because we used a small mapping panel. Moreover, this estimate also assumes a random sampling of molecular marker loci, an assumption that may not always be met (Becker *et al.* 1995; Kiem *et al.* 1997; Saliba-Colombani *et al.* 2000). For example, a recent analysis of tomato found that >50% of mapped anonymous molecular marker loci (AFLPs and RAPDs) were located in heterochromatic regions near centromeres (Saliba-Colombani *et al.* 2000). Our goal of mapping 500 additional protein-coding loci, which is underway, ultimately will provide a comprehensive genome map and a more accurate estimate of genome size.

Comparative gene mapping: Despite the relatively large size of the salamander genome, conserved syntenies were identified among Ambystoma, zebrafish, human, and mouse. Our data indicate that both tightly (*e.g.*, *PDGFRA-KIT*) and distantly (*HOXD8,11-AOX1*) linked genes in human are syntenic in Ambystoma. Moreover, our results show that *DLX3-COL1A1-HOXB13-THRA* are linked in Ambystoma as they are on Hsa 17. This finding provides support for the hypothesis that a large conserved block of Hsa 17 loci has been maintained in zebrafish and human (as well as cat, rat, and mouse) since their divergence from a common ancestor some 450 mya (Postlethwait *et al.* 2000). Our result suggests that Hsa 17 loci have remained linked for an additional 350 million years of independent evolution within the amphibian clade. Although linkage over this time frame might suggest some type of functional constraint, it is interesting to note that Hsa 17 loci are distributed among multiple chromosomes in chicken, indicating that if such a constraint exists it cannot be absolute (Groenen *et al.* 2000). Moreover, comparison of syntenic loci among chicken, human, and mouse suggests greater overall conservation between chicken and human than between chicken and mouse, despite the fact that human and mouse shared a more recent common ancestor (Burt *et al.* 1999). Clearly, the problem of reconstructing vertebrate genome evolution is complex and will undoubtedly require multiple representative genomes from each of the major vertebrate groups to distinguish lineage-specific patterns and processes (*e.g.*, genome duplications in fishes, translocations in mouse, chromosomal fissions in chicken) from true conservation and homology. The development of genetic linkage maps for anuran amphibians (*e.g.*, Rana, Hotz *et al.* 1997; Xenopus, http://www.virginia.edu/~develbio/trop/), will contribute to these efforts and provide an interesting comparison to salamander.

Extent of conserved synteny between salamander and human: Knowledge of the extent of conserved synteny between two species is informative for estimating rates of chromosome evolution and for identifying orthologous loci (International Human Genome Sequencing Consortium 2001). Using the human genome working draft (http://genome.ucsc.edu/index.html), we determined the physical distance between syntenic loci that presumably flank conserved chromosomal segments in salamander. Overall, the genetic linkage distances (centimorgans) of conserved segments in salamander were positively correlated to the physical distances (megabases) observed in human (Figure 2). For example, the linkage distance of *PDGFRA-KIT* was estimated at 2.60 cM in salamander and 0.36 Mb in human, while the linkage distance of *AOX1-HOXD* was estimated at 62.3 cM in salamander and 25.69 Mb in human. If large conserved syntenies are common between salamander and human, this would greatly simplify the search for orthologous candidate genes. However, we note that conserved syntenies reported in this article correspond

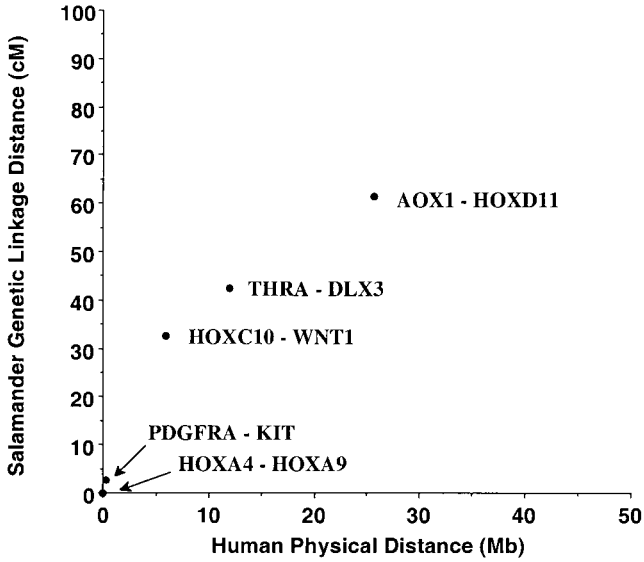

FIGURE 2.—Genetic linkage distance (cM) of conserved segments identified in salamander relative to physical distance (Mb) in human.

primarily to chromosomal segments containing *HOX* clusters, which may bias upward estimates of synteny conservation because of functional constraints associated with *cis*-regulatory elements (*e.g.*, VAN DER HOEVEN *et al.* 1996; KMITA *et al.* 2000).

Conclusions: Our study shows that genetic linkage analysis is an efficient method for identifying homologous chromosomal segments between ambystomatid salamanders and genomically well-characterized vertebrate models. Given the importance of Ambystoma as a research model (http://www.indiana.edu/~axolotl/) and its pivotal phylogenetic position for understanding evolutionary transitions from fishes to amniotes, additional protein-coding loci are being mapped to allow candidate gene identification within the ambystomatid system and to provide an amphibian perspective to vertebrate genome evolution.

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