Genome evolution and speciation genetics of clawed frogs (Xenopus and Silurana)

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1. ABSTRACT

Speciation of clawed frogs occurred through bifurcation and reticulation of evolutionary lineages, and resulted in extant species with different ploidy levels. Duplicate gene evolution and expression in these animals provides a unique perspective into the earliest genomic transformations after vertebrate whole genome duplication (WGD) and suggests that functional constraints are relaxed compared to before duplication but still consistently strong for millions of years following WGD. Additionally, extensive quantitative expression divergence between duplicate genes occurred after WGD. Diversification of clawed frogs was potentially catalyzed by transposition and divergent resolution - processes that occur through different genetic mechanisms but that have analogous implications for genome structure. How sex determination is maintained after genome duplication is fundamental to our understanding of why allopolyploidization is so prevalent in this group, and why clawed frogs violate Haldane's Rule for hybrid sterility. Future studies of expression subfunctionalization in polyploids will shed light on the role and purviews of *cis*- and *trans*- regulatory elements in gene regulation.

2. INTRODUCTION

Clawed frogs (genera Xenopus and Silurana) are model organisms for biology (1, 2). Considerable scientific resources have been developed for this group including DNA sequences of thousands of expressed genes in the tetraploid species X. laevis, a complete genome sequence of the diploid species Silurana tropicalis, transgene technology, and prefabricated expression microarrays. That multiple species of clawed frog are polyploid makes them useful models for studying evolution after vertebrate genome duplication. Sex-differences in reproductive barriers between species of clawed frog also make them compelling subjects for exploring the genetic basis of speciation. Their ability to form viable hybrid individuals facilitates the study of *cis*- and *trans*- acting factors in gene expression. This review will explore our current understanding of these issues as they relate to clawed frogs.

3. EVOLUTIONARY RELATIONSHIPS

Phylogenetic relationships are generally represented in terms of the hierarchy of monophyletic groups – or clades – wherein each group includes all descendants of their collective most recent common ancestor. However, the recent evolutionary history of clawed frogs is characterized by a combination of "regular" bifurcating speciation, in which one ancestral species splits into two descendant species, and also reticulating speciation via allopolyploidization, in which two ancestral species merge into one descendant species (Figure 1A). As a result, the phylogenetic tree of clawed frogs composed of branches that split and also merge as one goes from past to present. Speciation by allopolyploidization occurs when inter-species hybridization leads to fusion of their complete genomes into a new descendant species. The genome of a new allopolyploid species is therefore duplicated relative to that of each ancestral species. Each species of clawed frog is divergent on a genetic level (3, 4) and has a distinct male vocalization; X. laevis includes populations/subspecies with distinct male vocalizations (M. Tobias and D. Kelley, personal communication; 5, 6).

Multiple methods exist for estimating evolutionary relationships, and their implementation depends on the type of data being analyzed, the philosophical perspective of the researcher, and computational efficiency. Maximum parsimony is an optimality criterion that favors the evolutionary hypothesis (phylogenetic tree) that requires the fewest number of changes along its branches in order to account for the differences between the taxa. Maximum likelihood and Bayesian analyses favor an evolutionary hypothesis that maximizes the likelihood or posterior probability of the data, respectively, given a model of evolution and (for Bayesian analysis) prior distributions for each parameter in the model (reviewed in 7).

3.1. Pipid genera

Clawed frogs (genera Xenopus and Silurana) are aquatic anurans in the tongueless frog family Pipidae. This family includes four extant genera native to Africa: the African clawed frogs (Xenopus), the tropical clawed frogs (Silurana), the African dwarf frogs (Hymenochirus), and Merlin's clawed frogs (Pseudhymenochirus), and one extant genus from the New World: the Surinam toads (Pipa). Some phylogenetic relationships concerning pipid frogs are well established. The families Pipidae and the Mexican burrowing toad family Rhinophrynidae are a clade, Xenopus and Silurana are a clade, and Pseudhymenochirus and Hymenochirus are a clade (Figure 1B; 1, 3, 8-13). Monophyly indicates that Silurana could be subsumed by Xenopus and that Pseudhymenochirus could be subsumed hv Hymenochirus, but trenchant molecular, cytogenetic, and/or morphological differences argue for the independent recognition of each of these genera (3, 8). For instance, Silurana includes species multiples of 20 chromosomes (20 or 40) whereas Xenopus includes species with multiples of 18 chromosomes (36, 72, oe 108). Estimates of the time of whole genome duplication (WGD) in Xenopus, which occurred after the split with Silurana, have an upper limit of roughly 53-64 million years ago and a lower limit of roughly 21 million years ago (3, 14). In Silurana, WGD is estimated to have occurred about half as long ago (3).

Other relationships among pipid genera remain poorly resolved and vary depending on the dataset and analytical methods. Monophyly of (*Pipa* + *Hymenochirus* + Pseudhymenochirus). with respect to (Xenopus + Silurana) was supported by morphological data (1, 10). Monophyly of (Pipa + Hymenochirus) with respect to (Xenopus + Silurana) was weakly supported by molecular data from mitochondrial DNA and a portion of the RAG1 nuclear gene (3, 12). When analyzed using maximum parsimony, molecular data from linked mitochondrial genes and three (presumably) unlinked nuclear genes also supported this latter relationship (13). However, a sister relationship between Pipa and (Hymenochirus + Xenopus + *Silurana*) was recovered when the same data were analyzed using maximum likelihood and Bayesian methods (13). Another recent study recovered a third relationship among these genera: a sister relationship between Hymenochirus and (Pipa + Xenopus + Silurana) (11). That study was based on sequences from mitochondrial DNA and at least one nuclear gene from each of these genera, but the nuclear data were patchy in that no comparable nuclear sequences were obtained from all of these genera. One of the major challenges to resolution of relationships among pipid genera is the identification of the phylogenetic root (11).

Evolutionary relationships among these genera have implications for the calibration of a molecular clock for anurans based on continental drift of Africa and South America. This is because the distribution of pipid frogs spans the Atlantic Ocean and presumably was continuous before the breakup of Gondwanaland (3, 13). Resolution of these relationships is thus challenged by the advanced age of the unresolved phylogenetic relationships (Figure 1B), which are about 100 million years old or so. Ancestral polymorphism, which can lead to discordance between genealogical and phylogenetic relationships, could also contribute to the lack of concordance in these studies. An example of this is revealed by analyses of mitochondrial and nuclear DNA of clawed frogs which recover strongly supported but incongruent relationships in clawed frogs with respect to the placement of X. clivii (see next section; 3, 4, 12).

3.2. Clawed frog species, relationships, and inferred ancestors

Seventeen species of clawed frog have been described, and other species have been identified but are not fully documented. The only known diploid species of clawed frog is Silurana tropicalis; the genome of this species contains 20 chromosomes and is currently being Xenopus and Silurana both include sequenced (15). tetraploid species with 36 or 40 chromosomes respectively. In *Xenopus*, eight tetraploids have been documented: X. laevis, X. gilli, X. clivii, X. largeni, X. fraseri, X. pygmaeus, X. borealis, and X. muelleri. In Silurana, only one tetraploid has been described: S. epitropicalis. Xenopus also includes six octoploid species (which have 72 chromosomes): X. wittei, X. vestitus, X. andrei, X. boumbaensis, X. amieti, and "X. new octoploid" - whose description is in review (16) - and at least two dodecaploid species (which have 108 chromosomes): X. longipes and X. ruwenzoriensis. A few additional species have been



Figure 1. Speciation and evolutionary relationships of tongueless frogs (Pipidae). (A) Clawed frogs diversified by "regular" speciation where one ancestor diverges into two descendants, and by allopolyploid speciation where two ancestoral species are fused into one allopolyploid descendant species. (B) Some relationships are well resolved: Pipidae is sister to Rhinophrynidae but there is not a consensus concerning the relationships among three clades that together include five pipid genera. (C) Relationships among clawed frogs as reflected by autosomal DNA; modified from (4). Daggers indicate inferred ancestors for which an extant same-ploidy level descendant is not known. Blue lineages in (C) highlight a putative allopolyploidization event in *Xenopus*.

identified but are not vet described. In West Africa there is a widely distributed tetraploid that has been called "Xenopus muelleri West" (5, 17) or "Xenopus new tetraploid" (3, 4, 12). Also in west Africa are two other undescribed tetraploids that have been called "Silurana new tetraploid 1" and "Silurana new tetraploid 2" (3, 4, 12). Xenopus laevis and X. fraseri may include multiple species (12, 18). The evolutionary history of X. boumbaensis was recently re-evaluated as a result of a misidentified sample, leading to the identification of another undescribed but closely related dodecaploid individual, "X. cf. boumbaensis" (4). It is not yet clear whether X. cf. boumbaensis is in fact a species in terms of being an autonomous evolutionary lineage that persists through time (19), or just an individual allopolyploid progeny from a hybrid cross between X. fraseri and X. boumbaensis (4).

Within Xenopus, various "subgroups" have been defined (5, 20-22), but many of these groups do not contain all of the descendants of their most recent common ancestor (i.e. they are not monophyletic) or relationships among the species in the group remain unresolved. Following Kobel et al. (5), for example, the "laevis" subgroup includes the tetraploid species X. laevis, X. gilli, and X. largeni. Xenopus laevis and X. gilli are clearly sister species, but existing molecular data do not resolve the relationship of X. largeni with respect to (X. laevis + X.gilli) and some of the other species (Figure 1C; 3, 4, 12). The "muelleri" subgroup includes X. muelleri, X. borealis, X. new tetraploid / X. muelleri "west", and X. clivii (5). Analysis of two tightly linked autosomal genes indicate that this subgroup is a clade (4, 12), but analysis of mitochondrial DNA suggests that it is not (3). The "vestitus-wittei" group includes X. vestitus, X. wittei, and X. new octoploid, the "longipes" group includes only X. longipes, and the "fraseri" group includes the other species of Xenopus (5). The "vestitus-wittei", and "fraseri" groups are not monophyletic as a result of reticulating relationships among subgenomes of these allopolyploid species (Figure 1C). Genes carried by X. longipes, are also not monophyletic as a result of allopolyploidization, but this species now appears to be an independent evolutionary lineage.

Tetraploidization occurred once in Xenopus and once in Silurana to generate the most recent common ancestor of the tetraploids in each genus (12). Octoploids originated independently three times and dodecaploids originated independently two or three times, depending on the species status of X. cf. boumbaensis (4). An interesting aspect of this history is that multiple ancestral species are inferred even though there is not a known extant descendant species with this ploidy level (Figure 1C). These ancestral species may be extinct in terms of existing as an autonomous species, but their genomes persist in combination with others in extant allopolyploids. Alternatively, descendants of these ancestors might also exist today, providing motivation for further fieldwork and molecular studies. A scenario involving inferred but extinct ancestors contributing their genome to form an extant polyploid has also been suggested in the evolution of a tetraploid frog, Hyla versicolor (23).

4. SPECIATION

4.1. Bifurcation

Although most extant clawed frogs are allopolyploid, about twice as many instances of "regular" bifurcating speciation occurred during their evolution (Figure 1 $\overset{\circ}{C}$; 4). Bifurcating speciation can be initiated by physical barriers to dispersal between populations in different geographic locations (allopatric speciation) or by barriers to reproduction between populations that share a habitat (sympatric speciation). Variation within a species could be partitioned into geographically discrete regions by a barrier to dispersal such as a mountain, or its distribution could change gradually along a geographic cline if the barrier to dispersal were geographic distance. If a physical barrier to dispersal is present for long enough then genetic barriers to reproduction also will arise due to independent mutations that occur in each isolated lineage or population. Pipids once were more widely distributed in parts of the Middle East and North Africa (24, 25) but, probably as a combined consequence of northerly drifting of Africa and natural climatic fluctuations, the contemporary distribution of clawed frogs is restricted to sub-Saharan Africa. Within this region, they occur in slow moving or stagnant ponds over a striking diversity of altitudes, temperatures, habitat types, and pHs. There is evidence for barriers to dispersal over small geographic distances - perhaps as a consequence of marine inundation – such as the divergent western and eastern populations of X. gilli that are separated by only 100 km (3, 26, 27). There are also species whose distribution is extremely small, such as X. longipes, suggesting a recent origin and/or low dispersal and restrictive habitat requirements that could promote speciation. In contrast, there is also evidence for extensive gene flow or dispersal over long distances in some species - genetically similar populations of X. laevis in Nigeria and in Botswana, for example, are separated by thousands of kilometers.

Reproductive barriers maintain species autonomy, and these barriers can be complete or partial, prezygotic or postzygotic, and can be maintained by genetic or by environmental barriers. A key insight into understanding the genetic basis of speciation was the realization that (a) genetic incompatibilities generally involve new (derived) variation rather than ancestral variation in each individual, and that (b) this new variation is often at different but interacting loci (28-30). That epistatic interactions between loci (Dobzhansky-Muller incompatibilities) could explain the origin of reproductive barriers between individuals, population, or species overcomes problems with unfit heterozygotes in models where reproductive barriers arise from conflict between alleles at only one locus.

In addition to being caused by divergence, genetic barriers can also arise as a consequence of changes in genomic organization by transposition (Figure 2A; 31) or by divergent resolution – gene duplication followed by asymmetric pseudogenization (one duplicate becomes a pseudogene) in different populations (Figure 2B; 32, 33, 34). A variety of tandem repeats and transposable elements



Figure 2. Changes in genomic architecture can cause reproductive incompatibilities if a crucial gene is affected. (A) Transposition in a diverged population causes one sixteenth of F_2 backcross progeny to lack a gene. (B) Asymmetric pseudogenization of duplicate genes ("divergent resolution") also causes one sixteenth of the F_2 backcross progeny to lack a gene. In addition to their possible roles in speciation, transposition and divergent resolution can also facilitate switching of the sex chromosomes.

have been identified in clawed frogs (35-38) and activation of transposable element mobility (or de-repression of mobility) can change gene order and even genome size in hybrids (39). The genomic implications of transposition and of divergent resolution are analogous in that each one results in $1/16^{th}$ of the F2 hybrid progeny that completely lack a locus (Figure 2).

Substantial reproductive incompatibilities exist between species of clawed frogs, despite a comparatively high frequency of allopolyploidization in clawed frogs compared to most other vertebrates. Gametogenesis of F1 hybrids is defective - males are sterile and females produce unreduced eggs (Figure 3; 40, 41, 42). The proportion of unreduced eggs varies between individuals and between clutches and sometimes the unreduced eggs do not contain the full set of maternal chromosomes (40-42). Male F1 hybrids produce less sperm than do parental males, and hybrids have a higher proportion of undifferentiated spermatozoa (43). The reproductive success of hybridization also can be affected by the direction of the cross. For example, hybridization between X. laevis and X. *borealis*, produces more zygotes if the eggs are from X. laevis (44), perhaps due to the jelly coat of X. borealis eggs. Morphology could also play a role in the directionality of success of hybrid crosses due to the combined effects of sexual dimorphism and species dimorphism in size. Fertilization normally requires effective male clasping of the female (amplexus). The arm span (fingertip to fingertip) of adult X. gilli males is smaller than the inguinal girth of adult X. laevis females, suggesting that this direction of F1 hybrid cross would rarely produce progeny (unpublished data; 27). Consistent with this speculation, a naturally occurring putative F1 hybrid carried X. gilli mitochondria, suggesting that it was derived from a cross between an X. laevis male and an X. gilli female, which are more similar in size (26, 27). Barriers to specific directions of F1 hybrid crosses could have implications for speciation by allopolyploidization if gene expression is affected by epigenetic, maternal, or paternal effects (45, 46).

4.2. Reticulation

In clawed frogs, second generation backcrossed hybrid females can produce a clutch comprised of fertile polyploid individuals of both sexes (41, 42) and sympatric speciation could be essentially instantaneous if these polyploid siblings interbreed and if reproductive incompatibilities exist between them and the lower ploidy parental species. Assortative mating within a ploidy level could facilitate genetic isolation of polyploids and fixation of their duplicated genome in a new species (47). Whether WGD (by autopolyploidization or allopolyploidization) is advantageous, disadvantageous, or neutral is difficult to assess without knowing the rates of speciation and extinction of diploids and of polyploids. Polyploidization could be advantageous, for example, if new beneficial dominant alleles evolve frequently (48).

Allopolyploid as opposed to autopolyploid origin of WGD is evinced by duplicated genes (paralogs) within a species that each are evolutionarily more closely related to genes carried by another lower-ploidy level species than they are to each other. Except for the tetraploid ancestor of *Xenopus* tetraploids (tetraploids with 36 chromosomes), all polyploid clawed frogs are definitively derived from an allopolyploid ancestor (4, 12, 21, 22). The most recent common ancestor of *Xenopus* tetraploids probably also originated by allopolyploidization, but this has not been confirmed because diploid descendants of the putative 18 chromosome ancestors of this tetraploid have not been identified (4).

Allopolyploidization has genomic implications beyond those directly related to genome duplication as a result of interactions between diverged subgenomes that are inherited from each ancestral species. These interactions could include exchange of chromosome segments (49, 50), concerted evolution - where molecular variation of one copy of duplicated gene is homogenized by that of another (51, 52), recombination (53), and epistasis (54). Allopolyploidization can also be associated with substantial changes in genomic stability, chromatin, and expression (55-58), although it is clear that the extent of genomic rearrangement after allopolyploidization is variable (59-62). After allopolyploidization, co-adapted proteins that were inherited from one ancestor may function more efficiently with one another than with proteins that were derived from different ancestors (63). Furthermore, the impact on expression divergence of polyploidization and of hybridization in allopolyploid species may be unique, with the latter process potentially initially causing more divergence (64). A potential cost of allopolyploidization but not autopolyploidization is that Dobzhansky-Muller incompatibilities may be present in subgenomes resulting from the first type of genome duplication. In other words, genetically incompatible proteins derived from different parental species may be co-expressed in an allopolyploid. However, if these incompatibilities are not lethal and do not cause allopolyploid sterility, subsequent gene silencing (pseudogenization) or changes in the timing or location of gene expression could mitigate these conflicts.

Allopolyploidization could also offer benefits autopolyploidization. Both alloover and autopolyploidization can increase variation in dosage regulated gene expression, as well as epigenetic phenomena such as mobility of transposable elements, and molecular phenomena orchestrated by dimers, heterodimers, and protein complexes (58). However, these factors should vary to a greater degree immediately after allopolyploidization than after autopolyploidization because each half of an allopolyploid genome diverged in its respective parental species. Hybrid vigor is another potential advantage of allopolyploidization in that it could generate a genomic arena for heterosis, wherein interactions among proteins that were derived from different ancestral species are favored over those among proteins that were derived from the same species (4). Clawed frogs with higher ploidy levels, for example, are resistant to various parasites to which at least one of their ancestors was susceptible (65). Greater allelic diversity might also confer an advantage for allopolyploid genomes. It is also possibility that ecologically-intermediate



Figure 3. Generation of an allopolyploid individual by sequential backcrossing of unreduced hybrid eggs. F1 hybrid females generated from a cross between two disomic tetraploids can produce unreduced eggs – eggs that contain the entire genome of the mother. These eggs can be fertilized by sperm from one of the parental species to produce a fertile female with a genome that is 1.5 times larger than that of either parental species. These backcross females are also capable of producing unreduced eggs that, if fertilized by the sperm of the other parental species, produce a viable and fertile polyploid F3 backcross hybrid individual that carries a full complement of chromosomes from both parental species. In this figure, hybrids are colored according to the proportion of their genome that is derived from each of the blue and green parental species. The ploidy of each species or hybrid is indicated in terms of x, the number of haploid genomes and in terms of n, which is the number of chromosomes in half of a disomic genome in which bivalents rather than multivalents form during cell division. The genome of a diploid species, for example, is 2x and 2n and its normal reduced gametes are x and n. The genome of a disomic tetraploid genome is 4x but 2n and its reduced gametes are 2x and n.

conditions on the edge of the respective ranges of the parental species could favor a chimerical allopolyploid genome over the parental species (42). A benefit in the form of broad ecologically tolerance is suggested by the

large distribution of some tetraploid species such as X. *laevis* and the apparent out survival of 2n=36 tetraploids over 2n=18 diploids in *Xenopus*. However, some tetraploids such as X. *gilli* have very small ranges and



Figure 4. Comparative data can be used to dissect apart changes that occurred immediately following duplication from those that occurred more recently. A phylogeny and genealogy illustrates relationships among *X. laevis* (XL), *X. borealis* (XB), and *S. tropicalis* (ST), and paralogs α and β of *X. laevis* (XL α and XL β) and of *X. borealis* (XB α and XB β). Dotted lines represent diploid species or genes, red lines are tetraploid species or genes immediately after duplication, and blue lines are tetraploid species or genes at a later stage after duplication. Note that because a 2x=18 diploid is not available in *Xenopus* (indicated by daggers), a lineage between nodes 1 and 3 was compared to a lineage between 3 and the terminals. Modified from Chain et al. (71).

specific habitat requirements, and octoploid and dodecaploid species of *Xenopus* also generally have small ranges.

Both allo- and autopolyploid genomes have the potential for unequal chromosome segregation during gametogenesis and consequent reduction in reproductive success. In polysomic genomes each chromosome may have more than one homolog and multivalents or random bivalents form at meiosis. However, divergence can convert a polysomic genome into a disomic genome, such that each chromosome has only one homolog and only bivalents form during meiosis. After WGD, disomic inheritance may not evolve simultaneously in all loci (66-68). But because an allopolyploid genome is an amalgamation of the genomes of two diverged species. disomic inheritance might evolve immediately or more rapidly than in an autopolyploid one, avoiding problems associated with unequal chromosome segregation in a polysomic genome. In the dodecaploid X. ruwenzoriensis, the major histocompatibility complex class I and II shows evidence of polysomic inheritance, possibly as a consequence of recombination or gene conversion between duplicated genes (69). However, disomic inheritance of many alleles occurs immediately in laboratory generated hybrid Xenopus (70), many duplicated loci do not exhibit signs of extensive recombination between duplicated genes in tetraploids (4, 12, 71), and in most species, multivalents rarely form (72). Visible differences in secondary constrictions between duplicated pairs of chromosomes are present (72), though it is not clear whether these differences arose before polyploidization (i.e. in a diploid ancestor) or after the polyploid genome became disomic. Thus. disomic inheritance appears to be the more prevalent mode of gene inheritance in Xenopus tetraploids, and this is perhaps also true in clawed frogs of other ploidy levels.

5. MOLECULAR EVOLUTION AND EXPRESSION AFTER WGD

Whole genome duplication occurred during the evolution of several vertebrate lineages including the

ancestors of all jawed vertebrates (73), teleost fish (74), and salmonid fish (75). Similar to clawed frogs, some of these ancient WGDs may have occurred through allopolyploidization rather than autopolyploidization (76). It is difficult to directly study early genetic transformations in duplicates derived from these ancient WGDs because observable differences between them are the culmination of both early and more recent evolution. Moreover, changes in protein evolution and expression may have been dynamic after WGD, and the earliest changes (or periods of stasis) were likely pivotal in influencing the functional longevity of expressed duplicates (34, 77-79). In very ancient duplicates generated by WGD, substitutions at synonymous sites are often saturated because the same nucleotide position (80, 81). In contrast, divergence between Xenopus duplicates generated by tetraploidization is typically about 0.2 synonymous substitutions per synonymous site (71). The rate ratio of nonsynonymous to synonymous substitutions per site is therefore an informative metric of functional constraints after WGD in clawed frogs but not the older WGDs (14, 82-84).

Within an allopolyploid genome, divergence between paralogs can be greater than the divergence between one of them and an ortholog (two genes whose divergence is a result of speciation, Figure 4). For this reason, in *Xenopus*, because bifurcating speciation occurred after WDG, genetic changes that occurred immediately after WGD can be dissected apart from those that occurred later by analyzing data from multiple tetraploid species in a phylogenetic context (Figure 4; 71). Clawed frogs thus offer a promising model with which to explore the early stages of genome and transcriptome evolution after vertebrate WGD and to evaluate how genome evolution occurred in different stages after WGD.

5.1. Mechanisms for duplicate gene persistence after WGD

Duplication of a portion of a genome or an entire genome has a myriad of genetic implications including decreased pleiotropy, increased exon shuffling and microfunctionalization, buffering of genetic pathways



Figure 5. Genetic fates of duplicate genes include neofunctionalization, subfunctionalization, redundancy, and nonfunctionalization. These fates are the consequence of natural selection, mutation, and epigenetic processes that act on the regulatory and coding regions of the duplicates. In this figure, a protein is represented as a pair of pliers, each component of which constitutes a distinct subfunction of that tool. Neofunctionalization transforms one copy into a new tool (a hammer) with novel function. Subfunctionalization degrades complementary components of the duplicated protein (grayed and dashed) such that the combined activity of both paralogs is then necessary to recover the ancestral function. Non-functionalization completely degrades one of the duplicated proteins. Modified from Chain and Evans (14).

against null mutations, increased diversity of gene expression, increased biological complexity, increased specialization of genes and pathways, and catalysis of speciation by divergent resolution (33, 85-89). Understanding of the mechanisms behind duplicate gene persistence is therefore intertwined with the realization of these various genetic fates.

Unless natural selection acts to preserve functionality of both copies, the inevitable and most common fate of a duplicate pair is loss of function of one copy (pseudogenization; Figure 5). The rate of pseudogenization depends on the length and sequence composition of the duplicated genes, the rate and biases of mutation (including nucleotide substitutions and insertion/deletion mutations), the level of degeneracy of cis- and trans- acting regulatory factors, epigenetic phenomena, the action of transposable elements, and natural selection. The probability of pseudogenization may be influenced by gene function (90, 91) and pseudogenization after WGD can be non-stochastic, repeatable, and can occur immediately or within a few generations (51, 92-96).

Mechanisms that promote the persistence of duplicate genes after WGD - and particularly after WGD by allopolyploidization - may differ from or be more effective than those that operate on duplicates generated by segmental duplication. Whole genome duplication copies entire genetic networks whereas segmental duplication copies only a single gene, fragment of a single gene, or a portion of a network. In many organisms, the genomic scale of duplication is positively correlated with the functional longevity of the resulting copies (67, 97-100). Different functional types of genes tend to be retained after WGD versus after segmental duplication (91, 101). Segmental gene duplication occurs frequently and the resulting duplicates are generally are short lived, typically lasting on the order of only a few million years (34). However, thousands of duplicate genes generated by WGD are still expressed and functional in the tetraploid species X.

laevis, even though polyploidization occurred dozens of millions of years ago (3, 14, 71, 82-84).

After WGD, an incentive to preserve both copies of a duplicated gene can exist without mutation after duplication (Figure 5). For example, interacting duplicates may persist if pseudogenization of one copy disrupts a balanced stoichiometry (57, 102). Redundancy could also generate a novel and advantageous genetic buffer against deleterious mutations that is favored by natural selection (87, 103) or it could cause novel, advantageous dosagedependent phenotypes (104). Even before mutations occur, new beneficial phenotypes could arise if one copy has an incomplete coding or regulatory region, or if preexisting, differently-functioned alleles that were segregating at the ancestral locus independently fix at each paralogous locus (105).

Mutations that confer new function (neofunctionalization) or degrade paralogous function or expression in а complementary fashion (subfunctionalization), or a combination of these possibilities (Figure 5) may also promote the retention of functional copies of duplicated copies. Neofunctionalization can be realized by mutation in one or both paralogs at the level of protein expression or protein function, and it can occur by chance or be driven by positive selection (106-108). Neofunctionalization can also occur by expanding the expression domain of a paralog through recruitment of cis- acting regulatory elements (109). Mutations that degrade function may contribute to preservation of both paralogs if different subfunctions in each one are degraded such that they complement one another (110) or if they compromise activity of both paralogs such that their combined action is necessary to carry out the duties of the single-copy ancestor (111). Degradation of function after duplication (subfunctionalization) is possible at the level of protein function or in a quantitative, spatial, or temporal dimension paralogous expression (110. of 112). If neofunctionalization or subfunctionalization contribute to duplicate gene persistence, functional constraints after duplication should be dynamic in that duplicates should be initially subject to relaxed purifying selection (or even positive selection) but later under increased purifying selection.

Alternative explanations for how duplicate genes avoid pseudogenization can be examined by exploring how evolution and expression of duplicates differ from singlecopy genes and how these phenomena differ between paralogs. On a molecular level neofunctionalization might be associated with (a) asymmetrical rates of nonsynonymous substitution in paralogs, (b) a rate ratio of nonsynonymous to synonymous substitutions per site of one or both paralogs that is higher than a single-copy gene, (c) more radical amino acid substitutions in one or both paralogs, (d) different functional constraints at an early stage of duplicate gene evolution (when new function is being acquired) than at a later stage (after new function has been acquired), and/or (e) different paralogous expression domains. Degradation or enhancement of different

functional domains could be associated with a complementary distribution of new substitutions along the length of different paralogs (14, 79, 106, 113) or complementary paralogous expression domains that together match that of a single-copy ortholog (110, 114). Alternatively, activity compromising nonsynonymous substitutions could be randomly distributed along the length of a protein, but still more prevalent than in a single copy ortholog (14, 111). Understanding divergence of paralogous expression and molecular evolution is therefore key to dissecting apart the mechanisms that promote their persistence as functional genes. However, evidence of (either subfunctionalization altered function or neofunctionalization) could be difficult to detect if they are realized by few mutations. Of course, multiple mechanisms may operate within a genome or even sequentially or concurrently on the same pair of duplicates (14, 115, 116), making the mechanisms for duplicate gene preservation Evidence for dynamic challenging to pigeonhole. functional constraints also can be elusive because similar molecular footprints can be left by relaxed purifying selection and by positive selection, which respectively permit or favor amino acid changing substitutions.

5.2. Functional constraints on expressed duplicates in polyploid clawed frogs

Purifying selection on the coding region of expressed duplicate genes is often relaxed relative to single-copy genes but more constrained than the neutral expectation (34, 77, 78, 104). This has been demonstrated in clawed frogs by multiple independent studies (14, 71, 82-84). In particular, purifying selection on X. laevis paralogs is relaxed compared to single-copy genes in a closely related diploid frog, S. tropicalis (14, 71, 82, 84), compared to single-copy orthologs in mammals (82, 83), and compared to single-copy genes in X. laevis (82). Using different statistical methods, independent tests recover evidence for asymmetric amino acid substitution in 4-6% of expressed paralogs in X. laevis (14, 82). Retained duplicates and single-copy genes of X. laevis do not appear to be enriched for any particular functional category (82, 84) and the mechanisms that promote their retention do not appear to be biased by functional category of the expressed paralogs (14).

Using comparative data from X. borealis, X. laevis, and S. tropicalis, Chain et al. (71) tested whether functional constraints after WGD in Xenopus differ in an early versus a later stage of duplicate gene evolution (Figure 4). Molecular changes after WGD were separated into those that occurred after WGD but before tetraploid speciation and those that occurred after WGD and after tetraploid speciation (Figure 4). In contrast to comparisons in other organisms of young duplicates to older duplicates (77, 114), functional constraints in clawed frogs in the early stages did not differ from later stages of evolution. These comparisons are distinct because the first one compares differently aged and differently functioned duplicates whereas the second comparison compares evolution of the same duplicate genes at different intervals of time. The timescale for a "return to normalcy" - the point at which functional constraints on expressed duplicates match those



Figure 6. Expression divergence can occur between tissues, the sexes, duplicated genes (paralogs), and between species (orthologs), and is derived either from *cis*- or *trans*- acting factors, or potentially from both. Expression divergence between the sexes (that is not attributable to intraspecific polymorphism) and between tissues must be caused by *trans*- acting factors because the *cis*- acting factors are identical within a species. An exception to this, indicated by an asterisk, is expression divergence between the sexes that arises due to differences in allelic copy number or the sex determining locus. Because they share the same cellular environment, divergent expression of paralogs (α , β) that are co-expressed arises from *cis*- acting factors unless, as indicated by two asterisks, there are also paralog-specific *trans*-acting factors in each sub-genome as a result of allopolyploidization. Expression divergence between paralogs that are not co-expressed and between orthologs in different species (sp.1, sp.2) can arise by *cis*- or *trans*- acting factors, or both.

of a single-copy ortholog – thus appears drawn out in excess of the tens of millions of years since tetraploidization in *Xenopus*. In addition, evolution of most duplicates in the early stages of their existence may frequently differ substantially from the evolution of the few paralogous pairs that are destined to persist (71). This is also suggested in other organisms in that duplicates that initially evolve conservatively in terms of the rate of nonsynonymous substitutions tend to persist for longer periods (77, 117, 118).

5.3. Expression divergence after WGD by allopolyploidization

Regulatory plasticity is a crucial element of developmental and sexual differentiation, expression divergence and subfunctionalization after gene duplication, and the origin and divergence of species (86, 119, 120).

After allopolyploidization, completely novel expression patterns that were not present in either parental species can occur, perhaps as a result of dosage dependent gene regulation (121). Gene regulation is orchestrated by cisacting factors that are part of the expressed gene, such as a binding site for a transcription factor, and also by transacting factors that are not part of the expressed gene, such as a transcription factor. Cis-acting regulatory elements affect transcription initiation, abundance, or stability in an allele-specific manner whereas trans-acting factors affect regulation of both alleles by interacting (directly or indirectly) with cis-acting elements. Because they share a cellular environment, intraspecific expression divergence in co-expressed paralogs generated by segmental duplication or autopolyploidization is attributable to mutations on cisacting elements. However, when paralogs are generated by allopolyploidization, expression divergence of coexpressed paralogs can be driven by cis- and trans-acting factors because each paralog may respond to sub-genome specific *trans*-acting factors (Figure 6). Analysis of expressed duplicates in X. laevis indicates that in multiple tissue types and developmental stages, both paralogs are often co-expressed but at different levels. In particular, analyses of expression microarrays (71) and expressed sequence tag (EST) databases (82) suggest that 40-50% of co-expressed paralogs are quantitatively diverged, although another study based on EST data suggested only about 14% Expression microarray data from five are (84). developmental stages or tissue types suggest that spatial or temporal expression divergence is not common on a coarse scale; only 2-7% of these expressed paralogs were each expressed in a tissue type or developmental stage in which expression of the other paralog was not detected (71). Presumably, analysis of additional tissue types and developmental stages could increase this percentage as would a finer scale analysis of paralogous expression profiles using, for example, in situ hybridization (82).

6. SEX DETERMINATION

Variation in the identity of the primary locus for sex determination exists across animals (122-126) and variation in male versus female heterogamy occurs in frogs (127, 128). In clawed frogs, females are heterogametic (ZW) and males are homogametic (ZZ) (129). The sex chromosomes are not morphologically distinguishable but genetic sex determination has been determined with sex reversal experiments. Treatment with estradiol causes genetically male tadpoles to develop into fertile females (i.e. ZZ females). When crossed with normal ZZ males, the sex-reversed ZZ females produce progeny that are 100% male (ZZ). Additionally, implantation of testes causes genetically female frogs to develop male secondary sexual characteristics (130, 131).

So far, the locus or loci that govern primary sex determination in clawed frogs is unknown. Genes encoding cytosolic superoxide dismutase and glycerol-3phosphate dehydrogenase appear to be sex linked in clawed frogs (132). A previous study suggested that the primary sex determining locus (or loci) of clawed frogs was tightly linked to the malic enzyme locus (133), but subsequent research invalidated this assertion by demonstrating no sex linkage of this locus, and suggested that the allozyme assayed by Graf (133) was in fact mitochondrial malate dehydrogenase (134). Expression (or lack of expression) of the sex determination locus triggers sex-specific differentiation of gonads and secondary sexual characteristics such as body size and laryngeal morphology - phenotypes whose development is orchestrated by the action of steroid regulated genes (135). Interestingly, the early phases of secondary sexual differentiation do not require the presence of ovaries, suggesting that the homogametic sex need not be the one that exhibits the default program of sexual differentiation (135).

One regulator of primary sexual differentiation – the *doublesex-mab3* related transcription factors – is strikingly conserved across a diversity of animals including

fruit flies, nematodes, coral, and humans (136-138). In medaka, a teleost fish, a paralog of one of these transcription factors called DMY is located on the Ychromosome and is also the primary sex determining gene (139, 140), but this is not the case in other fish (141). In clawed frogs, the *doublesex-mab-3*-related transcription factor 1 (DMRT1) is expressed in the primordial gonads in tadpoles and in adult testes; this gene also acts as a transcription factor in cultured cells (142, 143). At least two divergent X. laevis transcripts of DMTR1, are expressed in X. laevis, raising the question of how expression of these paralogs is modulated to maintain sex ratio after WGD. Two DMRT1 sequences, accession numbers NM 001096500 and AB252634, are identical at the amino acid level and in the 3' untranslated region (UTR) but different in a portion of the 5' UTR, suggesting that they are splice variants. Another sequence, accession number NM 001085483, is divergent from the first two in the coding region and both UTRs. Relationships to X. borealis DMRT1 paralogs indicate that it has a paralogous relationship with respect to NM 001096500 and AB252634 that originated from tetraploidization in Xenopus (unpublished results).

6.1. Sex determination and allopolyploidization

Polyploidization is more common in plants than in animals (144-146), and more common in clawed frogs than in most other animal lineages. The question of how clawed frogs manage to maintain sex determination in the wake of genome duplication is central to our understanding of why polyploidization is so common in clawed frogs. A plausible explanation for the greater tolerance of WGD by plants and clawed frogs compared to most other animals is that WGD is rare in species that have evolved dosagecompensation to cope with sex chromosome degeneration (146). Y chromosome degeneration is generally more advanced in most animals than plants (147) and visible evidence of sex chromosome degeneration has not been detected in clawed frogs (72, 148). In fact, few frogs have sex chromosomes that have been distinguished by differences in size, banding pattern or replication time (127) and unlike mammals, bar bodies and other mechanisms of gene inactivation in the homogametic sex have not been observed in amphibians (127, 149). This suggests the possibility that in clawed frogs only a small region surrounding the sex determining locus (or loci) has a reduced level of recombination (150). If this is the case, WGD would not substantially disrupt aspects of gene dosage that differ between the sexes as a consequence of hemizygous sex chromosomes, and would not disrupt dosage compensation mechanisms that appear to be rudimentary or absent in amphibians (127, 149).

Sex chromosomes probably initially start out as autosomes that, if the ancestor is asexual, incurred a mutation that causes the switch to gonochorism. Subsequent switching of the sex chromosome can occur by duplication and divergent resolution of a pre-existing sex determination gene, by transposition of the sex-determining locus, or by mutation that transforms a gene into the primary sex-determining locus by either replacing or acting upstream of the ancestral one (151). If sex determination is based on presence or absence of a locus, as it is in humans, suppressed recombination prevents homogenization of the sex determination locus in the heterogametic sex. Degeneration of linked loci follows as either a consequence of Muller's ratchet turning over time due to the accumulation of deleterious mutations (152, 153) or as a result of selective sweeps at loci linked to the sex determination region, which incur a cost on linked genes (154). The level of degeneration is correlated with the time since recombination stopped, as well as other factors such as the rate of deleterious mutation, the fitness effect of these mutations, and the effective population size of the haploid locus (155).

That polyploidy is more common in clawed frogs than in other similarly-aged clades of frogs that also do not have extensively degenerated sex chromosomes, however, suggests a role for additional factors specific to clawed frogs that facilitate tolerance of or advantage to polyploidization. For instance, hybrid clawed frogs may be better equipped than other hybrid frogs to cope with sex determination after WGD by allopolyploidization. This could occur through environmental mechanisms for sex determination or dominance of the primary sex determining locus from one species over that of another (42). It is also possible that mechanisms such as nucleolar dominance (e. g. 156) – the transcriptional silencing of genes from only one parental species – facilitate tolerance of WGD in clawed frogs.

6.2. Sex determination and hybrid fertility; Haldane's rule

Haldane's rule for hybrid sterility posits that when one sex of a hybrid cross is absent, rare, or sterile, that sex is the heterogametic one (157). The broad applicability of Haldane's rule suggests that the genetic mechanisms of postzygotic reproductive isolation among many animals may be related to sexual determination and differentiation (158). Clawed frogs violate Haldane's Rule, however, because male but not female F1 hybrids are sterile (40, 159). This has interesting implications for understanding the genetic mechanisms of speciation and the origin of reproductive incompatibilities – implications that, like the prevalence of polyploidy in clawed frogs, are likely intertwined with the phenomena of sex determination and sexual differentiation.

Possible explanations for Haldane's rule for sterility in species with male heterogamy include (a) dominance, in that genes that cause sterility tend to be recessive and if sex-linked, more adversely affect the heterogametic sex because recessive sex-linked alleles are hemizygous when paired with a degenerate sex chromosome, (b) faster evolution of genes expressed mostly or only in males as a result of sexual selection, and (c) the complexity of spermatogenesis compared to oogenesis (158, 160-163). In species with female heterogamy that adhere to Haldane's Rule (heterogametic female hybrids are sterile or inviable), only the first of these explanations (dominance) applies. If sex chromosome degeneration is minimal, dominance is less likely to drive Haldane's Rule because hemizygous loci are

less common in the heterogametic sex than in species with a substantially degenerated sex chromosome. Exceptions to Haldane's Rule in reciprocal hybrid crosses could arise if there were partially dominant incompatibilities between autosomal loci of one species and loci on the sex chromosomes of the homogametic sex of another (161). An exception could also arise if there were partially dominant genetic incompatibilities between loci on the homogametic sex chromosome of different species (161). Furthermore, if dominant incompatibilities are between loci with sex biased expression, hybrid sterility could arise in hybrids of the sex that expresses them, irrespective of which sex is heterogametic, and irrespective of whether the incompatibility are physically located on a sex chromosome or an autosomal chromosome.

6.3. Sex-reversed hybrids

Recently it was reported that, like other hybrid males in Xenopus, sex reversed hybrid males are sterile, and that sex reversed hybrid individuals have similar gonadal expression profiles as non-sex reversed hybrids of the corresponding phenotypic sex (164). Three main conclusions were drawn from these observations. First, it was asserted that "the W chromosome in Xenopus bears few, if any, genes expressed in adult gonads and consists mainly of loci controlling the developmental regulation of physiological sex." Second, it was suggested that this result supports the faster male evolution hypothesis for Haldane's Rule. Third, on the basis of another study of gene expression between the sexes of different Xenopus species (165), it was proposed that hybrid male sterility is not due to "selection or divergence of male-specific genes". However, the authors did not consider another, more plausible scenario that is inconsistent with their first conclusion and that renders the others equivocal— namely, that the W chromosome of Xenopus includes a large pseudoautosomal region.

As discussed above, there is in fact no evidence that the W chromosome of *Xenopus* is particularly degenerate: no cytological differences between the Z and W chromosomes have been detected (72, 148), male versus female heterogamy is polymorphic in frogs (127), and there is not evidence for dosage compensation in frogs (127, 149). The W chromosome could therefore be indistinguishable from the Z chromosome except for a small sex-determining region (166). Under this scenario, the W and Z chromosomes contain similar (if not nearly identical) sets of loci, some of which are expressed in a sex-biased manner in either adult gonad. Expression of these sex-biased genes would be regulated by the endogenous hormones and transcription factors that produce normal ZZ males and ZW females and by exogenous ones triggered by testes implants or estradiol treatment that produce sex reversed ZW males and ZZ females respectively. It is no surprise then, that sexreversed males are sterile and that sex reversed individuals have similar gonadal expression profiles as non-sex reversed individuals of the same phenotypic sex. These observations tell us nothing new about what the mechanism of hybrid male sterility is in these animals. Sterility of hybrid males (whether sex-reversed or not) necessarily

implicates male expressed genes (on the sex chromosomes or elsewhere in the genome) that have diverged in sequence, expression, or both.

7. CONCLUSIONS AND PERSPECTIVES

Further information on the diversity of and evolutionary relationships among clawed frogs would enhance our understanding of genome evolution in this group, resolve species boundaries, and further catalyze study of interesting characteristics such as morphology, vocalization, and neuroethology (167-169). Evolutionary relationships and population structure among these frogs also have implications for toxological studies because there may be heterogeneous endocrine function within experimental species such as X. laevis (170). Morphological similarities among clawed frog species has the potential to obscure cryptic species and species identification and delineation can be assisted by the use of molecular markers and/or distinctive male advertisement songs that differentiate each species.

WGD in clawed frogs promises to offer perspective on the relative roles of cis- and trans-acting factors in gene regulation (171-176) in the wake of WGD, through development, between the sexes, in different tissue types, and between polyploid species. If two species can produce viable hybrids, the relative contributions of *cis*versus trans-acting factors in expression differences between species can be dissected apart by comparing the ratio of expression level of a gene in each parental species to the ratio of expression level of each parental allele in hybrid individuals (176). If exclusively cis-acting factors drive expression divergence, then the ratio of parental allele expression in hybrids should be the same as the expression ratio in each parental species, even if the overall level of expression of *trans*- acting factors is different in hybrids. If exclusively trans-acting factors drive expression divergence between species, then the level of expression of each parental allele in a hybrid should be equal because they share the same cellular environment. Other scenarios include changes that reinforce or oppose one another as a result of co-evolution are also possible (175).

Interesting examples of expression subfunctionalization after genome duplication have been identified through comparison of expression domains of X. laevis paralogs and the S. tropicalis ortholog. The cyclinassociated protein skp1a, for example, has identical amino acid sequence in both X. laevis paralogs and the S. tropicalis ortholog, but in situ hybridization indicates that the X. laevis paralogs are divergently expressed (82). Prefabricated microarrays are available for X. laevis and S. tropicalis and combined X. laevis/S.tropicalis chips are being developed (177), opening the door for high analysis of expression throughput. fine scale subfunctionalization across developmental time and different tissue types. Once subfunctionalized paralogs have been identified, analysis of polymorphism in their upstream regulatory elements could reveal the identity and purview of cis- regulatory elements (110) through comparison to the diploid species S. tropicalis. This

approach has identified putative subfunction partitioning in conserved noncoding regions in divergently expressed paralogs of zebrafish (112), and offers a compelling justification for an *X. laevis* genome sequencing project, if only at a low depth of coverage.

Additionally, identification of the primary locus (or loci) for sex determination will pave the way for study of how this crucial phenotype is maintained through multiple rounds of allopolyploidization. It will be fascinating to explore whether in polyploids all duplicated copies of the sex determining locus are expressed, whether these loci are or have been subject to positive selection, and whether dominance relationships exist among speciesspecific alleles of this locus in polyploid hybrids. This information, along with ongoing work on the phylogeny of amphibians, will also facilitate further research on sex Because their chromosome degeneration. sex chromosomes are not visually degenerate, this avenue of inquiry promises to offer unique insights into the earliest stages of sex chromosome evolution.

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9. REFERENCES

1. Cannatella, D. C. & R. O. de Sá: *Xenopus laevis* as a model organism. *Systematic Biology*, 42, 476-507 (1993)

2. Tinsley, R. C. & H. Kobel: The Biology of *Xenopus*. Clarendon Press (1996)

3. Evans, B. J., D. B. Kelley, R. C. Tinsley, D. J. Melnick & D. C. Cannatella: A mitochondrial DNA phylogeny of clawed frogs: phylogeography on sub-Saharan Africa and implications for polyploid evolution. *Molecular Phylogenetics and Evolution*, 33, 197-213 (2004)

4. Evans, B. J.: Ancestry influences the fate of duplicated genes millions of years after duplication in allopolyploid clawed frogs (*Xenopus*) *Genetics*, 176, 1119-1130 (2007)

5. Kobel, H. R., C. Loumont & R. C. Tinsley: The extant species. In: The Biology of *Xenopus*. Eds: R. C. Tinsley&H. R. Kobel. Clarendon Press, Oxford (1996)

6. Vigny, C.: The mating calls of 12 species and subspecies of the genus *Xenopus* (Amphibia: Anura) *J. Zool., London*, 188, 103-122. (1979)

7. Holder, M. & P. O. Lewis: Phylogeny estimation: traditional and Bayesian approaches. *Nature Reviews Genetics*, 4, 275-284 (2003)

8. Cannatella, D. C. & L. Trueb: Evolution of pipoid frogs: morphology and phylogenetic relationships of *Pseudhymenochirus. Journal of Herpetology*, 22, 439-456 (1988)

9. Ford, L. S. & D. C. Cannatella: The major clades of frogs. *Herpetological Monographs*, 7, 94-117 (1993)

10. Báez, A. M. & L. A. Pugener: Ontogeny of a new Palaeogene pipid frog from southern South America and

xenopodinomorph evolution. Zoological Journal of the Linnean Society, 139, 439-476 (2003)

11. Frost, D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. De Sá, A. Channing, M. Wilkinson, S. C. Donnellan, C. J. Raxworthy, J. A. Campbell, B. L. Blotto, P. Moler, R. C. Drewes, R. A. Nussbaum, J. D. Lynch, D. M. Green & W. C. Wheeler: The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 297, 1-370 (2006)

12. Evans, B. J., D. B. Kelley, D. J. Melnick & D. C. Cannatella: Evolution of RAG-1 in polyploid clawed frogs. *Molecular Biology and Evolution*, 22, 1193-1207 (2005)

13. Roelants, K. & F. Bossuyt: Archaeobatrachian paraphyly and Pangean diversification of crown-group frogs. *Systematic Biology*, 54, 111-126 (2005)

14. Chain, F. J. J. & B. J. Evans: Molecular evolution of duplicate genes in *Xenopus laevis* is consistent with multiple mechanisms for their retained expression. *PLoS Genetics*, 2, e56 (2006)

15. Joint Genome Institute: Xenopus tropicalis v4.1. (2005) 16. Evans, B. J., T. F. Carter, R. Hanner, M. L. Tobias, D. B. Kelley, R. Hanner & R. C. Tinsley: A new species of clawed frog (genus *Xenopus*), from the Itombwe Plateau, Democratic Republic of the Congo: implications for DNA barcodes and biodiversity conservation. *Zootaxa*, submitted, (2008)

17. Kobel, H., B. Barandun & C. H. Thiebaud: Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal*, 8, 13-17 (1998)

18. Measey, G. J. & A. Channing: Phylogeography of the genus *Xenopus* in southern Africa. *Amphibia-Reptilia*, 24, 321-330 (2003)

19. de Queiroz, K.: The general lineage concept of species, species criteria, and the process of speciation. In: Endless Forms: Species and Speciation. Eds: D. Howard&S. Berlocher. Oxford Press, New York (1998)

20. Mann, M., M. S. Risley, R. A. Eckhardt & H. E. Kasinsky: Characterization of spermatid/sperm basic chromosomal proteins in the genus *Xenopus* (Anura, Pipidae) *Journal of Experimental Zoology*, 222, 173-186 (1982)

21. Bürki, E. & M. Fischberg: Evolution of globin expression in the genus *Xenopus* (Anura: Pipidae) *Molecular Biology and Evolution*, 2, 270-277 (1985)

22. Graf, J. D. & M. Fischberg: Albumin evolution of polyploid species of the genus *Xenopus. Biochemical Genetics*, 24, 821-837 (1986)

23. Holloway, A. K., D. C. Cannatella, H. C. Gerhardt & D. M. Hillis: Polyploids with different origins and ancestors form a single sexual polyploid species. *American Naturalist*, 167, E88-E101 (2006)

24. Báez, A. M.: The fossil record of the Pipidae. In: The Biology of *Xenopus*. Eds: R. C. Tinsley&H. R. Kobel. Clarendon Press, Oxford (1996)

25. Henrici, A. C. & A. M. Báez: First occurrence of *Xenopus* (Anura: Pipidae) on the Arabian Penninsula: a new species from the Late Oligocene of the Republic of Yemen. *Journal of Paleontology*, 75, 870-882 (2001)

26. Evans, B. J., J. C. Morales, M. D. Picker, D. B. Kelley & D. J. Melnick: Comparative molecular phylogeography of two *Xenopus* species, *X. gilli* and *X. laevis*, in the

southwestern Cape Province, South Africa. *Molecular Ecology*, 6, 333-343 (1997)

27. Evans, B. J., J. C. Morales, M. D. Picker, D. B. Kelley & D. J. Melnick: Absence of extensive introgression between *Xenopus gilli* and *Xenopus laevis laevis* (Anura: Pipidae), in southwestern Cape Province, South Africa. *Copeia*, 1998, 504-509 (1998)

28. Dobzhansky, T.: Genetics and the origin of species. Columbia University Press, New York (1937)

29. Muller, H. J.: Isolating mechanisms, evolution and temperature. *Biol. Symp.*, 6, 71-125 (1942)

30. Orr, H. A.: The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics*, 139, 1805-1813 (1995)

31. Masly, J. P., C. D. Jones, M. A. F. Noor, J. Locke & H. A. Orr: Gene transposition as a cause of hybrid sterility in *Drosophila. Science*, 313, 1448-1450 (2006)

32. Taylor, J. S., Y. Van de Peer & A. Meyer: Genome duplication, divergent resolution and speciation. *Trends in Genetics*, 17, 299-301 (2001)

33. Werth, C. R. & M. D. Windham: A model for divergent, allopatric speciation of polyploid pteridophytes resulting from silencing of duplicate-gene expression. *American Naturalist*, 137, 515-526 (1991)

34. Lynch, M. & J. S. Conery: The evolutionary fate and consequences of duplicate genes. *Science*, 290, 1151-1155 (2000)

35. Greene, J. M., H. Otani, P. J. Good & I. B. Dawid: A novel family of retrotransposon-like elements in *Xenopus laevis* with a transcript inducible by two growth factors. *Nucleic Acids Research*, 21, 2375-2381 (1993)

36. Hikosaka, A., E. Yokouchi & A. Kawahara: Extensive amplification adn transposition of a novel repetitive element, Xstir, together with its terminal inverted repeat in the evolution of *Xenopus. Journal of Molecular Evolution*, 51, 554-564 (2000)

37. Lam, B. S. & D. Carroll: Tandemly repeated DNA sequences from *Xenopus laevis*. II. Dispersed clusters of a 388 base-pair repeating unit. *Journal of Molecular Biology*, 165, 587-597 (1983)

38. Unsal, K. & G. T. Morgan: A novel group of families of short interspersed repetitive elements (SINEs) in *Xenopus*: evidence of a specific target site for DNAmediated transposition of inverted-repeat SINEs. *Journal of Molecular Biology*, 248, 812-823 (1995)

39. Ungerer, M. C., S. C. Strakosh & Y. Zhen: Genome size evolution in three hybrid sunflower species is associated with retrotransposon proliferation. *Current Biology*, 16, R872-R873 (2006)

40. Kobel, H. R.: Reproductive capacity of experimental *Xenopus gilli x X. l. laevis* hybrids. In: The Biology of *Xenopus*. Eds: R. C. Tinsley&H. R. Kobel. Oxford University Press, Oxford (1996)

41. Kobel, H. R. & L. Du Pasquier: Genetics of polyploid *Xenopus. Trends Genetics*, 2, 310-315 (1986)

42. Kobel, H. R.: Allopolyploid speciation. In: The Biology of *Xenopus*. Eds: R. C. Tinsley&H. R. Kobel. Clarendon Press, Oxford (1996)

43. Malone, J. H., T. H. Chrzanowski & P. Michalak: Sterility and gene expression in hybrid males of *Xenopus laevis* and *X. muelleri*. *PLoS One*, 2, e781 (2007) 44. Brun, R. & H. R. Kobel: Observations on the fertilization block between *Xenopus borealis* and *Xenopus laevis laevis J. Exp. Zool.*, 201, 135-137 (1977)

45. Soltis, D. E., P. S. Soltis, J. C. Prires, A. Kovarik, J. A. Tate & E. Mavrodiev: Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biological Journal of the Linnean Society*, 82, 485-501 (2004)

46. Lee, H.-S. & Z. J. Chen: Protein-coding genes are epigenetically regulated in *Arabidopsis* polyploids. *Proceedings of the National Academy of Sciences*, 98, 6753-6758 (2001)

47. Husband, B. C. & D. W. Schemske: Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *Journal of Ecology*, 88, 689-701 (2000)

48. Otto, S. P. & J. Whitton: Polyploid incidence and evolution. *Annual Review of Genetics*, 34, 401-437 (2000)

49. Moscone, E. A., M. A. Matzke & A. J. M. Matzke: The use of combined FISH/GISH in conjunction with DAPI counterstaining to identify chromosomes containing transgene inserts in amphidiploid tobacco. *Chromosoma*, 105, 231-236 (1996)

50. Skalická, K., K. Y. Lim, R. Matyasek, M. Matzke, A. R. Leitch & A. Kovarik: Preferential elimination of repeated DNA sequences from the paternal, *Nicotiana tomentosiformis* genome donor of a synthetic, allotetraploid tobacco. *New Phytologist*, 166, 291-303 (2005)

51. Volkov, R. A., N. V. Borisjuk, I. I. Panchuk, D. Schweizer & V. Hemleben: Elimination and rearrangement of parental rDNA in the allotetraploid *Nicotiana tabacum. Molecular Biology and Evolution*, 16, 311-320 (1999)

52. Wendel, J. F., A. Schnabel & T. Seelanan: Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*) Proceedings of the National Academy of Sciences, 92, 280-284 (1995)

53. Zwierzykowski, Z., R. Tayyar, M. Brunell & A. J. Lukaszewski: Genome recombination in intergeneric hybrids between tetraploid *Festuca pratensis* and *Lolium multiflorum. Journal of Heredity*, 89, 324-328 (1998)

54. Jiang, C.-X., P. W. Chee, X. Draye, P. L. Morrell, C. W. Smith & A. H. Paterson: Multilocus interactions restrict gene introgression in interspecific populations of polyploid *Gossypium* (cotton) *Evolution*, 54, 798-814 (2000)

55. Adams, K. L. & J. F. Wendel: Novel patterns of gene expression in polyploid plants. *Trends in Genetics*, 21, 539-543 (2005)

56. Hegarty, M. J., G. L. Barker, I. D. Wilson, R. J. Abbott, K. J. Edwards & e. al.: Transcriptome shock after interspecific hybridization in *Senecio* is ameliorated by genome duplication. *Current Biology*, 16, 1652-1659 (2006)

57. Birchler, J. A., N. C. Riddle, D. L. Auger & R. A. Veitia: Dosage balance in gene regulation: biological implications. *Trends in Genetics*, 21, 219-226 (2005)

 Osborn, T. C., J. C. Pires, J. A. Birchler, D. L. Auger, Z. J. Chen, H. Lee, L. Comai, A. Madlung, R. W. Doerge, V. Colot & R. A. Martienssen: Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics*, 19, 141-147 (2003)

59. Levy, A. A. & M. Feldman: Genetic and epigenetic reprogramming of the wheat genome upon

allopolyploidization. *Biological Journal of the Linnean* Society, 82, 607-613 (2004)

60. Liu, B., C. L. Brubaker, G. Mergeai, R. C. Cronn & J. F. Wendel: Polyploid formation in cotton is not accompanied by rapid genomic changes. *Genome*, 44, 321-330 (2001)

61. Lukens, L. N., P. A. Quijada, J. Udall, J. C. Pires, M. E. Schranz & T. C. Osborn: Genome redundancy and plasticity within ancient and recent *Brassica* crop species. *Biological Journal of the Linnean Society*, 82, 665-674 (2004)

62. Madlung, A., R. Masuelli, B. Watson, S. H. Reynolds, J. Davison & L. Comai: Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic *Arabidopsis* allotetraploids. *Plant Physiology*, 129, 733-746 (2002)

63. Comai, L.: Genetic and epigenetic interactions in allopolyploid plants. *Plant Molecular Biology*, 43, 387-399 (2000)

64. Hegarty, M. J., G. L. Barker, I. D. Wilson, R. J. Abbott, K. J. Edwards & S. J. Hiscock: Transcriptome shock after interspecific hybridization in *Senecio* is ameliorated by genome duplication. *Current Biology*, 16, 1652-1659 (2006)

65. Jackson, J. A. & R. C. Tinsley: Parasite infectivity to hybridising host species: a link between hybrid resistance and allopolyploid speciation? *International Journal for Parasitology*, 33, 137-144 (2003)

66. Allendorf, F. W. & R. G. Danzmann: Secondary tetrasomic segregation of MDH-B and preferential pairing of homeologues in rainbow trout. *Genetics*, 145, 1083-1092 (1997)

67. Ferris, S. D. & G. S. Whitt: Evolution of the differential regulation of duplicate genes after polyploidization. *Journal of Molecular Evolution*, 12, 267-317 (1979)

68. Gaut, B. S. & J. F. Doebley: DNA sequence evidence for the segmental allotetraploid origin of maize. *Proceedings of the National Academy of Sciences*, 94, 6809-6814 (1997)

69. Sammut, B., A. Marcuz & L. Du Pasquier: The fate of duplicated major histocompatibility complex class 1a genes in a dodecaploid amphibian, *Xenopus ruwenzoriensis. European Journal of Immunology*, 32, 1593-1604 (2002)

70. Müller, W. P.: Diplotene chromosomes of *Xenopus* hybrid oocytes. *Chromosoma*, 59, 273-282 (1977)

71. Chain, F. J. J., D. Ilieva & B. J. Evans: Duplicate gene evolution and expression in the wake of vertebrate allopolyploidization. *BMC Evolutionary Biology*, in press, (2008)

72. Tymowska, J.: Polyploidy and cytogenetic variation in frogs of the genus *Xenopus*. In: Amphibian cytogenetics and evolution. Eds: D. S. Green&S. K. Sessions. Academic Press., San Diego (1991)

73. Dehal, R. & J. Boore: Two rounds of whole genome duplication in the ancestral vertebrate. *PLoS Biology*, 3, e314 (2005)

74. Taylor, J. S., I. Braasch, T. Frickey, A. Meyer & Y. Van de Peer: Genome duplication, a trait shared by 22,000 species of ray-finned fish. *Genome Research*, 13, 382-390 (2003)

75. Allendorf, F. & G. Thorgaard: Tetraploidy and the evolution of salmonid fishes. In: Evolutionary genetics of fishes. Ed: B. J. Turner. Plenum Press, New York (1984)

76. Spring, J.: Vertebrate evolution by interspecific hybridization – are we polyploid? *FEBS Letters*, 400, 2-8 (1997)

77. Jordan, I. K., Y. I. Wolf & E. V. Koonin: Duplicated genes evolve slower than singletons despite the initial rate increase. *BMC Evolutionary Biology*, 4, 22 (2004)

78. Nembaware, V., K. Crum, J. Kelson & C. Seoighe: Impact of the presence of paralogs on sequence divergence in a set of mouse-human orthologs. *Genome Research*, 12, 1370-1376 (2002)

79. Zhang, P., Z. Gu & W. H. Li: Different evolutionary patterns between young duplicate genes in the human genome. *Genome Biology*, 4, R56 (2003)

80. Taylor, J. S., Y. Van de Peer, I. Braasch & A. Meyer: Comparative genomics provides evidence for an ancient genome duplication event in fish. *Proceedings* of the Royal Society of London, 356, 1661-1679 (2001)

81. Van de Peer, Y., J. S. Taylor, I. Braasch & A. Meyer: The ghost of selection past: rates of evolution and functional divergence of anciently duplicated genes. *Journal of Molecular Evolution*, 53, 436-446 (2001)

82. Hellsten, U., M. K. Khokha, T. C. Grammer, R. M. Harland, P. Richardson & D. S. Rokhsar: Accelerated gene evolution and subfunctionalization in the pseudotetraploid frog *Xenopus laevis*. *BMC Evolutionary Biology*, 5, (2007)

83. Hughes, M. K. & A. L. Hughes: Evolution of duplicate genes in a tetraploid animal, *Xenopus laevis*. *Molecular Biology and Evolution*, 10, 1360-69 (1993)

84. Morin, R. D., E. Chang, A. Petrescu, N. Liao, M. Griffith, R. Kirkpatrick, Y. S. Butterfield, A. C. Young, J. Stott, S. Barber, R. Babakaiff, M. C. Dickson, C. Matsuo, D. Wong, G. S. Yang, D. E. Smailus, K. D. Wetherby, P. N. Kwong, J. Grimwood, B. C. P., M. Brown-Jogn, N. D. Reddix-Dugue, M. Mayo, J. Schnmutz, J. Beland, M. Park, S. Gibson, T. Olson, G. G. Bouffard, M. Tsai, R. Featherstone, S. Shand, A. S. Siddiqui, W. Jang, E. Lee, S. L. Klein, R. W. Blakesley, B. R. Zeeberg, S. Narasimban, J. N. Weinstein, S. P. Pannacchio, R. M. Myers, E. D. Green, L. Wagner, D. S. Gerhard, M. A. Marra, S. J. M. Jones & R. A. Holt: Sequencing and analysis of 10,967 full-length cDNA clones from Xenopus laevis and Xenopus tropicalis reveals post-tetraploidization transcriptome remodeling. Genome Research, 16, 796-803 (2006)

85. Bailey, J. A., Z. Gu, R. A. Clark, K. Reinert, R. V. Samonte, S. Schwartz, M. D. Adams, E. W. Myers, P. W. Li & E. E. Eichler: Recent segmental duplications in the human genome. *Science*, 297, 1003-1007 (2002)

86. Gu, Z., S. A. Rifkin, K. P. White & W. H. Li: Duplicate genes increase gene expression diversity within and between species. *Nature Genetics*, 36, 577-579 (2004)

87. Gu, Z., L. M. Steinmetz, X. Gu, C. Scharfe, R. W. Davis & W. H. Li: Role of duplicate genes in genetic robustness against null mutations. *Nature*, 291, 63-66 (2003)

88. Hancock, J.: Gene factories, microfunctionalization and the evolution of gene families. *Trends in Genetics*, 21, 591-594 (2005)

89. Li, W.-H., Yang, J, Gu, X.: Expression divergence between duplicate genes. *Trends in Genetics*, 21, 602-607 (2005)

90. Blanc, G. & K. H. Wolfe: Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* evolution. *The Plant Cell*, 16, 1679-1691 (2004)

91. Maere, S., S. DeBodt, J. Raes, T. Casneuf, M. Van Montagu, M. Kuiper & Y. Van de Peer: Modeling gene and genome duplication in eukaryotes. *Proceedings of the National Academy of Sciences*, 102, (2005)

92. Adams, K. L., R. Cronn, R. Percifeld & J. F. Wendel: Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proceedings of the National Academy* of Sciences, 100, 4649 (2003)

93. Adams, K. L. & J. F. Wendel: Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology*, 8, 135-141 (2005)

94. Adams, K. L., R. Percifeld & J. F. Wendel: Organspecific silencing of duplicated genes in a newly synthesized cotton allotetraploid. *Genetics*, 168, 2217-2226 (2004)

95. Ozkan, H., A. A. Levy & M. Feldman: Allopolyploidyinduced rapid genomic evolution of the wheat (*Aegilops-Triticum*) group. *Plant Cell*, 13, 1735-1747 (2001)

96. Shaked, H., K. Kashkush, H. Ozkan, M. Feldman & A. A. Levy: Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *The Plant Cell*, 13, 1749-1759 (2001)

97. Amores, A., A. Force, Y. L. Yan, L. Joly, C. Amemiya, A. Fritz, R. K. Ho, J. Langeland, V. Prince, Y. Wang, M. Westerfield, M. Ekker & J. H. Postlethwait: Zebrafish hox clusters and vertebrate genome evolution. *Science*, 282, 1711-1714 (1998)

98. Lynch, M.: The origins of genome architecture. Sinauer, Sunderland (2007)

99. Nadeau, J. H. & D. Sankoff: Comparable rates of gene loss and functional divergence after genome duplications early in vertebrate evolution. *Genetics*, 147, 1259-1266 (1997)

100. Wendel, J. F.: Genome evolution in polyploids. *Plant Molecular Biology*, 42, 225-249 (2000)

101. Davis, J. C. & D. A. Petrov: Do disparate mechanisms of duplication add similar genes to the genome? *Trends in Genetics*, 21, 548-555 (2005)

102. Freeling, M. & B. C. Thomas: Gene-balanced duplications, like tetraploidy, provide predictable drive to increase morphological complexity. *Genome Research*, 16, 805-814 (2006)

103. Gu, X.: Evolution of duplicate genes versus genetic robustness against null mutations. *Trends in Genetics*, 19, 354-356 (2003)

104. Kondrashov, F. A., I. B. Rogozin, Y. I. Wolf & E. V. Koonin: Selection in the evolution of gene duplications. *Genome Biology*, 3, RESEARCH0008 (2002)

105. Dulai, K. S., M. von Dornum, J. D. Mollon & D. M. Hunt: The evolution of trichromatic colour vision by opsin

gene duplication in New World and Old World primates. Genome Research, 9, 629-638 (1999)

106. Hughes, A. L.: The evolution of functionally novel proteins after gene duplication. *Proceedings of the Royal Society of London*, 256, 119-124 (1994)

107. Ohno, S.: Evolution by gene duplication. Springer-Verlag, Berlin (1970)

108. Zhang, J., H. F. Rosenberg & M. Nei: Positive Darwinian selection after gene duplication in primate ribonuclease genes. *Proceedings of the National Academy of Sciences*, 95, 3708-3713 (1998)

109. Papp, B., C. Pál & L. D. Hurst: Evolution of *cis*regulatory elements in duplicated genes of yeast. *Trends in Genetics*, 19, 417-422 (2003)

110. Force, A., M. Lynch, B. Pickett, A. Amores, Y. L. Yan & J. H. Postlethwait: Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, 151, 1531-1545 (1999)

111. Stoltzfus, A.: On the possibility of constructive neutral evolution. *Journal of Molecular Evolution*, 49, 169-181 (1999)

112. Postlethwait, J. H., A. Amores, W. Cresko, A. Singer & Y. Yan: Subfunction partitioning, the teleost radiation and the annotation of the human genome. *Trends in Genetics*, 20, 481-490 (2004)

113. Dermitzakis, E. T. & A. G. Clark: Differential selection after duplication in mammalian developmental genes. *Molecular Biology and Evolution*, 18, 557-562 (2001)

114. Lynch, M. & A. Force: The probability of duplicate gene preservation by subfunctionalization. *Genetics*, 154, 459-473 (2000)

115. He, X. & J. Zhang: Rapid subfunctionalization accompanied by prolonged and substantial neofunctionalization in duplicate gene evolution. *Genetics*, 169, 1157-1164 (2005)

116. Rastogi, S. & D. A. Liberles: Subfunctionalization of duplicated genes as a transition state to neofunctionalization. *BMC Evolutionary Biology*, 5, 28 (2005)

117. Davis, J. C. & D. A. Petrov: Preferential duplication of conserved proteins in eukaryotic genomes. *PLoS Biology*, 2, 318-326 (2004)

118. Shakhnovich, B. E. & E. V. Koonin: Origins and impact of constraints in evolution of gene families. *Genome Research*, 16, 1529-1536 (2006)

119. Cheng, Z., M. Ventura, X. She, P. Khaitovich, T. Graves, K. Osoegawa, D. Church, P. DeJong, R. K. Wilson, S. Pääbo, M. Rocchi & E. E. Eichler: A genome-wide comparison of recent chimpanzee and human segmental duplications. *Nature*, 437, 88-93 (2005)

120. Huminiecki, L. & K. H. Wolfe: Divergence of spatial gene expression profiles following species-specific gene duplications in human and mouse. *Genome Research*, 14, 1870-1879 (2004)

121. Wang, J., L. Tian, A. Madlung, H.-S. Lee, M. Chen, J. J. Lee, B. Watson, T. Kagochi, L. Comai & Z. J. Chen: Stochastic and epigenetic changes of gene expression in *Arabidopsis* polyploids. *Genetics*, 168, 2217-2226 (2004)

122. Haag, E. S. & A. V. Doty: Sex determination across evolution: connecting the dots. *PLoS Biology*, 3, e21 (2005)

123. Modi, W. S. & D. Crews: Sex chromosomes and sex determination in reptiles. *Current Opinion in Genetics and Development*, 15, 660-665 (2005)

124. Matsubara, K., H. Tarui, M. Toriba, K. Yamada, C. Nishida-Umehara, K. Agata & M. Matsuda: Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. *Proceedings of the National Academy of Sciences*, 103, 18190-18195 (2006)

125. Bull, J. J.: Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park (1983)

126. Charlesworth, B.: Sex determination: primitive Y chromosomes in fish. *Current Biology*, 14, R745-R747 (2004)

127. Hayes, T. B.: Sex determination and primary sex differentiation in amphibians: genetic and developmental mechanisms. *Journal of Experimental Zoology*, 281, 373-399 (1998)

128. Richards, C. M. & G. W. Nace: Gynogenetic and hormonal sex reversal used in tests of the XX-XY hypothesis of sex determination in *Rana pipiens. Growth*, 42, 319-331 (1978)

129. Mikamo, K. & E. Witschi: Masculinization and breeding of the WW in *Xenopus. Experientia*, 20, 622-623 (1964)

130. Mikamo, K. & E. Witschi: Functional sex-reversal in genetic females of *Xenopus laevis*, induced by implanted testes. *Genetics*, 48, 1411-1421 (1963)

131. Watson, J. T. & D. B. Kelley: Testicular masculinization of vocal behavior in juvenile female *Xenopus laevis* reveals sensitive periods for song duration, rate, and frequency spectra. *Journal of Comparative Physiology*, 171, 343-350 (1992)

132. Graf, J. D.: Genetic mapping in *Xenopus laevis*: eight linkage groups established. *Genetics*, 123, 389-398 (1989)

133. Graf, J. D.: Sex linkage of the malic enzyme in *Xenopus laevis. Experientia*, 45, 194-196 (1989)

134. Mácha, J., V. Tlapáková, V. Krylov & V. Kopsky: Xtir polymorphism and absence of sex linkage in *Xenopus laevis* ME2 gene. *Folia Biologica (Praha)*, 49, 115-117 (2003)

135. Kelley, D. B.: Sexual differentiation in *Xenopus laevis*. In: The Biology of Xenopus. Eds: R. C. Tinsley&H.
R. Kobel. The Zoological Society of London, Oxford (1996)

136. Raymond, C., J. Kettlewell, B. Hirsch, V. J. Bardwell & D. Zarkower: Expression of Dmrt1 in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sexual development. *Developmental Biology*, 215, 208-220 (1999)

137. Raymond, C., C. Shamu, M. Shen, K. Seifert, B. Hirsch, J. Hodgkin & D. Zarkower: Evidence for evolutionary conservation of sex-determining genes. *Nature*, 391, 873-881 (1998)

138. Miller, S. W., D. C. Hayward, T. A. Bunch, D. J. Miller, E. E. Ball, V. J. Bardwell, D. Zarkower & D. L. Brower: A DM domain protein from a coral, *Acropora millepora*, homologous to proteins inportant for sex determination. *Evolution and Development*, 5, 251-258 (2003)

139. Nanda, I., M. Kondo, U. Hornung, S. Asakawa, C. Winkler, A. Shimizu, Z. Shan, T. Haaf, N. Shimizu, A.

Shima, M. Schmid & M. Schartl: A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka *Oryzias latipes*. *Proceedings of the National Academy of Sciences*, 99, 11778-11783 (2002) 140. Matsuda, M., A. Shinomiya, M. Kinoshita, A. Suzuki, T. Kobayashi, B. Paul-Prasanth, E.-I. Lau, S. Hamaguchi, M. Sakaizumi & Y. Nagahama: DMY gene induces male development in genetically female (XX) medaka fish. Proceedings of the National Academy of Sciences, 104, 3865-3870 (2007)

141. Kondo, M., I. Nanda, U. Hornung, S. Askawa, N. Shimizu, H. Mitani, M. Schmid, A. Sihima & M. Schartl: Absence of the candidate male sex-determining gene dmrt1b (Y) of medaka from other fish species. *Current Biology*, 13, 416-420 (2003)

142. Yoshimoto, S., E. Okada, T. Oishi, R. Numagami, H. Umemoto, K. Tamura, H. Kanda, T. Shiba, N. Takamatsu & M. Ito: Expression and promoter analysis of *Xenopus DMRT1* and functional characterization of the transactivating property of its protein. *Develpment, Growth and Differentiation*, 48, 597-603 (2006)

143. Osawa, N., Y. Oshima & M. Nakamura: Molecular cloning of *Dmrt1* and its expression in the gonad of *Xenopus. Zoolog. Sci.*, 22, 681-687 (2005)

144. Mable, B. K.: 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. *Biological Journal of the Linnean Society*, 82, 453-466 (2004)

145. Muller, H. J.: Why polyploidy is rarer in animals in plants. *American Naturalist*, 59, 346-353 (1925)

146. Orr, H. A.: 'Why polyploidy is rarer in animals than in plants' revisited. *American Naturalist*, 136, 759-770 (1990)

147. Charlesworth, D.: Plant sex determination and sex chromosomes. *Heredity*, 88, 94-101 (2002)

148. Schmid, M. & C. Steinlein: Chromosome banding in Amphibia, XVI. High resolution replication banding patterns in *Xenopus laevis*. *Chromosoma*, 101, 123-132 (1991)

149. Schmid, M., S. H. Sims, T. Haaf & H. C. Macgregor: Chromosome banding in Amphibia X. 18S and 28S ribosomal RNA genes, nucleolus organizers and nucleoli in *Gastrotheca riobambae. Chromosoma*, 94, 139-145 (1986) 150. Hillis, D. M. & D. M. Green: Evolutionary changes of

hetergametic sex in the phylogenetic history of amphibians. Journal of Evolutionary Biology, 3, 49-64 (1990)

151. Wilkins, A. S.: Moving up the hierarchy: a hypothesis on the evolution of genetic sex determination pathway. *Bioessays*, 17, 71-77 (1995)

152. Felsenstein, J.: The evolutionary advantage of recombination. *Genetics* 78, 737-756 (1974)

153. Muller, H. J.: The relation of recombination to mutational advance. *Mutation Research*, 106, 2-9 (1964)

154. Bachtrog, D.: Evidence that positive selection drives Y-chromosome degeneration in *Drosophila miranda*. *Nature Genetics*, 36, 518-522 (2004)

155. Gordo, I., A. Navarro & B. Charlesworth: Muller's ratchet and the pattern of variation at a neutral locus. *Genetics*, 161, 835-848 (2002)

156. Cassidy, D. M. & A. W. Blackler: Repression of nucleolar organizer activity in an interspecific hybrid of the genus Xenopus. *Dev. Biol.*, 41, 84-96 (1974)

157. Haldane, J. B. S.: Sex-ratio and unisexual sterility in hybrid animals. *Journal of Genetics*, 12, 101-109 (1922)

158. Orr, H. A.: Haldane's rule. *Annual Review of Ecology and Systematics*, 28, 195-218 (1997)

159. Fischer, W. J., W. A. Koch & A. Elepfandt: Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae) *Journal of Zoology*, *London*, 252, 99-107 (2000)

160. Presgraves, D. C. & H. A. Orr: Haldane's rule in taxa lacking a hemizygous X. *Science*, 282, 952-954 (1998)

161. Laurie, C. C.: The weaker sex is hetermgametic: 75 years of Haldane's Rule. *Genetics*, 147, 937-951 (1997)

162. Wu, C.-I., N. A. HJohnson & M. F. Palopoli: Haldane's rule and its legacy: why are there so many sterile males? *Trends in Ecology and Evolution*, 11, 281-284 (1996)

163. Wu, C.-I. & A. W. Davis: Evolution of postmating reproductive isolation: the composite nature of Haldane's rule and its genetic bases. *American Naturalist*, 142, 187-212 (1993)

164. Malone, J. H. & P. Michalak: Physiological sex predicts hybrid sterility regardless of genotype. *Science*, 319, 59 (2008)

165. Malone, J. H., D. L. Hawkins & P. Michalak: Sex biased gene expression in a ZW sex determination system. *Journal of Molecular Evolution*, 63, 427-436 (2006)

166. Forsdyke, D. R.: Haldane's Rule: hybrid sterility affects the heterogametic sex first because sexual differentiation is on the path to species differentiation. *Journal of Theoretical Biology*, 204, 443-452 (2000)

167. Tobias, M. L., S. S. Viswanathan & D. B. Kelley: Rapping, a female receptive call, initiates male-female duets in the South African clawed frog. *Proceedings of the National Academy of Sciences*, 95, 1870-1875 (1998)

168. Tobias, M., R. O'Hagan, S. Horng & D. Kelley: Vocal communication between male *Xenopus laevis*; behavioral context and sexual state. *Animal Behavior*, 67, 353-365 (2004)

169. Vignal, C. & D. B. Kelley: Significance of temporal and spectral acoustic cues for sexual recognition in *Xenopus laevis. Proceedings of the Royal Society of London Series B*, 274, 479-488 (2007)

170. Du Preez, L. H., N. Kunene, R. Hanner, B. J. Evans, J. P. Giesy, K. R. Solomon, A. Hosmer & G. Van Der Kraak: Geographically structured occurrence of testicular ovarian follicles in *Xenopus laevis* from South Africa. *submitted* (2008)

171. Cowles, C. R., J. N. Hirschhorn, D. Altshuler & E. S. Lander: Detection of regulatory variation in mouse genes. *Nature Genetics*, 32, 432-437 (2002)

172. Kirst, M., C. J. Basten, A. A. Myburg, Z. B. Zeng & R. R. Seberoff: Genetic architecture of transcript-level variation in differentiating xylem of a eucalyptus hybrid. *Genetics*, 169, 2295-2303 (2005)

173. Morley, M., C. M. Molony, T. M. Weber, J. L. Devlin, K. G. Ewens, R. S. Spielman & V. G. Cheung: Genetic analysis of genome-wide variation in human gene expression. *Nature*, 430, 743-747 (2004)

174. Yan, H., W. Yuan, V. E. Velculescu, B. Vogelstein & K. W. Kinzler: Allelic variation in human gene expression. *Science*, 297, 1143 (2002)

175. Laundry, C. R., P. J. Wittkoop, C. H. Taubes, J. M. Ranz, A. G. Clark & D. L. Hartl: Compensatory cis-trans evolution and the dysregulation of gene expression in

interspecific hybrids of *Drosophila*. Genetics, 171, 1813-1822 (2005)

176. Wittkopp, P. J., B. K. Haerum & A. G. Clark: Evolutionary changes in cis and trans gene regulation. *Nature*, 430, 85-88 (2004)

177. Xenbase: http://www.xenbase.org/common/ (2007)

Abbreviations: WGD: Whole Genome Duplication

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