

REVIEW

Genome duplication in amphibians and fish: an extended synthesis

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Abstract

Whole genome duplication (leading to polyploidy) is widely accepted as an important evolutionary force in plants, but it is less recognized as a driver of animal diversification. Nevertheless, it occurs across a wide range of animals; this review investigates why it is particularly common in fish and amphibians, while rare among other vertebrates. We review the current geographic, ecological and phylogenetic distributions of sexually reproducing polyploid taxa before focusing more specifically on what factors drive polyploid formation and establishment. In summary, (1) polyploidy is phylogenetically restricted in both amphibians and fishes, although entire fish, but not amphibian, lineages are derived from polyploid ancestors. (2) Although mechanisms such as polyspermy are feasible, polyploid formation appears to occur principally through unreduced gamete formation, which can be experimentally induced by temperature or pressure shock in both groups. (3) External reproduction and fertilization in primarily temperate freshwater environments potentially exposes zygotes to temperature stress, which can promote increased production of unreduced gametes. (4) Large numbers of gametes and group breeding in relatively confined areas could increase the probability of compatible gamete combinations in both groups. (5) Both fish and amphibians have a propensity to form reproductively successful hybrids; although the relative frequency of autopolyploidy versus allopolyploidy is difficult to ascertain, multiple origins involving hybridization have been confirmed for a number of species in both groups. (6) Problems with establishment of polyploid lineages associated with minority cytotype exclusion could be overcome in amphibians via assortative mating by acoustic recognition of the same ploidy level, but less attention has been given to chemical or acoustic mechanisms that might operate in fish. (7) There is no strong evidence that polyploid fish or amphibians currently exist in more extreme environments than their diploid progenitors or have broader ecological ranges. (8) Although pathogens could play a role in the relative fitness of polyploid species, particularly given duplication of genes involved in immunity, this remains an understudied field in both fish and amphibians. (9) As in plants, many duplicate copies of genes are retained for long periods of time, indicative of selective maintenance of the duplicate copies, but we find no physiological or other reasons that could explain an advantage for allelic or genetic complexity. (10) Extant polyploid species do not appear to be more or less prone to extinction than related diploids in either group. We conclude that, while polyploid fish and amphibians share a number of attributes facilitating polyploidy, clear drivers of genome duplication do not emerge from the comparison. The lack of a clear association of sexually reproducing polyploids with range expansion, harsh environments, or risk of extinction could suggest that stronger correlations in plants may be driven by shifts in mating system more than ploidy. However, insufficient data currently exist to provide rigorous tests of these hypotheses and we make a plea for zoologists to also consider polyploidy as a possibility in continuing taxonomic surveys.

Introduction

Genome sequencing has revealed that across both plant and animal kingdoms, the vast majority of genes are organized in multiple sets rather than single copies (Wolfe & Shields, 1997; Blanc *et al.*, 2000; Edgell, Malik & Doolittle, 2000; Donoghue & Purnell, 2005; Wessler & Carrington, 2005). This extensive gene duplication is hypothesized to have arisen through multiple rounds of whole genome duplication (WGD), and is thought to be fundamental for speciation, diversification of gene functions and shaping genomic architecture across major eukaryotic groups (Lynch, 2002; Ramsey & Schemske, 2002; Blanc, Hokamp & Wolfe, 2003; Leitch *et al.*, 2004; Wessler & Carrington, 2005; Chen, 2007). It is now accepted that two rounds of WGD occurred during the early diversification of chordates and vertebrates, with strong evidence supporting a subsequent teleost fish-specific genome duplication (FSGD; Dehal & Boore, 2005; Hoegg & Meyer, 2005; Putnam *et al.*, 2008; Larhammar *et al.*, 2009; Van de Peer, Maere & Meyer, 2009). More recent WGD events have also occurred, resulting in species that are currently functionally polyploid. As some degree of diploidization and loss of duplicate gene expression inevitably follows WGD (Ferris & Whitt, 1977*d*; Adams, 2007; Flagel & Wendel, 2010), it is often difficult to distinguish 'ancient' (i.e. paleopolyploid) from 'recent' events. Nevertheless, in this review we define 'extant' polyploid species as those having twice the chromosome number of close relatives, sometimes retaining at least some pairing of multiple chromosome copies during meiosis and retaining evidence of duplicate gene expression distributed throughout the genome, with most having some recognizably distinctive features from their closely associated diploids. It is the distributions and characteristics of these extant polyploids that are the focus of this review.

There are no comprehensive surveys of the prevalence of polyploidy in animals, but it has been documented across a wide range of taxa, including insects, crustaceans, molluscs, fish, amphibians, reptiles and (to a lesser extent) mammals (reviewed in Bogart, 1980; Lewis, 1980; Otto & Whitton, 2000; Le Comber & Smith, 2004; Gregory & Mable, 2005). Polyploidy is most common in organisms that do not regulate their internal temperature (i.e. plants and ectothermic animals), and are therefore directly exposed to changes in their environments. However, because polyploidy is not widespread among ectothermic vertebrates in general, it begs the question of why some groups are polyploid and others not. Although it is possible that intrinsic mechanisms regulating genome integrity constrain polyploid establishment, it is also possible that ecological factors (i.e. living in habitats or conditions that favour polyploidy), in combination with the inherently stochastic nature of establishment of polyploid lineages [i.e. formation in the midst of diploid progenitors (Levin, 1975; Husband, 2000); producing balanced chromosome sets] are responsible.

In this review we question why, among vertebrates, polyploidy is most frequent among fish and amphibians.

Our purpose is to evaluate the phylogenetic and geographic distributions, hypothesized origins, reproductive biology and ecology of sexually reproducing polyploid fish and amphibians, to better understand the potential drivers of polyploidy. Kejnovsky, Leitch & Leitch (2009) recently contrasted differences between mammals and plants that might make the former less prone to polyploidy, but our objective is to emphasize what features shared by fish and amphibians might promote polyploid formation and establishment. We focus solely on bisexually reproducing polyploids in order to avoid confounding environmental and geographic effects related to breeding system variation rather than polyploidy, which has been a problem for interpreting patterns in plants, ostracods and insects (reviewed in Mable, 2003). We first set the historical context for polyploid discoveries in fish and amphibians, with an overview of the types of data that have been used to identify them and summarize the current phylogenetic distribution of extant polyploid fish and amphibians, before focusing on factors that favour polyploid formation and establishment.

We thus surveyed the distribution of polyploidy in anurans (frogs and toads) compared with fishes and exploited comprehensive databases summarizing their taxonomy and distribution [Amphibian Species of the World, (Frost, 2010) <http://research.amnh.org/vz/herpetology/amphibia/>], ecology and life history (Amphibiaweb, <http://amphibiaweb.org/>) and conservation status (International Union for Conservation of Nature Redlist of Endangered species, <http://www.iucnredlist.org>). For fishes, genome size and karyotype data were obtained from the animal genome size database (Gregory 2005a) (<http://www.genomesize.com/>), while taxonomic, ecological and biogeographic information was mined from Fishbase (Froese & Pauly, 2008) (<http://www.fishbase.org>). Because ploidy state is not an attribute that is available for most species in these databases, we first compiled a list of known extant polyploid species from the primary literature (please see supporting information Tables S1 and S2, where the relevant references are cited), before using the databases to update species designations and phylogenetic distributions, examine geographic distributions and ecological preferences, and assess endangered species status.

We conclude that the most striking feature shared by polyploid fish and amphibians is external reproduction in freshwater environments, predominantly in regions where temperature fluctuations during the breeding season are common. It would thus be tantalizing to speculate that this could support previous hypotheses that rates of polyploid formation and establishment are associated with periods of climatic change and/or currently unstable or extreme environments (e.g. Hagerup, 1932) because polyploids are genetically more resilient than their diploid progenitors or able to exploit more extreme habitats. We do not find evidence that bisexual polyploids have broader ecological ranges or distributions or are at lower risk of extinction than their diploid relatives but insufficient data currently exist to provide robust tests and to fully understand the potential impacts of climate change on rates of polyploid speciation.

Historical context

While polyploid animals are now well documented, the existence of the first polyploid vertebrate (the *Ambystoma jeffersonianum* complex of salamanders) was not accepted by the scientific community until 1964 (Uzzell, 1964). The first polyploid frogs (*Odontophrynus americanus* and *Ceratotophrys ornata*) were described in 1966 (Saez & Brum-Zorrilla, 1966), but despite providing clear figures showing multiple sets of chromosomes and multivalent formation during meiosis, the authors rather remarkably concluded that 'it does not mean that we believe in the existence of polyploidy'. Bogart (1967) later confirmed that these were both octoploid species that reproduced bisexually. Earlier research on fish also suggested that polyploidy played a major role in the evolution of the Salmonidae (Svärdson, 1945) and the genus *Coregonus* (Kupka, 1948) but these were discounted by some researchers, and polyploidy in Salmonidae as of 1967 was 'not considered to be a biological fact' (reviewed by Bogart, 1967). Despite the initial excitement of these early discoveries, polyploidy has never really emerged to the forefront of attention by animal evolutionary biologists.

In a thorough and insightful review based on experimental induction of polyploidy in amphibians compared with plants and insects, Gerhard Fankhauser (an embryologist from Princeton University) predicted that polyploidy was likely to be evolutionarily important in vertebrates (Fankhauser, 1945). He suggested that 'the following data should be gathered: (1) occurrence and frequency of polyploidy in different groups of animals; (2) origin of these exceptional individuals, in other words, the mechanisms that are responsible for their production; (3) the effectiveness of methods for the experimental induction of polyploidy; (4) the general effects of polyploidy on cell size, body size and viability, and on the general physiology and biochemistry of the organism; (5) the occurrence of qualitative effects, which are added to the more obvious quantitative consequences'. Half a century later, the answers to Fankhauser's queries remain largely unanswered. This is partly a result of the view that polyploidy could not be important in animals because: (1) they have too much developmental complexity compared with plants; (2) sex determining mechanisms in vertebrates and *Drosophila* are expected to be disrupted by changes in dosage (Muller, 1925; Orr, 1990; Mable, 2004); (3) regulation of body size in the developing ovum could be altered by cell size increases and dosage (Manevto, 1945).

The possibility that polyploidy has played an important role in animal evolution has recently received more attention (Donoghue & Purnell, 2005; Gregory & Mable, 2005; Volff, 2005) but discoveries of unrecognized polyploids remain rare, possibly because few researchers look for them, but also due to the difficulty of identifying cryptic polyploids (see 'Identification of Polyploids'). Thus, there is likely to be a considerable underestimate of the distribution and frequency of polyploidy in animals. In contrast, polyploid plants are the focus of modern genomic research not only due to their economic importance, but also due to the much

larger than expected genomic signatures of ancient WGD events, which opens opportunities for studying changes in gene expression following polyploidization. A special issue on polyploidy in *New Phytologist* (Ainouche & Jenczewski, 2010) emphasizes the contributions of rapid advances in genome sequencing technologies to understanding such genomic consequences of polyploidy. However, even in plants, we still do not have a complete understanding of the factors that promote the formation and establishment of polyploidy in the wild, the role that ecology plays in polyploid speciation, and whether polyploidy accelerates diversification rates or is an evolutionary dead end (reviewed in Levin, 2002; Soltis, Buggs, Doyle *et al.*, 2010).

Identification of polyploids

Extant polyploids have been identified using a variety of techniques, including chromosome counts, detection of multivalent formation during meiosis, banding patterns in markers such as allozymes, cell size comparisons and genome size estimations. However, none of these techniques are incontrovertible and controversies over polyploid status often remain unresolved [e.g. Viscacha rat (Gallardo *et al.*, 2004; Svartman, Stone & Stanyon, 2005)]. This is reflected in the wide range of estimates of polyploid frequencies in plants (Jenkins & Rees, 1991; Hilu, 1993; Masterson, 1994; Soltis & Soltis, 1999; Otto & Whitton, 2000). Most recently, based on detailed phylogenetic comparisons, it has been estimated that 15% of flowering plant and 31% of fern speciation events have been accompanied by a ploidy increase (Wood *et al.*, 2009). However, despite increased rigour of analytical methods, this study relied on inferring ploidy level primarily from chromosome data, which can be problematic (Otto & Whitton, 2000). No single method works for all groups, with differences among plants, fish and amphibians in the predominant methods used to infer ploidy status. Most documented cases of polyploidy in animals have used a pluralistic approach, rather than relying on a single method. Below we survey the major types of methods that historically have been used to identify polyploids.

Cytogenetics

The oldest methods for identifying polyploids are through cytogenetic assessment of chromosome numbers, banding and meiotic configuration patterns. Theory suggests that allopolyploids segregate disomically, as they present two sets of homeologous chromosomes that are unlikely to pair at meiosis, while autopolyploids segregate polysomically because their chromosomes pair at random and form multivalents during meiosis (Chenuil, Galtier & Berrebi, 1999). In practice, the reestablishment of disomic inheritance in ancient polyploids (deWet, 1980) or polysomic inheritance in allopolyploids arising from close relatives sharing partly homologous chromosomes (Stebbins, 1950), means that there is likely to be a continuum between strictly disomic and strictly polysomic inheritance (see review by Soltis *et al.*,

2010). In general, the demonstration of tetravalents during meiosis and tetrasomic inheritance provide good evidence of polyploid status but lack of tetravalent formation does not necessarily mean that a species is diploid, because even young polyploids experience some degree of diploidization (e.g. Le Comber *et al.*, 2010). Additional difficulties arise when chromosomal rearrangements (through Robertsonian fissions and fusions) lead to changes in chromosome number that are not related to genome duplication. Because increases in genomic content can be achieved through other means than duplication (particularly in noncoding regions), genome size also is not always a reliable estimate of WGD. Genome sequencing studies have confirmed that transposable elements (which can result in large differences in genome sizes without changes in chromosome numbers) may confound relationships between DNA content and chromosome numbers (e.g. Parisod *et al.*, 2010).

Nevertheless, at least in anurans, karyotyping remains the most reliable method of detecting polyploidy. This is facilitated by the conservation of basal chromosome number (range 9–13) and the presence of large chromosomes that allow banding patterns and rDNA distributions to be used to indicate changes in chromosome morphology. In fact, Bogart (1991) noted that species groups where Robertsonian changes in chromosomes (i.e. fusions or fissions) are common do not tend to include polyploids and polyploid species often share a high degree of apparent synteny (based on chromosome banding patterns) as their diploid progenitors. All known polyploid anurans have an even replication of chromosome number compared with closely related diploids (supporting information Table S1), often with highly similar chromosome morphologies, based on banding patterns and rDNA locations (Bogart, 1980; Stöck *et al.*, 2005).

In fishes, the situation is more complicated (supporting information Table S2). Recent analyses of whole genome sequences and ancestral reconstruction suggest that the ancestor of all teleost fishes had a haploid chromosome complement of 12–13 chromosomes that increased to 23–24 chromosomes after the teleost-specific duplication (Jaillon *et al.*, 2004; Kasahara *et al.*, 2007; Nakatani *et al.*, 2007). The modal diploid chromosome number for acanthopterygian fishes is 48 (Mank & Avise, 2006), with counts ranging from 22 to 250 and counts of 'diploid' species ranging between 22 and 78. Genome size is correlated with chromosome number when all species are investigated, but this relationship is weaker (although remains significant) when polyploids are removed from the analysis. Mank & Avise (2006), suggest that seven to 20 polyploidization events have occurred in extant ray-finned fish lineages based on analysis of chromosome numbers, although this is almost certainly an underestimate, as many polyploid fishes have yet to be karyotyped and within polyploid lineages there is often evidence of multiple independent duplications (M. A. Alexandrou and M. I. Taylor, pers. obs.). Nevertheless, chromosome numbers have been used to identify polyploid fish species and even entire families. For example, the Catastomidae are all tetraploids, with a basal chromosome complement of 100, which is twice that of diploid cyprinids (Ferris, 1984).

Marker-based methods

Allozymes have been widely used to identify suspected polyploids (based on number of copies or dosage of bands), to assess modes of origin of polyploid taxa (allopolyploid or autopolyploid) based on patterns of duplicate gene expression and sharing of alleles with putative ancestors, to identify genomic composition of hybrids and to make inferences about fates of duplicate genes. Because allozymes compare the protein products of expressed genes, they also have been used to assess the degree of duplicate gene expression across loci and tissue types (Ferris & Whitt, 1977*d*; Allendorf, 1978; Bailey, Poulter & Stockwell, 1978). In fact, Susumo Ohno (Ohno, Wolf & Atkin, 1968) based his precocious predictions that vertebrates had experienced multiple rounds of WGD on patterns of duplicate gene expression in allozymes. Despite the advent of more advanced technologies, allozymes remain one of the clearest methods for cheaply and rapidly characterizing polyploid genomes.

Because expertise in cytogenetics was also at its peak among evolutionary biologists when allozymes were popular, combining the two approaches is likely to have been responsible for a peak in discovery of new polyploid amphibian species in the 1970s (supporting information Fig. S1). Allozymes have been used to characterize the cryptic parental origins of previously identified polyploid amphibians [e.g. *Tomopterna tandyi* complex (Channing & Bogart, 1996); *Hyla versicolor* complex (Ralin, 1978; Romano *et al.*, 1987)], discover that disomic, tetrasomic and intermediate patterns can be found within tetraploid families depending on the locus and tissue type examined [*Hy. versicolor* (Danzmann & Bogart, 1983)], and to identify which sexual species contribute to hybrid unisexual lineages [*Ambystoma laterale* complex (Bogart *et al.*, 1985; Bi & Bogart, 2006)].

In fishes, allozymes have been used to identify polyploid lineages [e.g. Salmonidae (Allendorf & Thorgaard, 1984)], to infer hybrid composition [e.g. tetraploid loaches (Slechtova *et al.*, 2003)], to identify duplicated loci based on tissue-specific expression patterns (Ohno *et al.*, 1968; Ohno, 1970, 1993; Ferris & Whitt, 1975, 1977*c,b*, 1978), to infer preferential expression of parental alleles in experimental crosses [e.g. crosses between carp and goldfish (Danzmann & Down, 1982)] and preferential pairing of homeologues [e.g. rainbow trout (Allendorf & Danzmann, 1997)], and to assess the proportion of duplicate genes that remain expressed in 'old' polyploids [e.g. in the Salmonidae c. 50% of duplicated allozyme loci remain detectable (Allendorf & Thorgaard, 1984); a similar proportion to other tetraploid fishes, which retain between 25 and 70% of duplicated loci (Li, 1980)]. An interesting point to note is that the study of the fate of duplicate genes based on allozymes in Cypriniformes (Ferris & Whitt, 1977*a*) provided evidence for the separation of function of duplicate copies by tissue type or developmental stage (now known as subfunctionalization), along with evolution of new functions or loss of functions.

DNA-based markers such as microsatellites can be used directly to provide evidence of duplicate genes rather than

duplication of expressed products. However, fluorescent peak heights or stained-band intensities in microsatellites are not always directly proportional to dosage of starting products due to uneven amplification of alleles in PCR, so that copy numbers can be more difficult to infer than for allozymes. Nevertheless, the large numbers of alleles at microsatellite loci may make dosage less important, as polyploidy can be identified by the number of peaks rather than the differences in strength. Their higher levels of variation can also be useful for inferring parental origin and segregation of alleles to assess inheritance patterns; in carp, around 60% of microsatellite loci still amplify duplicate copies despite the duplication event having occurred around 12 mya (David *et al.*, 2003). Microsatellites also have been used in conjunction with flow cytometry to establish ploidy levels and provide evidence of occasional sex in supposed unisexuals (Ramsden, Beriault & Bogart, 2006; Bi, Bogart & Fu, 2007, 2009; Bogart *et al.*, 2007).

Genome size

Variation in genome size has the potential to uncover polyploidy, but for many organisms, there is no relationship between DNA content and chromosome number (Gregory, 2005a,b), so it is important to confirm estimates with chromosome counts. There are also a variety of methods available to estimate genome sizes (e.g. flow cytometry, feulgen image analysis densitometry) and there can be difficulties in calibrating estimates from different laboratories (Hardie, Gregory & Hebert, 2002; Gregory, 2005a,b; Leitch, 2007; Smith & Gregory, 2009). Methods that provide relative, rather than absolute, measures of DNA content can be less problematic for identifying suspected polyploids, particularly if they are conducted in the same laboratory.

In amphibians, absolute genome size is not a good predictor of ploidy level, particularly in salamanders, which have a large range in genome sizes, even among diploids (Gregory, 2005a,b) (Animal Genome Size Database, <http://www.genomesize.com>). In anurans, using genome size alone to infer ploidy is also equivocal. Diploid genome sizes vary widely, with diploids in the genus *Hyla* (c. 5.0 pg) having a genome size similar to tetraploid *Neobatrachus*, despite having the same basal chromosome number ($2n = 24$). At least in salamanders, differences in genome size of species with the same chromosome number have been found to be due to differences in repetitive DNA, rather than WGD (Baldari & Amaldi, 1976; Bozzoni & Beccari, 1978). Insufficient genomic data exist to assess whether this is also true in anurans or whether lineage-specific paleopolyploidy has also occurred. Comparisons of genome size between previously identified diploid and tetraploid species pairs may be more illuminating with regards to the potential to use such data to infer ploidy levels. Data for the five diploid–tetraploid pairs where data are available show an average tetraploid:diploid genome size ratio of 1.93 (supporting information Table S1). However, the same does not appear to be true for higher ploidy levels. In the genus *Ceratophrys*,

for example, there are two octoploid species with the same chromosome number ($8n = 104$) but one (*Ceratophrys aurita*) has a genome size of 6.34 pg, whereas the other (*Ce. ornata*) has 13.4 pg. Although a diploid or tetraploid progenitor has not been identified, a closely related diploid (*Ceratophrys calcarata*) has a genome size of 2.3 pg; neither octoploid has $8 \times$ its DNA content. This could suggest that the genome size of the progenitor diploid was much higher or that cryptic ancient polyploidy occurred in one ‘octoploid’ lineage, but might also be due to differences in genome size estimation by different authors. For *Xenopus*, genome size estimates for tetraploid species range between 3.0 and 4.1 pg, with an average ratio compared with *Silurana tropicalis* (the only extant diploid species) of 2.03 (range 1.8–2.3). However, this is not the likely progenitor, as it has a basal chromosome number of $2n = 20$ compared with $4n = 36$ in *Xenopus*. Again, higher ploidy levels show a nonlinear increase in DNA content, with ratios of 3.5 in the octoploid *Xenopus vestitus* and only 4.6 in the dodecaploid *Xenopus ruwenzoriensis* compared with *Si. tropicalis*.

Fish genome size varies considerably, with C-values ranging between 0.35 and 132.83 pg. C-values for teleosts range between 0.35 and 4.9 pg, with the average around 1.2 pg. However, the majority of species have C-values in the range 0.5–2.0 pg. The genome sizes of polyploid teleosts range from 1.36 to 3.75 pg, with a mean of 2.5 pg (Smith & Gregory, 2009). Smith & Gregory (2009) suggest that genome sizes are usually greater than 2.5 pg if polyploidy has occurred. Using genome size to infer polyploidy is more complicated in more basal groups of fishes, in which estimates may be confounded by transposable elements. For instance the cartilaginous fishes (Chondrichthyes) have C-values in the range 1.51–17 pg; although polyploidy has been suspected to have played a role in their early evolution (Kendall *et al.*, 1994; Stingo & Rocco, 2001), repetitive elements are also likely to have led to increases (Olmo *et al.*, 1982; Kellogg *et al.*, 1995) or decreases (Leitch & Bennett, 1997) in genome size. Moreover, chondrichthyans and sarcopterygians have only four Hox clusters (Venkatesh, 2007; Amemiya *et al.*, 2008; Putnam *et al.*, 2008), whereas actinopterygians have seven or eight Hox clusters (Crow *et al.*, 2006) which suggests that the large genome size of chondrichthyans may not be the result of polyploidization. In summary, comparing genome size to chromosome counts can be informative for inferring ploidy status in fishes, but the more extensive rearrangements and lineage-specific polyploidy makes the classification of extant polyploidy even less certain than for anurans (supporting information Table S2).

Cell size

Nucleus size is positively correlated with chromatin amount in polyploids (reviewed in Manevto, 1945). Cell size, however, is not always directly proportional to nuclear volume. In yeast, for example, whereas haploid cells tend to be smaller than diploid cells under reduced nutrient conditions, they can be the same volume when grown in rich medium

(Weiss, Kukora & Adams, 1975). The same can be true of higher ploidy levels (Mable, 2001) – for animals with nucleated red blood cells, the erythrocyte volume of tetraploids is consistently higher than in diploids but is often less than the factor of two that might be expected with the doubling of DNA content. In frogs, tetraploid *Hy. versicolor* (Hylidae) can be reliably distinguished from their diploid progenitors, *Hyla chrysoscelis* (Matson, 1990) based on differences in erythrocyte size, but the volume of tetraploid cells is less than that expected from the DNA content (ratio 1.3–1.5). This means that triploids can be difficult to distinguish because the ranges in volume overlap (Mable, 1989). For octoploid *Od. americanus*, erythrocyte size varies between juveniles and adults, with those of juveniles comparable to those of adult erythrocytes of the diploid *Odontophrynus cordobae* (Grenat *et al.*, 2009).

In fishes, genome size and erythrocyte size are also correlated in both teleosts and cartilaginous fishes (Hardie & Hebert, 2003, 2004). Interestingly, cold water fish have larger cell sizes than warm water species when controlling for genome size (Hardie & Hebert, 2003). However, distinguishing whether this is primarily due to polyploidy or nonduplication based genome expansion is difficult. Nevertheless, cell size may be useful for differentiating between closely related diploid and polyploid species or forms (Felip *et al.*, 2001).

Phenotypic characteristics

Polyploidy may lead to an increase in the overall size of organisms. Such ‘gigantism’ is prevalent among plants and insects but is not apparent in fish and amphibians. Polyploidy in fishes and amphibians appears to result in a reduction in the number of cells, so that even though cell size is increased, overall body size remains the same as in diploids (reviewed in Bogart, 1980). Most bisexually reproducing amphibians are found in close association with related and morphologically similar diploids, which have often been implicated in their formation (reviewed by Bogart, 1980). Polyploid anurans tend to have similar body size as the diploids (Fankhauser, 1945; Bachman & Bogart, 1975) and do not seem to experience radical changes in physiology; for example, *Hy. versicolor* have similar metabolic rates as their diploid progenitors (Kamel, Marsden & Pough, 1985). However, species-specific mating calls used by females to choose mates have been used to identify cryptic polyploids, based on polymorphism in mating calls among populations that were otherwise indistinguishable, with ploidy status subsequently confirmed by allozymes and/or chromosome counts (Wasserman, 1970; Bogart & Wasserman, 1972; Vigny, 1979; Barrio, 1980; Haddad, Pombal & Batistic, 1994; Roberts, 1997b; Stöck & Grosse, 2003). In grey treefrogs (*Hy. versicolor* complex), females prefer calls of their own ploidy level (Gerhardt, 1974, 1982, 2005a,b; Klump & Gerhardt, 1987), and some characters of the mating call (e.g. pulse rate) change as a direct consequence of the increase in cell size arising from polyploidy (Bogart & Wasserman, 1972; Bogart, 1980; Keller & Gerhardt, 2001; Holloway *et al.*, 2006), although this has not been found in all polyploid groups [e.g. *Neobatrachus* (Ro-

berts, 1997b)]. In some frog species, differences in colour patterns or morphometric measurements can also be used to distinguish some species that differ in ploidy (e.g. Mahony, Donnellan & Roberts, 1986; Castellano *et al.*, 1998; Stöck *et al.*, 2005), but such differences are likely to have arisen from adaptation postspeciation, rather than the genome duplication event itself.

There has been less focus in fish on using morphological or behavioural cues to identify polyploids, but there is the possibility that differences in quantity of chemical products that are produced in direct proportion to cell size or genome copy could be useful. As far as we are aware, no comparisons have been made between olfactory signal components in relation to ploidy in fish or amphibians that primarily use odour cues.

Identifying allo versus autopolyploids

Determining the origin of polyploid organisms, and whether they have arisen via autopolyploidy or allopolyploidy is crucial to our understanding of polyploid evolution. However, distinguishing between these mechanisms is difficult, due to the continuum between disomic and polysomic inheritance that exists in most polyploid species, regardless of whether they arose through hybridization or from a single progenitor species. As Soltis *et al.* (2010) eloquently discuss, there is also a different perspective between systematists (who are interested in whether polyploids arose from different species) and geneticists (who are interested in segregation patterns during meiosis). Nevertheless, considerable effort has been devoted to testing polyploid origins based on inheritance patterns and more rigorous statistical methodologies are in development (reviewed by Soltis, Soltis, Schemske *et al.*, 2007; Parisod, Holderegger & Brochmann, 2010). For example, by incorporating Bayesian statistics, Olson (1997) presented a method that allows simultaneous assessment of disomic versus tetrasomic inheritance, rather than performing goodness of fit tests separately for each model. Based on only two allozyme loci, they demonstrated disomic inheritance (allopolyploidy) in *Astilbe biternata* (Saxifragaceae) using a very small sample size. For DNA-based approaches such as microsatellites, Bayesian methods have been proposed that use large numbers of microsatellite loci and large numbers of individuals, to evaluate models of inheritance without use of progeny arrays (e.g. Catalan *et al.*, 2006).

More flexible statistical approaches that consider a range of inheritance patterns have also been proposed. For example, based on the number of homoeologous copies present in each species for a set of neutral markers, Chenuil *et al.* (1999) developed a method that does not require the assumption that allopolyploidy leads to multisomic inheritance. Based on this, using five microsatellite loci in eight cyprinid species (with one to three representatives of each) of the genus *Barbus* (Cypriniformes), the hypothesis that European tetraploid barbs originated through autopolyploidy was rejected. These tests have proven particularly useful in cases where hybridization between particular taxa is

known and well documented. Investigations of patterns of inheritance within the polyploid cyprinid *Cyprinus carpio* using 59 microsatellite markers suggested a hybrid origin (David *et al.*, 2003). Stift *et al.* (2008) describe a likelihood-based method incorporating intermediate inheritance patterns, as well as more complicated patterns due to double reduction, which provides a more realistic assessment of segregation patterns in polyploids.

Current taxonomy and phylogenetic distribution of polyploids

One feature shared by fish, amphibians and plants is their complex and dynamic taxonomic history. Ichthyologists, herpetologists and botanists historically have tended to share a passion for systematics, and it has been common for species to be reclassified multiple times not only among species and genera but also among families. This has been particularly true since molecular characters have been widely used to resolve phylogenies; whole genome studies will result in further revisions. It can, therefore, be difficult to sort through original reports of polyploidy in the face of changing taxonomy. Here we review the distributions of extant known polyploid anurans and fish, considering changing species designations and relationships among families or higher levels of classification. As a result of this review, it is interesting to note the absence of polyploid vertebrates from certain regions. Notably, there are no recorded polyploid fishes in Australia or Antarctica (possibly an artefact, as these areas are understudied), and in contrast to plants, very few extend their ranges into polar and arctic areas. There are polyploid frogs across all temperate and tropical continents, including Australia, but amphibians in general do not occur in the Antarctic or Arctic so their absence there is less surprising.

Anurans

Because polyploid frogs live in close proximity to their diploid relatives (Bogart, 1980), we provide a list of bisexual polyploids and their closely related diploids (supporting information Table S1). Wherever possible, credit has been given to the authors who first described a species as polyploid. Because the taxonomy of many anurans remains controversial (e.g. Roelants, Gower, Wilkinson *et al.*, 2007; Wiens, 2007), we provide the current classifications provided in the Frost Amphibian species of the world database [largely based on the revised taxonomy provided in Frost *et al.*, (2006)], as well as the species definitions at the time that polyploids were originally identified.

Polyploidy has arisen independently in multiple amphibian families. The majority of species in the basal family Pipidae are tetraploid or higher (Fig. 1). The model Pipid species *Xenopus laevis* was originally thought to be diploid but it is now recognized as an ancient tetraploid, with extensive, but incomplete diploidization across much of its genome (Kobel & Du Pasquier, 1986); octoploids and dodecaploids also occur in the family (see Evans *et al.*, 2004, 2005, 2008). Polyploidy has also been suggested in other basal groups (Leiopelmatidae Green, Kezer & Nussbaum, 1984) and the entire Sirenidae family may be ancient polyploids (Morescalchi & Olmo, 1974), but these reports remained unconfirmed. In the more derived groups (e.g. Hylidae, Ranidae, Microhylidae), polyploid species are more scattered. Bisexually reproducing polyploid anurans have been confirmed across eight traditional families (Gregory & Mable, 2005) but taxonomic revisions suggested in the Frost database (Frost, 2010) mean that the 43 polyploids are now distributed across 12 families, with 19 in the family Pipidae (plus some unnamed species); eight in Leptodactylidae (now divided into four families); four each

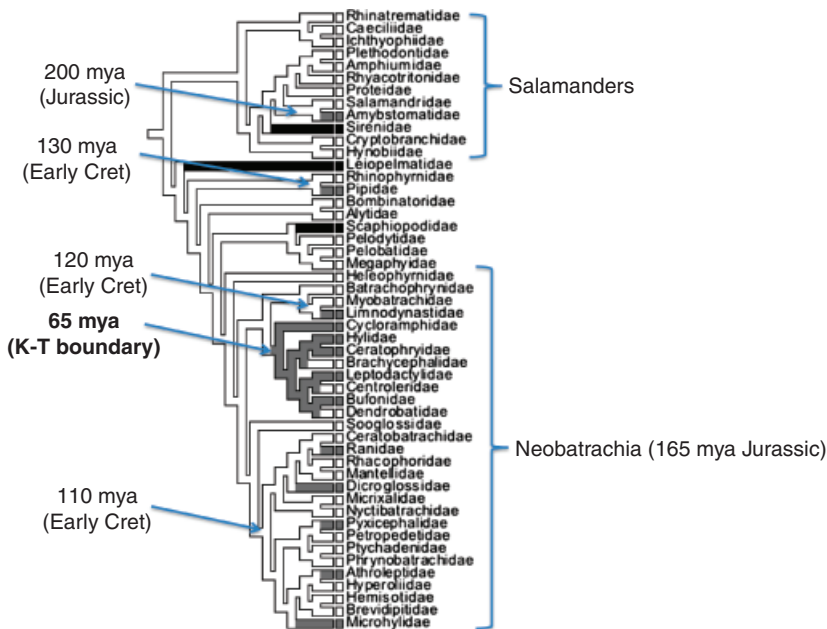


Figure 1 Family-level amphibian tree, redrawn from Roelants *et al.* (2007), indicating families where polyploid species are known and estimated dates of divergence of clades that include polyploid lineages. Suspected but unconfirmed cases of polyploid species have been reported in the Leiopelmatidae and Scaphiopodidae. The entire family Sirenidae has been reported as anciently polyploid but this has yet to be confirmed.

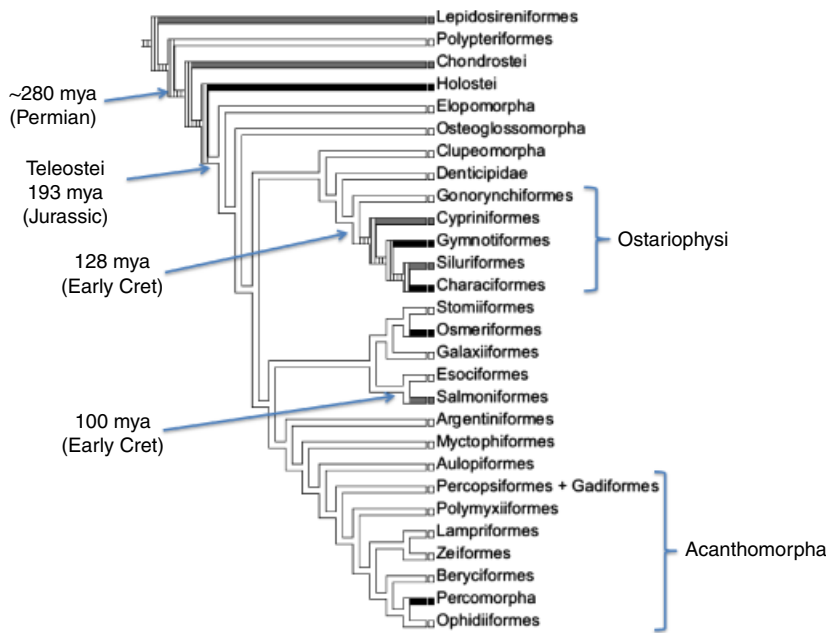


Figure 2 Phylogeny of fish orders, redrawn from Santini *et al.* (2009), indicating orders where polyplody species are known and estimated dates of divergence. A single family in the Percomorpha contains polyplody species (Characidae) but divergence of this lineage from relatives that do not include polyplody have not been clearly established. There is a notable absence of polyplody within the Acanthomorpha. Within the Ostariophysii, spontaneous polyplody have been reported within the Gymnotiformes and Characiformes.

in Myobatrachidae (now Lymnodynastidae) and Bufonidae; three in Microhylidae; two in Hylidae; and one each in Dicroglossidae, Arthroleptidae and Ranidae. Several new polyplody anuran species have been reported (*Chiasmocleis leucosticta*, *Cophixalus pansus*, *Scaphiophryne gottlebei*, *Ceratophrys joazeirensis*, *Pleurodema cordobae*) since the summary in Otto & Whitton (2000), along with a number of new *Xenopus* species (Evans *et al.*, 2004) and the surprising finding of sexually reproducing triploid toads in the *Bufo viridis* complex (Stöck *et al.*, 2002, 2006). Species status has also been given to diploids in what were previously mixed complexes of diploids and polyplody (e.g. *Od. americanus*, *Phyllomedusa burmeisteri* complex, *Bu. viridis* complex) and a cryptic octoploid (*Pleurod. cordobae*) has recently been discovered in populations of *Pleurodema kriegi* (see supporting information Table S1 for references).

Polyplody species (and their diploid progenitors) tend to be underrepresented in molecular phylogenies (e.g. Faivovich *et al.*, 2005; Frost *et al.*, 2006), possibly as a result of the practical difficulties of dealing with duplicated gene sequences, but also the fact that polyplody taxa are not strictly appropriate for phylogenetic analyses because they do not arise by cladogenesis. This, combined with lack of knowledge on the nature of origins for most species (auto or allopolyploid; single vs. multiple), makes it difficult to evaluate hypotheses about dates of origins of particular species pairs. There are also discrepancies between recent phylogenetic hypotheses for amphibians (e.g. Frost *et al.*, 2006; Roelants *et al.*, 2007). However, Roelants *et al.* (2007) present an analysis of diversification rates and predicted divergence dates within amphibians that allows some insights. Based on this family-level phylogeny, we plotted the phylogenetic distribution of families that include polyplody and highlight the approximate dates of divergence of those that include polyplody from their closest relatives (Fig. 2). It

is important to note that this is not an indication of when polyplody species arose, just when the families in which they are found diverged from families where polyplody has not been identified. The clustering of divergence times in different lineages that include polyplody could suggest that climatic conditions in the early Cretaceous and beginning of the Paleogene favoured speciation by polyplody. It is intriguing to note that the Pipidae (African polyplody in the genus *Xenopus*) and Lymnodynastidae (Australian polyplody in the genus *Neobatrachus*) both diverged in the early Cretaceous and both of these families include multiple polyplody species, some of which appear to have speciated as polyplody or have no extant diploid progenitors (Mahony *et al.*, 1986; Mable & Roberts, 1997; Evans *et al.*, 2004). Among the Ranoids (early Cretaceous diversification), polyplody are found in families within each of the major clades (Ranids, Dicroglossids, Pyxicephalids, Arthroleptids and Microhylids), which could suggest that conditions favourable for polyplody existed before their divergence. Particularly intriguing is the 65 mya divergence (corresponding to the Cretaceous-Tertiary boundary) of the clade including Hylids, Ceratophryids, Cycloramphids, Leptodactylids and Bufonids, all of which contain multiple independent diploid–polyplody species pairs. It would be tempting to speculate that the environmental instability during the K–T boundary promoted polyplody speciation, as has been suggested for plants (Fawcett, Maere & Van de Peer, 2009). Dates of divergence predicted for *Hy. versicolor* (last post-glacial period, c. 12 000–35 000 ya Otto *et al.*, 2007), and species in the Pipidae (maximum 65 mya and most species thought to have arisen before the Pleistocene (Evans *et al.*, 2004, 2005)), correspond to times when climatic conditions were likely highly variable. However, similarly to plants (Soltis, Soltis & Tate, 2004), many of the polyplody anurans are thought to have had multiple origins (Ptacek, Gerhardt

& Sage, 1994; Mable & Roberts, 1997; Espinoza & Noor, 2002; Gerhardt, 2005b; Stöck *et al.*, 2005; Holloway *et al.*, 2006) and so polyploidy may be an ongoing process. Even if complete phylogenies or genomic evidence for duplicate genes were available, dating origins precisely thus could remain difficult. As Doyle & Egan (2010) point out, the divergence of homoeologous copies (duplicate copies from each progenitor parent) in an allopolyploid tracks the divergence of diploid species, not the origin of the polyploid and autopolyploid origins could be even more difficult to infer, so that skepticism about estimated dates is warranted.

Fishes

For fishes, diploid ancestors of extant polyploids are often not identifiable but, unlike amphibians, there are entire polyploid families. Based on karyotyping and genome size analyses, extreme cytogenetic variation has been found in certain lineages of fish (Hinegard, 1968; Hinegard & Rosen, 1972; Venkatesh, 2003) and bisexually reproducing extant polyploids occur in a wide range of actinopterygian families, including the Acipenseridae (Birstein, Hanner & DeSalle, 1997; Ludwig *et al.*, 2001), Cyprinidae (David *et al.*, 2003), Catostomidae (Ferris, 1984), Callichthyidae (Oliveira *et al.*, 1992) and Salmonidae (Johnson, Wright & May, 1987). Using the Cyprinidae and Salmonidae to illustrate attributes of polyploidy in fishes, Le Comber & Smith (2004) conclude that polyploidy may have been of considerable importance in the evolution of fishes. Polyploidy has also evidently played a role in the evolution of some lungfish species (Lepidoseriniiformes), particularly within the genus *Protopterus*, with C-values surpassing 80 pg.

Although fish phylogenies also remain uncertain, we used the well-calibrated phylogeny presented in Santini *et al.* (2009) to illustrate relationships among the major fish orders that include polyploids and plot rough divergence times (Fig. 2). One large assemblage of fishes (primarily freshwater) referred to as the Ostariophysi encompass multiple examples of extant polyploid families (Cyprinidae, Catostomidae, Cobitidae, Callichthyidae), constituting a significant proportion of the known polyploid actinopterygian families within one massive clade. In addition, spontaneous polyploids have been reported in Gymnotiformes and Characiformes, which are also within the Ostariophysi (Fig. 2). Of the remaining polyploid fishes, all are brackish, anadromous, or strictly freshwater species. With few exceptions, polyploidy is more common among early diverging teleosts and relict bony fishes than later diverging teleost lineages such as the Perciformes (Leggatt & Iwama, 2003). Thus, after the original three rounds of ancient genome duplications, subsequent polyploidization events seem to have occurred within particular families, while the majority of fish genomes remain functionally diploid.

The order Acipenseriformes (sturgeons) is distributed across North America, Europe and Asia, and includes species with multiple levels of ploidy, with diploid species containing 120 chromosomes, tetraploids with 250 chromosomes and

even functional octaploids with 500 chromosomes (Birstein *et al.*, 1997). They are considered ancient relicts, with strikingly similar species preserved in the fossil record as far back as 200 million years (Bemis & Kynard, 1997). A study of genome duplication events and functional reduction of ploidy levels in sturgeon has revealed that gene silencing, chromosomal rearrangements and transposition events are likely to be the dominant mechanisms that have shaped Acipenseriform genomes (Ludwig *et al.*, 2001). Within this study, microsatellite analyses show that the maximum ploidy level for the Acipenseriformes is tetraploid and not octaploid, conflicting with original estimates (Birstein *et al.*, 1997). These differences may be due to the extinction of the original diploid Acipenseriform ancestor, as the oldest extant genus within the order (Polyodon) contains species whose chromosomes can be easily arranged into quartets (suggesting that Polyodon may actually be tetraploid). Furthermore, *Acipenser sturio* (presumed to be diploid with 116 chromosomes) has a C-value approximately double the size of its closest relatives. These basal species are thought to be ancient tetraploids functioning in a diploidized state (Vasil'ev, 2009). Thus, there are a number of unresolved issues within this ancient order of fishes that require further in-depth analyses, including complete taxonomic coverage, further karyotypes and genome size estimates.

The order Cypriniformes contains the largest diversity of fish polyploids known to date, with over 250 recognized polyploid species spread across North America, Europe, Africa and Asia. Most cypriniform polyploids are tetraploids or hexaploids, with chromosome complements ranging between 100 and 150, while one species (*Ptychobarbus dipogon*) has an amazing 446 chromosomes. The largest family of freshwater fishes, the Cyprinidae (Teleostei: Cypriniformes), contains many species and genera with great cytogenetic variation. Two other families contain a large number of tetraploid species, notably the Catostomidae and the Cobitidae; however, due to the complexity of Cypriniform systematics, this is likely to change in the future (Ferris, 1984; Suzuki & Taki, 1996; Saitoh, Chen & Mayden, 2010). A molecular phylogenetic study based on cytochrome *b* and rRNA sequence data has revealed that a single polyploidization event occurred in the lineage leading to the Botiinae (Cobitidae), suggesting a single origin for this monophyletic tetraploid assemblage (Slechtova *et al.*, 2006). Many cyprinid genera are composed of stable polyploid series, including *Barbus*, *Labeobarbus*, *Luciobarbus*, *Pseudobarbus*, *Spinibarbus*, *Diptychus*, *Carrasius*, *Capoeta*, *Tor*, *Cyprinus*, *Schizothorax*, *Sinocyclocheilus* and more. The genus *Barbus* consists of particular interest as it contains at least 350 species and is well known for its morphological variation, wide distribution and the existence of diploid, tetraploid and hexaploid species (Klose *et al.*, 1969; Berrebi, 1995; Machordom & Doadrio, 2001). In a study focusing on the evolutionary history and modes of speciation within the genus *Barbus*, Machordom & Doadrio (2001) reconstructed phylogenetic relationships based on three mitochondrial genes in an effort to infer patterns and processes of polyploidy. Although the authors focus on systematic relationships within *Barbus*, they propose that genome duplication

within this genus may be considered as a homoplastic character, since it must have occurred over at least three independent periods and/or in three independent African regions. However, the lack of nuclear markers within this study make it difficult to cross-validate relationships and potentially infer a history of hybridization through topological incongruence. Another phylogenetic study relying solely on cytochrome *b* comes to similar conclusions concerning the multiple origins of African barbs (Tsigenopoulos *et al.*, 2002); yet, once again, the authors focus more on the systematic relationships of the genus rather than hypotheses dealing with polyploidization events. Within Asia, the Yunnan province of China is home to an amazing diversity of cyprinid polyploids (Yu *et al.*, 1987), but the biogeography of these fishes remains understudied. Given the diversity of cypriniform polyploids, combining comprehensive molecular phylogenies with more karyotyping and genome size estimates and other techniques should help to resolve the complex history of genome duplications within the group.

Within the Siluriformes (Catfishes), the species rich genus *Corydoras* (Callichthyidae: Corydoradinae) from the neotropical region is the most well studied and diverse group of polyploids, with an impressive range of genome sizes and karyotypic variability (Hinegard & Rosen, 1972; Oliveira *et al.*, 1988, 1992, 1993*a,b*; Fenerich, Foresti & Oliveira, 2004; Shimabukuro-Dias, Oliveira & Foresti, 2004). The latter research supports the existence of multiple polyploid groups within the genus, with chromosomes ranging between $2n = 44-132$ and C-values between 0.65 and 4.5 pg. The primary mechanisms presumed to have shaped the complex genomic variability within these lineage include Robertsonian translocations, fissions, fusions, inversions and polyploidy followed by DNA loss (Oliveira *et al.*, 1992, 1993). Despite the recent publication of a comprehensive molecular phylogenetic framework (Alexandrou *et al.*, 2011), many species remain without cytogenetic information, making it difficult to infer ploidy levels within the genus. Given karyotypic variability, it is also possible that several species of *Hypostomus*, *Plecostomus*, *Trichomycterus* and *Wallago* are polyploid, but this remains to be confirmed (Rab, 1981; Fenerich *et al.*, 2004). Other polyploid catfish species that have been revealed at an intraspecific level include *Heteropneustes fossilis* (Pandian & Koteeswaran, 1999) and *Clarias batrachus* (Mustafa & Shams, 1982), yet these are isolated examples in comparison to the genus *Corydoras*.

Tetraploidy is an ancestral condition of the Salmonidae, originally shown via linkage analyses (Johnson *et al.*, 1987) and microsatellite data (Sakamoto *et al.*, 2000; Gharbi *et al.*, 2006), but also confirmed by PCR amplification of non-orthologous sequences in different taxa (Angers, Gharbi & Estoup, 2002). The Salmoniformes are all polyploid, having undergone a genome duplication after their separation from the Esociformes, between 45 and 100 mya (Allendorf & Thorgaard, 1984; de Boer *et al.*, 2007; Santini *et al.*, 2009). Most salmonids have a haploid C-value of *c.* 3 pg, while karyotypes range between $2n = 56-104$. Similar to the

Acipenseriformes, salmonids primarily occupy temperate regions; however, some species from the genus *Coregonus* can also be found within polar climates. The extreme migratory behaviour of salmonids might be related to the original ancestral shift in ploidy level. Recent evidence from molecular phylogenetic analyses supports the Esociformes (Esocidae and Umbridae composed strictly of freshwater species) as the sister group of the Salmonidae (Broughton *et al.*, 2010).

Finally, several species from the genus *Channa* (Channidae) are the sole polyploid representatives of the order Perciformes (Banerjee, Misra, Banerjee *et al.*, 1988); an extremely diverse lineage estimated to contain in excess of 10 000 species. Despite karyotypes of 78 and 104 chromosomes, genome size estimates for these species are lacking, making it difficult to accurately assess ploidy status (Rishi & Haobam, 1984). It is very likely that a bias in taxonomic sampling and a lack of more recent cytogenetic investigations have partially led to the pattern of imbalance in polyploidy among different taxonomic groups of fishes; and, given the diversity of Perciformes, species with duplicated genomes may yet be discovered.

What factors favour polyploid formation?

While polyploidy is not quite as rare as often suggested in animals, it is obvious that extant polyploidy is restricted to particular groups. In this section, we focus on the drivers that might promote formation of polyploid lineages, emphasizing traits shared by fish and amphibians. We specifically question whether there are intrinsic features or ecological preferences shared by fish and amphibians (or at least those that give rise to polyploid lineages) that make them more prone to the initial formation of polyploids. The majority of polyploidization events in both plants and animals are thought to have been the result of unreduced gamete formation (Winge, 1917; Hagerup, 1932; Rabe & Haufler, 1992; Ramsey & Schemske, 1998; Husband & Schemske, 2000; Ramsey, 2007) but other mechanisms, such as polyspermy are also possible. Unlike plants, somatic polyploidization has not been described in animals. Under conditions where the frequency of these types of events is highest, it seems probable that opportunities for formation of polyploids would be maximized. We also consider other factors that might increase opportunities for the formation of successful polyploid individuals, such as gamete production, reproductive environment and propensity for hybridization.

Frequency of unreduced gametes

Although unreduced gametes occur spontaneously in most vertebrates, they appear to produce viable progeny mainly in ectotherms. Artificial experimentation suggests that the ease of unreduced gamete formation is particularly high in fish and amphibians (Fankhauser, 1945). The absence of the pachytene checkpoint, which is a meiotic surveillance system

present in many animals that would normally prevent the formation of unreduced gametes, has been suggested as a possible reason for the high prevalence of polyploidy in plants (Li, Barringer & Barbash, 2009). The authors suggest that perhaps this system is absent or defective in the animals that do give rise to polyploids but this has not yet been evaluated. There also might be other features of gametogenesis or the fertilization process that increase the potential for producing unreduced gametes.

Oogenesis is basically the same in all amphibians: primary oocytes undergo meiotic division to yield secondary oocytes and the first polar bodies; activation of the egg by the sperm stimulates the second reduction division of the secondary oocyte (most amphibian eggs are arrested in metaphase II), resulting in an ovum and a secondary polar body (Duellman & Trueb, 1986). Polyploidy in amphibians can be experimentally induced by cold or pressure shock of females before fertilization. This is thought to disrupt spindle formation during meiosis, and result in retention of the second polar body, which would lead to a diploid ovum, producing triploid gametes if fertilized by haploid sperm. For example, in *Xenopus*, unreduced gametes are known to be produced at a rate of about 10% in artificial hybridizations between species (Tymowska, 1991) and this can be increased by cold or pressure shock (Kobel & Du Pasquier, 1986). It is apparently not as easy to induce the formation of unreduced (diploid) sperm experimentally and surveys of ploidy have not found as high a frequency of spontaneously formed diploid sperm as diploid eggs in natural populations of anurans (Bogart, 1980). In temperate anurans, sperm cells mature uniformly throughout the testes but in tropical species that breed throughout the year, testes contain sperm cells in various stages of maturation (Duellman & Trueb, 1986). Although the origins are unknown for most tetraploid taxa, one possibility is through an intermediate triploid stage (e.g. Fankhauser, 1945; Cunha *et al.*, 2011), because triploids only require unreduced gametes to be formed by one parent. Tetraploid gametes could be formed by disruption of the first mitotic division after fertilization. However, experimental attempts to synthesize tetraploid, rather than triploid, gametes in anurans have not been highly successful, so it has been inferred that an intermediate triploid stage is critical (Bogart, 1980).

Particularly in temperate or dry regions, anurans often breed during times when temperatures are unstable, which could increase the frequency of production of unreduced gametes. For example, the threshold for breeding in *Hy. chrysosecelis* (which is the diploid thought to have given rise to multiple independent lineages of *Hy. versicolor*) is at a water temperature of 15 °C, which normally occurs in the early spring, when low temperatures and frosts are still likely to occur. Nevertheless, other sympatric species do not produce tetraploid lineages, even though experimental induction of unreduced gametes has been demonstrated (e.g. Richards & Nace, 1977), suggesting that temperature variation alone cannot explain tetraploidy in the grey treefrogs. Experimental hybridization studies demonstrate that it is possible to produce viable polyploid offspring in a number

of frog species where natural polyploids do not exist (e.g. Fankhauser, 1945; Nishioka & Ueda, 1983), suggesting that there are not intrinsic blocks to production of polyploid lineages in other anuran species.

In fishes, although 37 different ways of inducing polyploidy have been described (Pandian & Koteeswaran, 1998), polyploids have most commonly been induced by either temperature or pressure shock, with cold shock typically favoured for warm water species and warm shock favoured for cold water species (Donaldson *et al.*, 2008). Temperature shock induces polyploidy by one of two mechanisms: (1) causing the retention of the second meiotic polar body or (2) blocking the first mitotic division (Tiwary, Kirubakaran & Ray, 2004). High pressure of between 400 and 600 atmospheres may also induce polyploidy. Polyploidization (triploids and tetraploids) has been induced in fishes through temperature shock in plaice *Pleuronectes platessa* (Purdom, 1972), common carp *Cy. carpio* (Gervai *et al.*, 1980), grass carp *Ctenopharyngodon idella* (Cassani & Caton, 1985), rainbow trout (Thorgaard, Jazwin & Stier, 1981), Channel catfish *Ictalurus punctatus* (Wolters, Libey & Chrisman, 1981), turbot *Scophthalmus maximus* (Piferrera *et al.*, 2003), tilapia *Oreochromis aureus* (Don & Avtalion, 1988) and a cyprinid loach *Misgurnus anguillicaudatus* (Chao, Chen & Liao, 1986). While pressure shock is not relevant to polyploidy formation in wild systems, cold or heat shocks may occur naturally through changes in thermoclines, water movements such as flooding or snow melting, heavy precipitation or rapid changes in seasonal temperatures (Donaldson *et al.*, 2008).

Polyspermy

One route to polyploidy that does not involve unreduced gametes is through polyspermy – the fertilization of a single egg with more than one sperm. In many fishes and amphibians there is ample opportunity for multiple sperm to come into contact with an egg. However, in fishes, with the exception of the elasmobranchs, only a single sperm actually penetrates. A range of physical and chemical mechanisms prevent polyspermy in animals, but the underlying mechanisms appear to be relatively conserved across the animal kingdom (Wong & Wessel, 2006). Physiological polyspermy is the condition where multiple sperm fuse with an egg, but only one male pronucleus is merged with the haploid egg nucleus. Polyspermy is common in many urodeles, where 90–100% of all eggs may be polyspermic (Elinson, 1986; Iwao, 1989). However, despite multiple sperm penetrating the egg, only a single sperm fuses with the pro-nucleus and additional cytoplasmic sperm nuclei are subsequently suppressed (Fankhauser, 1932; Elinson, 1986; Iwao, 1989; Iwao & Elinson, 1990). Anurans use a diverse variety of mechanical methods to block polyspermy including reorganization of the egg extracellular matrix and hydroscopic swelling of the outer jelly layers to create barrier against sperm (Elinson, 1986; Hedrick & Nishihara, 1991).

The micropile is the point of entry through the corian for sperm attempting to fertilize a teleost fish egg. In

teleosts there is a single micropile per egg (Hart, 1990); however, in the more ancient sturgeon and paddlefish (Acipenseriformes) there are several micropiles (Hart, 1990; Ciereszko, Dabrowski & Ochkur, 1996), which represents an evolutionary mid-point between sperm storage in the elasmobranchs and a single micropile in the teleosts. In fishes, the chorion is an important physical barrier to polyspermy, and unfertilized eggs will be polyspermic if this is removed. The width of the micropile in most teleosts (with the exception of carp, where the micropile diameter is two to three times the width of the sperm; Kudo, 1980) prevents multiple sperm entering the egg. Although sturgeons and paddlefish exhibit high levels of polyploidy and also have theoretically greater potential for polyspermy than teleosts due to multiple micropiles, polyspermy does not appear to be implicated in the genome duplications identified in other teleost lineages. There appears to be no difference in the mechanisms used by teleosts to prevent polyspermy in marine versus freshwater species, suggesting that polyspermy is not a major driver in the differences in rates of polyploidy between marine and freshwater teleosts.

Gamete production

Production of unreduced gametes does not necessarily lead to the formation of a viable polyploid individual. Problems tend to arise due to aneuploidy, imbalances in chromosome numbers, altered dosage of parental proteins, and incompatibilities of parental genomes. Duplicating the chromosome complement increases the risk of problems during meiosis, particularly if more than two copies of each chromosome can pair. Aneuploidy tends to be even more common for unbalanced chromosome sets (triploid or pentaploid), so if most polyploids arise through an intermediate triploid stage, aneuploidy would be expected to be an inhibitory factor. Generation of sustained polyploid lineages is thus limited by the ability to produce offspring with an appropriate copy number of each chromosome in both the diploid progenitors and the newly arising polyploids. Due to the stochastic nature of chromosome pairing, it might be expected that, although a small proportion of gametes would end up with balanced sets, if they were the only forms that were viable, selection for full chromosome complements would be strong. This means that organisms (such as mammals) that produce few female gametes at one time would not be expected to form stable polyploid lineages (Mable, 2004). Fish and amphibians both tend to produce large numbers of both male and female gametes, which could facilitate generation of viable polyploid progeny.

Annual fecundity in amphibians ranges from one to potentially more than 80 000 offspring (Salthe & Mecham, 1974). Clutch sizes are not available for most of the polyploid anurans or, perhaps more importantly, their diploid counterparts, but Duellman & Trueb (1986) report that while small clutches are produced by some species with terrestrial egg development and in ovoviviparous species, those with aquatic reproduction typically have clutch sizes

in the range of 100's to many thousands (ranging to over 47 000 in *Rana catesbeiana*). The tetraploid *Tomopterna (Pyxicephalus) delalandii* has a clutch size of 2500, and a survival rate of 19% (Wager, 1965), but this report was when it was still synonymized with its diploid progenitor so it is not clear on which ploidy level the study was performed. Multiple mating opportunities per year increase the combinatorial aspects of fertilization and so could further increase the probability of producing viable, balanced gametes. However, because the vast majority of anurans produce large numbers of eggs, egg number alone cannot explain the success of polyploid formation in some groups. Nevertheless, high gamete numbers could enhance the probability of formation of balanced combinations of parental genomes.

The fecundities of fish can be equally impressive; egg numbers range from relatively few large eggs found in mouthbrooding cichlids (Kellogg *et al.*, 1995; Taylor *et al.*, 2003) to many millions of small eggs found in Atlantic sturgeon (Ryder, 1888) and cod (Thorsen *et al.*, 2010). For example, egg numbers in *Esox lucius* can be as high as 300 000–400 000 per female (Billard, 1996) whereas salmonids have fewer eggs, with numbers ranging from 200 to 12 700 eggs per female (Scott & Crossman, 1973). Egg number and size is closely linked to breeding strategy and parental care (Wootton, 1984; Sargent, Taylor & Gross, 1987; Kolm & Ahnesjö, 2005). For example, no parental care is provided by either sturgeons or paddlefishes, which produce hundreds of thousands to millions of eggs. The number of eggs produced by female fish is highly correlated with body size and additional trade-offs may be found with egg size versus egg number. If female gamete number is related to the propensity to form polyploids, we might expect to find higher egg numbers in the ancestors of polyploid lineages or in the closest relatives of polyploid species than in lineages that do not include polyploids. However, while no exhaustive phylogenetic treatment has been conducted, this does not appear to be the case. While many freshwater groups where polyploidy occurs have large numbers of eggs, so do marine species such as the Gadidae, where no polyploid species have been identified. Additionally, in general, clutch sizes are much larger in marine fishes than in freshwater species, with freshwater fish producing low numbers of large eggs and marine fish produce large numbers of small eggs (Elgar, 1990). As all known polyploid teleosts reproduce in freshwater, the relationship does not appear to hold. It could be that there is a threshold number of eggs that would be required to favour the production of stable polyploid lineages.

Reproductive environment

The most obvious feature shared by polyploid fish and amphibians is that, nearly without exception, they reproduce in freshwater environments. Reproduction in aquatic environments in general exposes ectotherms to fluctuations in environmental conditions during the breeding season and freshwater habitats are known to be more variable than marine environments. During times of environmental instability such as postglacial periods, variation in

temperature during the breeding season thus could be substantial and large numbers of individuals could be exposed to temperature fluctuations in a local area (reviewed in Mable, 2004). External fertilization in an aquatic environment also enhances mixing of male and female gametes, which would facilitate the probability of producing offspring with balanced chromosome set combinations. Broadcast sperm deposition in aquatic environments, when multiple individuals breed at the same time, also promotes multiple paternity, which could further allow selection of favourable gamete combinations or polyspermy.

In contrast to salamanders and caecilians (where no bisexually reproducing polyploids have been found), virtually all anurans reproduce using external fertilization; internal fertilization is only known in a few genera and none of the species are known polyploids (Duellman & Trueb, 1986). Although there is a trend towards terrestrialization of fertilization in anurans (Duellman & Trueb, 1986), and there is variation in reproductive modes within the families that include polyploids, as far as can be ascertained, all of the known polyploid species and their diploid progenitors use the generalist mode of reproduction, thought to be ancestral: eggs deposited and larvae developed in a lentic (still water) environment. It is interesting that in the Pipidae, while the genus *Xenopus* is exclusively polyploid and deposits eggs communally into an aquatic environment, polyploids are not known in the genus *Pipa*, which has indirect development via eggs deposited to the dorsum of the female. In a survey of 5828 amphibian species, Vences & Kohler (2008) found that 4117 species live in an aquatic environment during at least one life history stage, with 177 more being water dependent.

All of the known polyploid anurans are communal breeders, where multiple males and females congregate simultaneously to breed. This can be particularly dramatic in amphibians that live in dry environments, where breeding opportunities are limited by occasional periods of extensive precipitation. For example, in the Australian frog genus *Neobatrachus*, individuals remain buried in sandy soil even across the central deserts for most of the year and breed only during cyclones, which means that breeding does not occur every year but breeding choruses are large when conditions are appropriate (Roberts & Majors, 1993). This type of group breeding strategy would enhance the potential for exposure of individuals to the same extreme conditions during breeding.

Many of the polyploid fish also breed communally and nearly all live in or return to freshwater to breed. The Salmonidae are anadromous and reproduce in freshwater, where females lay eggs in redds and these are fertilized by multiple males (Hutchings & Myers, 1988). Sturgeons migrate upstream in rivers to spawn if they are marine, or to shallow areas of lakes if they live in freshwater. Typically, several males spawn with a single female (Bruch & Binkowski, 2002). Some species of Cypriniformes form breeding aggregations, as observed in the genus *Barbus*, where communal spawning in the Lake Tana barbs occurs after a migration upstream from the lake (de Graaf, 2003). In the

Corydoradinae, reproduction is also a communal process and can often be triggered under aquarium conditions by reducing the temperature of the water (Fuller, 2001), thereby simulating the effects of sudden rainfall after a long dry season. Again, the preponderance of communal breeding in freshwater environments across fish and anurans does not explain why some species are polyploid and others not, but it does enhance the probability of formation of viable gamete combinations.

Propensity for hybridization

Although estimates of the relative frequency of autopolyploidization and allopolyploidization events remain rare, even in plants, polyploidy is often associated with hybridization. Both hybridization and polyploidy can involve dramatic and immediate changes in genome structure (McClintock, 1978; Gaeta *et al.*, 2007; Landry, Hartl & Ranz, 2007; Buggs *et al.*, 2010; Chelaifa, Monnier & Ainouche, 2010; Gaeta & Chris Pires, 2010; Marmagne *et al.*, 2010), which could alter adaptive responses to environmental change, so it is unclear whether it is hybridization or polyploidy that might allow invasion of 'harsh' environments. A recent special issue on polyploidy in plants emphasized that it is predominantly hybridization and not genome doubling that explains the dramatic changes documented in recent studies (Ainouche & Jenczewski, 2010). Although many cases of hybrid animals have been linked to polyploidy, many have also been associated with asexual reproduction (reviewed by White, 1973; Dowling & Secor, 1997). It has been suggested that autopolyploidy could be rarer in animals that reproduce strictly sexually because they would be too similar to their diploid counterparts to gain a competitive edge without the reproductive assurance and potential for increasing numbers of their own cytotype provided by self-fertilization, and so, hybridization would be required to produce competitively different lineages (White, 1973). As our tools for evaluating genome structure advance, footprints of hybridization are becoming more broadly apparent in animals as well as plants (Bi & Bogart, 2006; Baack & Rieseberg, 2007) and similar numbers of well-supported cases of homoploid hybrid speciation (i.e. without a change in genome copy number) have now been documented in both taxonomic groups (Gross, Turner & Rieseberg, 2007; Mallet, 2007; Mavarez & Linares, 2008). Whitney *et al.* (2010) estimated that plant hybrids in the wild occur in 40% of families and 16% of genera (including polyploids), with a frequency of 0.09 hybrids per nonhybrid taxa. They found that hybridization propensity tended to be consistent across regions, suggesting that hybridization behaviour may be determined more by intrinsic properties of a group rather than environmental conditions. While this would reflect the rate of 'successful' hybrid establishment, it does not necessarily mean that opportunities for hybridization would not be increased by climatic shifts; both habitat fragmentation and changes in temperature during times of environmental instability are likely to change species interactions by altering distributions and bringing new

combinations of individuals together, which can increase rates of hybridization (Seehausen *et al.*, 2008). In plants, polyploidization has been found to 'rescue' otherwise incompatible combinations of hybrids in diploids, possibly due to the possibility of preferential pairing of homeologous chromosomes, which could more easily allow incompatible allelic combinations to be eliminated or down-regulated (Martinez-Perez, Shaw & Moore, 2001; Mestiri *et al.*, 2010) or induce epigenetic silencing due to the increase in chromosome number (Mittelsten Scheid *et al.*, 1996). There is some evidence that the extent of genomic divergence between hybridizing species influences the likelihood of diploid versus polyploid hybrid speciation, with more divergent genomes more often giving rise to the latter (Chapman & Burke, 2007; Paun *et al.*, 2009). However, recent analyses in plants suggest that the pattern might be driven more by restriction of parental divergence in the production of viable diploid hybrids rather than polyploidy 'rescuing' more distant hybrids (Buggs *et al.*, 2008, 2009).

In the frog genus *Hyla*, whereas hybridization between diploid taxa tends to be limited by genetic distance (Ralin, 1970), experimental crosses involving tetraploid females have been found to be more successful with distantly related than closely related diploids (Mable & Bogart, 1995). This might be due to inability to recognize 'foreign' chromosomes and alter regulatory controls accordingly to reduce incompatibilities. Because the male genome is not expressed until post-gastrula in anurans, diploid hybrid combinations often fail until after this point, when incompatibilities between parental genomes would become apparent (Mecham, 1965). Some amphibians are quite prone to hybridization [e.g. *Bufo* (Blair, 1972; Malone & Fontenot, 2008)] but it is interesting that in the genus *Rana*, where hybridization is not as common (Green, 1985) no polyploids have been described, except for the *Rana esculenta* complex, which has a complex reproductive system (hybridogenesis; Vinogradov *et al.*, 1990). Although autopolyploidy has been suggested for some species based on tetrasomic inheritance and limited genetic distinction from closely related diploids [e.g. *Hy. versicolor* (Bogart, 1980)], hybridization between closely related diploids cannot be ruled out in most cases (e.g. Ptacek *et al.*, 1994; Dowling & Secor, 1997; Holloway *et al.*, 2006) and hybridization among tetraploid lineages is ongoing (Espinoza & Noor, 2002). Most species of *Xenopus* are thought to have arisen through hybridization (Evans *et al.*, 2005; Evans, 2008) and hybridization occurs naturally within ploidy levels of extant species (Kobel, Dupasquier & Tinsley, 1981; Fischer, Koch & Elefant, 2000). In addition, evidence for allopolyploid origins has been provided for species in the Bufonidae (Bogart, 1980; Stöck *et al.*, 2005, 2006), Microhylidae (Vences *et al.*, 2002), Ranidae (Channing & Bogart, 1996) and Myobatrachidae (Roberts, Maxson & Plummer, 1996; Mable & Roberts, 1997; Roberts, 1997*a,b*). Based on the production of polyploid individuals, there is some evidence that unreduced gamete formation is higher in artificially produced hybrids between distantly related parents (Bogart, 1980), but whether this is a cause (i.e. hybridization promotes unreduced gamete formation) or a consequence (i.e. unreduced gamete

formation allows survival of otherwise incompatible hybrid combinations) of polyploidy remains unclear.

Of the known groups of polyploid fish, the Salmonidae are thought to be autopolyploid due to multivalents at some loci, particularly in males (Wright *et al.*, 1983; Allendorf & Thorgaard, 1984; Hartley, 1987). However, most other examples indicate allopolyploid origins. For example, the tetraploid form of the Japanese spined loach (*Cobitis* 'yamato complex') appears to have an allopolyploid origin (Kitagawa *et al.*, 2003), resulting from the hybridization of *Cobitis biwae* and *Cobitis striata* (Saitoh *et al.*, 2010), while other species of polyploid *Cobitis* are gynogenetic (Juchno & Boron, 2006). Other groups likely to have had allopolyploid origins include the Catostomidae (Uyeno & Smith, 1972; Ferris & Whitt, 1977*b*; Ferris, 1984), which is comprised of more than 60 species. In the Cobitidae there are three independent groups of tetraploids; however, assigning allopolyploid origin to them is not certain (Vasil'ev, Vasil'eva & Osinov, 1990). The European polyploid *Barbus* spp. (Cyprinidae) appear to have an allopolyploid origin (Chenuil *et al.*, 1999), as does the common carp [*Cy. carpio* (David *et al.*, 2003)]. The Botiidae is comprised of diploid (Leptobotiinae) and tetraploid (Botiinae) taxa, with a single origin of tetraploidy (Slechtova *et al.*, 2006); however, the origins of the polyploidy event are not known. Polyploid origins of the Corydoradinae remain unknown and most certainly merit further investigation. Thus, although propensity for hybridization is not a limiting factor for fish or amphibians in the formation of allopolyploids, the proportion of polyploids with hybrid origins remains unclear.

What factors favour polyploid establishment?

Theoretical explanations for the existence of independently reproducing polyploid species have focused on the presumed difficulty in establishing reproductively independent lineages when initially outnumbered by their diploid progenitors (minority cytotype exclusion principle; Levin, 1975; Husband, 2000). The ability of a polyploid organism to occupy a new niche is crucial because otherwise competition with the presumably well-adapted diploid progenitor will be particularly pronounced. Moreover, newly formed polyploid populations are likely to be small, potentially allowing drift to fix ecologically relevant traits rapidly (although selection will be weaker). Possible advantages have been thought to be due to increased genetic flexibility provided by extra genome copies and the potential for regulatory innovation provided by extensive gene duplication (e.g. Levin, 1983; Soltis & Soltis, 2000; Beçak & Kobashi, 2004), which could broaden ecological tolerances and result in polyploids being able to survive harsher conditions than their diploid progenitors. This competitive edge might be expected to increase during times of climatic instability or allow polyploids to invade harsher environments (e.g. Stebbins, 1950, 1971; Lumaret, 1988). Dynesius and Roland (2000), for example, suggested greater rates of polyploid formation during times of climatic oscillations due to their high

adaptive potential to rapidly changing environmental conditions. An intriguing hypothesis based on comparative genomics in plants is that genome duplication events tended to be clustered around the Cretaceous–Tertiary boundary (K–T boundary), when many plant species went extinct (Fawcett *et al.*, 2009), suggesting that polyploids might have had increased survival during times of environmental upheaval or were the best adapted to expand into vacant niches exposed by extinction events. Although insufficient genomic data currently exist for animals to allow the same test to be conducted, fossil evidence suggests that neither freshwater fish nor amphibians experienced the scale of mass extinctions during this time that many endotherms did (e.g. Milner, 1998). We thus might expect to find larger numbers of polyploid taxa in extreme environments, at the edge of ranges, or generally in more variable environments. We might also expect polyploid taxa to be more resilient to both abiotic and biotic pressures (e.g. pathogens, predators), and to show lower rates of extinction than their diploid relatives. Because the extent of genomic novelty would be expected to be higher in hybrid lineages, such patterns should be most pronounced for allopolyploids.

It is also possible that other factors that do not require intrinsic genetic advantages (such as assortative mating) have played a role. Moreover, there has been a contrasting view in the literature that polyploidy represents an evolutionary dead end and that the prevalence of diploidization reflects maladaptation of duplicated genomes (Stebbins, 1950). In this section, we evaluate attributes of polyploid fish and anurans that might promote the establishment of polyploid lineages and question whether there is evidence that genome duplication events have been related to times of climatic change or that polyploids show advantages (or disadvantages) relative to their related diploids in terms of ecological ranges, pathogen tolerance, or extinction rates.

Assortative mating

Assortative mating by cytotype (i.e. prezygotic isolation from diploid progenitors) could enhance the probability that newly arising polyploid lineages will become established. Based on reproductive barriers between diploid and autotetraploid individuals of the perennial plant *Chamerion angustifolium*, simulations indicated that prezygotic isolation will reduce the strength of minority disadvantage acting on tetraploids and increase the importance of differences in viability and fertility between ploidy levels in regulating polyploid establishment (Husband & Sabara, 2004). In anurans, premating and/or postmating isolating mechanisms may arise automatically with the change in cell size or gene copy number resulting from genome doubling (Bogart, 1980; Keller & Gerhardt, 2001; Holloway *et al.*, 2006). Because most diploid–polyploid species pairs differ by mating call, assortative mating by call type could enhance the potential of newly arising polyploids to find mates, as the hearing mechanism in the female changes correspondingly and females choose males of their own ploidy level (Keller & Gerhardt, 2001).

In fish, mate choice experiments are not as extensive as in frogs and toads, but production of specific olfactory cues has the potential to provide signals that could vary in direct proportion to ploidy level and so allow assortative mating. There is also evidence that sound production is more common in fish than previously suspected, including in *Corydoras* catfish (Pruzsinszky & Ladich, 1998; Kaatz & Lobel, 1999, 2001), which include multiple independently derived polyploids. It would be interesting to determine whether mate choice by ploidy exists in fish and to identify the underlying mechanisms. In mammals, major urinary proteins [which are linked to the major histocompatibility complex (MHC)] have been demonstrated to be involved in mating decisions (Knapp, Robson & Waterhouse, 2006) and orthologues of MHC-linked odorant receptor genes have been identified in *Xenopus* and zebra fish (Santos *et al.*, 2010). However, the MHC does not appear to be retained in duplicate in polyploid *Xenopus* (Kobel & Du Pasquier, 1986; Shum *et al.*, 1993; Sammut, Marcuz & Pasquier, 2002; Du Pasquier, Wilson & Sammut, 2009), or salmon (Kruiswijk *et al.*, 2004; Shiina *et al.*, 2005) so it is not clear whether there would be ploidy-related signals.

Increased ecological tolerance

Even with assortative mating, establishment rates would be expected to be high only if polyploids had an initial fitness or competitive advantage over their diploid parents (Ramsey & Schemske, 2002). A long-standing observation is that polyploidy occurs more frequently at higher latitudes and higher altitudes, possibly because polyploids are genetically or physically more robust than their diploid counterparts (Löve & Löve, 1943; Stebbins, 1971; Ehrendorfer, 1980; Levin, 1983). However, in plants and some animals that show this distributional bias (e.g. *Daphnia* Dufresne & Hebert, 1997), polyploidy has also been associated with a shift in mating system towards autogamous reproduction (either through self-fertilization or parthenogenesis), which is thought to enhance dispersal abilities into novel environments because of relaxation of the need to find a suitable mating partner (reviewed in Mable, 2003). It also would provide newly arising polyploids with a higher chance of increasing to sufficient numbers that they could gain a competitive edge over their diploid progenitors. Whether polyploidy or the ability to reproduce without finding appropriate mating partners allows invasion of these potentially harsh environments is thus difficult to disentangle. This is particularly difficult in plants, where transitions from outcrossing to selfing modes of reproduction have been described as the most common evolutionary transition among angiosperms (Bateman, 1952). There has also been suggestion that polyploids avoid competition following establishment by diversifying their ecological niches (Stebbins, 1950); again, invasiveness to new habitats has also been associated with shifts to selfing in plants. As early as 1940, Clausen, Keck & Hiesey, (1940; Clausen, Keck & Hiesey, 1945) suggested that it was by no means a general rule that polyploids occupy more extreme habitats than

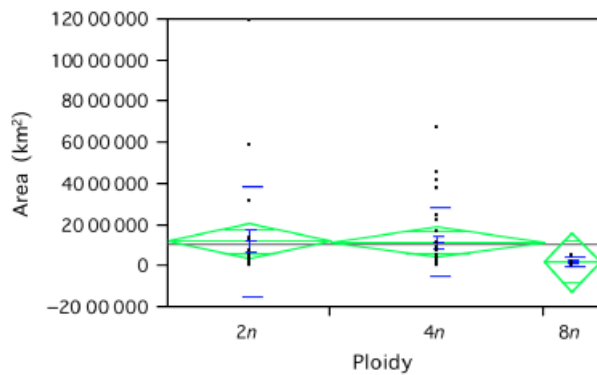


Figure 3 Range areas (km²) for polyploid anurans compared with their closest diploid relatives, calculated by digitizing distribution maps provided through the AmphibiaWeb database <http://amphibiaweb.org/index.html>. There are no significant differences in range areas, although octoploid taxa tend to have smaller range areas than their diploid or tetraploid relatives, and several species have only been reported from single sites.

their diploid relatives but this view has held, at least partly because of the confounding effects of mating system. Soltis *et al.* (2010) also point out that it is difficult to define what ‘success’ means in evolutionary terms and so whether or not polyploids are more successful than their progenitors is not a straight-forward question.

Nevertheless, vertebrates with strictly sexual reproduction may be better models to assess whether polyploidy, rather than mating system, allows range extensions to harsher environments. Based on digitizing areas in maps available through AmphibiaWeb, we found no significant difference in range sizes of polyploid anurans compared with their closest diploid relatives (Fig. 3). While this is somewhat confounded by misclassification before ploidy was confirmed in cryptic diploid–polyploid species pairs, there is not a consistent pattern that would suggest an overall colonization advantage for polyploids. As for plants, some anuran species with higher ploidy levels are found in smaller ranges than their diploid counterparts, while some have similar ranges and some larger. In addition, while some disjunction of ranges occurs, polyploids tend to exist in close geographical proximity to their diploid relatives. For example, for grey treefrogs, although tetraploids extend further north into colder environments and diploids extend further southeast into hotter environments, they overlap for most of their range. No differences in freeze tolerance have been demonstrated between the ploidy levels (Irwin & Lee, 2003), but the distribution of only the tetraploids north of the Great Lakes in eastern North America would be consistent with invasion of novel but more variable habitats after the last glacial period. It could, however, suggest that polyploidization initially occurred at the northern edge of the range after the last glacial maximum when environments were unstable and that the polyploids then expanded in both directions as the glacier receded, which would be consistent with estimates of speciation associated with the Wisconsin glaciation (12 000–35 000 ybp). Otto *et al.* (2007) found that the tetraploid species occupies areas where climatic conditions

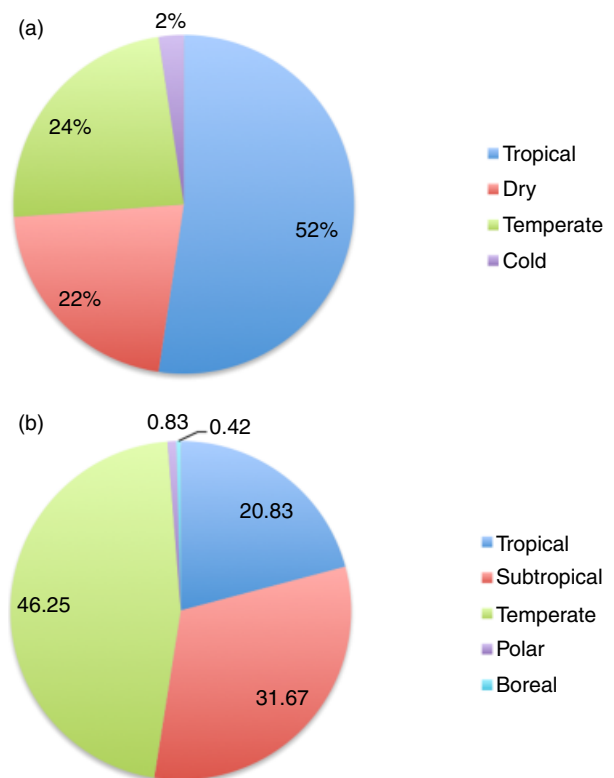


Figure 4 (a) Distribution of polyploid anurans by major climatic zones, based on Köppen classification schemes. Note that there are no polyploid amphibians found in polar regions but there are not any amphibians in general there and few species are found in cold regions. (b) Distribution of polyploid fish by major climatic zones. There are few polar species but a smaller proportion of tropical species than in amphibians.

are relatively severe (colder, drier, greater annual variation) whereas the diploid is more restricted in range, suggesting that large-scale climatic conditions have played a role in the establishment of the polyploid, in at least some portions of its range. For octoploid *Od. americanus*, a complex distribution pattern of populations with different ploidy levels exist, including areas of syntopy and sympatry, and cytogenetic variability, suggesting multiple origins of polyploids (Rosset *et al.*, 2006). Overall, no obvious pattern emerges about relative distribution of diploid and polyploid species in amphibians that would suggest that polyploids expand into new environments or have broader ecological niches.

If polyploidy were associated with extreme environmental conditions, we might expect to find a higher proportion of polyploids in regions where climates are unstable or unpredictable. Because for amphibians, dry and cold environments could be considered harshest, we used the Köppen classification of climatic zones to evaluate whether polyploids tend to be found in extreme environments. Although 24% of polyploid anurans are found in temperate regions, 22% in dry regions and 2% in cold regions, the majority (52%) are found in the tropics (Fig. 4a). However, in all cases, polyploids are found in the same climatic zones as

their close diploid relatives. Of the species found in the tropics, two species are from Madagascar (*Cophixalus*, *Scaphiophryne*), where they are found at high elevations, where climatic conditions would likely be variable, even in the tropics. The remaining polyploids from tropical regions are in the family Pipidae, where polyploids occur at a range of elevations and habitat types. However, polyploidization within this group is relatively ancient (ranging from 2.7 mya for octoploid *X. wittei* and 42 mya for the ancestor of the current tetraploids in the genus *Xenopus*), so climatic conditions during times of speciation might have been very different than what they are currently (Evans *et al.*, 2004, 2005). Few amphibians survive in cold environments so it is not surprising that only a single polyploid species (*Hy. versicolor*) is found under these conditions. Because in most cases polyploids do not have completely disjunct ranges from their diploid progenitors, there does not appear to be a dramatic advantage in terms of habitat exploitation.

For fish (Fig. 4b), 46% of polyploidy species occur in temperate, 32% in subtropical and 21% in tropical regions. Mirroring the amphibians, only a small fraction of polyploid fishes occur in boreal (0.42%) or polar (0.83%) regions. It is also worthwhile to consider the habitat and life history strategies of polyploids. The vast majority of polyploid fish are dependent on freshwater: either living exclusively in freshwater (55%), migrating from marine to freshwater to breed (anadromous: 18%), or completing their entire lifecycle within rivers (potamodromous: 21%; supporting information Fig. S2a). A small percentage are associated with brackish water (5.8%) and only a very few (0.4%) are catadromous (live in freshwater but migrate to a marine environment to breed). In addition, most polyploids either live near the bottom surface (benthopelagic: 61%) or in the bottom part of the water column (demersal: 36%) rather than on the surface (pelagic: 2.9%; supporting information Fig. S2b). As niche shifts associated with ploidy change have been observed in some cases, it is interesting to note the possibility that the ancestors of sturgeons (Birstein, Doukakis & DeSalle, 2002) and salmonids (McDowall, 2001) were both strict freshwater inhabitants (Broughton *et al.*, 2010), with subsequent species occupying a broader niche (anadromous behaviour) and exhibiting greater environmental tolerance. While association with freshwater may indicate greater tolerance to fluctuating conditions, population sizes tend to be smaller in freshwater than marine fishes, thus potentially allowing for drift to maintain ploidy shifts at a greater rate than in marine species. Because most polyploid fish are not easily paired with diploid progenitors, it is more difficult to assess whether polyploids have larger ecological niches, but the complete lack of polyploids in more stable marine environments is consistent with association of polyploidy with environmental variability.

Pathogen pressures

It is possible that polyploids also might have increased adaptation to biotic pressures, such as those posed by pathogens. Because pathogen pressures are likely to change

with environmental fluctuations and shifts to novel niches, newly arising polyploid lineages would likely be exposed to both novel and established pathogens. Particularly in the face of current concerns that global amphibian declines are related to changes in pathogen pressures (e.g. Green, Converse & Schrader, 2002; St-Amour *et al.*, 2008) there has been surprisingly little focus on resistance or response of polyploid taxa in relation to diploids. Frogs in the genus *Xenopus* have been implicated as reservoirs (i.e. they carry the pathogens but are not themselves greatly damaged by them) of the two most high profile disease agents currently thought to be threatening amphibians on a global basis: viruses in the family Iridoviridae (ranaviruses; Robert *et al.*, 2007) and the chytrid fungus *Batrachochytrium dendrobatidis* (Weldon *et al.*, 2004). For ranaviruses, this is due to the ability of adults to mount an effective immune response and clear the viruses (Robert *et al.*, 2007). The main vector is thought to be one of the tetraploid species, *X. laevis*, which has been used as an experimental developmental model for many years and has been commercially distributed worldwide. Although the only extant diploid species (*Si. tropicalis* $2n = 20$) has been found to be more susceptible than the tetraploid *X. laevis* ($2n = 36$) to the type strain frog virus 3 (FV3; J. Robert, pers. comm.), it is not clear that this is due to polyploidy because the two species have different basal chromosome numbers. Although increased tolerance to pathogens has been suggested theoretically as a potential advantage of polyploids relative to their diploid progenitors (Guegan & Morand, 1996), this is also somewhat confounded by hybridization. The consequences of gene duplication for particular pathogen response genes have been investigated in economically important plants such as cotton (Wright *et al.*, 1998), but because many cultivars arose through hybridization between species rather than within a single species, both effects of combining genomes and cultivation history can obscure effects due to polyploidy itself. Hybridization has been considered in the transmission of pathogens between species (Cleaveland *et al.*, 2007; Gonthier *et al.*, 2007) but few studies have examined the consequences of genome interactions for pathogen response. A notable exception is the suite of studies focusing on host–parasite co-evolution of monogeneans in relation to allopolyploid origins of their *Xenopus* hosts (Jackson & Tinsley, 1998, 2001, 2003). However, the long history of polyploidy in the host species makes it difficult to determine whether hybridization, polyploidy or other host factors are the most important in regulating this. Changes in pathogen distribution and virulence have also been linked to habitat and environmental changes (Bosch, Carrascal, Duran *et al.*, 2007; Dionne *et al.*, 2007; Laine, 2007) and so it is possible that at times when polyploid formation is most likely, there might also be exposure to new types of pathogen pressures. In these cases, it may be likely that newly formed polyploids benefit from increased pathogen resistance compared with diploid progenitors (Chevassus & Dorson, 1990; McDowall, 2001). Somewhat surprisingly, genes associated with immunity (at the MHC), which might be expected to benefit from higher diversity, are not always retained in duplicate in

polyploid frogs (Kobel & Du Pasquier, 1986; Shum *et al.*, 1993; Sammut *et al.*, 2002; Du Pasquier *et al.*, 2009), or fish (Kruiswijk *et al.*, 2004; Shiina *et al.*, 2005), so the relationship between pathogen response and ploidy remains unclear. In fish, despite focus on diseases of economically important salmonids, often in relation to MHC variation (e.g. Harris, Soleng & Bakke, 1998; Langefors *et al.*, 2001; McClelland, Penn & Potts, 2003; Dionne *et al.*, 2007; Dionne, 2009; Evans & Neff, 2009), there has been little emphasis on how polyploidy might influence pathogen responses.

Genomic flexibility

Successful polyploid lineages might be those that can tolerate dramatic changes in genomic structure and regulatory divergence. Although duplicated genomes are thought to provide greater genetic flexibility and broader adaptive responses in general, reversion to effectively diploid segregation is apparent in most 'old' polyploids and few polyploids retain duplicate gene expression across their genomes. Nevertheless, in animals rates of loss of duplicate gene expression have been far below expectations of neutral models (Ohno, 1970; Allendorf, 1978), so there is still potential that greater genomic flexibility exists in polyploids. For example, catostomid and salmonid fish retain *c.* 50% duplicate gene expression, despite up to 100 million years of divergence as polyploids (Ferris & Whitt, 1977*d*; Bailey *et al.*, 1978) and many genes are retained in duplicate in polyploid series of *Xenopus* frogs (Hughes & Hughes, 1993). It has been suggested that genes involved in regulatory processes will be retained most frequently (Birchler & Veitia, 2007, 2010) and that selection for expression divergence is stronger than protein sequence divergence (Chain, Ilieva & Evans, 2008). There is also increasing evidence that epigenetic changes are abundant following polyploidization and hybridization (Mittelsten Scheid *et al.*, 1996; Matzke, Scheid & Matzke, 1999; Rodin & Riggs, 2003; Beçak & Kobashi, 2004; Chen, 2007; Paun *et al.*, 2007; Soltis *et al.*, 2007; Bacquet *et al.*, 2008; Xu *et al.*, 2009; Jackson & Chen, 2010), increasing the potential plasticity of duplicated genomes.

Because hybrid ancestry itself might affect retention of duplicate genes and changes in expression (Evans, 2007), it is again difficult to separate the effects of genome duplication from the 'genome shock' of hybridization. For example, Semon & Wolfe (2008) compared the expression profiles in 11 tissues of 1300 genes retained in duplicate in the tetraploid *X. laevis* relative to those in single copy in the diploid *Si. tropicalis* and found a set of 68 genes that have undergone significant reduction in expression in at least two tissues. They found that slowly evolving genes tended to be more prone to subfunctionalization, which they concluded is due to allopolyploidization. They also found that the same orthologues found in zebrafish also tended to be retained in duplicate after the WGD at the base of the teleosts, suggesting that duplication of some types of genes could have selective advantages (or that some types of genes are not tolerated in duplicate). Polyploidy might be restricted to organisms with genomes flexible enough to tolerate the

dramatic changes in genome structure and gene regulation that follow WGD and/or hybridization. Pandian & Koteeswaran (1998) remarked on the amazing ability of fish to tolerate genomes from haploid to heptaploid, genomic contributions from the male or female parent alone, and unequal contributions from parents belonging to the same or different species.

The first amphibian genome sequence has recently been published (Hellsten *et al.*, 2010) (described as *Xenopus tropicalis* rather than *Si. tropicalis*) but no tetraploids have yet been sequenced. Genome resources are more advanced in the fishes compared with many other orders and so there is promise of assessing the consequences of WGD in detail. Draft genome sequences are available for *Fugu* (or Takifugu Aparicio *et al.*, 2002) and Medaka (Kasahara *et al.*, 2007) and have been used to identify paleopolyploid events and the proto-karyotype of vertebrates (Panopoulou *et al.*, 2003; Jaillon *et al.*, 2004; Dehal & Boore, 2005; Panopoulou & Poustka, 2005; Nakatani *et al.*, 2007; Putnam *et al.*, 2008). The availability of an increasing number of fish genomes is allowing a better understanding than ever before about the role of gene and genome duplication in the evolution of fishes. There is now substantial evidence from remnant duplicate gene pairs suggesting that an ancient genome duplication event of tetraploidization (followed by diploidization) enabled the diversification of gene functions necessary to promote the explosive speciation in fish (Volf, 2005; Luo *et al.*, 2007). The loss of certain genes, their subdivision, and acquisition of novel functions over evolutionary time seem to be linked with the evolution of fish variability (Vogel, 1998; Meyer & Schartl, 1999; Lynch, 2007; Siegel *et al.*, 2007). The genomic complexity and plasticity of the teleosts might be the reason for their evolutionary success and astounding biological diversity (Meyer & Schartl, 1999; Luo, Yang & Zhang, 2000), although this genomic plasticity might also come at a cost to diversity (Luo *et al.*, 2000). Yet, despite the indirect evidence, a link between a specific genome duplication event and an increase in overall complexity and diversity remains to be established (Otto & Whitton, 2000; Donoghue & Purnell, 2005). Crow & Wagner (2006) suggest that the probability of extinction was reduced by a factor of at least 5.5 in the lineages following the FSGD. However, correlations between specific duplications and increased diversity are problematic, as the genetic signature of single duplication events tends to be obscured by extensive genomic expansion, contraction and subsequent gene loss (Blanc & Wolfe, 2004; Crow & Wagner, 2006). Nevertheless, rapid increase in genomic information should enable more precise evaluation of whether genomic constraints to polyploidy can explain why some species are polyploid and some not.

Risk of extinction

If polyploids have some inherent competitive advantage compared with diploids, one might expect that they would be less at risk of population declines and extinction than their diploid counterparts. Alternatively, if WGD comes at a cost, they might be more at risk. In plants, a positive

Table 1(a) Endangered status in relation to ploidy based on data available on the IUCN red list of threatened species database <http://www.iucnredlist.org/>

Status	Frogs		Fish	
	Diploid	Polyploid	Diploid	Polyploid
Critically endangered	2.8	2.4	4.8	14.9
Endangered	7.1	4.9	6.4	12.9
Near threatened	4.9	9.8	3.9	7.9
Vulnerable	4.2	2.4	18.0	19.8
Least concern	55.5	70.7	37.9	37.6
Data deficient	25.4	9.8	24.8	4.0
Extinct	0.0	0.0	4.2	3.0

Percentages are indicated in the tables but calculations were based on observed numbers. Contingency χ^2 found no significant differences between the number of diploids and polyploids for frogs ($P=0.2$ with data deficient, 0.48 without) but there was a significant difference from expected values for polyploid fish ($P<0.00001$ when data deficient included but $P=0.003$ when excluded), where there were more critically endangered, endangered and near threatened species relative to diploids.

correlation between risk of extinction and C-value has been found, but this was attributed to repetitive DNA elements rather than polyploidy (Vinogradov, 2003). Extinction risk with increased genome size has also been implicated in the species-poor group of lungfishes, which have very large genomes littered with transposable elements (Kraaijeveld, 2010). We searched the IUCN redlist database for all known polyploid anurans and fish, in comparison to the diploids in the genera in which they occur. The list is proportionately more complete for anurans than for fish; nevertheless there were 41 polyploids and 283 diploids available for anurans and 101 polyploids and 311 diploids listed for fish. The number of species that were listed in each category was compared with the relative frequency of the ploidy types, using contingency χ^2 (percentages are shown in supporting information Table S1, for more direct comparison). For anurans, there was no significant deviation from expectations ($P=0.2$), and there were similar proportions of critically endangered diploids and polyploids (Table 1a). There were more polyploids of least concern, fewer vulnerable and fewer endangered, but more near threatened species than for diploids. None of the genera in which polyploids have been identified have extinct taxa listed on the IUCN database. Excluding data deficient taxa (of which there were more in diploids) did not change conclusions ($P=0.48$). In contrast, for fish, there was a significant deviation from expectations ($P<0.00001$), with more critically endangered, endangered and near threatened polyploid than diploid species but similar proportions of vulnerable, least concern and extinct species (Table 1b). Again, excluding data deficient taxa (of which there were again more in diploids) did not change conclusions ($P=0.003$). In terms of population trends, there were no differences between polyploids and diploids for fish or amphibians ($P=0.11$ and 0.54 for amphibians and fish, respectively),

Table 1(b) Population trends (i.e. stable, decreasing, increasing) in relation to ploidy

Population Trend	Frogs		Fish	
	Diploid	Polyploid	Diploid	Polyploid
Decreasing	20.2	26.8	32.2	36.0
Stable	46.9	53.7	6.4	8.0
Increasing	1.1	4.9	1.0	2.0
Unknown	31.8	14.6	60.4	54.0

No significant differences were found for either frogs or fish ($P=0.11$ and 0.54, respectively); including unknowns made frogs marginally significant $P=0.048$ but not fish $P=0.63$).

but few were increasing in either group and there were many more diploids and polyploids with unknown status among fish and a smaller proportion of species classified as stable. For anurans, the only critically endangered polyploid was *X. longipes*, which has only been described from the type locality and it was listed as stable at the population level; the two endangered species (*X. gilli* and *Sc. gottlebei*) are both listed as decreasing; all near threatened species are decreasing (*Ce. ornata*, *Pleurodema bibroni*, *Pl. kriegi*, *X. amieti*) as is the one vulnerable species (*Astylosternus diadematus*). These data suggest that there is not an overall advantage of being polyploid, in terms of risk of decline and extinction. In fact, in fish, there is some evidence that polyploids are at higher risk than diploids (Sturgeons); however, this pattern is complicated by the fact that some of the most 'successful' polyploid lineages (e.g. salmonids and catostomids) do not include diploids for comparison. Overall, polyploidy does not seem to be a major factor explaining variation in risk of extinction in extant fish or amphibians.

Conclusions

Our updated survey of polyploidy in fish and anurans suggests that, even in these vertebrates where it is relatively common, it is restricted to certain groups. However, where it occurs, multiple origins are often apparent within certain lineages. In amphibians, except for the exclusively polyploid genus *Xenopus*, polyploidy seems to be restricted to individual species across a wide range of families (Fig. 1; supporting information Table S1) and with no particular geographic pattern. In contrast, for fish, polyploidy seems to be more phylogenetically clustered (Fig. 2), but where it occurs, it tends to be found in multiple species (supporting information Table S2). Closely associated diploid ancestors are not often apparent, perhaps due to the high dispersal rates of fishes. In addition, the identification of polyploidy in fish is complicated by multiple rounds of lineage-specific WGD and large variation in chromosome morphology, which means that a combination of genome size, chromosome counts, marker-based assessment of duplicate gene expression, and more fine-scale genomic information is often necessary to confirm polyploidy. In contrast, there is high conservation of chromosome morphology and numbers in anurans, and most polyploid species are still found in

association with their diploid progenitors, making identification of polyploids more reliable based on karyotypes. Detection of polyploids in anurans has also been facilitated by differences in mating calls that can be used to identify otherwise cryptic species. Nevertheless, because cytogenetic surveys are no longer commonly performed, it is quite likely that we currently have an underestimate of the true frequency of polyploidy in both fish and amphibians.

The most striking feature shared by anurans and fish is that they breed in freshwater environments. They also tend to produce large number of gametes, have external fertilization and communal breeding and their type of gametogenesis enables the production of unreduced gametes. They also both have a propensity for hybridization, which is often involved in polyploid formation. These factors all should promote polyploid formation, particularly if environmental variability during the breeding season increases the production of unreduced gametes (Mable, 2004). Nevertheless, diploids giving rise to polyploid lineages in anurans have similar breeding tactics as those that are exclusively diploid and there are many freshwater fish that are not polyploid. So, these factors might facilitate the formation of polyploids but cannot explain the establishment of successful polyploid lineages in only certain taxa.

In anurans, establishment of polyploid lines could be enhanced by direct changes in the mating calls, which would enable assortative mating by cytotype that would decrease the potential barriers to polyploid speciation presented by being initially outnumbered by diploid progenitors. If this only occurred for particular species, this could explain why polyploidy occurs and often has multiple origins in some diploid–polyploid complexes, but not all polyploids seem to have this attribute. Polyploids don't seem to be restricted to certain geographic regions or climatic zones but there is some suggestion that they tend to be formed during times of climatic instability. However, the absence of dates for most polyploidization events and the exclusion of polyploid taxa from phylogenetic analyses (particularly in anurans) does not allow a rigorous test of this hypothesis. Comparing current distributions of polyploid anurans with their diploid progenitors does not suggest that polyploids have broader ranges or have always invaded harsher habitats than their diploid progenitors. Although this test is not possible for fish because their diploid progenitors often no longer exist, there also does not seem to be an overwhelming pattern based on distributional notes. It is possible that diploids that are able to invade harsh habitats are those with sufficient genomic flexibility to tolerate polyploidy, which would obscure differences between ploidy levels. It is also impossible to distinguish whether small range size reflects recent origins and exploitation of new habitats or contraction of previously larger ranges.

However, the occurrence of diploids that give rise to polyploids in the same habitats as closely related species where polyploidy does not become established (despite experimental demonstration that unreduced gamete formation can be induced), does not support this. In contrast, the phylogenetic clustering of polyploidy in fish suggests that

genomic constraints could be important for successful polyploid establishment. We also find no evidence that polyploid species are more or less at risk of extinction than their diploid relatives, suggesting that the stronger trends found in plants could be driven largely by mating system, rather than polyploidy. Although there are not as many species to compare, it would be interesting to repeat these tests using unisexual vertebrates.

We conclude that, while there is a tantalizing suggestion that rates of polyploid formation and establishment might be increased during times of environmental change, the data do not currently exist to fully evaluate this hypothesis. We also still do not have a very clear idea of what factors determine whether a given diploid can give rise to polyploid lineages or what determines the success of nascent polyploids. Particularly given concerns about climate change, experimental approaches to investigate whether tolerances are altered in polyploids compared with diploids (in terms of changes in both biotic and abiotic pressures) seem warranted. Re-initiating a focus on standardly measuring genome size and karyotyping in species surveys would help to determine the full extent of polyploidy, and including polyploid taxa in robustly dated family level phylogenies would enable evaluation of how often polyploidy is associated with drastic environmental change.

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Supplementary information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Glossary of Terms.

Figure S1. Distribution of descriptions of new polyploid anuran species by decade. The first naturally occurring polyploid amphibians were described in 1964 (*Ambystoma Jeffersonian* complex of salamanders) and the first polyploid anuran species (*Odontophrynus americanus*) was reported in 1966. The peak of new descriptions was in the 1970's, when allozymes and cytogenetics were at their peak.

Figure S2a. Distribution of polyploid fish by habitats and breeding site for migratory species. The vast majority of polyploid fish are dependent on freshwater: either living exclusively in freshwater, migrating from marine to freshwater to breed (anadromous) or completing their entire lifecycle within rivers (potamodromous). A small percentage are associated with brackish water and only a very few are catadromous (live in freshwater but migrate to a marine environment to breed).

Figure S2b. Distribution of polyploid fish by niche type. Most polyploids either live near the bottom surface (benthopelagic) or in the bottom part of the water column (demersal) rather than on the surface (pelagic).

Table S1. Summary of known polyploid anurans (bold face type), along with their closest known diploid relatives, indicating original taxonomy (genus and family) at the time that the polyploids were described, as well as revised

taxonomy. References are provided for the first report of polyploidy for each species, as well as those recommending changes to the original taxonomy. Chromosome numbers were taken from the original ploidy descriptions; genome sizes were obtained from the Animal Genome Size database (Gregory 2005a; <http://www.genomesize.com/>) but are not available for most of the species listed. Notes on origins of the polyploids were taken from the primary literature. Coordinates for the centre of distributions of polyploid taxa were taken from the maps available through Amphibiaweb (<http://amphibiaweb.org/>); Krüppen classifications were used to characterize the climates in the relevant regions. Endangered species status and population trends were obtained from the IUCN database (International Union for Conservation of Nature Redlist of Endangered species, <http://www.iucnredlist.org>). Descriptions of species distributions were obtained from the Amphibian Species of the World database (Frost *et al.* 2010; <http://research.amnh.org/vz/herpetology/amphibia/>).

Table S2. Summary of known polyploid fish, along with a list of species where polyploidy has been suspected but not confirmed. A description of higher level classifications is provided for comparison with Fig. 2. References are provided for the first report of polyploidy for each species. Chromosome numbers were taken from the original ploidy descriptions; genome sizes were obtained from the Animal Genome Size database. Endangered species status and population trends were obtained from the IUCN Red list database. Notes on distributions, environment, and climate were obtained from Fishbase (Froese *et al.* 2008; www.fishbase.org).

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