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A database of amphibian karyotypes

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Abstract One of the first characteristics that we learn about the genome of many species is the number of chromosomes it is divided among. Despite this, many questions regarding the evolution of chromosome number remain unanswered. Testing hypotheses of chromosome number evolution using comparative approaches requires trait data to be readily accessible and associated with currently accepted taxonomy. The lack of accessible karyotype data that can be linked to phylogenies has limited the application of comparative approaches that could help us understand the evolution of genome structure. Furthermore, for taxonomists, the significance of new karyotype data can only be determined with reference to records for other species. Here, we describe a curated database (karyotype.org) developed to facilitate access to chromosome number and sex chromosome system data for amphibians. The open web interface for this database allows users to generate customized exploratory plots and tables of selected clades, as well as downloading CSV files for offline analyses.

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Abbreviations
cf   for the Latin *conformis* or *conferre*
aff   for the Latin *affinis*

Introduction

Cytogenetic data provide some of the most basic information about a genome (i.e., how many discrete chromosomes and chromosomal arms it is divided among). Despite the fundamental nature of this data, many questions about genome evolution at this scale remain unanswered (Voss et al. 2011). Explanations for change in chromosome number are as varied as the organisms studied and include roles for meiotic drive, selection, drift, and even differences in chromosome number impacting the evolution of other traits. Much of the early study of chromosome number assumed that changes in chromosome number were deleterious or underdominant and that as such may be important in the process of speciation (White 1977). However, support for these models of chromosome evolution has received mixed support (Freyman and Höhna 2017; Lande 1985). In mammals, large comparative studies have shown that the evolution of chromosome number may be primarily driven by changes in the bias of female meiotic drive (Blackmon et al. 2019). In contrast, it was long thought that chromosome number evolved in response to indirect selection on recombination rate in Hymenopteran insects (Sherman 1979), but recent work has shown that this

likely only explains a fraction of the change seen in this clade (Ross et al. 2015). Theoretical and empirical work has shown that sexually antagonistic variation on autosomes may lead to changes in chromosome number through fusions (Charlesworth and Charlesworth 1980; Kitano et al. 2009; Pennell et al. 2015; Blackmon and Demuth 2015), and recent studies have shown that chromosome number may play a direct role in the evolution of haplodiploidy in Acari (Blackmon et al. 2015). In any case, it is clear that much of our current understanding of karyotype evolution is largely biased towards specific taxonomic clades, such that even basic summaries and studies of chromosomal characteristics and evolution are lacking for many organisms.

Amphibians have a rich history in the field of cytogenetics that began before we even fully understood the hereditary role of chromosomes. The first karyotype of any organism was described in 1882 for the salamander *Salamandra infraimmaculata* (Flemming 1882), which predates the chromosomal theory of inheritance by 20 years. Since this first report, interest in the cytogenetics of amphibians has continued (Hillis 1991; Kezer 1964; Morescalchi 1973). Given their enormous diversity of genome sizes (~ 1.4–120 Gb), amphibians have also become prominent model systems for studying genome gigantism (Mohlhenrich and Mueller 2016; Sun et al. 2011) and its impacts on cell biology (Gregory 2001; Roth et al. 1994). Despite such longstanding interest in the cytogenetics of amphibians, the last synthesis of amphibian karyotypes was completed in 1990; in this compilation, Max King published records for 1022 amphibians (851 Anura, 155 Caudata, and 16 Gymnophiona) (King 1990). The following year, a book focusing on amphibian cytogenetics and evolution was published with chapters devoted to select groups, but lacking an amphibian-wide synthesis or dataset (Green and Sessions 1991). More taxonomically or geographically narrow syntheses and collections have been published more often (Schmid et al. 2010). Thus, available cytogenetic data for large clades are often scattered among many journals and frequently restricted behind paywalls. Additionally, due to the popularity of amphibians among systematists, many groups have experienced extensive taxonomic revisions leading to new synonymies and name combinations. As a result, no resource allows free and easy access to all cytogenetic data for researchers studying amphibians or chromosome variation across large clades.

Here, we present the amphibian karyotype database that is designed to facilitate research into many fundamental questions about the evolution of genome structure at broad taxonomic scales. Alongside existing or new phylogenies, researchers can use these publicly available datasets to test hypotheses with comparative approaches that can reveal driving forces of amphibian chromosome evolution. For taxonomists, this database will offer a single easily searched resource that includes all currently available records for any amphibian group under study. This accessibility will accelerate future work and will make it possible to synthesize older records into a more complete picture of amphibian karyotype evolution as new data become available. Finally, as we move into an era of ever more affordable sequencing, karyotypes are critical preliminary information that should be evaluated and may even suggest particularly attractive targets for future sequencing efforts. For instance, within this curated database are many examples of closely related species with radically remodeled karyotypes. This rapid change in genome structure could play an important role in speciation and is still poorly understood. Species pairs like this are particularly appropriate targets for whole genome sequencing since they may reveal genomic characteristics that predispose some lineages to higher rates of genome structure evolution.

Methods

To facilitate investigation of these questions, we have built the Amphibian Karyotype Database (karyotype.org). We first collected all data from an existing compilation (King 1990). We then searched for new data by using Google Scholar searches combining order names with "chromosome number," "karyotype," "cytogenetic," "sex chromosome," "b chromosome," and "microchromosome." Records were also located by tracking citations to earlier studies. This search strategy revealed 371 sources that contained unique karyotype records that are now available in the database. Due to conflicts between the Open Tree of Life Database (Rees and Cranston 2017) and the most widely accepted amphibian taxonomic work (Amphibian Species of the World Database (Frost 2018), we manually searched for each name in our dataset using the Amphibian Species of the World Database. In cases where a name was found to be invalid, we identified the currently valid

name. We also collected higher-level taxonomic data (family and order) for each species in our dataset. Where applicable, we link records to both the original name used in the initial report as well as the currently accepted name in the database. In four cases, a species name was unable to be resolved because an invalid species name was a junior synonym for multiple valid names and geographic/range information was insufficient to resolve the name. These four names have been omitted from the database to avoid the application of erroneous data in downstream analyses. In one case, a species name is absent from the name database. For this species, we have retained the record but noted that the name is invalid. A total of 31 species in the database were initially reported with tentative species-level identifications indicated by use of the abbreviations *cf.* for the Latin *conformis* or *conferre* or the abbreviation *aff.* for the Latin *affinis*; we retain these abbreviations in the database. In nine species in the database, these initial reports with tentative species-level identifications were under species names that are not valid. In these cases, we list the currently valid name and the *cf.* or *aff.* abbreviations are retained with the valid name. The name resolution process led to the identification of 768 records where the initial cytogenetic report required alteration to a currently valid name.

For each species in the database, we have scored four taxonomic fields (order, family, genus, and species) and have made every effort to score seven data fields Table 1. (1) Diploid number—the number of chromosomes of a typical cell if sexes differ the value is for the homogametic sex. (2) Fundamental number—the number of chromosomal arms present in a typical cell. We considered chromosomes as bibrachial if the ratio of the lengths of the long to short arm was less than 7. (3) Sex chromosomes—the identity of cytogenetically identifiable sex chromosomes. (4) Ploidy—the number of homologous copies of each chromosome present in the genome. (5) Microchromosomes—the number of microchromosomes in the genome. There is no widely accepted heuristic to classify chromosomes as micro or macro, and therefore, we include the classification reported in the original investigations. (6) B chromosomes—the maximum number of B or accessory chromosomes reported. (7) Notes—any additional information of importance from the original report. (8) Original listing—any names that a record has previously been associated with in a previous report. (9) Citation— the full citation for the source of the report. Scripts were used to identify possible errors in the manual entry of data (e.g., a fundamental number that is more than twice the diploid number). We used exploratory plots of diploid number for families, and outliers that had significantly lower or higher chromosome number were checked a second time to confirm the originally reported values had been correctly entered in the database.

We used the R package Shiny to create a dynamic, interactive web interface for the database (Chang et al. 2018). Users generate database queries by

Table 1 Data fields in the Amphibian Karyotype Database

Trait	State
Order	Valid order for the record
Family	Valid family for the record
Genus	Valid genus for the record
Species	Valid species for the record
Diploid number	The number of chromosomes in a typical cell
Fundamental number	The number of chromosomal arms in a typical cell
Sex chromosomes	Cytogenetically identified sex chromosome system (e.g., XY, ZW) multiple sex chromosome systems are denoted with numerals before one or both chromosomes (e.g., 2XY)
Ploidy level	The number of homologous copies of each chromosome
Microchromosomes	The number of microchromosomes present in the typical cell
B chromosomes	The maximum number of B chromosomes reported for a species
Notes	Additional notes describing the record
Original listing	Invalid name that was originally reported for the record
Citation	The original publication that data was drawn from

making selections at up to three taxonomic levels (order, family, or genus). Once a user has defined the taxonomic group of interest, they can then explore the data through interactive plots or customizable tables that can be downloaded as CSV files for offline analyses. We also include a flexible text search function that can be used to search for all records that contain a particular term. For instance, if we search the term "bufo," a genus that taxonomists have revised extensively, the database not only returns all records whose valid genus is Bufo (17 records) but also returns an additional 99 records which have been listed in previous publications under this genus.

Our database allows open access to all available cytogenetic data. The Amphibian Karyotype Database currently contains 2124 records, which is more than a two-fold increase since the last compilation published nearly 30 years ago. With 1833 records, the vast majority of current data is comprised of frogs (Anura). In contrast, Caudata and Gymnophiona have only 246 and 45 records, respectively. Our goal is to provide this database as a permanent resource that we will expand as new data become available with updates yearly. All data underlying the online database as well as the scripts used to generate the database are available as a plain text CSV file and an R script from a FigShare repository (https://doi.org/10.6084/m9.figshare.7525523). Data downloaded from the database may be imported into common comparative analysis R packages like *ape* (Paradis et al. 2018), *diversitree* (Fitzjohn 2012), *chromploid* (Zenil-Ferguson et al. 2018), or *phytools* (Revell 2012).

Results and discussion

The Amphibian Karyotype Database enables insight into amphibian chromosomal characteristics and diversity. As an example demonstration, we used the database to characterize a number of broad evolutionary trends in amphibian chromosomal characteristics. To visualize the chromosome number data in our database, we have mapped haploid number for 1187 species onto a recent time-calibrated phylogeny of amphibians (Liedtke et al. 2018). This mapping was performed using a simple Brownian motion model for the evolution of haploid chromosome number allowing internal branches to be painted with inferred ancestral states (Revell 2012). This

plot illustrates several striking features of chromosome evolution. First, in Gymnophiona, the family Ichtyophiidae exhibits higher chromosome number (mean haploid number = 20.4) than is typical for all other members of Gymnophiona (mean haploid number = 13.4). Likewise, in Caudata, the family Hynobiidae exhibits higher chromosome number (mean haploid number = 29.6) than is typical for other members of Caudata (mean haploid number = 14.1). Second, we can see that the vast majority of Anura has a haploid chromosome number between 11 and 13 (1409 of 1826; 77%). The distribution of chromosome number within genera suggests that most clades have relatively low rates of evolution. Just over 90% of anuran genera (265 of 294) have haploid numbers between 11 and 13 for all sampled species. Despite this relative homogeneity, we can also see that some genera exhibit far greater diversity. The genera *Sclerophrys*, *Pristimantis*, *Strabomantis*, *Eleutherodactylus*, *Engystomops*, *Pipa*, and *Xenopus* all contain species that have a haploid number of 10 or fewer and other species with a haploid chromosome number of 15 or greater. In fact, the range of haploid chromosome number in the genus Xenopus is 10 to 54 which captures almost the entire range of haploid chromosome number observed in Anura (7 to 54). The disparification of chromosome number in these clades suggests that they may be useful in identifying the forces that lead to high rates of chromosome evolution versus relative stasis.

Looking across all of the amphibians, we find extraordinary diversity in chromosome number (Fig. 1). The lowest counts are in the anuran genus *Athroleptis* with reports for four species with a haploid chromosome number of 7. The highest chromosome number is reported in the anuran genus *Xenopus* where four species are dodecaploids and have a haploid chromosome number of 54. In contrast to groups like insects where polyploids are almost always parthenogenic, these *Xenopus* species reproduces bisexually. Polyploidy has been reported in 85 amphibians in the database. The most common of these higher ploidy levels is tetraploidy with 45 species. Octoploids and dodecaploids are also well represented with 13 and 4 records respectively. Much of the diversity in chromosome number is found at relatively low taxonomic levels. The database includes records for 372 genera and 91 of these contain species with different chromosome number
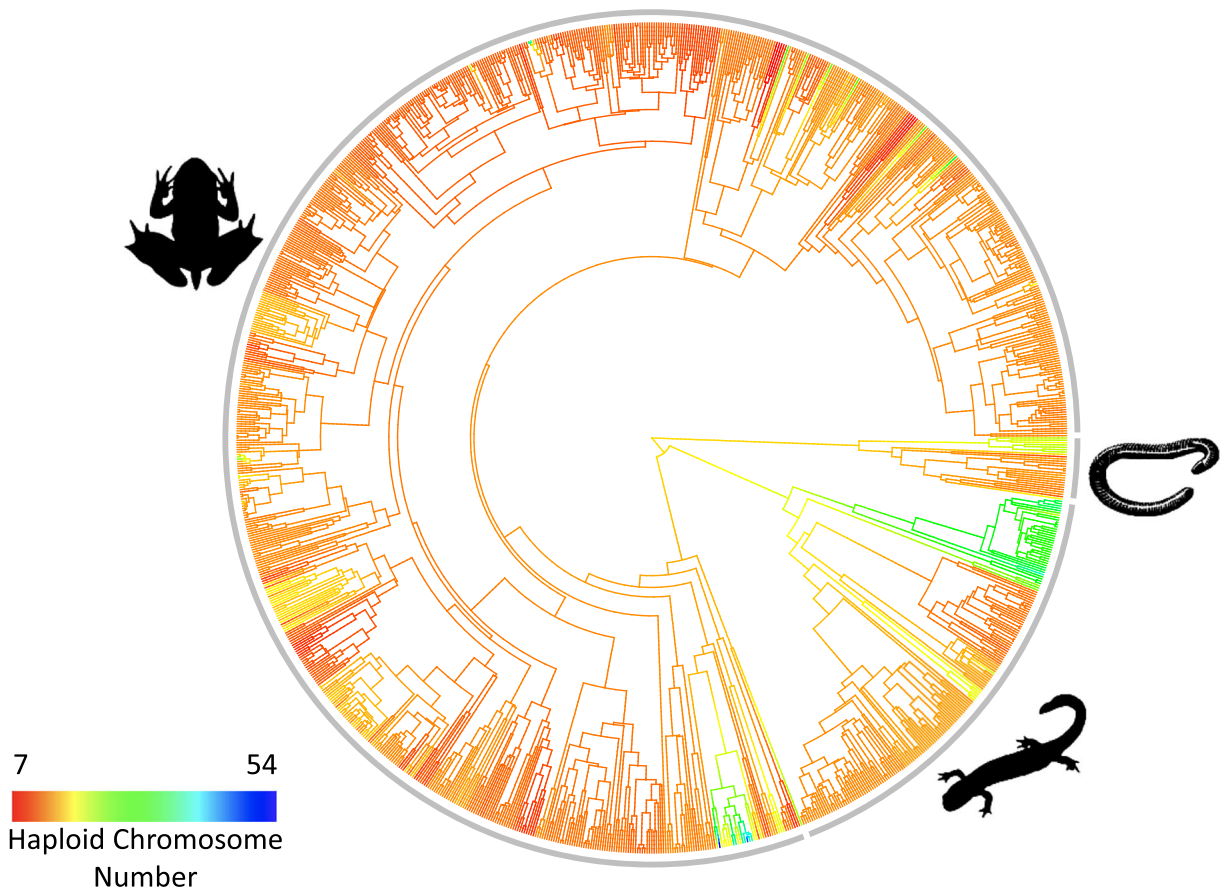
Fig. 1 Haploid chromosome number in amphibians. The color of branches indicates the ancestral state reconstruction of haploid chromosome number based on a Brownian motion model of trait evolution. The three major clades are indicated by the gray bar around the edge of the phylogeny

and 170 genera contain species with variation in fundamental number. We have also included sex chromosome data in the database. The XY sex chromosome system is most common, with reports from 66 species. An additional 30 species in the database have reports of ZW sex determination systems, and 2 species have reports of multiple sex chromosome systems (e.g., XXY). Reports for accessory chromosomes or B chromosomes range from 1 to 15 in a total of 40 species. Of these, 17 have a single accessory chromosome while the highest 15 is reported in the anuran *Leiopelma hochstetteri*. While microchromosomes are common among reptiles, in amphibians, they are restricted to Caudata and Gymnophiona. In Caudata, 8–19 microchromosomes have been reported in 12 species (5% of Caudata species) spread among 6 genera. In contrast, 19 species or 42% of

Gymnophiona have reports of microchromosomes (range 2–30). Gymnophiona also exhibits a larger number of microchromosomes with a high of 30 in *Ichthyophis beddomei*.

One of the simplest types of karyotype remodeling that is possible is combining two monobrachial chromosomes into one bibrachial chromosome reducing haploid chromosome number by one (e.g., Session et al. 2016). The reverse process with one bibrachial chromosome breaking into two monobrachial chromosomes will increase haploid chromosome number by one. If this is the only process involved in changing chromosome number, then the proportion of chromosomes that are monobrachial should be strongly predictive of the chromosome number—a pattern that is widespread among Diptera (Sved et al. 2016). However, as shown in Fig. 2, when we plot the
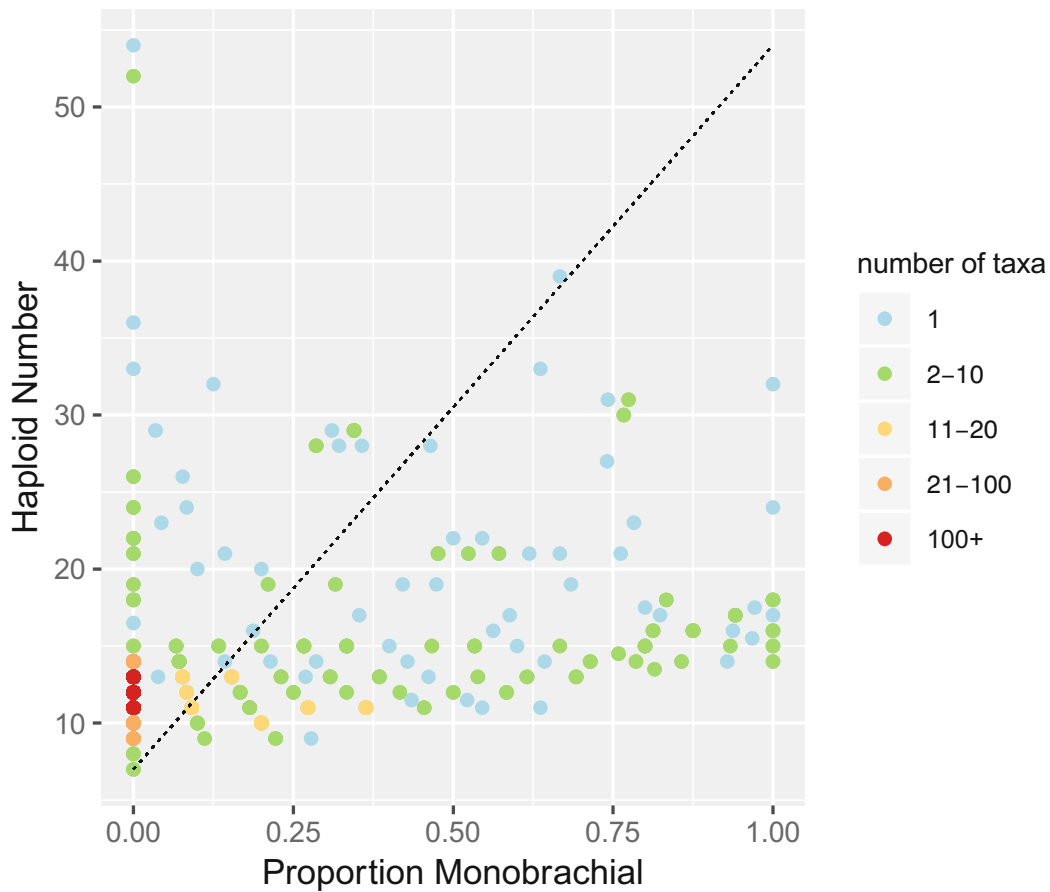
Fig. 2 Relationship between haploid number and proportion of chromosomes that are monobrachial. The color of each point indicates the number of records sharing that pairing of a haploid number and a proportion of monobrachial chromosomes. Deviations from the diagonal in black suggest processes other than simple fusions and fissions must be responsible for changes in chromosome number

haploid chromosome number of amphibians against the proportion of chromosomes that are monobrachial we find only a weak pattern. Fitting a linear model to this relationship, we find that the relationship is significant (p value < 0.001, $\beta = 7.6$, df = 1525). Despite this significant pattern, this relationship explains only 17.8% of the variation in haploid chromosome number among amphibians. We interpret this as support that more complex rearrangements (e.g., gain and loss of centromeric and telomeric sequences) are common among amphibians. Whether these gain and loss events are due to dysploidy or de novo generation of these structural elements cannot be resolved with our data in isolation, but the database can facilitate multiple roads forward to explore the dynamics of chromosome number evolution.

A variety of comparative approaches could be used to determine the drivers of chromosome evolution across amphibians. First, because the database contains the most recently valid name for all species, this data could be mapped onto phylogenies where biologically realistic probabilistic models of chromosome change could be fit allowing exploration of the rate of polyploidy and dysploidy across large clades. The database also allows the identification of many sister species where whole-genome sequencing could answer this fundamental question. For instance, some Dendrobatidae exhibit reductions in fundamental number but not diploid number. These species could be prioritized in sequencing projects since they can offer insights into the genomic characteristics associated with rapid structural remodeling of genomes, a process that is poorly understood but potentially important in speciation.

Authors' contributions HB and RHA conceived of the database and developed the ontology used. HB and RP both contributed to writing the manuscript, and collection of records. Research towards name resolution was performed by all authors and all authors edited the manuscript.

Compliance with ethical standards

Competing interests The authors declare no conflict of interest.

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