Rapid Changes in Chromosome Counts in Fishes from the Spiral Egg Clade within the Gourami Family (Osphronemidae).

Brendan Mobley and Andrew P. Anderson

Biology Department, Reed College, Portland, Oregon 97202, USA

Abstract

Identifying clades with numerous and noticeable changes in chromosome counts is an important step in unraveling the evolutionary mechanisms that shape cytogenetic processes. Here, we describe low chromosome counts in a group of teleost fishes delimited by their unique spiral egg structure and with a species with a known low chromosome count within the labyrinthine clade (Osphronemidae). We sampled seven of nine known species within this spiral egg clade, reporting novel chromosome counts for five species and confirming two others. Overall, we find high variability in both chromosome count and arm number, which suggests a rapid loss of chromosomes during the emergence of the clade and numerous large-scale mutations occurring across evolutionary time. Lastly, we offer some possible explanations for these changes based on current and ongoing empirical and theoretical research. These data provide important information in cataloguing rapid chromosomal shifts in teleost fishes and highlights this group for further study in chromosomal and genomic evolution due to their karyotypic heterogeneity.

Keywords

Cytogenetics, evolution, genomics, karyotype, fish

Introduction

Variability of chromosome numbers across vertebrates and the evolutionary mechanisms that create it is an active point of inquiry for evolutionary biologists (Martinez et al. 2015). Finding clades with high variance or rapid changes presents valuable data points in resolving the various hypotheses on how karyotypes change over time. Some patterns can include changes due to long stretches of repeat content creating opportunities for mismatched recombination (Amores et al. 2014) such as found in mammals which range in chromosome counts from 2N=6 in the female muntjac deer (*Muntiacus muntjac*) (Wurster and Benirschke 1970; Graphodatsky et al. 2020) to 2N=102 in the plains viscacha rat (*Tympanoctomys barrerae*) (Gallardo et al. 2006; Stanyon and Graphodatsky 2012; Lebeda et al. 2020). Other patterns can involve microchromosomes as typically seen in birds which have a range from 2N=40 in *Ceratogymna bucinator* to 2N=136–142 in *Corythaixoides concolor* (Christidis 1990; Kretschmer et al. 2018). Conversely, non-avian reptiles are karyologically heterogeneous and exhibit distinct evolutionary trends between lineages (Deakin and Ezaz 2019) and have a narrower range of diploid chromosome counts than other groups (2N=24–70, Olmo 2005). Teleost fishes have been found to have notable karyotype evolution patterns, particularly with regard to extreme chromosome counts, rapid cytogenetic changes, or both (e.g. *Nothobranchius* 2N=16–50:Krysanov et al. 2023 and *Corydoras* 2N=40–134: Shimabukuro-Dias et al. 2004) and have the widest 2N range of all vertebrates, ranging from 2N=12 in the marine species *Gonostoma bathyphilum* to 2N=446 in the freshwater species *Ptychobarbus dipogon* (Lebeda et al. 2020). Paradoxically, teleost fishes have a strong trend of conserved karyotypes (Galetti et al. 2000; Mank and Avise 2006; Nakatani et al. 2007) with over half of all karyotyped fish species having

diploid chromosome counts of 48 or 50 (Mank and Avise 2006; Arai 2011), which has changed little from the proposed karyotype for the ancestor of all teleost fish (2N=52, Nakatani et al. 2007). With such diverse patterns across vertebrates, finding clades with unusual changes in chromosome counts is valuable to understanding chromosome evolution as whole.

An intriguing group of fishes with apparent chromosomal variance is within the family Osphronemidae, commonly called gouramis. The chocolate gourami, *Sphaerichthys osphromenoides*, has the lowest recorded chromosome count among freshwater fishes (Lehmann et al. 2021) with 2n=16 (Calton and Denton 1974). A species in the neighboring genus, the pikehead gourami, *Luciocephalus pulcher*, was reported to have 2n=20 (Arai 2011). Chromosome counts this low are exceedingly rare in fishes, as there are only thirteen fish species with a diploid chromosome count lower than 2N=22 (Lehmann et al. 2021). Furthermore, these counts are highly derived from the other Osphronemidae species, which generally have 2N values between 46 and 48 (Supplemental Table 1).

Both *S. osphromenoides* and *L. pulcher* are members of the “spiral egg” clade, a monophyletic group within the family Osphronemidae that includes the genera *Sphaerichthys*, *Luciocephalus*, *Parasphaerichthys*, and *Ctenops*. The monophyly was proposed based on the unique morphology of their eggs, which are covered in projections arranged in a spiral pattern, and later confirmed and refined with molecular evidence (Britz et al. 1995; Rüber et al. 2006). Another differentiating feature of the spiral egg clade is an angular jaw shape, which is taken to the extreme in the highly derived pike-like morphology of the piscivorous genus *Luciocephalus*. The spiral egg clade is also notable for having the only species, *S. osphromenoides* and *S. selatanensis*, in the family Osphronemidae with female broodcare via mouthbrooding compared to the overwhelmingly male mouthbrooders or bubble nesters in the family (Rüber et al. 2006), although recent evidence has called into question the sex of caring parent in *S.*

osphromenoides (Zworykin et al. 2024). Chromosomes of the spiral egg clade remain largely uninvestigated; besides *S. osphromenoides* and *L. pulcher*, only one other species has been studied cytogenetically (*Ctenops nobilis*, 2N=44: Rishi et al. 1997). Given the low chromosome counts of *S. osphromenoides* and *L. pulcher* and the large 2N decrease relative to the wider family, we aim to characterize the karyotypes of additional members within the spiral egg clade. With this information we will describe the karyotypic diversity and evolutionary history for this remarkable group of fishes, thereby adding an extraordinary example to the chromosome count diversity in fishes specifically and animals in general.

Methods

Fishes were sourced from the aquarium trade (Wet Spot Tropical Fish, Portland, Oregon, USA; Nationwide Aquatics, Tinley Park, Illinois, USA; Aqua Imports, Boulder, Colorado, USA), then held in species-specific tanks (110 liters) on a shared flow-through system (pH 7.0, GH 30 ppm, KH 40 ppm) with a 12/12 hour light/dark cycle with 30 minutes of dim light to simulate dawn and dusk. Specimens were housed for a minimum of one week before sampling to ensure good health for optimal cell proliferation.

Chromosome preparations were made following Kligerman and Bloom (1977) with the indicated modifications. Specimens were incubated in 0.005% colchicine solution for 6-7 hours, then euthanized and dissected to remove gill arches. Sex determination was conducted by gross examination of gonads with pictures taken throughout. Dissected specimens were stored in -80 °C for future molecular analyses. Gill arches were incubated in 0.4% KCl solution for 20-30 minutes, then fixed in two changes of 3:1 ethanol:acetic acid fixative for at least 30 minutes each, followed by an overnight fixation period at 4 °C. To prepare slides, tissue was homogenized into suspension by mincing in 50% acetic acid, then dropped onto a slide warmed

to 30-40 °C and air dried. Slides were examined under phase contrast microscopy for quality control, then aged for at least one day at room temperature before being stained for 10 minutes in 10% Giemsa in pH 6.8 phosphate buffer (Gibco™ Gurr Buffer Tablets) and air-dried.

Chromosomes were examined under a Nikon Eclipse Ti-E microscope driven by Nikon NIS Elements AR software, then photographed with an oil immersion objective at 100x magnification and green color filtering using a Hamatsu ORCA-Flash4.0 camera. Digital images were optimized, then homologous chromosomes were paired by size and morphology and arranged in decreasing order of size using ImageJ v1.52v and Adobe Photoshop 24.3.0. At least 35 complete metaphase spreads were photographed from each specimen with completeness defined as the highest consistently observed chromosome count. Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), or acrocentric (a) according to their arm ratios (Levan et al. 1964). Chromosome arm number (Fundamental Number, FN) was calculated by considering metacentric and submetacentric chromosomes as biarmed and subtelocentric and acrocentric chromosomes as uniarmed.

Results

We describe karyotypes for the first time in six species (Fig 1): *S. selatanensis*, *S. vaillanti*, *S. acrostoma*, *L. aura*, and *P. ocellatus*. We also confirmed the karyotypes of an additional two species (*S. osphromenoides*, *L. pulcher*) which matched those established in the literature (Calton and Denton 1974; Arai 2011). All species in the genus *Sphaerichthys* had different chromosome counts, with 2N ranging from 16–28 (Table 1). The number of chromosome arms (fundamental number, FN) showed less variation, with a range of 30–38. Notably, the sister species *S. osphromenoides* and *S. selatanensis* had a primarily biarmed and primarily uniarmed karyotype respectively, resulting in a nearly identical FN despite a 2N difference of 12. The

other two sister species in the genus (*S. acrostoma* and *S. vaillanti*) had karyotypes that were a mix of biarmed and uniarmed chromosomes and had different values for both 2N and FN. We confirmed that *L. pulcher* had an entirely uniarmed karyotype of 20 acrocentric chromosomes. *Luciocephalus aura* had a primarily biarmed karyotype with six fewer chromosomes and eight more chromosome arms. *Parasphaerichthys ocellatus* had a primarily uniarmed karyotype with higher 2N and FN that were higher than any *Sphaerichthys* or *Luciocephalus* species, but lower than was reported for *C. nobilis* in the literature (Arai 2011).

The two *S. selatanensis* that we sampled had different karyotypes (Supplemental Table 2). One had 28 uniarmed chromosomes (2N=28, karyotype 2st+26a), while the other had 26 uniarmed chromosomes and an unpaired metacentric chromosome (2N=27, karyotype 1m+2st+24a). The unpaired metacentric chromosome was approximately twice the size of the largest acrocentric chromosomes and may have been caused by a fused acrocentric pair. We cannot say whether this is a sex chromosome because we could not confidently determine the sex of either individual.

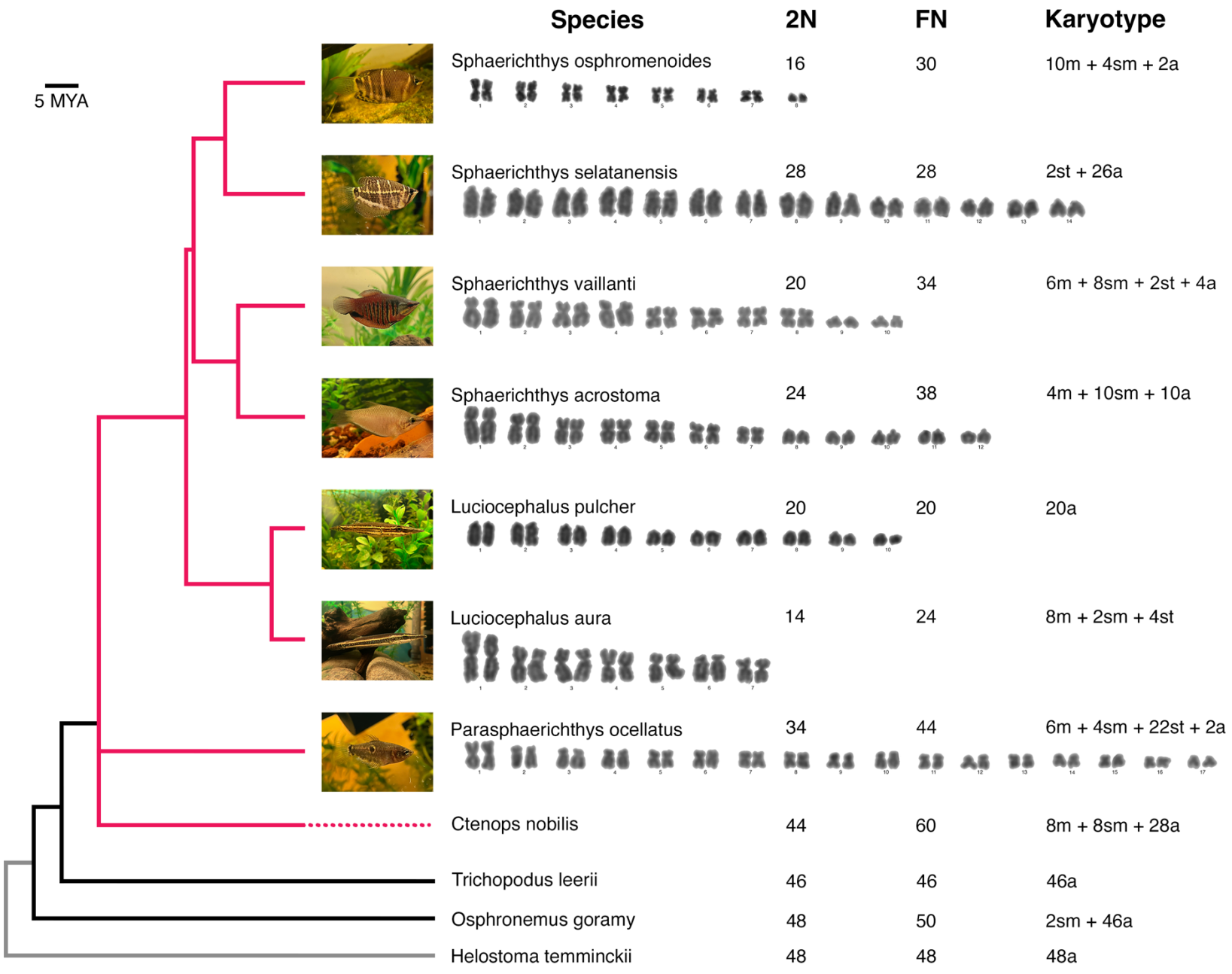
5 MYA
Species
2N
FN
Karyotype
Sphaerichthys osphromenoides 16 30 10m + 4sm + 2a
Sphaerichthys selatanensis 28 28 2st + 26a
Sphaerichthys vaillanti 20 34 6m + 8sm + 2st + 4a
Sphaerichthys acrostoma 24 38 4m + 10sm + 10a
Luciocephalus pulcher 20 20 20a
Luciocephalus aura 14 24 8m + 2sm + 4st
Parasphaerichthys ocellatus 34 44 6m + 4sm + 22st + 2a
Ctenops nobilis 44 60 8m + 8sm + 28a
Trichopodus leerii 46 46 46a
Osphronemus goramy 48 50 2sm + 46a
Helostoma temminckii 48 48 48a

Figure 1. Selected anabantoid karyotypes. Phylogenetic relationships are from Ruber et al. (2006) and are shown to scale for the spiral egg clade (red) but not the selected species in the family Osphronemidae (black) or the outgroup (Helostomatidae, grey). Values for 2N, FN, and Karyotype for four species not generated in this study can be found in Arai (2011) and Grazyna et al. (2008). Karyotype formula describes number of metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a) chromosomes. Note that chromosome sizes vary widely between spreads and are thus not directly comparable except within the same spread. Live specimens were photographed with iPhone 13.

Discussion

We found that the genera *Sphaerichthys*, *Luciocephalus*, and *Parasphaerichthys* have low chromosome counts (2N≤34) with high intra-genus variation in both 2N and FN. These trends are not observed in the karyotypes of the broader family Osphronemidae, which mostly have karyotypes similar to the hypothesized ancestral state for all teleost fishes (2N=52, Nakatani et al. 2007), thereby suggesting that rapid karyotype evolution occurred since the divergence of the spiral egg clade about 25 million years ago (Rüber et al. 2006). Karyotype evolution of this rate and magnitude has not been reported in teleost fishes. The drastic differences between karyotypes within the *Sphaerichthys* and *Luciocephalus* genera suggests that karyotype evolution may have played a role in speciation process by creating post-zygotic isolation (Canitz et al. 2016; Jackson et al. 2016; Mezzasalma et al. 2017; Romanenko et al. 2018), but it is also possible that the observed karyotype diversity happened alongside the speciation process instead of driving it (Krysanov et al. 2023). The observed karyotype pattern could have been created by the influence of genetic drift or other forms of neutral selection, extremely strong meiotic drive, the evolution of a trait that stimulates chromosome evolution, or a combination of these factors.

Genetic drift has been proposed to be the driving force behind the fixation of highly differentiated karyotypes in some clades of freshwater fishes, including the annual killifishes in the genera *Nothobranchius* (2N=16-50) (Krysanov et al. 2016, 2023; Krysanov and Demidova 2018) and *Aphyosemion* (2N=20-40) (Völker et al. 2005, 2007, 2008). These species tend to live in small, biogeographically isolated populations and frequently experience genetic

166 bottlenecks and founder effects due to the ephemeral nature of their habitats (Völker et al. 2006;
167 Krysanov and Demidova 2018; Krysanov et al. 2023), which may have sped up the
168 accumulation of both intra-and inter-chromosomal mutations, with centric fusions being
169 responsible for most decreases in chromosome count (Völker et al. 2005, 2008; Krysanov et al.
170 2016). *Sphaerichthys* and *Luciocephalus* species have very limited geographical ranges and,
171 given that most of them are threatened or endangered according to the IUCN Red List, these
172 populations are likely small; however, it is difficult to precisely estimate the strength of genetic
173 drift on the evolution of the karyotypes in our study group because there is little information
174 about their distribution and population structure.
175
176 An alternative explanation to genetic drift is meiotic drive. Species evolving under the influence
177 of strong meiotic drive will tend to reach karyotypes of predominantly biarmed or uniarmed
178 chromosomes (de Villena and Sapienza 2001; Molina et al. 2014). This has been observed in
179 fishes and mammals, with the note that groups with high rates of mismatched karyotypes
180 tended to have higher rates of chromosome evolution (Blackmon et al. 2019). The decrease in
181 chromosome number in our study group relative to the rest of the family may have been caused
182 by a meiotic drive toward biarmed chromosomes that rapidly fixed fusion mutations.
183 Additionally, the near-complete inversions in biarmed proportion between sister species (*S.*
184 *osphromenoides* and *S. selatanensis*, *L. aura* and *L. pulcher*) are consistent with an inversion in
185 the directionality of meiotic drive after or during the divergence of these species from their
186 common ancestor, such that either fusions or fissions were preferentially fixed in one species
187 but not the other. The karyotypes of *S. vaillanti* and *acrostoma*, which have a mix of biarmed
188 and uniarmed chromosomes, may be partway through a shift to a completely biarmed or
189 uniarmed karyotype. Additionally, changes in the arm number may have been caused by
190 pericentric inversions, which may also be subject to the force of meiotic drive (Molina et al.
191 2014). The large differences in karyotype between recently diverged species indicates that the

192 karyotypes were fixed extremely quickly, suggesting that meiotic drive would have to have been
193 extremely strong if it was driving these changes. There are several counterbalancing forces that
194 we would expect to weaken the strength of meiotic drive, including the relatively stronger force
195 of genetic drift, as well as the general trend of changes in chromosome count tending to be
196 slightly deleterious (King 1995).
197
198 Chromosomal rearrangements can have phenotypic impacts, particularly inversions, which can
199 suppress recombination by capturing multiple locally adapted alleles (Kirkpatrick and Barton
200 2006; Berg et al. 2016; da Silva et al. 2021). Additionally, low chromosome number has been
201 found to have correlations with phenotypic effects related to genome size (Gold 1979), including
202 specialization (defined as being highly phenotypically derived from their close evolutionary
203 relatives), tightening linkage groups, and occupying a narrower ecological niche (Gold 1979;
204 Hardie and Hebert 2004). The observed rearrangements and reductions in the spiral egg clade
205 may have played a role in acquiring highly specialized adaptations such as the ability of
206 *Sphaerichtys and Luciocephalus* to live in peat swamp forests and the associated blackwater
207 habitats, which are oligotrophic, sparsely inhabited, and highly acidic (pH < 4) (Polgar and
208 Jaafar 2018). By contrast, *P. ocellatus* and *C. nobilis* are not adapted to such harsh conditions
209 and are typically found in small muddy streams and pools. Additionally, it is possible that the
210 rapid genomic rearrangements observed in this group may have contributed to the observed
211 phenotypic differences in this subfamily, such as the highly derived morphology in
212 *Luciocephalus*.
213
214 The spiral egg clade presents an excellent opportunity to understand how these exceptionally
215 differentiated karyotypes arose and could give insight into larger patterns of chromosomal
216 evolution. Advanced cytogenetic techniques could help clarify which types of chromosomal
217 rearrangements occurred (ex. Ag-NOR staining, c-banding, FISH, etc.) as has been done in

other species in the family Osphronemidae (Grazyna et al. 2008; Pazza et al. 2009; Chaiyasan et al. 2021; Supiwong et al. 2021), and measuring genome size of our study species would allow testing for non-conservative mechanisms of chromosome evolution. To test the influence of meiotic drive, work could be done to examine kinetochore protein levels during meiosis (Chmátal et al. 2014), as well as the amount of minor satellite DNA repeats on the centromere , which have been associated with the action of meiotic drive in the western house mouse (Iwata-Otsubo et al. 2017; Dudka and Lampson 2022). Other factors could be investigated that are known to stimulate chromosomal rearrangements such as repetitive DNA content (King 1995; Martinez et al. 2017) which was also implicated in the high incidence of chromosomal mutations in *Nothobranchius* (Krysanov et al. 2023). There are other monophyletic clades in the family Osphronemidae that have unusually differentiated chromosomes (Srisamoot et al. 2021), suggesting that the underlying mechanism driving karyotypic change in the spiral egg clade may be a shared ancestral trait and allowing for comparative genomic studies between the spiral egg clade and closely related groups. Finally, it is also worth noting that most Osphronemidae species have not been examined cytogenetically, hence karyotyping more species in the family Osphronemidae could reveal more clades with high karyotype differentiation. Further attention should be pair to this cytogenetically diverse group, as they could help resolve outstanding evolutionary questions of chromosomal rearrangements and diversity.

AUTHOR CONTRIBUTIONS

Mobley: Conceptualization; Investigation; Methodology, Data analysis and curation; Writing – original draft. **Anderson**: Conceptualization; Methodology; Data analysis and curation; Writing – review and editing; Supervision.

ACKNOWLEDGEMENTS

This work was supported by National Science Foundation Grant #2147567. AP Anderson is supported by the NSF Postdoctoral Research Fellowship in Biology Grant #2010841. B Mobley was supported by a REP supplement to NSF grant #2147567.

DATA AVAILABILITY

Raw images of chromosome spreads and finalized karyotype images can be found here: https://github.com/AndersonDrew/Gourami_Chromosome Contact authors for any additional data/information.

CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no competing interests.

References

Amores, A., J. Catchen, I. Nanda, W. Warren, R. Walter, M. Schartl, and J. H. Postlethwait. 2014. A RAD-tag genetic map for the platyfish (Xiphophorus maculatus) reveals mechanisms of karyotype evolution among teleost fish. Genetics 197:625–641. Oxford University Press.

Arai, R. 2011. Fish karyotypes: a check list. Springer Science & Business Media.

Berg, P. R., B. Star, C. Pampoulie, M. Sodeland, J. M. Barth, H. Knutsen, K. S. Jakobsen, and S. Jentoft. 2016. Three chromosomal rearrangements promote genomic divergence between migratory and stationary ecotypes of Atlantic cod. Sci. Rep. 6:23246. Nature Publishing Group UK London.

Blackmon, H., J. Justison, I. Mayrose, and E. E. Goldberg. 2019. Meiotic drive shapes rates of karyotype evolution in mammals. Evolution 73:511–523. Blackwell Publishing Inc Malden, USA.

Britz, R., M. Kokoscha, and R. Riehl. 1995. The anabantoid genera Ctenops, Luciocephalus, Parasphaerichthys, and Sphaerichthys (Teleostei: Perciformes) as a monophyletic group: evidence from egg surface structure and reproductive behaviour. Jpn. J. Ichthyol. 42:71–79.

Calton, M. S., and T. E. Denton. 1974. Chromosomes of the Chocolate Gourami: A Cytogenetic Anomaly. Science 185:618–619.

Canitz, J., F. Kirschbaum, and R. Tiedemann. 2016. Karyotype description of the African weakly electric fish Campylomormyrus compressirostris in the context of chromosome evolution in Osteoglossiformes. J. Physiol.-Paris 110:273–280. Elsevier.

CHAIYASAN, P., B. MINGKWAN, S. JANTARAT, C. SUWANNAPOOM, M. CIOFFI, T. LIEHR, S. TALUMPHAI, A. TANOMTONG, and W. Supiwong. 2021. Classical and molecular

cytogenetics of Belontia hasselti (Perciformes: Osphronemidae): Insights into the ZZ/ZW sex chromosome system. Biodiversitas J. Biol. Divers. 22.

Chmátal, L., S. I. Gabriel, G. P. Mitsainas, J. Martínez-Vargas, J. Ventura, J. B. Searle, R. M. Schultz, and M. A. Lampson. 2014. Centromere strength provides the cell biological basis for meiotic drive and karyotype evolution in mice. Curr. Biol. 24:2295–2300. Elsevier.

Christidis, L. 1990. Chordata 3. B. Aves. Schweizerbart'sche Verlagsbuchhandlung.

da Silva, S. A. S., P. A. de Lima-Filho, C. C. da Motta-Neto, G. W. W. F. da Costa, M. de B. Cioffi, L. A. C. Bertollo, and W. F. Molina. 2021. High chromosomal evolutionary dynamics in sleeper gobies (Eleotridae) and notes on disruptive biological factors in Gobiiformes karyotypes (Osteichthyes, Teleostei). Mar. Life Sci. Technol. 1–10. Springer.

de Villena, F. P.-M., and C. Sapienza. 2001. Female meiosis drives karyotypic evolution in mammals. Genetics 159:1179–1189. Oxford University Press.

Deakin, J. E., and T. Ezaz. 2019. Understanding the evolution of reptile chromosomes through applications of combined cytogenetics and genomics approaches. Cytogenet. Genome Res. 157:7–20. S. Karger AG.

Dudka, D., and M. A. Lampson. 2022. Centromere drive: model systems and experimental progress. Chromosome Res. 30:187–203. Springer.

Galetti, P. M., C. T. Aguilar, and W. F. Molina. 2000. An overview of marine fish cytogenetics. Mar. Genet. 55–62. Springer.

Gallardo, M. H., C. A. González, and I. Cebrián. 2006. Molecular cytogenetics and allotetraploidy in the red vizcacha rat, Tympanoctomys barrerae (Rodentia, Octodontidae). Genomics 88:214–221. Elsevier.

Gold, J. R. 1979. Fish cytogenetics. Fish Physiol. 7–405. Academic Press.

Graphodatsky, A. S., P. L. Perelman, and S. J. O'Brien. 2020. Atlas of mammalian chromosomes. No Title.

Grazyna, F.-S., D. Fopp-Bayat, M. Jankun, S. Krejszeff, and A. Mamcarz. 2008. Note on the karyotype and NOR location of Siamese fighting fish Betta splendens (Perciformes, Osphronemidae). Caryologia 61:349–353. Taylor & Francis.

Hardie, D. C., and P. D. Hebert. 2004. Genome-size evolution in fishes. Can. J. Fish. Aquat. Sci. 61:1636–1646. NRC Research Press Ottawa, Canada.

Iwata-Otsubo, A., J. M. Dawicki-McKenna, T. Akera, S. J. Falk, L. Chmátal, K. Yang, B. A. Sullivan, R. M. Schultz, M. A. Lampson, and B. E. Black. 2017. Expanded satellite repeats amplify a discrete CENP-A nucleosome assembly site on chromosomes that drive in female meiosis. Curr. Biol. 27:2365-2373. e8. Elsevier.

Jackson, B., R. K. Butlin, A. Navarro, and R. Faria. 2016. Chromosomal rearrangements and speciation. Encycl. Evol. Biol. Elsevier Sci. Technol. Amst. Neth. 149–158.

King, M. 1995. Species evolution: the role of chromosome change. Cambridge University Press.

Kirkpatrick, M., and N. Barton. 2006. Chromosome inversions, local adaptation and speciation. Genetics 173:419–434. Oxford University Press.

Kligerman, A. D., and S. E. Bloom. 1977. Rapid chromosome preparations from solid tissues of fishes. J. Fish. Board Can. 34:266–269. NRC Research Press Ottawa, Canada.

Kretschmer, R., M. A. Ferguson-Smith, and E. H. C. De Oliveira. 2018. Karyotype evolution in birds: From conventional staining to chromosome painting. Genes 9:181. MDPI.

Krysanov, E., and T. Demidova. 2018. Extensive karyotype variability of African fish genus Nothobranchius (Cyprinodontiformes). Comp. Cytogenet. 12:387. Pensoft Publishers.

Krysanov, E., T. Demidova, and B. Nagy. 2016. Divergent karyotypes of the annual killifish genus Nothobranchius (Cyprinodontiformes, Nothobranchiidae). Comp. Cytogenet. 10:439. Pensoft Publishers.

329 Krysanov, E. Y., B. Nagy, B. R. Watters, A. Sember, and S. A. Simanovsky. 2023. Karyotype
330 differentiation in the Nothobranchiusugandensis species group (Teleostei,
331 Cyprinodontiformes), seasonal fishes from the east African inland plateau, in the context
332 of phylogeny and biogeography. Comp. Cytogenet. 17:13. Pensoft Publishers.
333 Lebeda, I., P. Ráb, Z. Majtánová, and M. Flajšhans. 2020. Artificial whole genome duplication in
334 paleopolyploid sturgeons yields highest documented chromosome number in
335 vertebrates. Sci. Rep. 10:19705. Nature Publishing Group UK London.
336 Lehmann, R., A. Kovařík, K. Ocalewicz, L. Kirtiklis, A. Zuccolo, J. N. Tegner, J. Wanzenböck, L.
337 Bernatchez, D. K. Lamatsch, and R. Symonová. 2021. DNA transposon expansion is
338 associated with genome size increase in mudminnows. Genome Biol. Evol. 13:evab228.
339 Oxford University Press.
340 Levan, A., K. Fredga, and A. A. Sandberg. 1964. Nomenclature for centromeric position on
341 chromosomes.
342 Mank, J. E., and J. C. Avise. 2006. Phylogenetic conservation of chromosome numbers in
343 Actinopterygiian fishes. Genetica 127:321–327. Springer.
344 Martinez, P. A., U. P. Jacobina, R. V. Fernandes, C. Brito, C. Penone, T. F. Amado, C. R.
345 Fonseca, and C. J. Bidau. 2017. A comparative study on karyotypic diversification rate in
346 mammals. Heredity 118:366–373. Nature Publishing Group.
347 Martinez, P. A., J. P. Zurano, T. F. Amado, C. Penone, R. Betancur-R, C. J. Bidau, and U. P.
348 Jacobina. 2015. Chromosomal diversity in tropical reef fishes is related to body size and
349 depth range. Mol. Phylogenet. Evol. 93:1–4. Elsevier.
350 Mezzasalma, M., F. Andreone, G. Aprea, F. Glaw, G. Odierna, and F. M. Guarino. 2017. When
351 can chromosomes drive speciation? The peculiar case of the Malagasy tomato frogs
352 (genus Dyscophus). Zool. Anz. 268:41–46. Elsevier.
353 Molina, W. F., P. A. Martinez, L. A. C. Bertollo, and C. J. Bidau. 2014. Evidence for meiotic drive
354 as an explanation for karyotype changes in fishes. Mar. Genomics 15:29–34. Elsevier.

Nakatani, Y., H. Takeda, Y. Kohara, and S. Morishita. 2007. Reconstruction of the vertebrate ancestral genome reveals dynamic genome reorganization in early vertebrates. Genome Res. 17:1254–1265. Cold Spring Harbor Lab.

Olmo, E. 2005. Rate of chromosome changes and speciation in reptiles. Genetica 125:185–203. Springer.

Pazza, R., K. F. Kavalco, P. R. Penteado, S. A. F. Kavalco, and L. F. de Almeida-Toledo. 2009. Gene mapping of 18S and 5S rDNA genes in the karyotype of the three-spot gourami Trichogaster trichopterus (Perciformes, Osphronemidae). Zebrafish 6:219–222. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA.

Polgar, G., and Z. Jaafar. 2018. Endangered Forested Wetlands of Sundaland. Springer.

Rishi, S., P. K. Gill, and K. K. Rishi. 1997. Karyotypic studies in eight species of fishes belonging to family Belontidae (Pisces, Perciformes). Chromosome Sci. 1:55–59. The Society of Chromosome Research.

Romanenko, S. A., N. A. Serdyukova, P. L. Perelman, V. A. Trifonov, F. N. Golenishchev, N. S. Bulatova, R. Stanyon, and A. S. Graphodatsky. 2018. Multiple intrasyntenic rearrangements and rapid speciation in voles. Sci. Rep. 8:14980. Nature Publishing Group UK London.

Rüber, L., R. Britz, and R. Zardoya. 2006. Molecular phylogenetics and evolutionary diversification of labyrinth fishes (Perciformes: Anabantoidei). Syst. Biol. 55:374–397.

Shimabukuro-Dias, C. K., C. Oliveira, and F. Foresti. 2004. Cytogenetic analysis of five species of the subfamily Corydoradinae (Teleostei: Siluriformes: Callichthyidae). Genet. Mol. Biol. 27:549–554. SciELO Brasil.

Srisamoot, T., W. Supiwong, N. Sriwattanarothai, B. Panijpan, N. Srisamoot, and A. Tanomtong. 2021. Karyotype of four mouth-brooding Betta fishes in Thailand. Food Agric. Sci. Technol. FAST 7:22–31.

Stanyon, R., and A. Graphodatsky. 2012. Evolutionary dynamics of mammalian karyotypes. Cytogenet Genome Res 137:79–286.

Supiwong, W., P. Wongchantra, W. Thongnetr, B. Mingkwan, P. Chaiyasan, S. Pinmongkhonkul, K. Pinthong, and A. Tanomtong. 2021. Comparative cytogenetic analysis of fishes in the genus Trichopodus (Osphronemidae) in Thailand. Biodiversitas J. Biol. Divers. 22.

Völker, M., P. Ráb, and H. Kullmann. 2005. Karyotype differentiation in Chromaphyosemion killifishes (Cyprinodontiformes, Nothobranchiidae). I: Chromosome banding patterns of C. alpha, C. kouamense and C. lugens. Genetica 125:33–41. Springer.

Völker, M., P. Rab, and H. Kullmann. 2008. Karyotype differentiation in Chromaphyosemion killifishes (Cyprinodontiformes, Nothobranchiidae): patterns, mechanisms, and evolutionary implications. Biol. J. Linn. Soc. 94:143–153. Oxford University Press.

Völker, M., R. Sonnenberg, P. Ráb, and H. Kullmann. 2006. Karyotype differentiation in Chromaphyosemion killifishes (Cyprinodontiformes, Nothobranchiidae). II: Cytogenetic and mitochondrial DNA analyses demonstrate karyotype differentiation and its evolutionary direction in C. riggenbachi. Cytogenet. Genome Res. 115:70–83.

Völker, M., R. Sonnenberg, P. Ráb, and H. Kullmann. 2007. Karyotype differentiation in Chromaphyosemion killifishes (Cyprinodontiformes, Nothobranchiidae). III: Extensive karyotypic variability associated with low mitochondrial haplotype differentiation in C. bivittatum. Cytogenet. Genome Res. 116:116–126. S. Karger AG.

Wurster, D. H., and K. Benirschke. 1970. Indian Muntjac, Muntiacus muntjak: A Deer with a Low Diploid Chromosome Number. Science 168:1364–1366. American Association for the Advancement of Science.

Zworykin, D., J. Müller, H. Grundmeijer, and E. Pavlov. 2024. Paternal mouthbrooding in the chocolate gourami Sphaerichthys osphromenoides (Osphronemidae). Environ. Biol. Fishes 107:381–389.