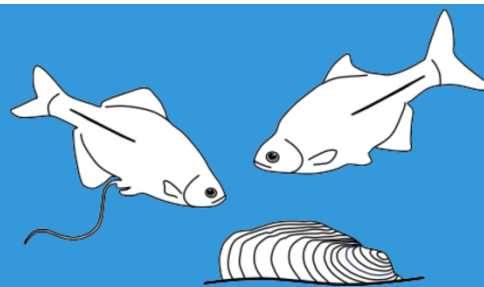


SYMPOSIUM ON FISH
MATING SYSTEMS

21-23 January 2026,
Brno, Czech Republic



Symposium on Fish Mating Systems

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Unedited abstracts of contributed talks

- Listed alphabetically based on the presenting author
- Posters marked after presenting author name
- Plenary lectures: Suzanne Alonzo, John Fitzpatrick, Chiara Benvenuto

Partners:



Andjel

Rapid Evolution of Sex Chromosomes and Its Association with Hybrid Mating Patterns in Spined Loaches (Cobitis)

Lucija Andjel, Vladimir Trifonov, Stephen A. Schlebusch, Zuzana Halenková, Marharyta Klianitskaya, Dmitrij Dedukh, Tomáš Tichopád, Anatolie Marta, Radka Reifová, Jan Pačes, Karel Janko

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Reproductive strategies in fishes display remarkable diversity, spanning clonal lineage formation, recurrent polyploidy, rapid speciation, frequent interspecific hybridisation, and adaptation across diverse ecological niches. Loaches, particularly spined loaches (*Cobitis*) exemplify the extraordinary breadth of fish mating systems, combining frequent interspecific hybridisation with the formation of asexual lineages, emergence of polyploid forms, rapid speciation, and adaptation to contrasting environments. Crosses show a striking sex bias: hybrid males are invariably sterile, whereas hybrid females reproduce asexually by gynogenesis, producing clonal eggs via premeiotic endoreplication and occurring as diploids, triploids, and tetraploids. Here, we aimed (I) to identify and comparatively analyse genetic sex-determination (GSD) systems across *Cobitis* species and (II) to link chromosomal pairing behaviour in hybrid males to the mechanistic basis of meiotic failure.

We produced high-quality, chromosome-level genome assemblies for three parental species (*C. elongatoides*, *C. taenia*, *C. tanaitica*) using Oxford Nanopore sequencing polished with Illumina reads and superscaffolded with Hi-C to resolve chromosomal architecture. Pooled whole-genome resequencing (Pool-Seq) across sexes per species was used to detect sex-linked regions via coverage and sex-biased SNP patterns. Candidate X- and Y-linked loci were converted into PCR/qPCR assays and validated against independently sexed individuals and natural/laboratory hybrids. Chromosomal pairing in *C. elongatoides*, *C. tanaitica*, *C. taenia*, and their hybrids was visualised using colchicine-treated kidney and testis cells prepared for mitotic and meiotic metaphase analysis following established cytogenetic protocols.

Our analyses uncovered distinct, species-specific GSD signals. In *C. elongatoides*, a robust XY signature spans chromosome 1 (Ch01A), whereas in *C. taenia* a younger, minimally differentiated Y-linked segment localizes to chromosome 5 (Ch05). In *C. tanaitica*, a candidate region on chromosome 6 (Ch06) shows moderate sex differentiation without clear coverage shifts. Three primer sets reliably distinguish Y- from X-linked markers in *C. elongatoides* and detect Y-linked signal in *C. taenia*. Testing of diploid and triploid hybrids largely matched expectations (Y present in males, absent in females) but revealed exceptions—some wild F1s lacked parental signals, and some females carried Y-linked markers—consistent with structural variation and/or sex-chromosome turnover across lineages. Our results support this hypothesis, revealing

that orthologous chromosome pairs (Ch01A, Ch01B, Ch05, and Ch20) show markedly different rates of bivalent formation in hybrid males. Pairing was particularly reduced for Y-linked Ch01A and other chromosomes, with far fewer bivalents observed than expected, and notable interindividual variation detected for Ch01B.

Together, these data indicate rapid turnover of sex chromosomes in closely related *Cobitis* species and tie GSD evolution to the characteristic hybrid outcomes of male sterility and female clonality. We discuss how divergence of sex-linked regions, together with altered meiotic pairing in hybrids, can be associated with the emergence of asexual reproduction via premeiotic endoreplication in *Cobitis*.

Blabolil (poster)

Healthy brown trout populations, example from the Bohemian Forest

Petr Blabolil, Michal Tušer, Milan Muška, Vladislav Draštík, Daniel Bartoň, Tomáš Jůza

Biology Centre CAS, Institute of Hydrobiology

The watercourses of the Bohemian Forest region represent a typical trout zone. Brown trout inhabited these waters for centuries until acidification led to their disappearance from many upper reaches. Currently, their return to the headwaters is being observed. However, the status of present populations remains uncertain. Between 2023 and 2025, a field survey was carried out at more than 170 locations in the Czech and Bavarian parts of the Bohemian Forest. During electrofishing, over 8,000 brown trout – the most frequently found species – were caught. Sampling took place in September and October, relatively shortly before spawning, which allowed the sex of larger individuals (>100 mm standard length) to be determined based on morphological characteristics. The ratio of males to females was nearly equal, with a slight predominance of females. The poster summarises the influence of trout size, altitude, and stream size on sex ratio. The research was supported by the Interreg Bayern – Česko 2021–2027 project [No. BYCZ01–020].

Blazek

Mate recognition mechanisms in African annual killifish

Milová T., Polačik M., Vrtílek M., Blažek R.

Institute of Vertebrate Biology, Czech Academy of Sciences

Killifish belonging to the genus *Nothobranchius* are short-lived species that follow an annual life cycle, residing in temporary pools in Eastern Africa. Despite the presence of multiple species within a single pool, there is no evidence of interspecific breeding occurring in nature, suggesting that effective reproductive isolation mechanisms are in place. Although these killifish display notable sexual dimorphism, the often turbid waters of their habitats raise questions about the factors influencing mate selection and whether cues beyond visual signals play a role. Our study aimed to investigate the role of olfactory communication and individual experience in partner recognition, as well as to examine the pre- and postzygotic reproductive barriers that prevent hybridization, particularly in relation to the age of the fish in sympatric populations of *Nothobranchius furzeri* and *N. orthonotus*. We found that olfactory communication is a crucial method of mate selection, while individual experience with other species had little impact. Females showed a reduced inclination to mate with males of different species, leading to smaller brood sizes, especially in the youngest fish. As the fish aged, the performance of heterospecific pairings declined across all measures. The most significant postzygotic barrier was noted in pairings of *N. orthonotus* females with *N. furzeri* males, where embryo survival rates were the lowest, with no embryos hatching. Conversely, embryos from the reverse pairing had better survival rates, resulting in a significantly female-biased F1 hybrid population.

Bohlen

A new case of hybridogenesis in fish

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Hybridogenesis or semi-clonal reproduction combines clonal with sexual reproduction and is restricted to animals of hybrid origin. While the genome of one of the parental species is inherited in a clonal form to all gametes, the complete genome of the second parental species is not inherited at all. Breeding with a sexual male of the second species brings a second genome to the offspring and restores the F1 status of the hybridogenetic lineage. Hybridogenesis is the most rare among the non-sexual modes of reproduction, and in the pure form found in only three fish genera. In the present study we introduce a newly detected case of hybridogenetic fish, that has a great potential as future model organism for studies on semi-clonal reproduction.

Boryshpolets

Basics of sperm motility and environmental factors affecting spermatozoa behaviour during reproduction in different species

Boryshpolets Serhii; Kholodnyy Vitaliy; Sotnikov Anatolii; Bondarenko Olga; Mueni Laurine Mule; Rodina Marek; Dzyuba Viktoriya; Dzyuba Borys

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Spermatozoa represent the most diverse eukaryotic cell type known, playing a crucial role in delivering male genetic material to the oocyte. Fertilization success and species survival are tightly linked to the physiological state and functional competence of individual sperm cells. Typically, males produce numerous small spermatozoa that compete to fertilize large, nutrient-rich ova, which provide a protected environment for embryonic development. Evolution has shaped sperm morphology, size, and structure in response to species-specific reproductive strategies and fertilization environments. Understanding sperm morphology and motility is therefore essential not only for elucidating evolutionary adaptations but also for improving assisted reproductive technologies across taxa.

The fertilization environment—closely linked to fertilization mode—plays a central role in shaping both sperm structure and function and is broadly categorized as internal or external. Internal fertilization is considered an ancestral trait among gnathostomes, from which external fertilization later evolved in modern bony fishes and amphibians. In external fertilization, males release sperm into the environment simultaneously (or shortly after) females release eggs, with fertilization occurring outside the female body. Both gametes are activated immediately upon contact with the external medium (typically water), and the process is highly time-sensitive, often lasting only a few minutes.

In contrast, internal fertilization—observed in mammals, chondrichthyans, reptiles, and some amphibians and teleosts—occurs within the female reproductive tract following direct sperm transfer. This can reduce the sperm limitation effect and buffer the sperm and zygote from environmental stress. Moreover, in this fertilization mode, sperm and egg physiological functioning must be temporally synchronized for successful fertilization. This is often reflected in the prolonged duration of sperm motility, specific sperm storage mechanisms within the female body, and capacitation and navigation processes adapted to the physical complexity of the female reproductive tract. These two contrasting environments—one unpredictable and rapid, the other stable but selective—exert distinct selective pressures that have been key drivers in the evolution leading to diversity of sperm morphology and motility characteristics across species.

Regardless of fertilization mode, the microenvironment surrounding the sperm is critical in determining sperm behaviour and fertilization outcomes. Microenvironmental factors—such as pH, ion concentration, temperature, fluid dynamics, and the chemical

composition of reproductive fluids (e.g., seminal or oviductal fluid)—can significantly influence sperm physiology, motility, and, as a result, reproductive success.

This communication summarises recent findings on sperm physiology and behaviour in response to various environmental signals. Using classical models of freshwater fish species, as well as examples from mammals and early-diverging Elasmobranchii species, we aim to better understand the interaction mechanisms between sperm and oocyte across different reproductive strategies.

Bose

Strong reproductive competition in a social fish is weakened by anthropogenic pollutants

Aneesh P. H. Bose, Paul Nührenberg, Alex Jordan, Kristina M. Sefc, Tomas Brodin, Erin S. McCallum

Swedish University of Agricultural Sciences

Neolamprologus multifasciatus, a group-living shell-dwelling cichlid endemic to Lake Tanganyika in East Africa, is an emerging model organism for social evolution. Much of the ecology and behaviour of this fish shows similarities with many other social animals. For example, i) it lives in restricted-entry groups on fixed territories centered around resource clusters, ii) its groups contain both dominant and subordinate individuals with a strict dominance hierarchy, and iii) reproductive partitioning within its group is dictated by the most dominant and competitive individuals. By analyzing how this fish's social traits and mating patterns are perturbed by exposure to pharmaceutical pollutants, this fish is also helping us uncover the ultimate consequences of behaviour-altering chemicals on social animals at large. In this talk, I will present what we know about the mating system of *N. multifasciatus* under natural conditions in the wild, as well as under controlled pharmaceutical exposures – revealing how these pollutants can reduce the opportunity for selection by weakening reproductive skew.

Cattelan

The influence of demographic history on reproductive patterns in Nothobranchius species

Cattelan, Silvia; Baskaran Jhanya; Valenzano, Dario Riccardo

Fritz Lipmann Institute (FLI) - Leibniz Institute on Aging

African annual killifishes evolved in fragmented, temporary habitats shaped by alternating rainfall and drought, resulting in frequent population bottlenecks. The limited population sizes caused by repeated bottlenecks have favoured the accumulation of genome-wide deleterious genetic variants, which in turn contributed to the evolution of short lifespans at both species and population levels. Populations from arid regions tend to have shorter lifespans than those from semi-arid regions, likely due to higher genome-wide mutation load. However, whether demographic history affected reproductive patterns across lifespan remains unclear. To address this, we investigated age-dependent reproductive output across multiple Nothobranchius species using a combination of functional, physiological, and -omics approaches throughout the lifespan. Consistent with life-history theory, short-lived species generally exhibit higher reproductive output early in life compared to long-lived species. Long-lived species, in contrast, reach the reproductive peak later but experience a steeper subsequent decline. However, irrespective of lifespan, all species reach their minimum reproductive output at the same age. Finally, by analysing age-dependent de novo mutations across species, we aim to determine whether germline mutation rates reflect demographic history and contribute to the evolution of lifespan and reproductive strategies. This work provides new insights into how life-history traits and genome evolution interact across contrasting evolutionary backgrounds.

Consuegra

Sex in the mangroves: what is the role of epigenetics on the evolution of fish sex-determination and mating systems?

Sofia Consuegra, Waldir Miron, Amy Ellison, Wei Huang, Ethan Friis, Carlos Garcia de Leaniz

Swansea University (UK)/ IIM-CSIC (Spain)

No abstract available.

Daupagne

Rethinking inbreeding avoidance mechanisms in Poeciliid fishes

Daupagne Léa, Living Amanda & John Fitzpatrick

Stockholm University

Inbreeding depression is expected to favor the evolution of mechanisms that prevent matings between relatives, yet empirical evidence for such mechanisms, particularly beyond the precopulatory stage, remains limited. Although theoretical models increasingly emphasize the potential importance of post-copulatory processes, most empirical studies have focused on behaviors expressed before or during mating. Here, we integrated pre- and post-copulatory assays across five poeciliid fishes to evaluate how selection on inbreeding avoidance operates across multiple stages of mate choice. Using dichotomous mate-choice tests, we found no evidence of precopulatory kin discrimination in either males or females, regardless of mating status. We then tested whether ovarian fluid, a known enhancer of sperm performance in most fishes, biases sperm velocity toward unrelated males. Across all species, ovarian fluid significantly enhanced sperm swimming speed, yet its effect was independent of genetic relatedness, indicating an absence of cryptic female choice via ovarian fluid. Overall, our results indicate that neither behavioral nor molecular mechanisms reliably promote inbreeding avoidance in these species, suggesting that inbreeding avoidance may be weaker or absent across multiple stages of mate choice and highlighting the need to reassess expectations about its prevalence in natural systems.

Franěk

From development to decline: testicular remodeling and germline aging in the turquoise killifish

Roman Franěk, Tomáš Tichopád, Diógenes Henrique de Siqueira-Silva, Essaikiammal Sodalai Muthu Konar, Aarón Torres-Martínez, Hana Sehadová, Radek Šindelka

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The male germline undergoes profound developmental and age-related transformations that ultimately determine reproductive capacity and longevity of fertility. However, vertebrate models allowing these processes to be studied across the full lifespan within a short timeframe remain limited. The turquoise killifish (*Nothobranchius furzeri*), an annual teleost with an exceptionally rapid life cycle, provides a unique opportunity to characterise testicular development and aging in a compressed temporal window.

Here, we present a comprehensive histological atlas of killifish testicular maturation, delineating germ cell compartments and their somatic microenvironments from hatching to late adulthood. Through cell proliferation assays and bulk RNA sequencing of young, mid-aged, and old testes, we demonstrate a pronounced mid-life transition marked by reduced spermatogonial proliferation, increased fibrosis, and transcriptional activation of apoptotic, immune, and extracellular matrix remodeling pathways.

Our findings establish the turquoise killifish as a tractable vertebrate model for dissecting the cellular and molecular mechanisms of testis development and germline aging. The study provides a framework for understanding how intrinsic aging processes shape reproductive potential and may influence the evolution of reproductive timing and life-history strategies in short-lived fish species.

Reproductive strategies shape sexual trait evolution and body morphology in livebearing fishes

Presenting author: K. Natividad García-Cabello – Università degli studi di Padova Co-Authors: J. Jaime Zúñiga-Vega- Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México Jason Pienaar- Department of Biological Sciences, Florida International University Nabila Saleh-Subaie- Universidad Nacional Autónoma de México Jesualdo A. Fuentes-González- Department of Biological Sciences, University of Alabama, Tuscaloosa Maren G. Callaway-Department of Biology, Brigham Young University, Provo,Utah Jerald B. Johnson-Department of Biology, Brigham Young University, Provo,Utah

Università degli studi di Padova

Reproductive strategies can reshape evolutionary trajectories, and poeciliid fish offer a striking example of how shifts in maternal investment affect both natural and sexual selection. Here we explore how two key reproductive traits, superfetation and placentotrophy, interact to shape patterns of maternal resource allocation, and how these reproductive strategies are associated with variation in body morphology and male genital diversification. Using phylogenetic comparative analyses across a broad set of species, we find a consistent pattern: increases in superfetation tend to arise first, potentially facilitating the subsequent evolution of more complex placentation. These reproductive strategies are also associated with a recognizable morphological footprint. Superfetation is repeatedly associated with the evolution of slender, hydrodynamic female bodies, likely easing the locomotor costs of carrying multiple broods. Male genital evolution shows a different dynamic: diversification rates are highest in lecithotrophic, non-superfetating lineages, while superfetating lineages evolve longer and more serrated gonopodia, consistent with intensified sexual conflict. Overall, these findings highlight strong associations between reproductive load, embryo-production strategies, and the diversity of fish mating systems. These findings highlight how reproductive load and embryo-production strategies shape the diversity of fish mating systems.

Glavaschi

Sperm – female reproductive fluid interactions in a fish with a parasitic reproductive strategy

Institute for Vertebrate Biology, CAS

No abstract available.

Graziano

From microbiome to sperm motility traits: An inside out perspective

Marco Graziano, Gian Luigi Garbini, Alessandro Devigili, Livia Pinzoni, Andrea Quagliariello, Emanuele Bosi, Clelia Gasparini, Marco Fondi, Maria Elena Martino

University of Padova

Growing interest in the relationship between microbiome composition and host biology has revealed the many ways host-associated microbes influence physiology, ecology, and evolution. However, microbial communities associated with reproductive organs - and their roles in reproduction - remain poorly understood. Here, we characterized the skin- and ejaculate-associated microbiomes in an internally fertilizing fish and tested whether microbial diversity and specific bacterial taxa correlate with sperm motility traits key for reproductive success. We used the guppy (*Poecilia reticulata*), a well-established model in ecology and evolutionary biology with well-characterised reproductive physiology. In guppies, sperm velocity is a validated predictor of male reproductive performance, making them a powerful system for exploring microbiome–fertility interactions. Our analyses reveal a correlation between skin microbiome diversity and sperm performance. Notably, increased skin microbiome total richness is associated with reduced sperm velocity, whereas no significant associations were detected for ejaculate-associated microbiomes. We also identified bacterial taxa across both tissues that were positively or negatively linked with sperm performance. These findings suggest that, while the ejaculate-associated microbiome may directly influence sperm traits, the skin microbiome could serve as a proxy for reproductive potential by reflecting systemic physiological and immunological states associated with fertility.

Héjja-Brichard

Comparative analysis reveals assortative mate preferences in darters independent of sympatry and sex

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University of Veterinary Medicine Vienna

A preference for mating with conspecifics over heterospecifics is fundamental to the maintenance of species diversity in sexually reproducing organisms. This type of positive assortative preference results in sexual isolation, a reduction in gene flow between species due to differences in mate choice. The proximate and ultimate causes of sexual isolation therefore constitute active areas of research in evolutionary biology. Sexual isolation is often stronger between closely related sympatric species as compared to allopatric species, because of processes such as reinforcement. In addition, traditional theories of sexual selection suggest that because reproduction is more costly to females, they should be the choosier sex and play a more central role in sexual isolation. We conducted a comparative analysis of assortative mate preferences in males and females of sympatric and allopatric species pairs of darters (fish genus *Etheostoma*). We performed a meta-analysis of 17 studies, encompassing 21 species, in which assortative preference was measured when fish were (in most cases) allowed only visual information. As expected, we found stronger preferences for conspecifics over heterospecifics across studies and species. However, we did not find an effect of sympatry or sex on the strength of preference for conspecifics, but rather remarkable variation across species. Several testable hypotheses can be explored to explain the variation we observed in the strength of assortative preference.

Höffle (poster)

Comparing potential and realized fecundity of North East Arctic cod (*Gadus morhua*) and their relationships to recruitment

Hannes Höffle, Frode B. Vikebø, Olav S. Kjesbu

Havforskningsinstituttet

In recent years, North East Arctic (NEA) cod (*Gadus morhua*), has suffered low recruitment success at 3 years of age, reinvigorating interest in the drivers of recruitment. An early influence on recruitment is stock reproductive potential (SRP) of adult females estimated either from potential fecundity of the stock or from realized fecundity in egg surveys. Further modification of recruitment success happens during transport of spawning products to nursery areas and the subsequent juvenile phase, depending on prevailing environmental conditions. Here we employ a 27-year (1985-2012) long time series, augmented by environmental data going back to 1971, to test for parallelism in the SRP of NEA cod derived from total potential and total realized fecundity estimates. Furthermore, we aim to relate recruitment to these estimates as well as demographic and environmental variables using glmmTMB. Results show a good match between potential and realized fecundities for most years, with greater variability on the offshore side of the Lofoten archipelago than between the islands and the Norwegian mainland and with significant differences on the fine scale, indicating rapid dispersal. The relationships of recruitment to environmental conditions and stock structure three years earlier, took the shape of a third-order polynomial, corroborating the assumption of a dome-shaped relationship between temperature and recruitment and suggesting a negative effect on recruitment at very large stock size. The only variable showing a better fit as a linear term was the number of observed pelagic eggs. The final model excludes fecundity in favor of including temperature on the feeding grounds during gonad maturation as well as bottom temperature on the spawning grounds. Potential drivers for deviations between different methods of estimating SRP as well as the pros and cons of the reproductive strategy of NEA cod compared to other species are discussed.

Keywords: North East Arctic cod, realized fecundity, potential fecundity, egg production models, reproductive strategies, recruitment

Janko

Evolution of Non-Mendelian Reproductive Modes through Interspecific Hybridization and Their Eco-Evolutionary Consequences in Fishes

Karel Janko

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Interspecific hybridization is a widespread evolutionary mechanism, particularly common among fishes. Beyond its traditionally emphasized roles in generating novel phenotypes or adaptive potential, hybridization also frequently disrupts canonical gametogenesis and gives rise to unexpected reproductive outcomes. Our research demonstrates that the transition from sexual to asexual reproduction is not a rare anomaly, but a recurring response to genomic incompatibilities between diverging species.

Here, I present a comparative framework showing that interspecific crosses among multiple pairs of sexually reproducing species tend to converge on a common outcome: hybrid females frequently abandon meiosis and reproduce clonally via premeiotic endoreduplication (PMER) — a cytological modification that duplicates chromosomes prior to meiosis, ensuring pairing between sister chromatids. These females thereby clonally transmit only their own genetic material but require a developmental stimulus from sperm of co-occurring sexual males, forming the so-called sperm-parasitic lineages.

Strikingly, PMER is female-specific, whereas hybrid males remain sterile due to unpaired chromosomes, explaining the pervasive sex bias in hybrid asexuality. Moreover, these reproductive aberrations are sensitive to genomic dosage. Diploids typically reproduce clonally via PMER, whereas inseminated eggs give rise to triploids that undergo premeiotic genome elimination of one parental haploid set followed by normal meiosis among the remaining diploid genome.

Such dosage-dependent switches profoundly influence evolutionary trajectories and biodiversity. For instance, asexual hybrids may preserve the genome of a parental species that has since gone extinct, maintaining it clonally in diploid lineages. Yet, when becoming triploid, they can mediate gene flow from extinct ancestors into extant sexual hosts, transferring ancient alleles through asexual intermediates — a process we term “zombie gene flow.” This mechanism highlights how non-Mendelian inheritance can preserve and reintroduce genetic diversity across deep timescales.

Finally, sperm-dependent asexuals (pseudogams) exert strong ecological and behavioral feedbacks on their sexual hosts. Acting as sperm parasites, they divert male reproductive effort, skew operational sex ratios, and reduce effective population sizes of sexual species. This imbalance alters mate-choice dynamics—often promoting stronger male selectivity—and reshapes the spatial coexistence and competition between sexual and asexual populations. Such interactions can generate metapopulation turnover, promote reproductive isolation, and ultimately reshape the spatial structure of biodiversity.

By integrating cytogenetic, ecological, and evolutionary perspectives, I outline a unifying framework showing how hybridization between sexual species generates qualitatively novel evolutionary phenomena, in which reproductive aberrations reshape the formation of species, their spatial distributions, and the adaptive diversification of mating systems.

Kvarnemo

Molecular, behavioural and morphological comparisons of sperm traits between alternative reproductive tactics of the sand goby

Charlotta Kvarnemo, Leon Green, Ola Svensson, Kai Lindström, Sofie Schöld, Martina Griful-Dones, Jonathan N. Havenhand, Erica H. Leder

University of Gothenburg

In species with alternative reproductive tactics, there is much empirical support that parasitically spawning males have larger testes and greater sperm numbers as an evolved response to a higher degree of sperm competition, but support for higher sperm performance (motility, longevity and speed) by such males is inconsistent. We used the sand goby (*Pomatoschistus minutus*) to test whether sperm performance differed between breeding-coloured males (small testes, large mucus-filled sperm-duct glands; build nests lined with sperm-containing mucus, provide care) and parasitic sneaker-morph males (no breeding colouration, large testes, rudimentary sperm-duct glands; no nest, no care). We compared motility (per cent motile sperm), velocity, longevity of sperm, gene expression of testes and sperm morphometrics between the two morphs. We also tested if sperm-duct gland contents affected sperm performance. We found a clear difference in gene expression of testes between the male morphs, with several mucin genes upregulated in breeding-coloured males and two ATP-related genes upregulated in sneaker-morph males. There was also a partial evidence of higher sperm velocity in sneaker-morph males, but no difference in sperm motility. Presence of sperm-duct gland contents increased sperm velocity, and tended to increase sperm motility, but equally so for the two morphs. The sand goby has remarkably long-lived sperm, with only small or no decline in motility and velocity over time (5 min vs. 22 h), but again, this was equally true for both morphs. Sperm length (head, flagella, total and flagella-to-head ratio) did not differ between morphs and did not correlate with sperm velocity for either morph. Thus, other than a clear difference in testes gene expression, we found only modest differences between the two male morphs, confirming previous findings that increased sperm performance as an adaptation to sperm competition appears not to be a primary target of evolution.

Lamatsch

The Prussian carp – imperfect sperm-dependent parthenogenesis as success strategy?

Lamatsch Dunja K., Dedukh Dmitry, Trifonov Vladimir, Wanzenböck Josef, Manfred Scharl

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The invasive Prussian carp, *Carassius gibelio*, occurs in two reproductive forms as sexual allotetraploids or gynogenetic hexaploids. Both forms employ homo- and heterospecific mating systems. While heterospecific matings are non-adaptive for the sexual form they are almost mandatory for the gynogens in the majority of their natural habitats because of the absence of conspecific sperm-donors. Conversely, heterospecific matings are rare for the sexual forms. To better understand the mechanisms of these two reproductive modes and mating systems as well as their ecological consequences, we have performed crossing experiments of both forms with sympatric Cyprinid species. Crosses of sexual *C. gibelio* with goldfish (*C. auratus*) and Crucian carp (*C. carassius*) resulted in fertile offspring.

The same species were crossed to hexaploid asexuals generating exclusively hexaploid offspring. Also crosses with tetraploid and hexaploid *C. gibelio* males gave the same result. An intriguing question is how males can occur in the otherwise all-female lineage. A current hypothesis proposes a link between the presence of certain B-chromosomes and a switch in sexual development from female to male in gynogens. We used molecular markers to trace B-chromosomes in European populations of Cyprinids. Genotyping revealed a correlation between the rare male phenotype in populations of hexaploid Prussian carps and one repetitive DNA marker sequence.

How a B-chromosome could possibly interfere with the genomic determinants of sexual development is unclear and requires at high quality genomic resources. To this end, we have generated a chromosome-level fully annotated assembly of a gynogenetic female with resolving all six haplotypes, long-read transcriptomes of gonads of both sexes of both ploidy and reproductive types and an assembly of a allotetraploid sexual male. We are following the inheritance of the B chromosomes.

Mueni (poster)

Effect of viscosity on spermatozoa

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Sperm motility is a crucial determinant of sperm quality and fertilization capability, yet the influence of fluid viscosity of the fertilization environment on sperm performance remains understudied. The viscosity creates diverse complex microenvironments that interact with sperm morphology and swimming mechanics, and these conditions differ across reproductive systems. In externally fertilizing species, sperm released in water encounter a rapid change in viscosity from the viscous ovarian fluid near the egg and micropyle, whereas internally fertilizing species, sperm encounter viscosity gradients along the female tract as they traverse to the egg. Sperm, being the most diversified cell, the species-specific morphological traits may influence sperm hydrodynamic and propulsion responses to viscosity.

In the study, methylcellulose (0-2%) was used at different concentrations to differentiate the viscosities and sperm from externally fertilizing fish species (trout, sterlet, and common carp) and internally fertilizing elasmobranch (shark, ray, and skates), and amphibians (axolotl) were used in the study for their diversity in sperm morphology and fertilization strategies. In externally fertilizing fish, the motility declined with an increase in viscosity, while in elasmobranchs, sperm exhibited progressive motility only at higher viscosities. Axolotl sperm similarly achieved forward progressive motility above 0.5%MC, whereas low viscosities resulted in circular motion, non-progressive swimming. Flagella responses varied across taxa: trout and carp displayed stable amplitude and wavelength across the viscosities; sterlet displayed decreasing wavelength and increasing amplitude at high viscosities. Elasmobranchs generally exhibited reduced lateral beating, with both amplitude and wavelength decreasing except in rays, where they were less affected by an increase in viscosity. These findings demonstrate that viscosity differentially influences sperm behaviors across species with distinct morphologies and fertilization modes. Further comparative studies across additional taxa are needed to elucidate the evolutionary and ecological importance of viscosity-driven sperm motility responses.

Keywords: motility, progressive motility, viscosity, amplitude, wavelength

Paulin

Dance like no predator's watching: kinematics of sigmoid displays in natural populations

Ignacio Paulin, Noori Choi, Alex Jordan

Max Planck Institute of Animal Behavior

Sexual selection produces some of the most extreme forms of signals in the natural world, yet understanding how these forms evolve and are maintained remains a central challenge in evolutionary biology. This is especially true for behavioural signals which, due to their multidimensional nature, cannot be quantified in the same manner as colour or auditory signals.

Guppies (*Poecilia reticulata*) in Trinidad offer a powerful natural system to study how variation in signalling environments and predation risk drives rapid adaptation, not only in life history and ornamentation but also in the evolution of complex courtship behaviours such as the sigmoid display.

Here, we use posture tracking analyses of paired interactions between wild-caught fish from high and low predation populations across two rivers in the Northern Range of Trinidad to characterise variation in courtship postural dynamics. This allows us to create a behavioural morphospace in which all movements and interactions during courtship are quantitatively mapped.

We find direct support for predictions from signalling theory. Our results show that (i) sigmoid displays occupy a distinct and more extreme region of the behavioural space compared to all other movements, and (ii) sigmoid displays from high versus low predation populations occupy different regions of this behavioural space, mirroring differences that have been previously observed in ornamental traits.

Together, these findings quantify the kinematic structure of courtship behaviour itself, demonstrating that behavioural signals evolve to extreme values and can be shaped by selection in ways similar to visual and auditory traits.

Pšenička (poster)

Species-Specific Sensitivity of Salmonid Embryos to Blue Light

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Early fish embryos vary widely in their sensitivity to light, yet the mechanisms underlying these differences remain unclear. We investigated the effects of visible light (415–590 nm) on the embryos of four salmonid species—rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), and Atlantic salmon (*Salmo salar*)—and compared them with zebrafish (*Danio rerio*). Embryonic survival, whole-embryo reactive oxygen species (ROS), mitochondrial ROS, and mitochondrial absorbance spectra were assessed across early developmental stages.

Among trout species, exposure to 415 nm blue light induced species-specific mortality, which declined progressively with developmental stage. Rainbow trout embryos exhibited near-complete mortality, brown trout and brook trout showed substantial but incomplete mortality, while higher wavelengths (475–590 nm) caused no mortality or developmental defects in any species. Mitochondria isolated from all three trout species displayed similar absorbance spectra, with maximum absorbance at 415 nm and a declining absorbance trend during development, matching the narrowing window of embryonic sensitivity. Rainbow trout showed the strongest increase in both whole-embryo and mitochondrial ROS under 415 nm, whereas brown trout and brook trout showed elevated embryonic ROS but weaker or non-significant mitochondrial ROS responses.

In contrast, zebrafish embryos exhibited neither mortality nor ROS elevation under any wavelength, and Atlantic salmon embryos remained completely resistant even at much higher 415 nm doses. This resistance may be partly attributed to the high pigmentation of salmon eggs, which can limit light penetration and reduce photochemical stress. Additionally, the burial of salmonid eggs in gravel likely provides a general physical barrier restricting light exposure during early development.

These findings identify a species- and stage-specific vulnerability to blue light among salmonids, driven by differential ROS responses despite similar mitochondrial absorbance profiles. The results offer mechanistic insight relevant for optimizing light management in aquaculture.

Rosenthal

From mate choice to mating systems: what are we measuring, and how?

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Mating systems emerge from networks of social interactions among individuals. These interactions have historically been studied through the lens of male agency and male traits. In particular, the evolution of female mating decisions has usually been framed in the context of sexual selection on extreme or species-typical male phenotypes. Yet mate-choice mechanisms and their fitness consequences extend far beyond their effects on male reproductive success. Incorporating mating preferences into a total selection framework facilitates conceptual integration with reproductive allocation and broader life-history theory. The male-centric framing extends to the way we empirically measure sexual behavior, prioritizing precise control of courter stimuli over biologically meaningful measures of chooser sexual response. Moving forward requires not only shifting to a female-centric point of view, but also recognizing the social and cognitive complexity of fishes. New technologies can revive classic ethological approaches for quantifying mating decisions from naturalistic social interactions.

Genome Evolution of the Gynogenetic Amazon Molly, *Poecilia formosa*

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Unisexual reproduction, which generates clonal offspring, is an alternative strategy to sexual breeding and has evolved independently and repeatedly in several branches of the animal tree of life. The Amazon molly, *Poecilia formosa*, is a gynogenetic lineage derived from a single hybridization event of two distantly related species, the Atlantic molly (*P. mexicana*) and the sailfin molly (*P. latipinna*). It needs to mate with males of related species for obtaining sperm to trigger parthenogenetic development of diploid ameiotic eggs. Fertilization occurs without karyogamy, followed by degradation of the paternal DNA. Theoretically, non-recombining genomes will accumulate maladaptive mutations (Muller's ratchet), and clonal reproduction counteracts genetic diversity for adaptation to changing environments (Red Queen). Thus, such lineages are expected to be prone to extinction. However, the Amazon molly has existed much longer than predicted from modelling these scenarios, and it is a prolific species in its natural range. We are interested to find out how this fish defies the grim prognosis of the consequences of its reproductive mode.

In rare cases the destruction of the paternal DNA is incomplete, and introgression occurs in the form of a microchromosome. To evaluate if and how such additional genomic components contribute functional genes thereby increasing genetic diversity we sequenced a microchromosome and analyzed transcriptomes of Amazon mollies with and without supernumerary chromosomes. We show that the introgressed genes are expressed and can even lead to phenotypic changes.

To uncover the genomic consequences of clonal reproduction we produced a haplotype-resolved genome of the Amazon molly and compared it to the genomes of the sexual ancestral species. We find that although the Amazon molly has accumulated mutations faster than its progenitor species (Meselson effect), this has not led to functional decay of genes. Instead, gene conversions reverting mutations to the ancestral state have been preferentially retained, thereby limiting mutational load. Moreover, population genomics uncovered the existence of multiple genetic clones over the whole range of the species. Clonal competition favoring well-adapted genomes and eliminating others would be a mechanism for adaptation and long-term survival of this species.

Svensson

How salinity shapes sperm competition: A meta-analysis of sperm longevity and alternative reproductive tactics

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Adaptive responses to sperm competition are often studied through mating modes (external vs. internal fertilization, sperm casting) and male mating roles—typically an advantageous 'bourgeois' and a disadvantageous 'parasitic' strategy. However, sperm longevity in teleost fishes varies markedly with spawning environment: sperm generally live longer in hyperosmotic saltwater than in hypoosmotic freshwater. We hypothesize that these osmotic differences shape sperm adaptations to competition, through physiological constraints and altered trade-offs. To test this, we extract sperm longevity data from studies of species with alternative reproductive tactics and perform a phylogenetic multilevel meta-analysis, using salinity as a moderator. Our initial model includes 29 effect sizes from 25 studies across 18 species. Salinity significantly predicts effect size: in saltwater species, 'parasitic' males exhibit longer-lived sperm than 'bourgeois' males, whereas the opposite pattern occurs in freshwater. Most variance occurs at the effect ID and species levels, not at the study or phylogenetic level. Substantial residual heterogeneity remains. We are currently updating our literature review, and at least one additional effect size and species will be included. We also explore further moderators, including methodology (e.g. sperm collection method), test temperature, and biological traits like exclusive paternal care. In a preliminary analysis, the measure of longevity (time to 5% motility vs. percent motility at a given time) did not significantly influence effect size. We will conduct a systematic review and further meta-analyses to clarify how environmental and methodological factors shape sperm competition adaptations across diverse reproductive systems.

Early selection allows for efficient and rapid introgression of reporter construct into a transparent zebrafish line

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The primary goal of our research is to understand sexual development of vertebrates using the zebrafish model. During the development of zebrafish gonad, a 'juvenile ovary' forms in each individual. In males, it goes through an apoptosis and transforms into a testis [1]. This process is not fully understood yet. We are using the tg{ddx4::egfp} (earlier tg{vas::egfp}; [2-3] transgenic line to visualize the gonads during their development and transformation. However, the presence of pigmentations interferes with in vivo observation of Egfp signals derived from ovaries. For this reason, we utilized the double pigment mutant transparent zebrafish line, casper [4], in our study.

Typically, the introgression of a transgene into a double mutant, recessive line would take at least three generations [5,6]. Therefore, we developed an efficient selection protocol that would allow us to identify homozygous transgenic casper individuals in somewhat more than two generations. The procedure is based on five selection steps. Based on the presence of black and reflective pigments, individuals with the casper phenotype can be selected as early as the third day of development. In the third generation, homozygous individuals are identified by Egfp signal and PCR based analysis. To make the selection more efficient, we determined the insertion site of the tg{ddx4::egfp} transgene by re-sequencing the genome of the transgenic line. Detailed analysis of the integration site revealed a 3 kb deletion in tg{ddx4::egfp} line. Primers designed to the deleted region allow for a direct PCR-based selection of homozygous individuals without their propagation.

The new homozygous transgenic line on casper background was named gáspár (pronounce: ghaashpar). We are currently working on detailed characterization of the gáspár line that includes a thorough study of the gonadal transformation. Our selection procedure will be potentially useful for introgressing other transgenes into transparent mutant zebrafish lines.

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Timing is the key: daily and seasonal reproductive migration in cypriniform fish

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High reproductive performance is the key attribute of male fitness, especially due to the high reproductive skew among the males of most fish species. Males of long-lived iteroparous fishes have opportunities to improve upon their previous reproductive failures with increasing age and reproductive experience. At the same time, mortality risks of their eggs have to be considered, and reproductive timing adapted to the migration of predatory fishes. We collected reproductive data on a cypriniform fish, the asp (*Leuciscus aspius*), from 2015 to 2021, and evaluated predation risks of the eggs by monitoring them during drift and adhesive phase. We tested whether males changed their performance over time using a unique dataset where individual performance was recorded yearly with passive telemetry. Fish were scored by measures of quality (first arrival time, number of visits per season, time spent in the reproductive grounds and encountered operational sex ratio). A subset of those that arrived for at least four reproductive seasons were chosen for the analysis. In general, the fish improved in the first three metrics with increasing age and experience. More experienced individuals encountered a relatively low sex ratio, which was probably due to their increasing ability to compete for early-arriving females. An important parameter was the tagging length, which indicated a higher initial competitive ability. Mortality of drifting eggs was avoided by predominantly nocturnal spawning, a pattern that breaks down at the end of the spawning season. Mortality of already attached eggs was avoided through spawning habitat selection, which was possible only at low water level in the reservoir. This study demonstrated the importance of experienced and aged individuals in the mating competition of a long-lived fish and intense predation effects in the human-modified environment.

Taboada

Female colouration in the pipefish *Nerophis ophidion*, competitive and geographical differences

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Animal ornamentation is a common venue of communication among animals. However, the function of female ornamentation is still poorly understood. In the straight-nosed pipefish, *Nerophis ophidion*, females show an iridescent-blue breeding colouration that males use to select mating partners. Since mate choice can be modulated by abiotic and biotic factors, we investigated geographical differences in female sexual ornamentation at Klubban (Skagerrak), Gotland and Tvärminne (both Baltic Sea). Females were photographed and dissected, and ovary, liver and spleen mass were used as proxies for fecundity and possible energy and immune costs, respectively. The results show that at all sites, the relative blue ornamental area reflected overall good female condition rather than a physiological cost.

Females were more ornamented at Klubban than the other sites, but the blue area was not associated with fecundity. We hypothesize that the blue colouration is used as a status badge” in intrasexual competition, rather than intersexual context, since Klubban had a female-biased operational sex ratio. In contrast, both Baltic locations showed positive covariation between ornamentation and fecundity. A more turbid environment in the Baltic Sea and a male-biased operational sex ratio at Gotland, might ease intrasexual competition among females. These results suggest that the same ornament might serve different functions depending on ecological conditions.

Thünken

Optimal inbreeding in a cichlid fish

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Animal mating systems are generally thought to be characterized by outbreeding and active avoidance of inbreeding. However, recent studies suggest that inbreeding avoidance is rarer than expected, and theory predicts fitness benefits from inbreeding. *Pelvicachromis taeniatus*, a biparental cichlid fish from West Africa, has been shown to prefer kin as mating partners. Inbreeding is adaptive because related parents cooperate more during brood care. We did not find evidence of inbreeding depression in F1 inbred offspring. Here, we examined the impact of multigenerational inbreeding on assortative and kin mating preferences. We conducted dichotomous mate choice experiments in which inbred and outbred individuals were given the choice between their own species versus a foreign species and between kin versus non-kin. Reinforced assortative mating preferences in inbred groups could lead to divergent tendencies. Conversely, emerging inbreeding depression could result in inbreeding avoidance in highly inbred fish. Highly inbred individuals showed similar assortative mating preferences for their own species as outbred individuals did. Regarding kin-mating preferences, outbred fish exhibited similar inbreeding preferences to those observed in prior studies. However, highly inbred fish preferred unrelated mates from different inbred lines over related ones, suggesting that a certain level of inbreeding triggers outbreeding preferences. Next, to test this hypothesis, we examined kin-mating preferences in outbred crosses of different inbred lines. As predicted, these outbred fish showed kin-mating preferences. Our studies indicate that inbreeding preferences and avoidance are linked to one's own inbreeding level. Kin-mating preferences seem to oscillate between preference and avoidance of kin, according to the associated costs and benefits, leading to an optimal level of inbreeding.

How Life History Shapes Oogenesis: A Cross-Species Comparison in Killifish

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No abstract available.

Structural Bases of Lecithotrophic and Matrotrophic Nutrition in Embryos of Two Viviparous Teleost Lineages, *Gambusia affinis* (Poeciliidae) and *Xenotoca eiseni* (Goodeidae)

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Viviparous teleosts possess a reproductive system characterized by the absence of oviducts, resulting in intraovarian gestation, a condition unique among vertebrates. Depending on the species, gestation may occur either intraluminally, with embryos developing freely within the ovarian lumen, or intrafollicularly, where early development proceeds within the follicle and embryos are released into the ovarian cavity before birth. During gestation, the close association between the embryo and maternal tissues facilitates maternal provisioning, giving rise to placenta-like structures of variable complexity. Viviparity in teleosts encompasses two contrasting nutritional strategies: lecithotrophy, in which embryos rely entirely on yolk reserves accumulated during oogenesis, and matrotrophy, in which reduced yolk deposition during vitellogenesis requires supplemental maternal nutrient transfer once yolk stores are depleted. Consequently, maternal investment is concentrated during oogenesis in lecithotrophic species, whereas matrotrophic species allocate a substantial proportion of maternal resources during gestation. These divergent strategies offer valuable opportunities to study the evolution of maternal-embryonic nutritional systems. In this study, we compare two viviparous teleosts that exemplify these distinct modes of provisioning: *Gambusia affinis* (Poeciliidae), which produces large, yolk-rich oocytes, and *Xenotoca eiseni* (Goodeidae), which produces small, yolk-poor oocytes. Using detailed histological analyses, we examined the maternal and embryonic structures responsible for nutrient exchange. In *G. affinis*, the highly vascularized yolk sac encloses a voluminous yolk mass and facilitates efficient transfer of yolk-derived nutrients. In *X. eiseni*, nutrient uptake occurs through the trophotaeniae, elongated, flattened, and vascularized extensions of the posterior intestine that project into the ovarian lumen and mediate the absorption of histotrophe. These observations reveal how lecithotrophy and matrotrophy rely on fundamentally different structural strategies to sustain embryonic development. By comparing the yolk-based system of *G. affinis* with the trophotaenia-based placental analogue of *X. eiseni*, this study highlights the diversity of maternal provisioning mechanisms in teleosts and contributes to a deeper understanding of the evolutionary pathways that underlie viviparity and placentation in fishes.

Trifonov

Reproductive Strategies and Polyploid Evolution in Fish: Mechanisms, Consequences, and Insights from Genomics

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Fishes exhibit an extraordinary diversity of reproductive strategies, including the formation of clonal lineages, the emergence of polyploid species, rapid speciation, frequent interspecific hybridization, and adaptation to a wide range of ecological niches. Some fish lineages are characterized by frequent polyploid formation, for example, loaches of the order Cypriniformes. Recent advances in sequencing technologies have produced an increasing number of high-quality, chromosome-level genomes across major fish lineages. Our new assemblies of three *Cobitis* (Cypriniformes, Cobitoidea) species—often forming triploid and tetraploid gynogenetic lineages—contribute to this growing resource and reveal extensive interspecific variation in fine-scale structural rearrangements, even among closely related species. These data indicate that species diversification in fishes has been driven by lineage-specific evolutionary processes acting primarily on largely conserved karyotypes. Sex chromosomes in many fish species appear to have originated repeatedly from different ancestral syntenic groups, even among closely related taxa, with little evidence of convergence. Collectively, these findings underscore the critical importance of chromosome-level assemblies for elucidating the molecular and cytological mechanisms underlying reproductive diversity and polyploid evolution in fishes, and highlight the need for broad taxonomic sampling and high-quality genomic resources in comparative genomics.

Żegota

Sexual isolation among ecotypes of the three-spined stickleback

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To understand how mating systems contribute to the evolution of reproductive barriers, we examined the pre- and post-zygotic reproductive barriers between ecotypes of the three-spined stickleback (*Gasterosteus aculeatus*) from North Uist, where the species has undergone an adaptive radiation. Using an experimental design that separated the effects of ecotype, population of origin, and body size, we identified a pre-zygotic barrier attributed solely to ecotype; pairings within ecotypes were more likely to result in mating than those between ecotypes, regardless of population origin or relative body size of males and females. This effect was associated with reproductive incompatibilities linked to the failure of armoured females to enter the unusually small nests of armourless males, along with high levels of aggression from armoured males directed at armourless females. In vitro crosses did not reveal post-zygotic reproductive barriers associated with ecotype or population of origin. These findings indicate that sexual isolation, linked primarily to male traits and involving the coupling of barrier effects, is a significant obstacle to gene flow among the ecotypes, supporting an emerging view that pre-zygotic sexual isolation tends to evolve rapidly and at an early stage during ecological divergence.

Zimmermann

Mating Strategies in a Brood-Parasitic Fish: Insights from Behavioural and Genetic Analyses

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Assessing mating patterns in animals is often challenging, particularly when reproductive behaviours is hidden. In brood parasites, oviposition typically occurs covertly and, in avian taxa, fertilisation takes place internally before parasitic laying, limiting opportunities for direct observation. In avian brood parasites, a further obstacle arises because offspring from a single female are typically dispersed across many host nests, complicating attempts to reconstruct complete clutches and infer individual reproductive output. The Lake Tanganyika cuckoo catfish (*Synodontis multipunctatus*), the only confirmed vertebrate brood parasite outside birds, presents a contrasting yet tractable system. Spawning in the wild has never been observed, but several biological features enable detailed study under controlled conditions. The species thrives in captivity allowing systematic observations of reproductive behaviour. Fertilisation is external, which requires simultaneous presence of males and females at parasitic spawning events and provides direct opportunities to study mating interactions. Moreover, multiple parasitism from one parasite female is common, enabling the reconstruction of sibship structures within reproductive bouts more easily than in avian brood parasites. Taken together, these features make *S. multipunctatus* an unusually powerful system for investigating mating strategies in brood parasitic vertebrates. Here, I synthesise evidence from a series of behavioural and genetic studies to provide an integrative summary of current knowledge on the cuckoo catfish mating system.