Switches, stability and reversals: the evolutionary history of sexual systems in fish

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Abstract

Sexual systems are highly diverse and have profound consequences for population dynamics and resilience. Yet, little is known about how they evolved. Using phylogenetic Bayesian modelling on 4740 species, we show that gonochorism is the likely ancestral condition in teleost fish. While all hermaphroditic forms revert quickly to gonochorism, protogyny and simultaneous hermaphroditism are evolutionarily more stable than protandry. Importantly, simultaneous hermaphroditism can evolve directly from gonochorism, in contrast to theoretical expectations. We find support for predictions from life history theory that protogynous species live longer than gonochoristic species, are smaller than protandrous species, have males maturing later than protandrous males, and invest the least in male gonad mass. The large-scale distribution of sexual systems on the tree of life does not seem to reflect just adaptive predictions and thus does not fully explain why some sexual forms evolve in some taxa but not others (William's paradox). We propose that future studies should take into account the diversity of sex determining mechanisms. Some of these might constrain the evolution of hermaphroditism, while the non-duality of the embryological origin of teleost gonads might explain why protogyny predominates over protandry in this extraordinarily diverse group of animals.

Keywords

Hermaphroditism, phylogeny, Williams' paradox, Dollo's law, developmental plasticity, sex determination

Introducction

Sexual reproduction is a unifying feature of Eukaryotes[1] and yet it is extremely diverse[2]. Sexual systems, defined as the distribution of the male and female function among the individuals of a given species, vary from separate fixed sexes (known as gonochorism in animals and dioecy in plants) to simultaneous hermaphroditism (each individual produces both male and female gametes at the same time). These two sexual systems can be viewed as the extremes in a sexually plastic gradient[3] of intermediate (sequential hermaphroditism) and mixed (coexistence of males and/or females with hermaphrodites)[4,5] systems. Sexual systems have a profound influence on individuals' mating success and fitness[6], population sex ratios and effective sizes[7], and colonization events and habitat use[8]. As a result, sexual system influence the population dynamics and resilience to natural and anthropogenic stressors of ecologically and commercially important species that are often endangered or overexploited[9].

Hermaphroditism is predominant in flowering plants, i.e., angiosperms[10], where 94% of the species have male and female sex organs in the same individual/flower, and it is widespread in invertebrates and fish, totalling 5% of animal species or up to ~30% if insects are excluded[11]. While this diversity suggests multiple evolutionary transitions between sexual systems in response to selection, current evolutionary models on the adaptive advantage of different sexual systems explain little about how and why sexual systems evolve and thus their large-scale distribution across the tree of life (Williams' paradox[4,12]). Thus, unravelling the evolutionary history of sexual systems and quantifying how frequently and in what direction transitions occur, is key to revealing which sexual systems are evolutionarily labile or stable, elucidating how one changes into another over evolutionary time, and identifying the environmental, genetic and developmental drivers favouring or opposing these changes. Yet, our understanding of how sexual systems evolve is still limited, particularly in animals.

Theoretical models, initially developed for plants, suggest that simultaneous hermaphroditism and dioecy are evolutionary stable conditions that are retained over long evolutionary time and unlikely lost once evolved, while mixed sexual systems represent evolutionary intermediate stages[4,5,13] (Fig. 1).

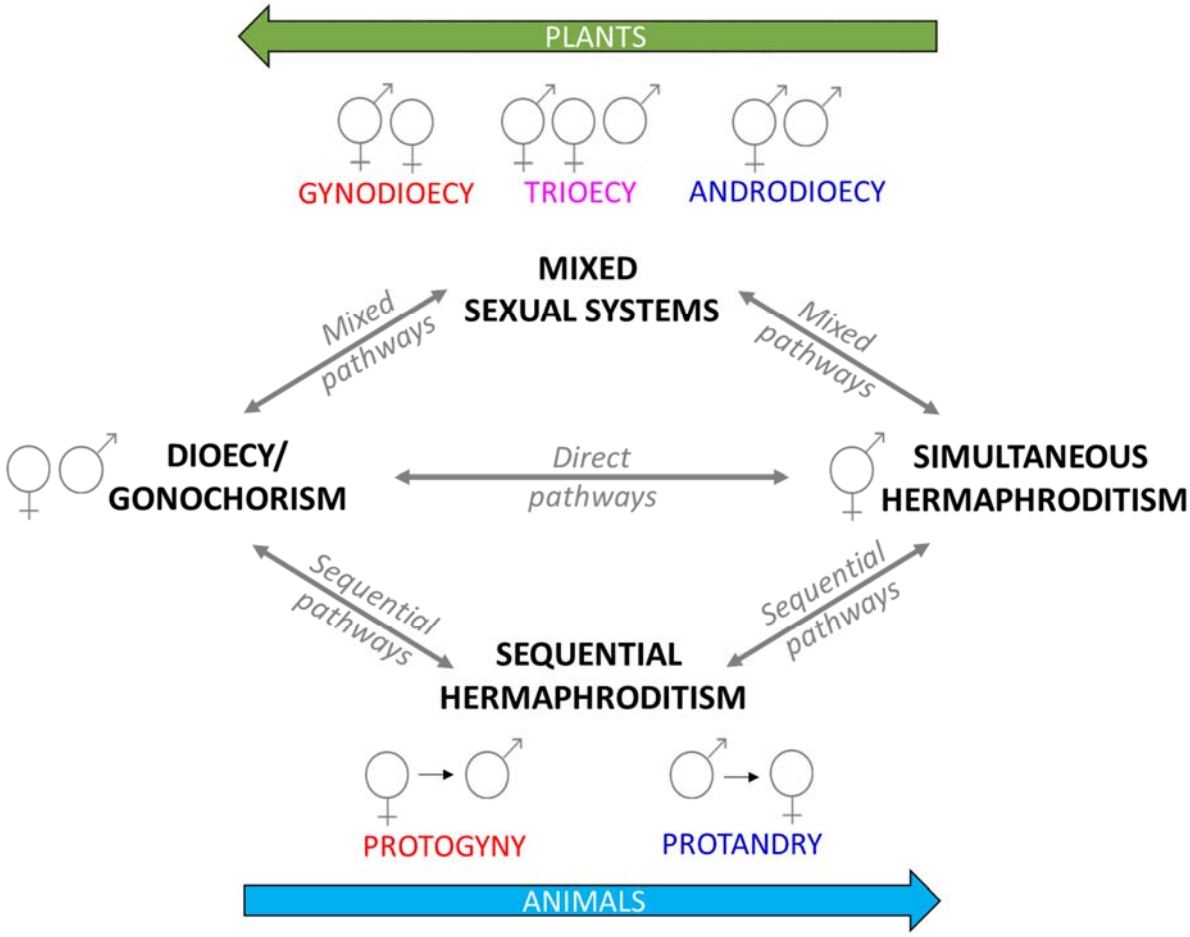

Fig. 1. Theoretical framework for the evolution of sexual systems: potential evolutionary transitions between gonochorism and simultaneous hermaphroditism via mixed systems (mixed pathways) as described in plants and some animals; via sequential hermaphroditism (sequential pathways) as recently suggested[4]; or without intermediate states (direct pathways) as proposed for plants[6]. Double-headed arrows indicate theoretical pathways. Large arrows identify the directionality more frequently investigated for plants (green) and animals (blue), from the most common to the rarest state.

Simultaneous hermaphroditism is likely the ancestral state in angiosperms from which dioecy, a rare sexual system in plants[6], has evolved independently several times, possibly to avoid inbreeding[14,15]. Theoretical models predict that separate sexes in plants evolve from hermaphroditism in different ways: 1) primarily through the intermediate state of gynodioecy[16], a sexual system common in plants that occurs when a male-sterile mutant invades an hermaphroditic population resulting in the coexistence of hermaphrodites and females; 2) androdioecy, where the mutation results in female sterility, resulting in the coexistence of hermaphrodites and males, which is less common in plants[17,18]; 3) possibly via trioecy, i.e., the coexistence of hermaphrodites, males and females, which is very rare; and 4) less frequently, via a direct transition[6,10] (Fig. 1). However, in animals no evidence of a direct transition exists.

Once gained, dioecy was believed to be an irreversible condition[19], a conclusion based on the assumption that returning to a simultaneous expression of male- and female-specific genes would likely produce contrasting effects on sex-specific physiology. Recent studies, however, reject this claim in plants, as phylogenetic reconstructions of direct transitions from dioecy/gonochorism to simultaneous hermaphroditism have been documented[10,20].

The same theoretical framework with mixed pathways has been proposed also for animals where, in contrast to plants, gonochorism is the most common sexual system, androdioecy is more common than gynodioecy[5,13] and trioecy is very rare[21]. However, several reproductive characteristics in plants are quite different from the ones evolved in animals[22], albeit similarities can be found in some invertebrates[23]; hence, different theoretical frameworks are required (Fig. 1). Furthermore, evolutionary transitions between sexual systems in fish, the only vertebrates to exhibit hermaphroditism[24], might be less likely to occur preferentially via a mixed pathway (Fig. 1) given that in fish (>30000 species) only a few killifish species in the genus *Krytpolebias* (formerly *Rivulus*) are truly androdioecious[5,25,26] while the presence of gynodioecy and trioecy is still debated in the hagfish *Myxine glutinosa*[13,27]. Recently, sequential hermaphroditism has been suggested as a possible intermediary state that may facilitate evolutionary changes between gonochorism and simultaneous hermaphroditism[4] (Fig. 1). However, we currently have no robust study on the evolution of sexual systems in animals and thus we currently do not know whether sequential hermaphroditism represents an evolutionary intermediate stage between gonochorism and simultaneous hermaphroditism, whether protogyny and protandry act equally as transitional forms between the two, and whether gonochorism and simultaneous hermaphroditism are evolutionary stable conditions in animals as they are in plants.

The evolution of hermaphroditism in animals has mostly been interpreted in the context of its adaptive advantages relative to gonochorism, as proposed by the low density and the size advantage models[28]. The former predicts that simultaneous hermaphroditism evolves under low population densities and/or low dispersal capacity as, in these conditions, individuals with this sexual system can maximize their chances of securing a mate than sex-changing or gonochoristic individuals[29]. Of note, the possible advantages of self-fertilizing simultaneous hermaphrodites would be offset by increased risk of inbreeding. The size advantage model proposes adaptive explanations for the evolution of sequential hermaphroditism based on the relationship between size and fecundity[28,30–32]. Specifically, since sequential hermaphrodites have indeterminate growth[33], sex change should be favoured when the reproductive value of an individual depends on size (and thus age), and this affects in particular one of the sexes.

Thus, individuals change from a smaller first sex to a larger second sex and the direction of sex change depends on the sex that maximizes its reproductive value with a larger size[34]. The interdependence between size, fecundity and fitness is also affected by the mating system of the species, defined as the pattern of sexual interactions that take place considering the number of males and females involved in reproduction (Table 1). Therefore, protandry (male-to-female sex change) is expected in species that reproduce in monogamous or random pairs and where individuals switch from small males to large, highly fecund females, achieving higher reproductive potential. Conversely, protogyny (female-to-male sex change) is expected in polygynous/group-mating species, where small females become large dominant males that monopolize females, often grouped in harems (Table 1). In both systems exceptions exist with a few individuals born directly as second sex (primary females in digynic protandrous species[35] and primary males in diandric protogynous species[36]).

Crucially, life history traits underpin the size advantage model and life history theory is central to the study of sexual systems evolution since it allows to derive clear predictions about how and when individuals should allocate energy among different life history traits, including sexual functions, to optimize fitness[37]. However, life history traits are surprisingly not explicitly incorporated in the theory, nor tested in empirical studies[12]. Longevity, maximum size and age/size at maturity are key life history traits because they determine individual fitness, influence demographic parameters of populations[38] and impact on the population's genetic diversity[39]. Since sequential hermaphrodites achieve higher fitness when reproducing as the second sex[34], they should benefit more from increased longevity or larger size than gonochoristic and simultaneous hermaphroditic species. Alternatively, sequential hermaphrodites could mature earlier as the first sex compared to the same sex in gonochoristic species and capitalize on reproduction as second sex. These predictions, however, remain to be tested.

The size advantage model has been supported in crustaceans[40], molluscs[41] and fish[42]. Spawning behaviour, i.e., how the two sexes interact to release the gametes, can be classified in fish as pair spawning, involving only two individuals at the time, and spawning aggregations, comprising large breeding groups[43] (Table 1). Mating system and spawning behaviour together determine the intensity of direct male-male competition and sperm competition (i.e., the competition between the sperm of two or more males for fertilization of the same eggs), and thus certainty of paternity. Sperm competition is a key selective force shaping male reproductive anatomy, physiology and behaviour across diverse animal groups[44,45] and is assumed to be low in haremic systems[42,46,47] where large dominant males can better monopolize

166 groups of females (pair spawning) with no or limited competition by other males[48]. Likewise,
167 low sperm competition is expected under monogamy. Group spawning is found in promiscuous
168 mating or aggregations, leading to intense sperm competition[49] as many males try to fertilize
169 the eggs of multiple females. The intensity of sperm competition has been incorporated in the
170 size advantage model[50] as it can play a significant role in the advantage of protogyny: changing
171 sex from female to male should be more advantageous when paternity assurance is high due to
172 reduced sperm competition[51]. Consistent with these predictions, the gonadosomatic index
173 (GSI), defined as the percentage of body mass devoted to the gonads[52] and a reliable indicator
174 of the intensity of sperm competition[53], is significantly lower in protogynous teleost species
175 than in gonochoristic congeners[47,48,54]. However, protandrous teleost fish do not always
176 conform to theoretical expectations, exhibiting higher GSI as males than expected[48]. We have
177 recently proposed that, at least in the family Sparidae, high male GSI in protandrous fish can
178 be explained not only by spawning aggregations and high sperm competition in some species,
179 but also because high investment in the gonads represents a compensatory mechanism that
180 allows small males to fertilize highly fecund females much larger than themselves[54].
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Table 1. Predictions of associations between sexual systems, mating systems, sex ratio, size of mates and spawning behaviour in teleosts.

Sexual System	Mating system	Sex ratio	Size of mates	Spawning behaviour
GONOCHORISM [G] Individuals reproduce as one sex throughout their lifetime (male or female)	♂ ↔ ♀ ♂♂♂ ↔ ♀♀♀ ♂ ↔ ♀♀♀	1:1	♂ = ♀ ♂ > ♀ ♀ > ♂	♂♀ ♂♂♂♀♀♀
PROTOGYNY [PG] Female-first sequential hermaphroditism: individuals first reproduce as females, change sex once with increasing size/age and then reproduce as males	♂ ↔ ♀♀♀ ♂♂♂ ↔ ♀♀♀	♀ Biased	♂ > ♀	♂♀ ♂♂♂♀♀♀
PROTANDRY [PA] Male-first sequential hermaphroditism: individuals first reproduce as males, change sex once with increasing size/age and then reproduce as females	♂ ↔ ♀	♂ Biased	♀ > ♂	♂♀ ♂♂♂♀♀♀
BIDIRECTIONAL [BD] Individuals can change sex more than once, in either directions throughout their lifespan usually from PG	♂ ↔ ♀ ♂ ↔ ♀♀♀	♀ Biased	♂ > ♀	♂♀
SIMULTANEOUS [SH] Individuals produce gametes of both sexes at the same time or in a short period of time	♂ ↔ ♀	1:1	♂ = ♀	♂♀

Mating system: ♂ ↔ ♀ = monogamy (pair bond) or random pairing; ♂♂♂ ↔ ♀♀♀ = promiscuity; ♂ ↔ ♀♀♀ = harem polygyny or temporary lek-like systems (many females in a territory defended by a male). Spawning behaviour: ♂♀ = pair spawning (pair of individuals); ♂♂♂♀♀♀ = group spawning/spawning aggregations.

Fish account for more than 50% of the extant species of vertebrates and are characterized not only by their extraordinary diversity in morphology, physiology, ecology and habitat, but also by different sexual systems, including gonochorism, different forms of hermaphroditism —the only group among vertebrates— and unisexuality (all-female populations)[24,55,56]. Hermaphroditism in fish is broadly divided into simultaneous (synchronous) and sequential (consecutive) hermaphroditism, the latter in the form of protandry, protogyny and bidirectional sex change (Table. 1). This remarkable diversity in sexual systems in fish, particularly in the infraclass teleosts accounting for 96% of species, makes them an ideal group in which to study the evolution of different forms of

hermaphroditism. Here, we investigate the evolutionary origin and transitions among sexual systems across 4740 teleost species belonging to 294 families using a recent time calibrated phylogeny and modern phylogenetic comparative approaches. Our large-scale approach allows us to fully unravel how sexual patterns evolved and identify which ones represent evolutionary stable conditions. We demonstrate that gonochorism is the likely ancestral condition in teleosts and it is an evolutionarily stable state from which not only protogyny and protandry, but also simultaneous hermaphroditism evolve at a moderate evolutionary rate. In contrast to theoretical predictions, we show for the first time that simultaneous hermaphroditism can evolve directly from (and be directly lost back to) gonochorism, without the need of intermediary evolutionary steps. Further, we expand the theoretical framework of the evolution of hermaphroditism investigating how life history traits and male GSI differ between sexual systems, as predicted by life history theory. In support of predictions, we found longer lifespan in protogynous species compared to gonochoristic, larger size in protandrous species compared to protogynous, and smaller GSI in protogynous males. However, contrary to predictions, we found no differences in age or size at maturity across sexual systems, with the exception of late maturing protogynous males. Finally, we discuss how our results should be incorporated in an even broader framework that includes sex determining mechanisms and gonadal plasticity as possible constraint and facilitation mechanisms, respectively, to gather a better understanding of the evolution of sexual systems and possibly resolve Williams' paradox.

Results

Evolutionary history of sexual systems

Our dataset includes 4740 extant teleost species, of which 403 hermaphroditic (protogynous: n = 285; protandrous: n = 57; bidirectional sex change: n = 14; simultaneous hermaphroditism: n = 47; see Methods, Data collection). We used Discrete models of evolution to reconstruct the evolutionary history of sexual systems using Reversible Jump (RJ) Markov chain Monte Carlo (MCMC) in *BayesTraits* (Methods, Phylogenetic comparative analysis). Treating sexual systems as a two character states (gonochoristic or hermaphroditic) our analysis revealed that gonochorism is the most likely ancestral character state in teleosts (Fig. 2; Supplementary Table 1) and that hermaphroditism slowly evolves from, and reverts very quickly and multiple times back to, gonochorism (Fig. 2). This indicates that gonochorism is an evolutionarily stable state in teleosts (Supplementary Fig. 1).

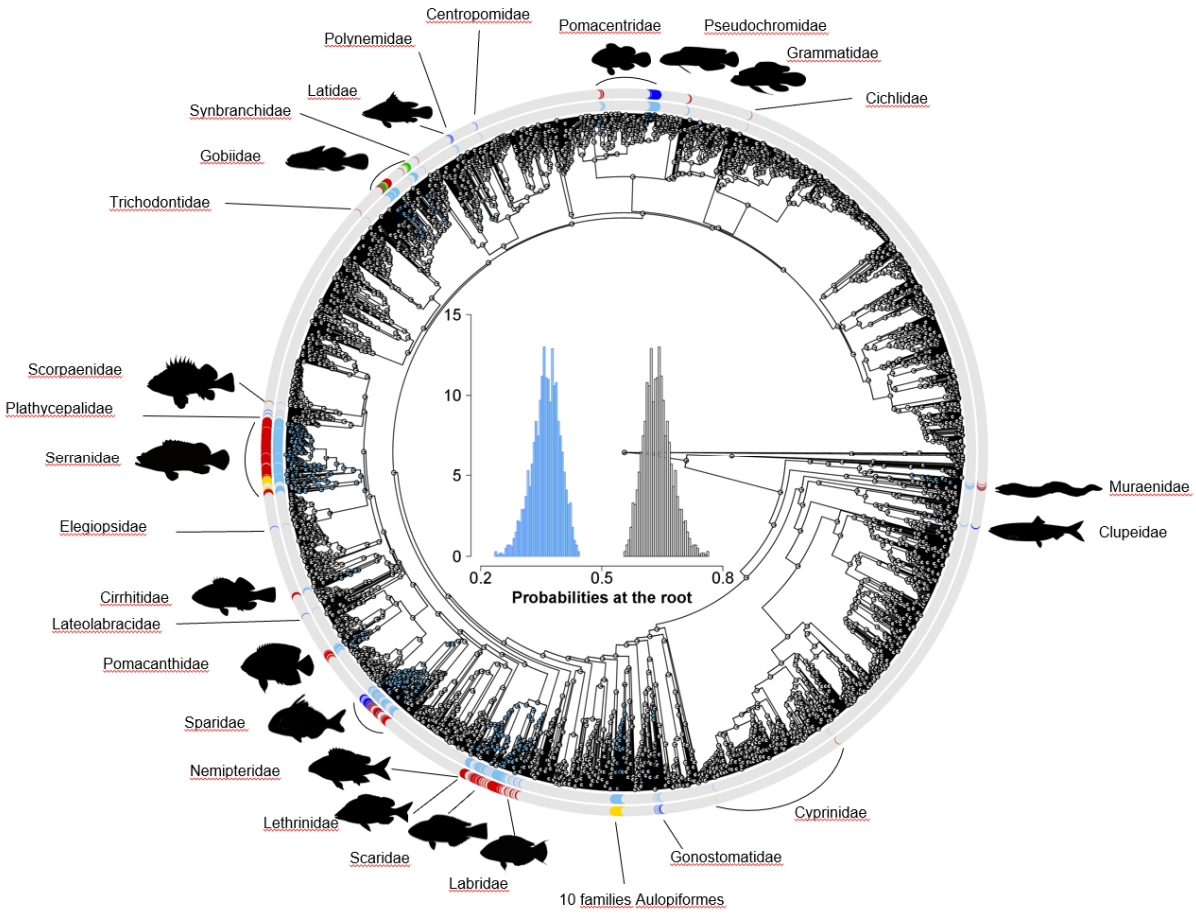

Fig. 2. Teleost phylogeny with the evolutionary history of sexual systems in maximum likelihood using the R package Ape v4. In the inner circle and in the internal nodes of the phylogeny, the sexual systems of extant species and their ancestors are colour coded for gonochorism (grey) and hermaphroditism (light blue). In the external circle, the sexual systems of extant species are colour coded for gonochorism (grey), protogyny (red), protandry (blue), bidirectional hermaphroditism (green) and simultaneous hermaphroditism (yellow). The density plots in the inset show the estimated probability of character state at the root of the phylogeny (RJ-MCMC Multistate) and colour coded for gonochorism (grey) and hermaphroditism (light blue).

Treating sexual system as four character states (gonochoristic, protandric, protogynic, simultaneous hermaphroditic) our analysis revealed that all three types of hermaphroditism evolve at a very low rate from gonochorism (Fig. 3, Table 2). Protogyny and protandry revert very rapidly back to gonochorism. Furthermore, protogyny is as likely to evolve from gonochorism as it is to switch to simultaneous hermaphroditism or protandry, although nearly 20% of the models estimate the latter transition rate to be equal to zero. Conversely, protandry is lost as quickly to protogyny as it is to gonochorism and, to a much smaller extent, to simultaneous hermaphroditism given that 38% of the models estimated this latter transition rate to be zero. Finally, simultaneous hermaphroditism evolves directly from and is lost back to

gonochorism at similar low rates, with 15% of the models estimating these transition rates to be equal to zero. Therefore, simultaneous hermaphroditism can evolve at similar rates directly from gonochorism or from protogyny, but less likely from protandry. Conversely, while simultaneous hermaphroditism is also lost to protogyny and protandry at similar low rates as to gonochorism, transitions from simultaneous hermaphroditism to sequential hermaphroditism are much less likely given that over 45% of the models estimated them to be equal to zero. Altogether, these results clearly indicate that in teleosts gonochorism is an evolutionarily stable state; protogyny is evolutionarily more stable than protandry, while simultaneous hermaphroditism evolves rarely but is evolutionarily stable, being lost slowly to gonochorism, and possibly to protogyny and protandry (Fig. 3, Table 2).

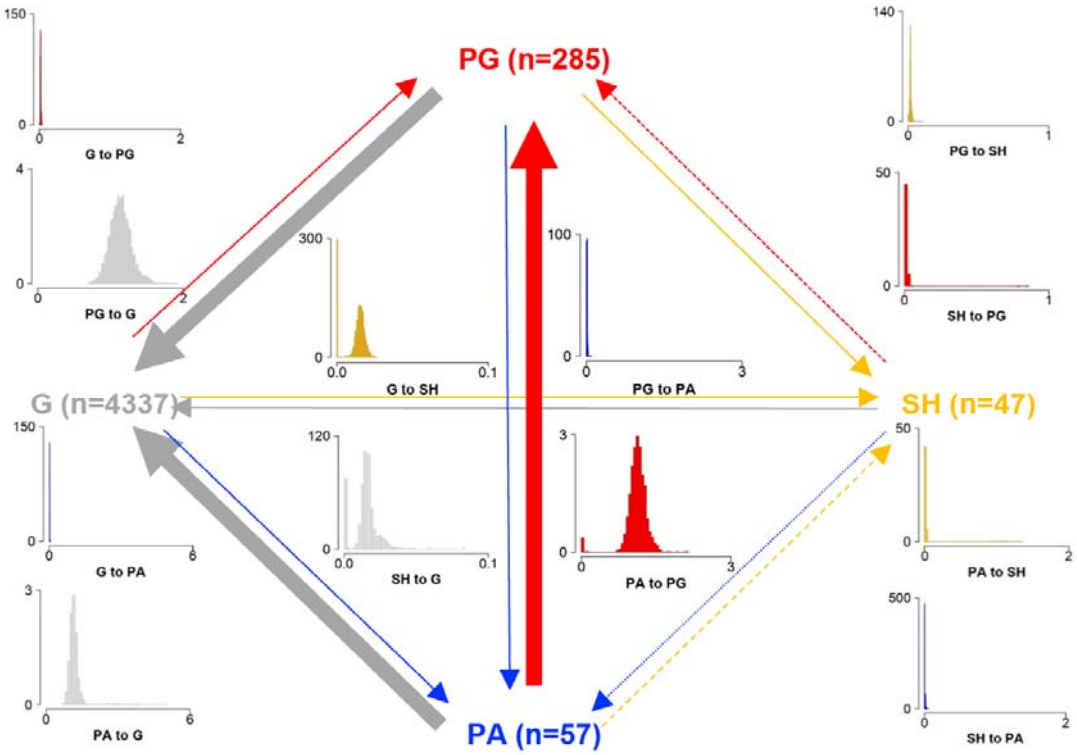

Fig. 3. Summary of RJ-MCMC Multistate analysis with density plots of the posterior distributions of the transition rates to gonochorism (G, in grey), protogyny (PG, in red), protandry (PA in blue), and simultaneous hermaphroditism (SH, in yellow). Note, only x-axis, but not y-axis, are the same for each pair of gain and loss between two character states. The thickness of the arrows is roughly proportional to the mean magnitude of the transition rates from the posterior distribution. Dotted lines indicate transition rates estimated to be equal to 0 in over 30% of the models in the posterior distributions. Sample sizes of extant species for each sexual system category are indicated between parentheses.

Table 2. Results of the RJ-MCMC Multistate analysis in BayesTraits of sexual systems as a four state categorical variable: gonochorism (G), protogyny (PG), protandry (PA) or simultaneous hermaphroditism (SH). For each posterior distribution, we report the effective sample size (ESS), the mean and 95% high density intervals (95-HPD), the mode and the percentage of models in which the parameter is estimated as zero. This analysis is based on 4726 extant species (G: n = 4337; PG: n = 285; PA: n = 57; SH: n = 47). Note: 14 species of bidirectional sex change were not included due to their low number.

Transition rates	ESS	Mean	95-HPD	Mode	% Zero
G → PG	2000	0.0172	0.0097 – 0.0291	0.0156	0.2
PG → G	2000	1.1370	0.8381 – 1.4181	1.1096	0
G → PA	2000	0.0172	0.0097 – 0.0291	0.0156	0
PA → G	2000	1.1770	0.8427 – 1.4866	1.1096	0
G → SH	2000	0.0132	0 – 0.0201	0.0156	15
SH → G	2000	0.0147	0 – 0.0275	0.0162	15
PG → PA	2000	0.0142	0 – 0.0277	0.0161	19
PA → PG	2000	1.1160	0 – 1.4893	1.1096	0.3
PG → SH	2000	0.0170	0.0089 – 0.0309	0.0156	2
SH → PG	2000	0.0102	0 – 0.0252	0	45
PA → SH	2000	0.0611	0 – 0.0589	0	38
SH → PA	2000	0.0090	0 – 0.0247	0	48
Root probabilities					
G	2000	40.7	35.7 – 48.2	39.2	0
PG	2000	29.9	25.5 – 32.8	30.3	0
PA	2000	29.3	23.7 – 37.0	30.4	0
SH	2000	<0.1	0 – 0.9	<0.01	2.3

Life history traits and sexual systems in teleosts

The phylogenetic generalized least square (PGLS) analysis revealed that protogynous, but not protandrous, species live longer than gonochoristic species (Fig. 4a; Table 3). Protandrous species reach larger maximum adult size than protogynous species (Fig. 4b; Table 3). Female and male age at maturity do not differ across species with different sexual systems (Fig. 4c-d; Table 3) nor does sex-specific length at maturity (Table 3). However, when accounting for allometry, males in protogynous systems mature later than males in protandrous systems (Fig. 4e). Finally, protogynous males have lower GSI values than gonochoristic and protandrous ones, but GSI does not differ significantly between gonochoristic and protandric males (Fig. 4f; Table 3).

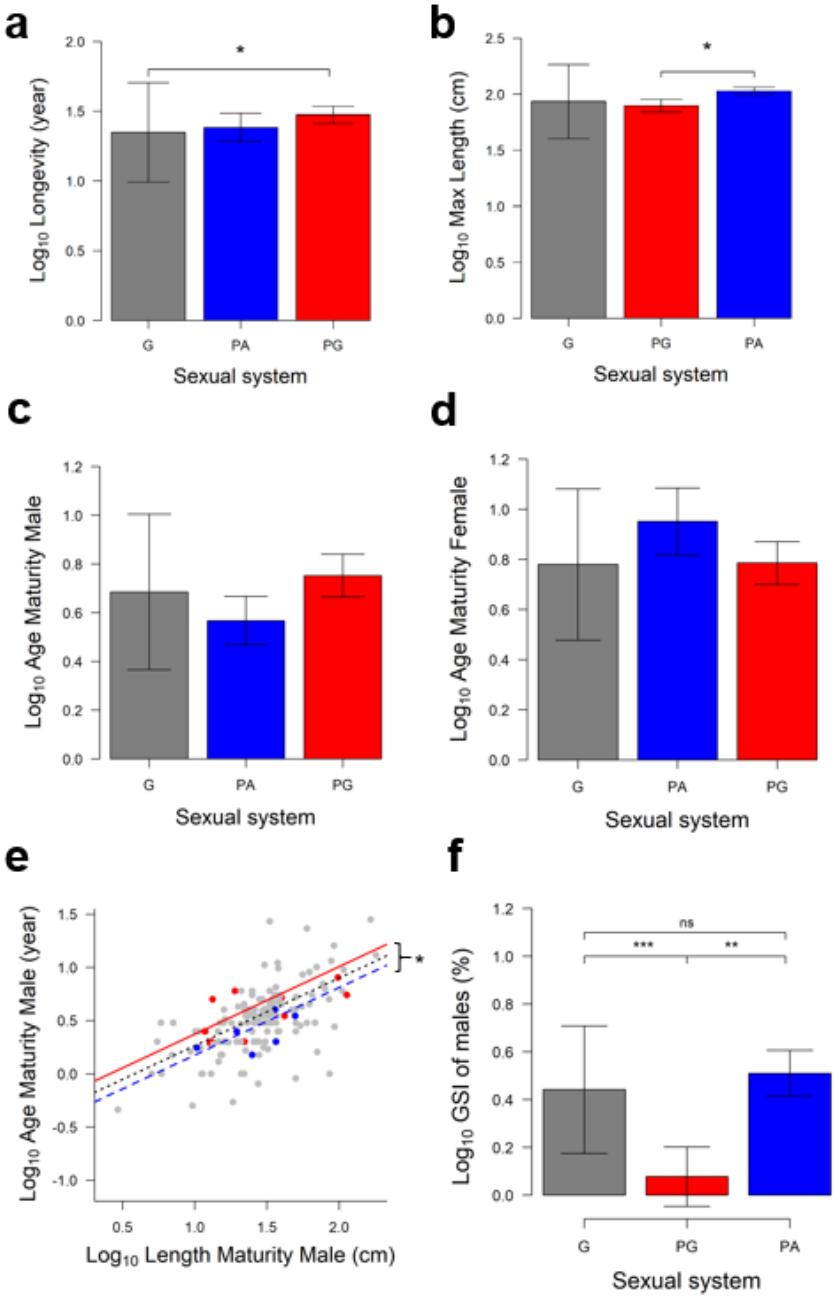

Fig. 4. Selected life history traits by sexual system. Phylogenetic estimated mean and standard error from the PGLS results of: **a)** longevity (G: n = 759; PG: n = 88; PA: n = 19); **b)** maximum length (G: n = 2624; PG: n = 243; PA: n = 36); **c)** male age at first maturity (G: n = 258; PG: n = 20; PA: n = 12); **d)** female age at first maturity (G: n = 281; PG: n = 33; PA: n = 5); **e)** male age at first maturity corrected for male length at first maturity (G: n = 136; PG: n = 12; PA: n = 9); **f)** male gonadosomatic index, GSI (G: n = 45; PG: n = 45; PA: n = 15). In all panels gonochorism (G) in grey, protogyny (PG) in red and protandry (PA) in blue.

Table 3. Results of phylogenetic generalized least square (PGLS) model of longevity (year; log_{10} transformed), length at first maturity (cm; log_{10} transformed) across sexual systems: gonochorism (G); protogyny (PG); protandry (PA). For each independent variable we report the parameter estimate (Beta), t-statistics (T), *P*-value (P), and the model statistics including the degrees of freedom (df), the maximum likelihood estimation of the phylogenetic signal (λ) and R^2. Refer to supplementary table 2 for sexual system and sex-specific data. Significant differences are indicated in bold.

Variable		Beta	T	P	Df	Model statistics	
Dependent	**Independent**					**λ**	**R^2**
Longevity	Sexual system - PA[1]	0.035	0.345	0.730	2; 863	0.909	0.005
	Sexual system - PG[1]	0.127	2.079	**0.038**			
	Sexual system - PG[2]	0.092	0.878	0.380			
Max length	Sexual system - PA[1]	0.094	1.615	0.106	2; 2900	0.973	0.001
	Sexual system - PG[1]	-0.037	-1.111	0.267			
	Sexual system - PG[2]	-0.131	-2.025	**0.043**			
Age at maturity ♂	Sexual system - PA[1]	-0.117	-1.183	0.237	2; 287	0.865	0.009
	Sexual system - PG[1]	0.067	0.763	0.446			
	Sexual system - PG[2]	0.184	1.595	0.112			
Age at maturity ♀	Sexual system - PA[1]	0.172	1.288	0.198	2; 316	0.861	0.005
	Sexual system - PG[1]	0.006	0.070	0.944			
	Sexual system - PG[2]	-0.166	0.138	0.231			
Length at maturity ♂	Sexual system - PA[1]	-0.031	-0.468	0.640	2; 368	0.973	0.001
	Sexual system - PG[1]	-0.033	-0.601	0.548			
	Sexual system - PG[2]	-0.003	-0.034	0.973			
Length at maturity ♀	Sexual system - PA[1]	0.030	0.344	0.731	2; 348	0.970	0.0003
	Sexual system - PG[1]	0.013	0.199	0.842			
	Sexual system - PG[2]	-0.017	-0.174	0.862			
Age at maturity ♂	Length at maturity ♂	0.581	8.186	**<0.001**	3; 153	0.782	0.318
	Sexual system - PA[1]	-0.145	-1.680	0.095			
	Sexual system - PG[1]	0.091	1.184	0.238			
	Sexual system - PG[2]	0.236	2.427	**0.016**			
Age at maturity ♀	Length at maturity ♀	0.535	8.203	**<0.001**	3; 168	0.831	0.287
	Sexual system - PA[1]	0.032	0.243	0.808			
	Sexual system - PG[1]	0.008	0.112	0.911			
	Sexual system - PG[2]	-0.023	-0.181	0.856			
GSI ♂	Sexual system - PA[1]	0.072	0.553	0.581	2; 102	0.904	0.143
	Sexual system - PG[1]	-0.367	-3.722	**<0.001**			
	Sexual system - PG[2]	-0.433	-3.214	**0.002**			

[1] G as reference level; [2] PA as reference level

364 **Discussion**

365 Our large-scale phylogenetic study has, for the first time, tested the theoretical
366 predictions for how sexual systems have evolved and has revealed the evolutionary origin of
367 and transitions between different sexual systems in the highly diverse teleosts. We thus identify
368 gonochorism, and to some extent simultaneous hermaphroditism, as stable conditions over
369 evolutionary time, being lost at lower rates than they are gained. Protandry is evolutionarily
370 the most labile condition. In contrast to theoretical predictions, our study clearly demonstrates
371 that, while simultaneous hermaphroditism can evolve from protogyny and possibly from
372 protandry, it is as likely to evolve directly from —and revert directly back to— gonochorism.
373 We find partial support for the predictions derived from life history theory that sequential
374 hermaphrodites live longer and grow bigger than teleosts with other sexual systems.
375 Specifically, protogynous species live longer than gonochoristic species and protandrous
376 species attain a larger size than protogynous ones. Finally, protogynous males invest the least
377 in male gonad tissues (quantified by the gonadosomatic index) relative to males with other
378 sexual systems and mature later for their size than protandrous males. However, female age at
379 maturity does not differ between sexual systems. Combined, these results suggest that the two
380 forms of sequential hermaphroditism must be treated separately in theoretical and empirical
381 studies as protandry and protogyny are characterized by distinct life history strategies[34], even
382 though they both entail sex change.

383 Using the largest dataset ever collected with four sexual systems in teleost fish, our
384 study reveals a complex and dynamic way through which sexual systems evolve and switch
385 between one another. All forms of hermaphroditism can evolve directly from gonochorism (the
386 ancestral state in teleosts) and revert back to gonochorism even faster. Therefore, even though
387 gonochorism is an evolutionarily stable condition, gained faster than it is lost, our results refute
388 the assumption that the transition to gonochorism is irreversible[19] and represent another
389 example[57,58] against Dollo's law of irreversibility[59]. Conversely, in teleosts, all forms of
390 hermaphroditism are less evolutionarily stable than gonochorism. Importantly, our analysis
391 reveals that simultaneous hermaphroditism can originate directly from gonochorism or through
392 sequential hermaphroditism, but simultaneous hermaphroditism is lost preferentially to
393 gonochorism rather than either form of sequential hermaphroditism. Our results therefore
394 refute the conclusion of Pennell et al[60]. Specifically, using a much smaller dataset biased
395 towards a greater proportion of hermaphrodite than gonochoristic species than what is observed
396 in teleosts, and without discriminating between different types of hermaphroditism, Pennell et
397 al[60] found that the evolutionary transition from gonochorism to hermaphroditism is over twice

398 as fast than the reverse, suggesting rapid evolution of hermaphroditism from gonochorism, a
399 conclusion that these authors acknowledge is counterintuitive. Importantly, our analyses
400 demonstrate that no intermediate stages are strictly required to either gain or lose simultaneous
401 hermaphroditism from gonochorism. These results call into question theoretical predictions
402 (Fig. 1) that sex-specific gene expression in gonochoristic species prevents direct evolutionary
403 transitions between gonochorism and simultaneous hermaphroditism, and intermediate stages,
404 like sequential hermaphroditism, are required[4]. Androdioecy is considered an intermediate
405 stage from simultaneous hermaphroditism to gonochorism in plants and from gonochorism to
406 hermaphroditism in some invertebrates[5,13], but this sexual system is extremely rare in fish and
407 cannot explain the evolution of diverse sexual system in this vertebrate group. Overall, and
408 contrary to what had previously been surmised, our analyses suggest that the complexity of
409 sex-specific physiology and behaviour do not constrain transitions between sexual systems,
410 and that even changes to simultaneous expression of both sexual functions can be
411 evolutionarily gained and lost easily from gonochorism.

412 According to life history theory, sequential hermaphrodites should live longer, grow
413 bigger and mature earlier as the first sex than gonochoristic species. We find that protandrous
414 and protogynous species differ in their life history strategies: protandrous species attain a
415 bigger size than protogynous species while protogynous species live longer than gonochoristic
416 species. These differences reflect the fact that protandry and protogyny maximise their fitness
417 as the second sex[34] which differs between the two systems. Therefore, protandrous species
418 benefit primarily by achieving a larger size, as larger females (second sex) are more fecund
419 than smaller ones. Conversely, the longer life in protogynous species maximises the fitness of
420 the large successful males (second sex) that can monopolize females in harems or in spawning
421 grounds. In addition, male investment in gonad tissue (as quantified by the gonadosomatic
422 index) is lower in protogyny, as expected by theory[48,54], since large males can better
423 monopolize mating opportunities and face low levels of sperm competition in harems and
424 spawning aggregations (Table 2). Small-sized protandrous males in spawning aggregations
425 instead need to boost their investment in the gonads but even in the absence of sperm
426 competition (monogamy) they require large gonads to fertilize highly fecund females larger
427 than themselves[54]. Thus, sexual systems and mating strategies affect life history traits
428 differentially in protandrous and protogynous species. It is well known that in sequential
429 hermaphrodites the second sex always matures later and is larger than the first sex, so it is not
430 surprising that in protandrous species females are significantly larger than males when reaching
431 maturity, while in protogynous species males are significantly larger than females[51] (excluding

the cases primary females and primary males, respectively). Yet, no comparison has been made for size/age at first maturity for males and females across sexual systems. Life history theory predicts that the first sex of sequential hermaphrodites matures earlier than the same sex in gonochorism, but we find no evidence for this. However, future studies should re-evaluate these relationships as more data become available for a large number of sex-changing species, as currently sex-specific life history data are scarce for hermaphroditic fish species. Data are particularly necessary to fully assess theoretical predictions for size and time spent as females and as males in sequential hermaphrodites. Finally, when accounting for allometry, we find that protogynous species mature as males (second sex) at a later age than males of protandrous species. Such difference for males but not females in sequential hermaphrodites might reflect social regulation associated with sex change in protogyny. Specifically, protogynous females typically change sex when a dominant male is not present[36]; thus, social regulation might delay sex change in females until later in life.

While we have shown that life history theory can provide a major contribution to our understanding of sexual system evolution, we propose that the highly dynamic picture revealed by this study should be expanded using a more comprehensive approach that includes not only selection and adaptation, but also genetic and developmental mechanisms (Fig. 5). We propose that more data should be gathered on sex determining mechanisms to gain a better understanding of the evolution of sexual systems. Fixed, strongly canalized, genetic sex determination (culminating in the formation of fully differentiated and stable heteromorphic sex chromosomes) might constrain the evolution of hermaphroditism (acting as an evolutionary trap[2,61–63], but see Pennell et al[60]). Similarly, the peculiarities of the embryonic origin of the urogenital system in teleosts, compared to other vertebrates[64], may favour a certain sexual system (protogyny) over another (protandry) in this group. Sex determination in gonochoristic animals is determined either at fertilization by different genetic mechanisms, including male (XX/XY) or female (ZW/ZZ) heterogamety with homomorphic or heteromorphic sex chromosomes, or after conception by environmental factors, or by a combination of both[65,66]. Fish are characterized by an incredible diversity[67–69] and evolutionary plasticity[60,70] of sex determining mechanisms. Many fish do not have sex chromosomes[68] and when they are present, they might not always be clearly differentiated, with the sex determining loci often not easily identifiable[71]. In some cases, sex determination can be due to a difference in a single nucleotide[68]. Moreover, high turnover of sex chromosomes has been detected in some fish lineages (e.g., sticklebacks[72,73]), including reversal to autosomes[60]. Although data are currently scarce for formal analyses, sequential hermaphrodites do not appear to have sexually

differentiated chromosomes[74], but additional research on the underlying genetic basis of sex determination specifically linked to hermaphroditism in teleosts is clearly needed. Therefore, although complete genetic control of hermaphroditism is common in plants[75], a better knowledge of the genetic mechanisms of sex determination could be helpful to explain how hermaphroditism in teleosts has evolved in some taxonomic groups but not in others under similar ecological pressures. Previous attempts to connect the distribution of sexual systems and sex determining mechanisms have invoked morphological[76] and developmental[64] aspects. Furthermore, sequential hermaphroditism can be regarded as a clear example of phenotypic plasticity, and since epigenetics underlies phenotypic plasticity, common epigenetic mechanisms have been proposed to participate in the evolutionary transitions between different sexual systems and sex-determining mechanisms[77].

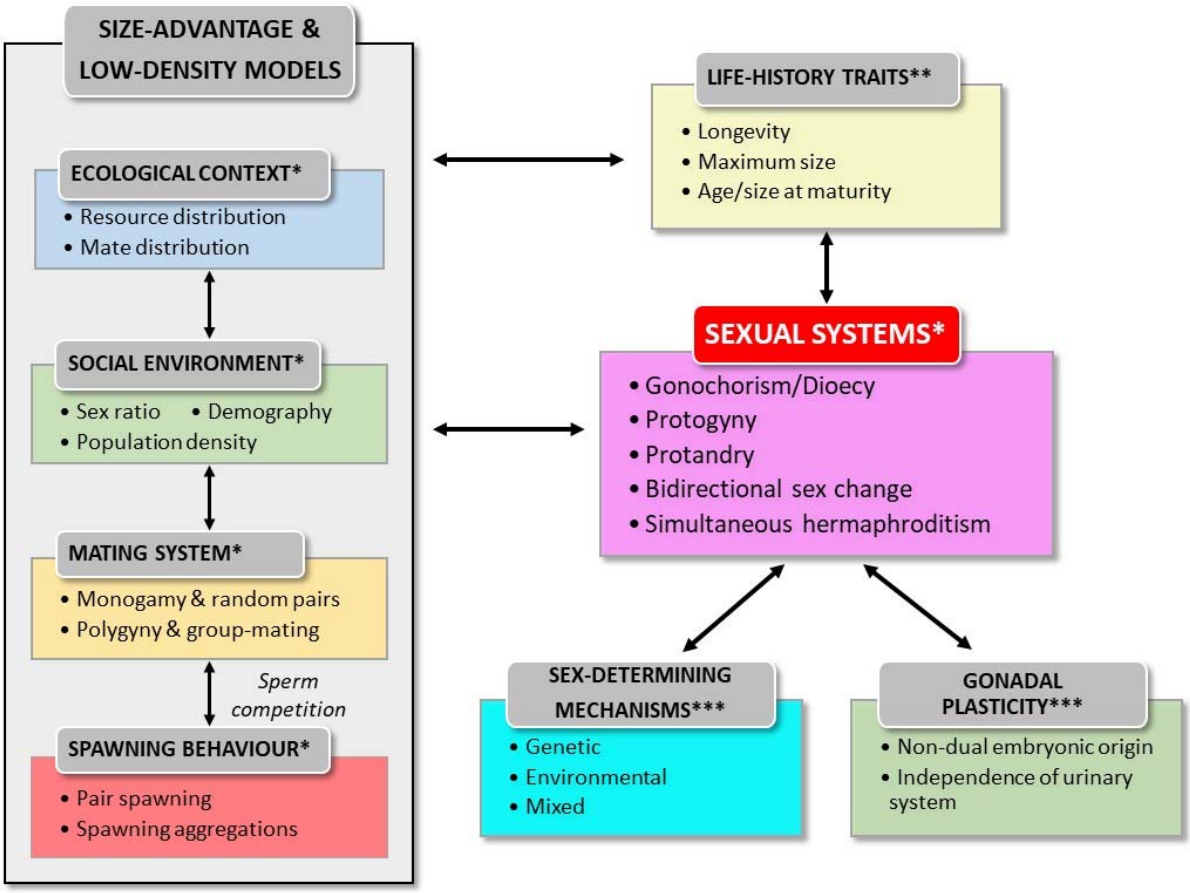

Fig. 5. Theoretical framework for the evolution of sexual systems: overview of parameters (with some examples) considered in the low density and the size advantage models (*), used in our analyses (**) and proposed in the present study (***).

Overall, sex determination is achieved through plastic switches that channel an individual's development towards one sex or the other, or both at different or at the same time, usually with the concurrence of biotic or abiotic influences[78]. Further developmental plasticity is uniquely documented in fish via the bipotential nature their gonads and gonoducts[64]. Thus, while in most vertebrate taxa gonads develop from two distinct germinal layers (medulla, endodermal in origin, which gives rise to the testes; and cortex, mesodermal in origin, which gives rises to the ovary), in teleosts the gonads consist entirely of the cortex homolog[79]. Therefore, anatomically all fish could be, in principle, hermaphrodites[64]. The transition to protogyny may be favoured by the peculiarities of gonadal development in many fish species, which develop a female gonad, complete with ovaries containing cysts of oocytes, during the initial stage of gonadal formation[80,81]. Only later testicular development is triggered and superimposed on this arrangement so that the individual ultimately matures functionally as a male[82–84]. Thus, protogyny might be favoured because female gonads are often the first to develop. Bidirectional sex change, a rarer system in fish (Table 1), further demonstrates the importance of gonadal plasticity. In most cases, the initial strategy is protogyny[85], but males can revert back to females when triggered by new social conditions. The retention of some female gonadal tissue in males facilitates a new change of sex, if and when required[86]. Thus, it has been argued that in bidirectional sex change it should be less expensive for a male to maintain a non-functional female gonad than for a female to maintain a non-functional male gonad[85]: females typically use the full gonad to produce eggs, while males could afford to spare some tissue as ovarian given that their mating success should not be greatly affected by a slight reduction in sperm production[86] (e.g., in some monogamous gobies of the genus *Trimma*). The maintenance of both gonadal tissues could facilitate a transition to simultaneous hermaphroditism. Consistent with this suggestion, our analyses indicate that evolutionary transition to simultaneous hermaphroditism are more likely from protogyny than from protandry: protogyny might change to bidirectional sex change and, with maintenance of both gonadal tissues, to simultaneous hermaphroditism (Fig. 3, Table 2).

In conclusion, our study reveals that gonochorism is the most likely ancestral state and the most evolutionary stable sexual system in teleosts. In contrast to theoretical predictions, we demonstrate that simultaneous hermaphroditism can evolve directly to and from gonochorism and, overall, the evolution of sexual systems is evolutionarily more dynamic than commonly assumed. Our results call into question theoretical assumptions that changes between sexual systems are constrained by sex-specific gene expression, physiology and behaviour. Instead we propose that the adaptive advantage of different sexual systems is underpinned in fish by

their extraordinary and unique developmental plasticity[87], including common and fast transitions among different sex determining mechanisms[60,65]. Our study also reveals that different sexual systems exhibit different life history strategies that allow species with sequential hermaphroditism to maximise fitness as the second sex[34], and highlights the need for more sex-specific life history data to gain a fuller and deeper understanding of the interplay between life history strategies and sexual system. Altogether we propose that a comprehensive framework that incorporates life history traits, sex determining mechanisms and gonadal plasticity into traditional theoretical models of sexual system adaptive value will be essential if we are to fully understand the evolution of sexual systems, their phylogenetic distribution and their implications for conservation and management.

Methods

Data collection and verification

We compiled the most comprehensive database on sexual systems in teleost fish to date (9005 species). Of these, 4740 (403 hermaphroditic) are included in the most recent and largest phylogeny for the class[88] (Supplementary Data). Information on sexual system were first extracted from FishBase[89] and then verified individually for the majority of the species against the primary literature available to date (Supplementary Methods). Life history traits were also collected from the primary literature and FishBase[90]: longevity (in years), maximum length (in cm); length (in cm) and age at maturity (in years) of males and females; male gonadosomatic index (GSI; Supplementary Table 2).

Phylogenetic comparative analyses

We investigated the evolutionary history of sexual systems of 4740 teleost species using Multistate models in BayesTraits V.3[91,92] in a Bayesian framework. Multistate estimates instantaneous transition rates between alternative character states of a single categorical variable (i.e., the rate of change between states along the branches of a phylogeny), based on a continuous-time Markov model of evolution for discrete traits[93,94]. A high transition rate from one state to another indicates that the first state changes rapidly to the second state over evolutionary time. Therefore, a character state is evolutionarily stable when it is lost more slowly than it is gained[95]. Multistate also produces posterior distributions of the ancestral character state at the root of the phylogeny. We scaled the tree by a default constant (mean of 0.1) in all analyses[95] and used an exponential prior whose mean was seeded from a uniform hyperprior ranging from 0 to 10 to reduce inherent uncertainty and biases of prior choice[92]. We

552 ran all Multistate analyses with Reversible Jump (RJ) Markov chain Monte Carlo (MCMC)
553 methods. MCMC samples models in direct proportion to their fit to the data, generating a
554 posterior distribution of parameter estimates for each transition rate, and RJ sets some
555 parameters equal to zero or equal to one another, thereby reducing model complexity and over-
556 parametrization[91,92,94]. As a result, posterior distributions of parameter estimates may not be
557 normal; we thus summarised results by presenting mean and mode of the posterior distributions
558 of each parameter estimate, 95% higher posterior density, and percentage of models with
559 parameters estimated to be 0. We ran all MCMC chains for 400 million iterations in addition
560 to a burn-in of half a million iterations, sampling every 200000 iterations. All chains converged
561 and showed good mixing as indicated by their effective sample sizes of 2000 and visual
562 inspections of their traces in Tracer v1.6[96]. All analyses were run in triplicate and the three
563 independent chains converged on very similar solutions, leading to qualitatively similar results.
564 Here we present the results from the first chain. We ran RJ-MCMC Multistate analysis on
565 sexual system (Supplementary Table 3) as a binary state (gonochoristic or hermaphrodite) and
566 as a four state categorical variable (gonochorism, protandry, protogyny, simultaneous
567 hermaphroditism). Bidirectional sequential hermaphrodites were excluded from the latter
568 analysis as the sample size of extant species was too low so that the chains failed to converge
569 and mix properly when sexual system was studied as a five states categorical variable. For the
570 analyses with two character state, we graphed the evolutionary history of sexual systems on
571 the phylogeny using maximum likelihood (ML) in the R package Ape v.5.3[97], which provided
572 a reasonably close approximation of the RJ-MCMC Multistate results (this was not the case
573 with the four character state analysis).
574 We used phylogenetic generalized least square (PGLS) models[98–100] to test for the
575 association of each life history trait, entered as dependent variables, with sexual systems
576 entered as independent discrete variable with three possible states (gonochorism, protogyny,
577 protandry), as not enough data were available for simultaneous hermaphroditic species and
578 bidirectional sex-changers. PGLS models were run with the R package *caper*[101] in Maximum
579 Likelihood. The parameter λ of PGLS models quantifies the strength of the phylogenetic signal
580 in the model residuals[98]. λ ranges between zero (there is no phylogenetic structure in the data)
581 and one (the species share similarity in trait values directly proportional to their common
582 evolutionary time, under Brownian motion model of evolution[98,100]). Continuous variables
583 were $\log_{10}$-transformed to meet assumptions of normality.
584

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Author contributions

F.P. conceived the project. S.P., C.B., I.C., and F.P. designed the study. S.P. collected data with assistance from C.B. Data analyses were performed by S.P., C.B., and I.C. and S.P., C.B., I.C., and F.P. wrote the manuscript.

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Switches, stability and reversals: the evolutionary history of sexual systems in fish

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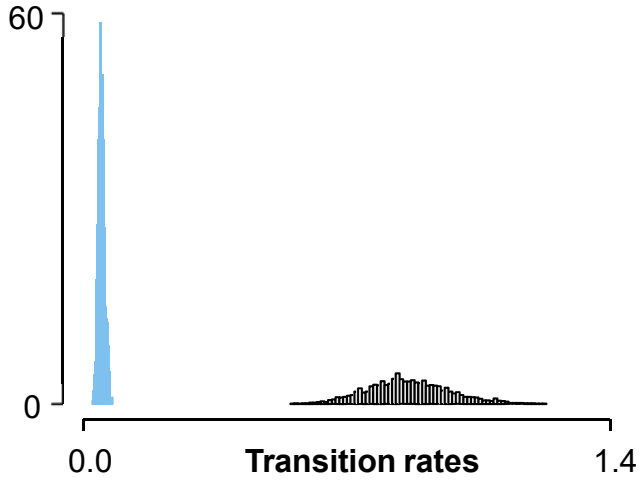
Supplementary information

- Supplementary figure (p 2)
- Supplementary tables (pp 3–5)
- Supplementary methods (p 6)
- Supplementary references (pp 7–27)
- Supplementary data are attached as an Excel file

Supplementary figure



Supplementary Fig. 1. RJ-MCMC Multistate posterior distributions of the transition rates (two character state) from gonochorism (n = 4337 species) to hermaphroditism (n = 403 species) in light blue and from hermaphroditism to gonochorism in grey.

Supplementary tables

Supplementary Table 1. Results of the RJ-MCMC Multistate analysis in BayesTraits of sexual systems as a binary trait: gonochorism (G) or hermaphroditism (H). For each posterior distribution, we report the effective sample size (ESS), the mean and 95% high posterior density (95-HPD) intervals, the mode, and the percentage of models in which the parameter is estimated as zero. This analysis is based on 4740 extant teleost species (G: n = 4337; H: n = 403)

Transition rates	ESS	Mean	95-HPD	Mode	% Zero
G → H	2000	0.047	0.032 – 0.065	0.045	0
H → G	2000	0.864	0.663 – 1.098	0.831	0
Root probabilities					
G	2000	63.8	57.3 – 70.7	64.5	0
H	2000	36.2	29.3 – 42.7	35.5	0

Supplementary Table 2. Sample sizes for longevity, maximum length, age at maturity and length at maturity for each sexual system with sex-specific male (♂) and female (♀), when data were available. In italics data not used in the analyses (due to the small male size). G = gonochorism; H = hermaphroditism; PG = protogyny; PA = protandry; SH = simultaneous hermaphroditism; BD = Bidirectional hermaphroditism

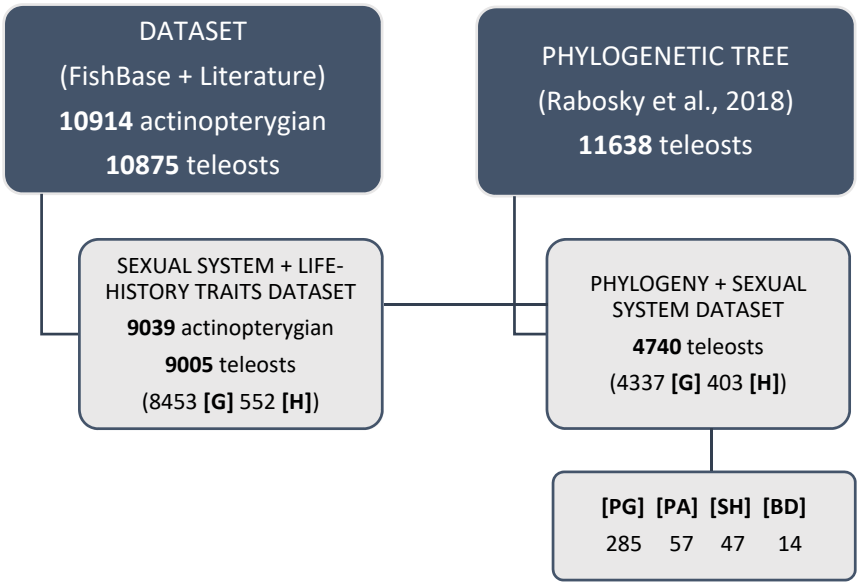
Life-history traits	Sex	G	PG	PA	Total for analyses	*SH*	*BD*
Longevity		759	88	19	866	*7*	*2*
Max length		2624	243	36	2903	*28*	*10*
Age maturity	♂	258	20	12	290	*1*	-
	♀	281	33	5	319	*2*	-
Length maturity	♂	303	50	18	371	*9*	-
	♀	295	43	13	351	*2*	-
GSI	♂	45	45	15	105	*3*	-

Supplementary Table 3. Number of species used to study the evolutionary transitions among different sexual systems, out of a total sample of 4740 teleosts. Note that androdioecious species are not included in the analyses

Sexual system	2 state [G, H]	4 state [G, PG, PA, SH]
Gonochorism [G]	4337	4337
Hermaphroditism [H]	403*	
Protogyny [PG]		285
Protandry [PA]		57
Simultaneous hermaphroditism [SH]		47
Total	4740	4726

*Includes 14 (13 verified against primary literature) species of bidirectional sequential hermaphrodites, which were not included in other analyses due to their small sample size as a separate category.

Supplementary methods



Data collection and verification The sexual system was extracted from FishBase (www.fishbase.org) and the classification of 403 hermaphroditic species was cross-checked against more recent primary literature: 67% of those species (i.e., more than two out of three) were confirmed to be functional hermaphrodites (see Supplementary Data and full references below).

The following life history traits were obtained from FishBase (www.fishbase.org; CD-ROM version) and updated with primary literature:

- Longevity (in years): also updated with data extracted with rfishbase (Boettiger et al., 2012; https://www.rdocumentation.org/packages/rfishbase/versions/3.0.4). When more than one value was present for species, we used the max value reported (in the wild);
- maximum length (TL: total length; in cm);
- sex-specific age (in years) and length (in cm) at maturity for each sex.

See supplementary Data for full dataset attached as an Excel file.

Supplementary references used to verify functional hermaphroditism in fish

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