

Twenty ways to lose your bladder: common natural mutants in zebrafish and widespread convergence of swim bladder loss among teleost fishes

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SUMMARY Convergence is an important evolutionary phenomenon often attributed solely to natural selection acting in similar environments. The frequency of mutation and number of ways a phenotypic trait can be generated genetically, however, may also affect the probability of convergence. Here we report both a high frequency of loss of gas bladder (swim bladder) mutations in zebrafish and widespread convergent loss of gas bladders among teleost fishes. The phenotypes of 22 of 27 recessive lethal mutations, carried by a sample of 26 wild-caught zebrafish, involve loss or noninflation of the gas bladder. Nine of these bladderless mutations showed no other obvious phenotypic abnormalities other than the lack of an inflated gas bladder. At least 19 of the 22 bladderless mutations are genetically distinct, as shown by unique morphology or complementation. Although we were not able to obtain eggs for all 21 required crosses to dem-

onstrate definitively that the remaining three mutations are different from all other bladderless mutations, all available evidence suggests that these mutants are also distinct. At least 79 of 425 families of extant teleosts include one or more species lacking a gas bladder as adults. Analysis of the trait's phylogenetic distribution shows that the gas bladder has been lost at least 30–32 times independently. Although adaptive explanations for gas bladder loss are convincing, a developmental bias toward bladderless phenotypes may also have contributed to the widespread convergence of this trait among teleosts. If gas bladder development in teleosts is as vulnerable to genetic perturbation as it is in zebrafish, then perhaps a supply of bladderless phenotypes has been readily available to natural selection under conditions for which it is advantageous not to have a gas bladder. In this way, developmental bias and selection can work together to produce widespread convergence.

INTRODUCTION

In the evolution of phenotypic diversity, the processes of sorting (selection, drift) and those which generate variation are equally important. However, when similar phenotypic traits are detected in different lineages, many evolutionary biologists address the role of natural selection in producing this convergence rather than investigating how similar phenotypic traits can be produced by different generative processes. Few interested in the distribution of characters on a phylogeny would consider the frequency that a particular mutation occurs in a population or the number of ways in which the same phenotypic trait may be generated genetically and the resultant frequency with which a particular phenotypic trait is therefore available to natural selection. Because natural selection can only affect existing variation, however, the frequency with which genetic and developmental processes generate a particular trait must also affect the probability of convergence. Whereas the generative processes underlying variation have been largely ignored by phylogeneticists (but see Brooks 1996) and many evolutionary biologists interested

in adaptation, understanding positive biases in the generation of variation is clearly a critical part of understanding evolutionary process (Waddington 1975; Gould 1980, 2002; Alberch 1982; Oster and Alberch 1982; Duboule and Wilkins 1998; West-Eberhard 1998; Wilkins 2002). Here we present data on gas bladder (swim bladder) mutations in zebrafish that suggest a macroevolutionary pattern of convergence may be attributable not only to the action of natural selection, but also to the frequency with which a phenotypic variant is exposed to the processes of selection.

Geneticists who work on zebrafish know well that individuals expressing a variety of different mutants, created through artificial mutagenesis, also often fail to develop a gas bladder (Haffter et al. 1996). Despite the functional importance of the gas bladder as a buoyancy organ, mutants lacking a gas bladder, absent other phenotypic abnormalities, have not been characterized (Haffter et al. 1996). Bladderless mutants in wild populations are fascinating in the context of vertebrate evolution because the gas bladder has been lost in many teleosts. Not having a gas bladder is generally considered adaptive for fishes living in benthic habitats (Steen 1970)

or deep sea environments (Marshall 1960) where fine-tuned buoyancy control may be irrelevant or even detrimental (e.g., because physiological control of gas bladder volume is too slow to accommodate rapid changes in depth).

Here we demonstrate that bladderless mutations are common in wild-caught zebrafish and that these multiple mutations are genetically distinct. At a macroevolutionary level, we compile data on loss of the gas bladder across all 425 families of extant teleosts. We show that the gas bladder has been lost independently in many lineages of teleosts that inhabit benthic or deep sea environments. We suggest that a macroevolutionary pattern of convergence in loss of gas bladders may be attributable not only to the action of natural selection, but also to the frequency with which a phenotypic variant involving loss of the gas bladder is exposed to the processes of natural selection.

After a brief note on terminology and background on the structure and function of the gas bladder, we present data on the frequency of bladderless mutations in wild-caught zebrafish. We then review the phylogenetic pattern of gas bladder loss in teleosts. Finally, we discuss the evolutionary implications of frequently occurring mutations resulting in the same phenotype.

Phylogenetic terminology

We follow what has become the conventional modern phylogenetic classification of vertebrates (Lauder and Liem 1983; Greene 1998) within which the Osteichthyes (traditionally and colloquially the bony fishes) includes two great clades (Rosen et al. 1981): (1) Sarcopterygii, the lobe-finned fishes, lungfishes, and all tetrapods (Lauder and Liem 1983; Nelson 1994; Cloutier and Ahlberg 1996), and (2) Actinopterygii, the ray-finned fishes, including bichir, sturgeon, paddlefish, gar, bowfin, and teleosts (Lauder and Liem 1983; Nelson 1994).

Background on the gas bladder

The gas bladder (also commonly known as the swim bladder) is a gas-filled sac that arises as an outpocketing of the foregut in actinopterygian (ray-finned) fishes and functions in many fishes as a hydrostatic organ. We follow Harder's (1975) preference for the term "gas bladder" rather than "swim bladder" because the functions of this gas-filled sac go well beyond buoyancy control and because the organ has more to do with being stationary than swimming. "Gas bladder" appears to be used more commonly in the post-1975 literature (by physiologists and ichthyologists) despite the nearly exclusive use of "swim bladder" in the zebrafish literature.

In basal actinopterygians (e.g., gars, bowfin) and a number of mostly basal teleosts (e.g., Osteoglossiformes, including fishes such as arawana and arapaima, tarpon), the gas bladder is vascularized and used in respiration (Helfman et al. 1998). Additionally, for some fishes the gas bladder may aid in

hearing by amplifying sound waves. Pressure waves are transmitted directly to the inner ear either by structural modifications of the bladder itself or by modified anterior vertebrae (Alexander 1970). In fact, zebrafish and 6000 of their closest relatives (the Otophysi) have such a connection made by modified vertebrae known as Weberian ossicles. Thus, the gas bladder in zebrafish functions in both buoyancy and hearing. In some lineages, the gas bladder is also involved in sound production (Ladich 1997).

In many relatively basal teleost fishes, such as the Ostariophysi (comprising approximately 6000 species of freshwater fishes and including the zebrafish, *Danio rerio*), the gas bladder is physostomous; that is, it retains a connection with the gut (Fink and Fink 1996). In zebrafish, inflation of the gas bladder occurs at about 4–5 days of development when fry gulp air at the surface of the water. The more derived condition in teleosts is that the connection between the gas bladder and gut is lost during ontogeny, resulting in a closed or physoclistous gas bladder. Addition and elimination of gas from the physoclistous gas bladder is achieved through the rete mirabile and ovale, respectively. The size and structure of the gas bladder varies considerably in different teleost lineages; some taxa, particularly those living in deep sea or benthic habitats, have lost the gas bladder altogether (Marshall 1960; Harder 1975).

MATERIALS AND METHODS

Characterization of mutations involving loss of the gas bladder in zebrafish

Frequency of bladderless mutations in zebrafish

We determined the frequency of recessive lethal mutations involving loss (or lack of inflation) of the gas bladder as part of a survey of all recessive lethals carried by a sample of wild-caught fish. Recessive lethals carried by parental fish were exposed in the F₂ generation using a classic genetic design (Timofeeff-Ressowsky and Timofeeff-Ressowsky 1927 in McCune et al. 2002). We first produced and reared 13 F₁ families derived from wild-caught parents. Within these 13 families, we mated brothers with sisters (3–11 sib pairs for each family) and scored the resulting F₂ offspring for mutant phenotypes, identified by abnormal morphology. With this design, recessive lethals present in the parental generation are revealed in the F₂ generation in a 3:1 ratio of normals to recessives (Fig. 1). Similar phenotypes exhibited by different sib crosses within a family were interpreted as being due to the same mutation. Mutations with similar phenotypes discovered in different families were subjected to complementation tests to determine genetic identity or distinctness (see below).

We obtained two batches of wild-caught parental zebrafish with the help of Asian Exports of Calcutta, India in March and June 2000. These zebrafish were collected from stagnant flood plains in their native Ganges River Basin, about 65 km east or 80 km north-east of Calcutta. Voucher specimens have been deposited in the Cornell University Museum of Vertebrates (CU 89303).

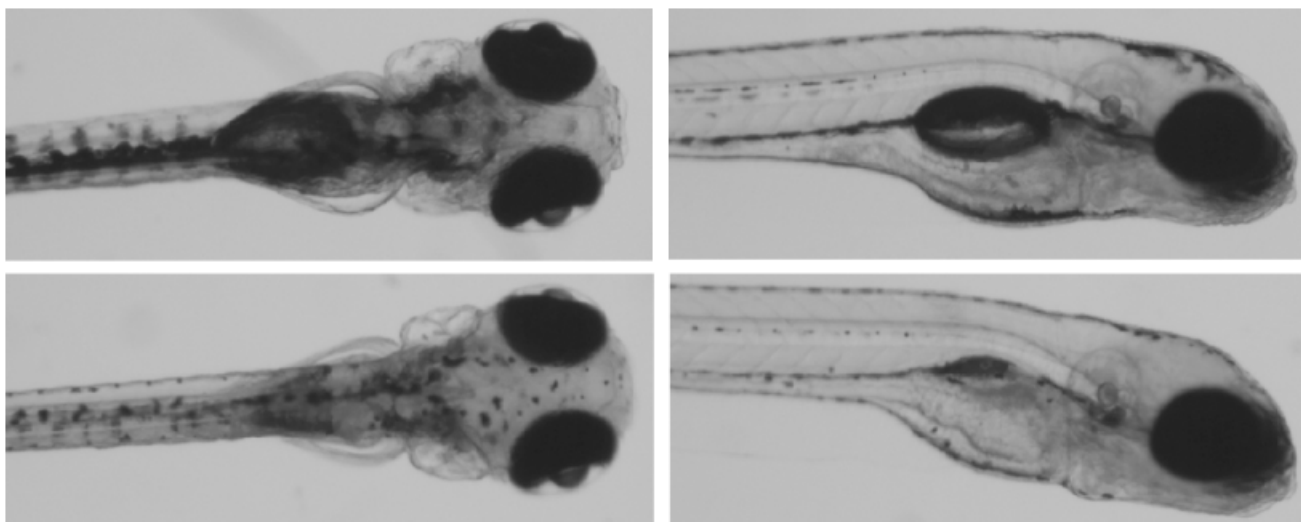


Fig. 2. Example of normal and bladderless individuals from the same clutch. (Left) Dorsal views of a normal phenotype (top) and a bladderless sibling expressing *exb* (bottom). (Right) Side views of the same normal individual (top) and bladderless (bottom) individual expressing *exb*. Magnification, 50 \times . (Photos by K. McMillan.)

Phylogenetic pattern of gas bladder loss in teleost fishes

We compiled data on the presence or absence of the gas bladder in the 425 extant families of teleosts using Nelson (1994) supplemented by the primary taxonomic literature. Although our survey is not exhaustive, it does provide a reasonable minimum estimate of the number of families in which the gas bladder is absent in at least one species (see Discussion). Even if the gas bladder is present in the larvae of a species, we considered the gas bladder to be absent if absent in the adults. Reduced gas bladders, found in a variety of fishes, were tallied as present. Within a given family, we considered the gas bladder to be absent if it is not present in at least one species. We did not attempt to detect multiple losses within a family because of the difficulty of obtaining reliable species-level phylogenies for many families. Thus, our estimate of the number of times the gas bladder has been lost in teleosts is certainly an underestimate.

Using MacClade (Maddison and Maddison 1997), we mapped the phylogenetic distribution of families in which the gas bladder is absent and assessed the minimum number of independent losses that could account for the observed distribution among these taxa. Character states were assumed to be unordered and equal in weight. When the gas bladder was absent in multiple closely related families, we counted a single loss for the most inclusive monophyletic group lacking a gas bladder until encountering a sister-group having a gas bladder.

RESULTS

Characterization of loss of gas bladder mutations in zebrafish

Of the 27 recessive lethal mutations detected in 26 individual wild-caught parental zebrafish, 80% (22/27 mutants) involved loss or noninflation of the gas bladder (Table 1). Other

features that characterized more than one mutant in the same data set were small eyes (6/27 mutants), curved spines (2/27 mutants), and absence of pectoral fin (2/27 mutants).

Of the 22 bladderless phenotypes, 13 involved various other drastic deformities (Table 1). Several sets of these drastic mutants appeared similar, but all five complementation tests between phenotypically similar mutants from different sibships (three combinations of *puo*, *ppo*, and *kno*; *sph* and *whr*; and *ved* and *blt*) produced only wild-type offspring.

Nine bladderless recessive lethals were not associated with other morphological deformities apparent on gross inspection (at 6–25 \times), although in some bladderless mutants (*bdd*, *btt*, *exb*, *lwt*; Table 1) we observed a transient chain of bubbles in the gut at approximately the time the gas bladder would ordinarily be filled. We are confident that these bladderless phenotypes are not the result of environmental effects because they did not occur in 16 control outcrosses (McCune et al. 2002).

To test whether phenotypically indistinguishable bladderless mutations found in different sibships were caused by the same alleles, we attempted complementation crosses of all 36 pairwise combinations between F₁ sibships carrying a bladderless mutation (sibships 1, 4, 7, 9, 10, 11, 13, 14, and 15) by crossing males and females from different sibships, both of which were known to carry a bladderless mutation (because as sib couples, they had produced bladderless offspring). We obtained eggs from 13 of 36 attempted crosses. All 13 crosses produced only wild-type individuals, showing that all 13 pairs of mutants tested complement each other and must affect different loci (Table 2).

Detailed morphological comparison of cleared and stained specimens of mutant and normal siblings revealed minor

Table 1. Presence/absence of the gas bladder and other phenotypic abnormalities caused by recessive mutations from wild-caught *Danio rerio*

Mutation (abbreviation)	Sibship	Gas Bladder	Other Phenotypic Abnormalities Detected by External Examination and Study of Cleared and Stained Specimens	Day First Seen
<i>anchor (anc)</i>	15	Absent	None	~ 4-5
<i>ballast (bll)</i>	13	Absent	Lower jaw narrow; notochord wavy	~ 4-5
<i>ballooneyes (boo)</i>	1	+	Edema around eyes	5
<i>bent (ben)</i>	7	Absent	None	~ 4-5
<i>big bladder (big)</i>	10	+	Gas bladder overfilled by day 5 and fish float at surface; by day 9, bladder has resumed normal size; a few big bladder individuals survived beyond day 20	5
<i>bladderless (bdd)</i>	9	Absent	Lower jaw narrow; notochord wavy	~ 4-5
<i>bloated (bli)</i>	13	+	Extensive edema ventrally, moves only intermittently	6-7
<i>blockhead (blk)</i>	13	Absent	Squared off head, eye and body edema	4
<i>bottom heavy (btt)</i>	4	Absent	None	~ 4-5
<i>candycane (cdc)</i>	14	Absent	Spine curved in candy cane shape; small eyes, heart edema; widespread edema	3
<i>darkguts (drk)</i>	1	+	Dark granular yolks; heart, eye, or body edema	1
<i>dividing unevenly (div)</i>	14	NA	Cells dividing unevenly (~1.5 h), yolk poking through cluster of cells at 5 h, dead by 24 h, long before gas bladder inflation	0
<i>extra bubbles (exb)</i>	14	Absent	None; name refers to transient air bubbles in the gut of mutants during the time that normals are filling their gas bladders	~ 4-5
<i>jaw deformity (jaw)</i>	13	Absent	Head stubby, snout does not project anterior to eyes, pronounced lower jaw, upper jaw not clear, lenses do not protrude from eyes, pectoral fins bent; edema around otic capsule	3
<i>jawless eyebulgers (jey)</i>	14	Absent	Small head, small eyes with large lenses, extensive eye edema, jaw small or absent, ventral edema, stubby tail	1
<i>kinky (knk)</i>	1	Absent	None	~ 4-5
<i>knockout (kno)</i>	10	Absent	Eyes small, head knobby anteriorly, lacks jaws, heart edema; lacks pectoral fins	2
<i>knucklehead (knu)</i>	3	Absent	Red spot on yolk; heart edema starting day 3; head flat anteriorly, head knobby dorsally, s-shaped spine	2
<i>large lens (lln)</i>	5	Absent	Eyes small with lens protruding from eye; jutting jaw develops by day 9	3-4
<i>lead weight (lwt)</i>	11	Absent	None	~ 4-5
<i>pseudopunchout (ppo)</i>	8	Absent	Eyes small and low on head; head knobby anteriorly; jaws and gill arches poorly defined or absent; s-shaped spine with poor differentiation of spinal column, somites, and muscles; pectoral fins absent; some grainy yolk still present day 5; poor circulation; head and heart edema	2
<i>punchout (puo)</i>	11	Absent	Eyes small; head knobby anteriorly; lacks jaws; gut poorly developed; heart and eye edema; head and yolk grainy on day 1	1
<i>sinker (skr)</i>	10	Absent	Pigmentation reduced; Meckel's cartilage and ceratohyal bent; notochord wavy; palatoquadrates and ceratohyals too close together	~ 4-5
<i>spirograph (sph)</i>	8	Absent	Spine curved, almost circular; moves by spinning in circles; develops severe head edema; pectoral fins absent	3
<i>stiffjaw (sff)</i>	14	Absent	Jaw appears to be frozen open; eyes and head slightly reduced; lower jaw curved and projects anterior to upper jaw; pigmentation reduced; some develop edema before death	3
<i>ventral edema (ved)</i>	8	Absent	Edema extensive ventrally, moves only intermittently	5
<i>whirly (whr)</i>	13	Absent	Almost circular curved spine; moves or spins in circles	2

Note that the gas bladder is present (or inflated) in 4 mutations and absent in 22 mutations. One mutation was lethal before the bladder would normally inflate. Mutants are registered at www.zfin.org, and photographs of many are included in the Zfin database. Fish carrying many of the mutants have been deposited in the Zebrafish International Resource Center at the University of Oregon.

skeletal deformities associated with three bladderless mutants. *Bladderless (bdd)* and *ballast (bll)* had unusually narrow lower jaws. *Sinker (skr)* had a deformed Meckel's cartilage and

ceratohyal. The palatoquadrates and ceratohyals in *bdd* mutants were abnormally close together. *Bdd*, *bll*, and *skr* mutants also had wavy notochords. Discovery of skeletal

Table 2. Results of complementation tests for bladderless-only mutations from nine different sibships

<i>knk</i> (1)	<i>btt</i> (4)	<i>ben</i> (7)	<i>bdd</i> (9)	<i>skr</i> (10)	<i>lwt</i> (11)	<i>bll</i> (13)	<i>cdc</i> (14)	<i>anc</i> (15)	Allele (sibship)
—	—	—	<i>bdd</i>	0 (117)	—	0 (84)	0 (67)	0 (50)	<i>knk</i> (1)
—	—	—	<i>bdd</i>	0 (39)	—	<i>bll</i>	0 (115)	—	<i>btt</i> (4)
—	—	—	<i>bdd</i>	<i>skr</i>	—	<i>bll</i>	—	—	<i>ben</i> (7)
—	—	—	—	0 (47)	<i>bdd</i>	<i>bdd</i>	<i>bdd</i>	<i>bdd</i>	<i>bdd</i> (9)
—	—	—	—	—	<i>skr</i>	0 (44)	0 (84)	<i>skr</i>	<i>skr</i> (10)
—	—	—	—	—	—	<i>bll</i>	0 (14)	0 (30)	<i>lwt</i> (11)
—	—	—	—	—	—	—	<i>bll</i>	0 (39)	<i>bll</i> (13)
—	—	—	—	—	—	—	—	0 (63)	<i>cdc</i> (14)
—	—	—	—	—	—	—	—	—	<i>anc</i> (15)

We attempted to cross all 36 pairwise combinations of fish from different sibships carrying bladderless mutations. Eggs were obtained from 13 pairwise combinations, all of which produced wild-type offspring. For these pairs, the number of bladderless individuals relative to clutch size is given in parentheses. Thus, for the cross *skr* × *knk*, 0/117 indicates that no individuals were bladderless in a clutch of 117 fertilized eggs. Of the remaining 23 possible pairwise combinations, 14 pairwise combinations of bladderless-carrying parents from different sibships were inferred not to be carrying the same bladderless mutation because a skeletal deformity was discovered in the phenotype of a bladderless mutant from one of the families. For these combinations, the abbreviation for the mutant carrying the additional deformity is given in the appropriate cell of the matrix (see text and Table 1 for morphological detail). Shaded, blank cells show the nine pairwise combinations for which no eggs have been obtained, and the bladderless mutants from these two families could not be distinguished by skeletal abnormalities. Note that all but one of these crosses involve either sibship 4 or 7 (light gray shading). If these two mutants are eliminated from the tally of genetically distinct bladderless mutants, then it is clear that there are six bladderless-only mutations (*bdd*, *skr*, *lwt*, *bll*, *cdc*, and *anc*) that are genetically distinct. Available evidence (for 5/6 of requisite crosses and morphological comparisons) suggests that *knk* might also be distinct. Thus, the 6–7 distinct bladderless-only mutants, plus the 13 bladderless mutants with drastic abnormal morphologies, sum to 19–20 genetically distinct mutations involving loss of the gas bladder.

deformities associated with *bdd*, *bll*, and *skr* and not with other bladderless mutations demonstrated distinctiveness of bladderless mutants for an additional 14 pairwise combinations (Table 2).

Despite repeated attempts to obtain eggs from the appropriate pairs, we failed to obtain eggs or evidence of morphological distinctiveness for 8 of the 15 required pairwise comparisons involving *btt* (sibship 4) and *ben* (sibship 7). We also failed to obtain eggs for one of six additional crosses required to show the distinctiveness of *kinky* (Table 2).

Combined results of complementation tests and detailed morphological study show with certainty that 19 of these bladderless mutants (*bdd*, *skr*, *lwt*, *bll*, *cdc*, *anc* plus the 13 with dramatic morphological abnormalities) are genetically distinct. Although we failed to obtain complete complementation data for the remaining three mutants (*knk*, *btt*, *ben*), available evidence suggests these mutants are also distinct (see Discussion).

Phylogenetic distribution of gas bladder loss in teleosts

Gas bladder loss occurred in 9 of the 14 extant teleost superorders. In 79 of the 425 extant families of teleost fishes, the gas bladder was absent in at least one species (Table 3). In most families there was either a single species or genus lacking a gas bladder or the bladder was absent in all members of the family. In 25 families, however, there were multiple species in at least two genera that lacked the gas bladder, suggesting the

possibility of multiple independent losses within a family. Interpreted in a phylogenetic context, the gas bladder was lost independently between 30 and 32 times within teleosts (Fig. 1). This estimate excludes multiple losses within families (including within-family losses, known to us even without a survey, would likely increase the number of independent losses to 57 or more). Whether the minimum number of independent losses is 30 or 32 depends on which of two equally parsimonious optimizations (two independent losses versus one loss and a subsequent reversal) was used for two cases. The first case occurred within the Lampridiformes and the second case depended on whether gas bladder loss occurred independently in the Cyclosquamata and Scopelomorpha or whether the gas bladder was lost at the level of Cyclosquamata+all more derived superorders and then gained for Lampridomorpha+all more derived superorders (Fig. 3).

Most taxa (60 families) that lack a gas bladder are either benthic (live on or in the bottom) or deep sea fishes (Table 3). The 19 families that are neither benthic nor deep sea are either nested within clades that are entirely bladderless or have lifestyles that are not compatible with having a gas bladder (see Discussion).

DISCUSSION

Bladderless mutations in zebrafish

We found that mutations involving loss or noninflation of the gas bladder occur commonly in wild-caught zebrafish.

Table 3. Families of teleost fishes that have lost the gas bladder

Taxon	Common Name	Ecology	Reference(s)
Elopomorpha			
Saccopharyngiformes ¹			
Cyematoidei			
Cyematidae	Bobtail snipe eels	Bathypelagic	Nelson 1994
Saccopharyngoidei			
Saccopharyngidae	Swallowers	Meso- and bathypelagic	Nielsen and Bertelsen 1985; Nelson 1994
Eurypharyngidae	Gulpers	Deep sea	Nielsen et al. 1989; Nelson 1994
Monognathidae	—	Deep sea	Nelson 1994
Ostariophysi			
Gonorhynciformes			
Gonorhynchoidei			
Gonorhynchidae	Beaked sandfishes	Benthic?	Lauder and Liem 1983
Ictaluridae ²	North American catfishes	Artesian wells	Fink and Fink 1996
Protacanthopterygii			
Osmeriformes			
Argentinoidei			
Bathylagidae	Deep sea smelts	Surface to bathypelagic	Ahlstrom et al. 1984; Kobylanskiy 1985
Opisthoproctidae ³	Barreleyes	Deep sea	Kobylanskiy 1985; Nelson 1994; Paxton and Eschmeyer 1998
Leptochilichthyidae	—	Deep sea	Nelson 1994; Paxton and Eschmeyer 1998
Alepocephalidae	Slickheads	Below 1000 m	Nelson 1994; Paxton and Eschmeyer 1998
Platyroctidae	—	Between 300 and 1000 m	Nelson 1994; Paxton and Eschmeyer 1998
Stomiformes			
Gonostomidae ⁴	Bristlemouths	Deep sea	Alexander 1993
Cyclosquamata ¹			
Aulopiformes			
Giganturoidei			
Giganturidae	Telescopefishes	Pelagic to bathypelagic	Johnson 1984a; Nelson 1994; Paxton and Eschmeyer 1998
Aulopoidei			
Aulopodidae	Aulopus	Benthic	Okiyama 1984; Nelson 1994
Chlorophthalmoidei			
Chlorophthalmidae	Greeneyes	Benthic	Okiyama 1984; Nelson 1994
Ipnopidae	—	Deep-sea benthic	Okiyama 1984; Nelson 1994
Scopelarchidae	Pearleyes	Oceanic/meso- and bathypelagic	Johnson 1984b; Nelson 1994
Notosudidae	Waryfishes	Oceanic midwater	Okiyama 1984; Nelson 1994
Alepisauroidi			
Synodontidae	Lizardfishes	Reef associated/benthic/ depths > 400 m	Robins and Ray 1986; Nelson 1994
Pseudotriconotidae	—	Pelagic to bathypelagic	Nelson 1994
Paralepididae	Barracudinas	Oceanic pelagic	Okiyama 1984; Nelson 1994
Anopteroidei	Daggertooths	Pelagic to bathypelagic	Nelson 1994
Evermannellidae	Sabertoothfishes	Mesopelagic	Nelson 1994
Omosudidae	—	Mesopelagic	Okiyama 1984; Nelson 1994
Alepisauridae	Lancetfishes	Pelagic to bathypelagic	Nelson 1994
Scopelomorpha ¹			
Myctophiformes			
Neoscopelidae ⁵	—	Deep oceanic midwaters	Nafpaktitis 1977; Nelson 1994
Myctophidae ^{6,7}	Lanternfishes	Deep sea pelagic/ benthipelagic	Nafpaktitis et al. 1977; Robins and Ray 1986; Nelson 1994
Lampridiomorpha			
Lampridiformes			
Stylephoridae	Tube-eyes	Between 300 and 800 m	Nelson 1994
Trachipteridae	Ribbonfishes	Epipelagic to abyssal depths	Nelson 1994; Wiley et al. 1998

Table 3. (Contd.)

Taxon	Common Name	Ecology	Reference(s)
Regalecidae	Oarfishes	Epipelagic to abyssal depths	Nelson 1994; Wiley et al. 1998
Paracanthopterygii			
Ophidiiformes			
Bythitoidei			
Aphyonidae	—	Between 2000 and 6000 m/benthic	Nelson 1994; Paxton and Eschmeyer 1998
Gadiformes			
Macrouridae ⁸	Rattails	Slope dweller	Merret et al. 1983; Nelson 1994
Melanonidae ⁹	Pelagic cods	Deep sea	Chiu and Markle 1990; Nelson 1994
Lophiiformes			
Antennarioidei			
Antennariidae ¹⁰	Frogfishes	Benthic	Robins and Ray 1986; Nelson 1994
Percomorpha			
Stephanobercyiformes			
Rondeletiidae	Redmouth whalefishes	Bathypelagic	Nelson 1994
Barbourisiidae ⁷	Red whalefishes	Bathypelagic	Nelson 1994
Cetomimidae	Flabby whalefishes	Bathypelagic	Nelson 1994
Mirapinnidae ⁷	Mirapinnids	Bathypelagic	Nelson 1994
Megalomycteridae	Largenose fishes	Bathypelagic	Nelson 1994
Gasterosteiformes			
Syngnathoidei			
Pegasidae	Seamoths	Benthic	Masuda et al. 1984; Nelson 1994
Scorpaeniformes			
Scorpaenoidei			
Scorpaenidae ¹¹	Scorpionfishes	Deep sea	Eschmeyer et al. 1983; Nelson 1994
Hexagrammoidei ¹			
Hexagrammidae	Greenlings	Shallow and benthic, up to 1830 m	Nelson 1994; Paxton and Eschmeyer 1998; Dean et al. 2000
Cottoidei			
Cottidae ¹²	Sculpins	Benthic	Nelson 1994; Dean et al. 2000
Agonidae	Poachers	Benthic	Robins and Ray 1986; Nelson 1994; Paxton and Eschmeyer 1998
Cyclopteridae ¹³	Lumpfishes	Benthic	Alexander 1993
Perciformes			
Percoidei			
Percidae ¹⁴	Perches	Benthic	Nelson 1994
Sillaginidae ⁶	Sillagos, whittings	Relatively shallow water	Nelson 1994
Echeneidae	Remoras	Reef associated/pelagic	Strasburg 1964
Pempferidae ¹⁵	Sweepers	Reef associated	Bohlke and Chaplin 1993; Nelson 1994
Zoarcoidei			
Zoarcaeidae	Eelpouts	Benthic	Robins and Ray 1986; Nelson 1994
Notothenioidei ¹			
Bovichthyidae	Temperate icefishes	Benthic/pelagic	Hardy 1988; Nelson 1994
Nototheniidae	Cod icefishes	Benthic/pelagic	Eastman 1991; Nelson 1994; Gutt and Ekau 1996
Harpagiferidae	Plunderfishes	Benthic	Wyanski and Targett 1981; Eastman 1991; Nelson 1994
Bathydraconidae	Antarctic dragonfishes	Benthic/deep sea	Eastman 1991; Nelson 1994; Gutt and Ekau 1996
Channichthyidae	Crocodile icefishes	Pelagic	Eastman 1991, 1999; Nelson 1994; Gutt and Ekau 1996
Trachinoidei			
Ammodytidae	Sand lances	Burrowers	Robins and Ray 1986; Nelson 1994
Blennioidei			
Blenniidae ¹⁶	Combtooth blennies	Benthic	Robins and Ray 1986; Nelson 1994
Gobiesocodoidei ¹			
Gobiesocidae	Clingfishes	Benthic	Nelson 1994

Table 3. (Contd.)

Taxon	Common Name	Ecology	Reference(s)
Gobioidei ¹			
Rhyacichthyidae ¹⁷	Loach gobies	Benthic	Nelson 1994
Odontobutidae ¹⁷	—	Benthic/pelagic	Nelson 1994
Eleotridae ¹⁷	Sleepers	Benthic/midwater	Merrick and Schmida 1984; Nelson 1994
Gobiidae ¹⁷	Gobies	Benthic	Robins and Ray 1986; Nelson 1994
Kraemeriidae ¹⁷	Sand gobies	Burrowers	Nelson 1994
Xenisthmidae ¹⁷	—	Benthic	Gill and Hoese 1993; Nelson 1994
Microdesmidae ¹⁷	Wormfishes	Reef associated/benthic	Robins and Ray 1986; Nelson 1994
Schindleriidae ¹⁷	—	Oceanic	Nelson 1994
Scombroidei			
Scombridae ¹⁸	Mackerels, tunas	Oceanic, deep diver	Gibbs and Collette 1967; Collette and Chao 1975; Gee 1983; Collette and Russo 1985; Collette and Gillis 1992; Alexander 1993; Carpenter et al. 1995
Pleuronectiformes ¹			
Psettoidoidei			
Psettodidae ¹⁹	Psettodids	Benthic	Nelson 1994
Pleuronectoidei			
Citharidae ¹⁹	Ciharids	Benthic	Nelson 1994
Bothidae ¹⁹	Lefteye flounders	Benthic	Nelson 1994
Achiropsettidae ¹⁹	Southern flounders	Benthic	Nelson 1994
Scophthalmidae ¹⁹	—	Benthic	Nelson 1994
Paralichthyidae ¹⁹	—	Benthic	Nelson 1994
Pleuronectidae ¹⁹	Righteye flounders	Benthic	Nelson 1994
Samaridae ¹⁹	—	Benthic	Nelson 1994
Achiridae ¹⁹	American soles	Benthic	Nelson 1994
Soleidae ¹⁹	Soles	Benthic	Nelson 1994
Cynoglossidae ¹⁹	Tonguefishes	Benthic	Nelson 1994
Tetradontiformes			
Tetradontoidei			
Molidae	Ocean sunfishes	Pelagic	Nelson 1994

All members of the listed families lack gas bladders except as noted (footnotes 2–19). Ecological notes apply only to those members of each family that lack a gas bladder.

¹Higher taxa in which the gas bladder has been lost in all families.

²Absent in *Trogloglanis* only.

³Absent in some genera.

⁴Absent in *Gonostoma elongatum* only.

⁵Absent in *Scopelengys* only.

⁶Absent in adults of a few species.

⁷Present in juveniles; regresses in adults.

⁸Absent in *Squalogadus* only.

⁹Absent in *Melanomus* only.

¹⁰Absent in subfamily Tetrabrachiinae and some Antennaridae.

¹¹Absent in *Plectrogenium* and *Sebastolobus* as well as subfamilies Minoinae, Choridactylinae, and Synanceinae.

¹²Absent in adults.

¹³Absent in *Cyclopterus* only.

¹⁴Absent in tribe Etheostomatini.

¹⁵Absent in *Pempheris poeyi* only.

¹⁶Absent in all genera except *Phenablennius*, *Omox*, and *Nemophil*.

¹⁷Absent in most species.

¹⁸Absent in mackerel, *Scomber scombrus*; in tunas, absent in *Thunnus tonggol*, degenerates with growth in *Euthynnus*, *Auxis*, and *Katsuwonus*; present in all bonitos except *Gymnosarda*; present in basal-most Spanish mackerel, *Scomberomorus sinensis* but absent in other 17 congeners.

¹⁹Absent in adults of most species.

Twenty-two of the 27 mutations recovered from wild-caught zebrafish involve loss of the gas bladder, and 9 of this bladderless class of mutations involve, at most, very slight morphological manifestations of a mutation other than the

absence (or noninflation) of the gas bladder. Despite the phenotypic similarity of these mutant phenotypes, all complementation crosses yielding eggs demonstrated that bladderless mutations in different sibships are genetically

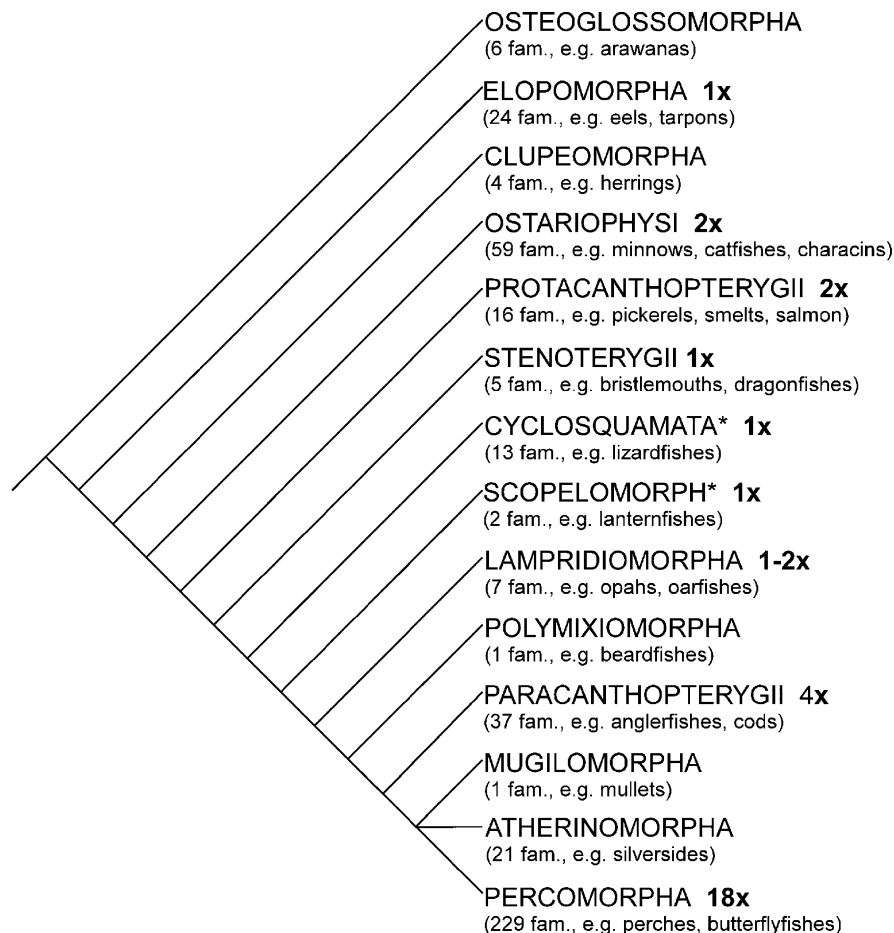


Fig. 3. Phylogenetic distribution of gas bladder loss in teleosts. Phylogeny is based on classifications in Lauder and Liem (1983), Nelson (1994), and Helfman et al. (1998). An asterisk indicates that all members of the superorder lack a gas bladder. Note that for Scopelomorpha and Cyclosquamata we have represented the loss of the gas bladder as two independent events. An equally parsimonious optimization would involve loss of the gas bladder at Cyclosquamata (their sister-group, the Stenopterygii, have gas bladders) and a gain of the gas bladder at Lampridomorpha. Multiple independent losses of the gas bladder below the family level, although known to occur, were not tallied.

distinct. Our data show that a minimum of 19–20 of these mutants are genetically distinct. Although we were only able to obtain eggs for half the requisite complementation tests needed to demonstrate that *btt* and *ben* are different from all the other eight bladderless-only mutants, all crosses yielding eggs produced only wild-type offspring. The fact that no individual lethal has been found in multiple sibships of this wild population demonstrates that particular lethals are rare and indicates a high effective population size with little inbreeding (Powell 1997; McCune et al. 2002). With such a large source population, we expect that the three bladderless mutants, for which we have only partial complementation data, are also rare and distinct mutants.

At this time, it is unclear what aspects of the development of the gas bladder make it so vulnerable to genetic disruption. We also do not know for any of the bladderless phenotypes whether they are due to absence or noninflation of the gas bladder; this would require histological study. However, absence and noninflation are functionally equivalent. Neither condition permits known functions of the gas bladder, including buoyancy, air breathing, or the production or reception of sound.

All the bladderless mutations in this study are lethals. Thus, the mutations we have identified are not the actual mutations that have led to loss of the gas bladder in living teleosts. However, because lethal mutations are a subset of all deleterious mutations, we suggest that if there are many lethal ways to disrupt gas bladder development, then there are likely to be many nonlethal ways to disrupt gas bladder development as well. In fact, we know that there must be nonlethal ways to disrupt gas bladder development because this has occurred a minimum of 30–32 times over the course of teleostean evolution.

Phylogenetic pattern of gas bladder loss in teleosts

The fact that at least one species in 79 of the 425 extant teleostean families lacks a gas bladder reminds us that the absence of a gas bladder itself does not need to be physiologically or ecologically lethal. Many teleosts make a successful living without a gas bladder. Based on the phylogenetic distribution of the 79 families lacking a gas bladder, we estimate that the gas bladder has been lost within teleosts a

minimum of 30–32 independent times. This number is an underestimate for two reasons. First, our tally is based primarily on Nelson's (1994) *Fishes of the World*, which does not purport to be comprehensive on the distribution of any particular character, including the absence of the gas bladder. When the compilation notes that a particular clade lacks a gas bladder, we can be confident that this is the case. In cases where there is no comment on the gas bladder, it may be because the taxon retains a gas bladder or simply that gas bladder presence/absence was not reported in the literature summarized. We know of a few specific cases (e.g., scombrids, eyeless catfish, *Gonostoma elongatum*, *Cyclopterus*) where the known absence of the gas bladder was not noted in Nelson (1994) and expect there are others. Second, we consider our tally to be a minimum estimate because we did not consider multiple losses within families. When all species in a given family lack the gas bladder (e.g., Synodontidae [lizardfishes]) or when one species lacks the gas bladder (e.g., *Pempheris poeyi*, Pempheridae [sweepers]), we counted a single loss. However, we also counted a single loss for the 25 families in which multiple, but not all, species lack the gas bladder even though we know that the gas bladder was lost more than once in at least some of these families. For example, the gas bladder has been lost independently at least four to five times within the Scombridae (e.g., tunas and mackerels; Gibbs and Collette 1967; Collette and Chao 1975; Collette and Russo 1985; Collette and Gillis 1992; Carpenter et al. 1995). If we were to include the 25 instances of multiple losses within families known to us even without having done a within-family survey, our minimum estimate could increase to 57 independent losses or more independent losses. Thus, we are confident that our estimate of 30–32 independent losses of the gas bladder is very much a minimum. The actual number of independent losses could be several times greater.

It has been argued that the most frequently lost feature during teleost evolution is the pelvic fin (Nelson 1989–1990). Absence of the pelvic fin, a condition that can easily be assessed in photos and drawings, occurs in one or more species of 92 teleostean families; the trait has been lost about 50 independent times, excluding multiple losses within families (Nelson 1989–1990). Despite the inherent difficulty of detecting loss of an internal feature like the gas bladder (which requires internal anatomical study) and the fact that our estimate (79 families and at least 32 independent losses) is very much a minimum, the high frequency of loss of pelvic fins and loss of gas bladder is remarkably similar.

Adaptive scenarios for loss of the gas bladder

Most of the 79 families of teleosts that include at least one species lacking a gas bladder are either benthic or deep sea fishes. Sixteen families include one or more species lacking a gas bladder but are neither benthic nor deep sea (e.g., catfishes

[Ictaluridae], smelt-whittings [Sillaginidae], sharksuckers [Echeneidae], sweepers [Pempheridae], lizardfishes [Synodontidae], wormfishes and dartfishes [Microdesmidae], waryfishes [Notosudidae], barracudinas [Paralepididae], Bovichthyidae [no common name, related to icefishes], icefishes [Nototherniidae], crocodile icefishes [Channichthyidae], Odontobutidae [no common name], sleepers [Eleotridae], Schindleriidae [no common name], tunas [Scombridae], and molas [Molidae]). These taxa apparently lack the gas bladder for other reasons. Some taxa that are neither benthic nor deep sea may lack the gas bladder because the trait is ancestral. For example, all notothenioids (icefishes) lack the gas bladder, but within this group, several nototheniids and bovichthyids, plus all channichthyids, are pelagic or dwell in shallow water. These taxa, apparently derived from bottom-dwelling species, have not regained the gas bladder (Ekau 1991). Instead, they have achieved a lower overall density and neutral buoyancy by retaining a cartilaginous skeleton (cartilage is nearly half the density of bone and only slightly greater than seawater) and increasing fat deposits (Ekau 1991), a common alternative to buoyancy control via a gas bladder (Gee 1983; Alexander 1993). Similarly, a few pelagic cyclosquamates, including several notosudids and paralepids, as well as some gobioids, such as schindleriids, eleotrotids, and odontobutids, may lack the gas bladder because it is an ancestral characteristic in that clade.

Although remoras (echeneids) are neither benthic nor deep sea dwellers, they may lack the gas bladder for ecological reasons. Remoras often attach to other marine animals (e.g., sharks, turtles, and whales) that move rapidly up and down in the water column. With this lifestyle, buoyancy control is irrelevant and potentially physiologically impossible. No living fish is capable of adding and eliminating gas through the pneumatic duct of a gas bladder fast enough to accommodate daily vertical migrations, let alone the rapid depth changes made by the remora's hosts. Likewise, in tunas the gas bladder is sometimes reduced or absent (Collette 1978). These fishes not only make rapid vertical movements, they also swim at the surface at high speed. It has been argued that it is energetically disadvantageous to have a gas bladder above a threshold swimming speed; scombrids often exceed this threshold (Alexander 1993). Some reef-associated taxa, including pempherids and sillaginids, are not benthic per se but do dwell in holes and crevices (Bohlke and Chaplin 1993) where having a gas bladder might be disadvantageous. As apparently pelagic fishes and members of a clade that otherwise have gas bladders, molas may be potentially perplexing because of their lack of a gas bladder. Like many other fishes lacking a gas bladder, the overall density of molids is relatively low due to their having a poorly ossified largely cartilaginous skeleton. Interestingly, new data from satellite tagging show that a mola may make as many as 40 dives to 600 m each day (Tierney Thys, personal communication). The

presence of a gas bladder would clearly hamper or preclude this behavior.

Convergence and developmental bias

Convergence is exceedingly common and has engendered considerable interest, albeit for very different reasons, among evolutionary biologists who study natural selection and adaptation, evolutionary developmental biology, or phylogenetics (Wake 1996). For evolutionary biologists focused on natural selection, convergence is usually attributed to adaptation by natural selection in similar environments. An alternative (but not mutually exclusive) hypothesis is that convergence may also reflect developmental constraint or bias in developmental systems (Gould 1980; Alberch 1982; Maynard Smith et al. 1985; for recent reviews see Gould 2002; Wilkins 2002).

Here we demonstrate a bias in the occurrence of natural phenotypic variants, involving loss of the gas bladder, in wild-caught zebrafish. In our sample, 22 of 27 mutants involved loss of the gas bladder, whereas other morphological traits found in more than one mutant occurred either six times (small eyes) or twice (curved spine; pectoral fins absent). The high frequency of gas bladder variants is paralleled at a higher taxonomic level by a strikingly high incidence of convergent gas bladder loss in teleost fishes. Eye size is also variable across teleosts, and pectoral fin loss has occurred several times (e.g., in the families Monognathidae, Nettastomatidae, Idiacanthidae). However, neither of these traits has been lost as frequently as the pelvic fins.

The generality of common gas bladder mutations and convergent loss of the gas bladder at higher taxonomic levels may extend beyond actinopterygian fishes into the sarcopterygian clade. If the gas bladder of ray-finned fishes and the lungs of sarcopterygians, including tetrapods, are homologous (Romer 1970; Rosen et al. 1981), then the reduction of one lung in snakes, caecilians, and amphibia; the loss of both lungs once in the caecilian, *Atretochoanba*; and the multiple occurrences of lung loss in salamanders (e.g., all plethodontids, three salamandrids, *Chioglossa*, *Salamandrina*, *Euproctus*, and a hynobiid, *Onchodactylus*) (Pough et al. 2001; Larson et al. 2003), might also have been facilitated by frequent mutations to the same (homologous) genetic-developmental system.

Based on this example of gas bladder loss in zebrafish and a macroevolutionary pattern of convergent losses of the gas bladder in many taxa, we suggest the following. First, a bias in developing systems at the organismal level may occur when the same phenotypic trait can be generated in multiple ways genetically. This is a developmental bias, not a mutational bias, because multiple genetic mutations have the same phenotypic result. It is striking that 80% of the mutants recovered from wild-caught zebrafish involved loss of the gas

bladder. Nine of these were essentially indistinguishable phenotypically, but there is no evidence of genetic identity for any of these mutants. Second, when the same phenotype results from multiple genetic pathways, this phenotypic variant will be more often exposed to natural selection, thus increasing the probability that some genetic version of the phenotypic trait could become fixed within a species. Third, if a phenotypic trait has potential adaptive value, the frequency with which a phenotypic trait occurs in a population/species will affect the probability of convergence at the macroevolutionary level because that particular phenotype will more often be exposed to natural selection. We suggest that widespread convergence of gas bladder loss in different teleost lineages may not be due to selection alone; multiple mutations yielding the same phenotypic variant can produce developmental pressure (analogous to mutation pressure) that would increase the general availability of the phenotype for possible sorting by selection. Only under circumstances where having a gas bladder is disadvantageous would a bladderless mutant become fixed in a population. Adaptation and developmental bias are not mutually exclusive alternatives but can work together to produce widespread convergence.

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