Chapter 19
Sperm-Dependent Parthenogenesis and Hybridogenesis in Teleost Fishes

Dunja K. Lamatsch and Matthias Stöck

> *… there is always some danger in using sperm: even if you do not want, you run the risk of being fertilized… (Dubois 1990)*

Abstract In so-called unisexual teleost fishes, a broad spectrum of evolutionary stages with varying amounts of sexual elements has evolved. These range from pure sperm-dependent parthenogenesis (gynogenesis) without or with different amounts of paternal leakage to hybridogenesis with hemiclonal diploid gametogenesis or genome elimination followed by meiosis (meiotic hybridogenesis). All of these phenomena are of hybrid origin.

Many of these fish form complexes which involve the coexistence of one or more sexually reproducing species with derived all-female forms that have various ploidy levels and reproductive modes, including gynogenesis, (meiotic) hybridogenesis and sexual reproduction. In teleosts, parthenogenetic reproduction is strictly dependent on sperm to initiate embryonic development. As opposed to true parthenogenesis, sperm-dependent parthenogenetic teleost lineages must primarily coexist with their "sperm donor", usually males from a parental sexual lineage or from a related sexual species. In some systems, gynogens were able to escape from their initial sperm donors ("host switch") and therefore, to enlarge their ranges and ecological niches. Sperm donors normally do not contribute genetically to the next generation. However, paternal leakage is observed in many systems contributing differing amounts of genetic material (from microchromosomes to entire chromosome sets) allowing interaction between genomes of different origin. Hybridogenesis is similar to gynogenesis in depending upon coexistence with sexual species but incorporates recombined genetic material by true fertilization. While hybridogens usually form clonal gametes, some triploids are capable of genome elimination followed by a normal diploid meiosis. Sperm-dependent parthenogenesis and hybridogenesis

---

D.K. Lamatsch (✉)
Institute for Limnology, Austrian Academy of Sciences, Mondseestrasse 9, A – 5310 Mondsee, Austria
e-mail: dunja.lamatsch@oeaw.ac.at

I. Schön et al. (eds.), *Lost Sex*, DOI 10.1007/978-90-481-2770-2_19,

combine disadvantages and advantages from both sexuality and asexuality. Here, we give an overview of sperm-dependent breeding complexes in fishes, discuss the evolutionary consequences of paternal leakage, and speculate about the evolutionary significance of intergenomic (re)combination.

19.1 Introduction

Fifteen years ago, the topic "unisexual fish" and their evolution have been reviewed to a great completeness (Vrijenhoek 1994). While the total number of newly discovered teleost complexes with unisexual bias has been limited, an enormous number of papers examining the different systems with new techniques have been published. The present chapter attempts to introduce to the various teleost complexes with some key papers and major recent findings.

19.1.1 Unisexual Reproduction

The need for at least some recombination seems to govern most eukaryotic life. This general rule may be also responsible for the fact that unisexual or all-female reproduction is very rare among vertebrates, comprising just <0.1% of all vertebrate species (Dawley 1989). Unisexuals seem exclusively to arise as a consequence of hybridization between sexually reproducing progenitors. It has been hypothesized that a particular combination of genomes shifts the sex ratio in an interspecific hybrid towards all-female and alters meiosis in the hybrids so they produce eggs without reduction in ploidy and supposedly without recombination. This was articulated by Wetherington et al. (1987) and called the "balance hypothesis" by Moritz et al. (1989). Genetic divergence of parental genomes has to be sufficiently large to cause a high proportion of unreduced gametes, but not too large to significantly decrease the viability or fertility of hybrids (Moritz et al. 1989). Hybridization is particularly common among fish (Scribner et al. 2000), in which it occurs more frequently than in other vertebrate groups. Fishes might hybridize more frequently because of their (usually) external fertilization, unequal abundance of parental species, competition for limited spawning habitats and susceptibility to secondary contact (Campton 2008). In fishes, unisexual reproduction occurs exclusively in the form of sperm-dependent parthenogenesis (i.e., gynogenesis) and hybridogenesis, forcing them into close ecological associations with their progenitor sexual species (Beukeboom and Vrijenhoek 1998).

19.1.2 Gynogenesis

In gynogenetic systems, unreduced eggs are normally produced by an all-female species, but egg development must be triggered by allospecific sperm from males of a related species (Fig. 19.1). Normally, the sperm does not contribute any genetic material to the offspring. Due to this exploitation of the host, gynogenesis has also been called "sperm parasitism" (Hubbs 1964). Although not a common mode of reproduction, sperm-dependent parthenogenesis has evolved multiple times within seven phyla (Beukeboom and Vrijenhoek 1998). However, regular gynogenesis is absent in some major groups of vertebrates, like birds and mammals. It is assumed that genomic imprinting plays a role in the absence of natural parthenogenesis in mammals (Georgiades et al. 2001; Scott and Spielman 2006), in which it leads to death during embryogenesis (Rougier and Werb 2001; see also Chapter 26).

19.1.3 Paternal Leakage

Gynogens are interesting models because they seem to combine some disadvantageous traits from both sexuality (e.g., finding mating partners, exposure to predation during mating, risk of diseases) and asexuality ("Muller's ratchet", Muller 1964; see also Chapter 5) including "mutational meltdown" (Lynch et al. 1993; for more details, see Schlupp 2005). Occasional leakage of genes from a paternal host into sperm-dependent clones may however provide a source of adaptive variation to circumvent the disadvantages of asexuality (Fig. 19.1). This additional genetic material may also cause the formation of a small proportion of males (Lamatsch et al. 2000). Expression of paternal genes may provide a local adaptive advantage in physiological or phenotypic sexual mimicry traits (Beukeboom and Vrijenhoek 1998). It has also been argued that paternal leakage leading to the expression of paternal genes plays a pivotal role to stop Muller's ratchet (Schartl et al. 1995a; Schlupp 2005; Loewe and Lamatsch 2008). However, the observation of paternal leakage should not be confused with true recombination, a reason for discussions of the ratchet-stopping potential of paternal leakage (Beukeboom et al. 1995; Beukeboom and Batenburg 1999). It has been speculated that the paternal genome might be used as a template for DNA repair, but its precise role remains unclear (Beukeboom and Vrijenhoek 1998). Despite its obscure role, paternal leakage enables interactions of a "frozen" (unrecombined) genome with a recently recombined one.

19.1.4 Hybridogenesis

Hybridogenesis is a hemiclonal form of reproduction with features of both, sperm-dependent parthenogenesis and sexuality (Fig. 19.1) (Schultz 1969; Vrijenhoek et al. 1977; see also Chapters 4, 16 and 18). Diploid hybridogenetic females (e.g., AB) transmit a haploid, non-recombinant, maternal genome (i.e., hemiclone A) to their ova. Diploidy is restored by true fertilization with sperm from males of species B. The hemiclonal A genome is combined with a new recombined B genome in each generation; therefore, only maternal genes and chromosomes are perpetuated across generations of the unisexual biotype. Although variation from species B is phenotypically expressed by a hybridogenetic lineage, it is substituted in each generation and is not heritable.

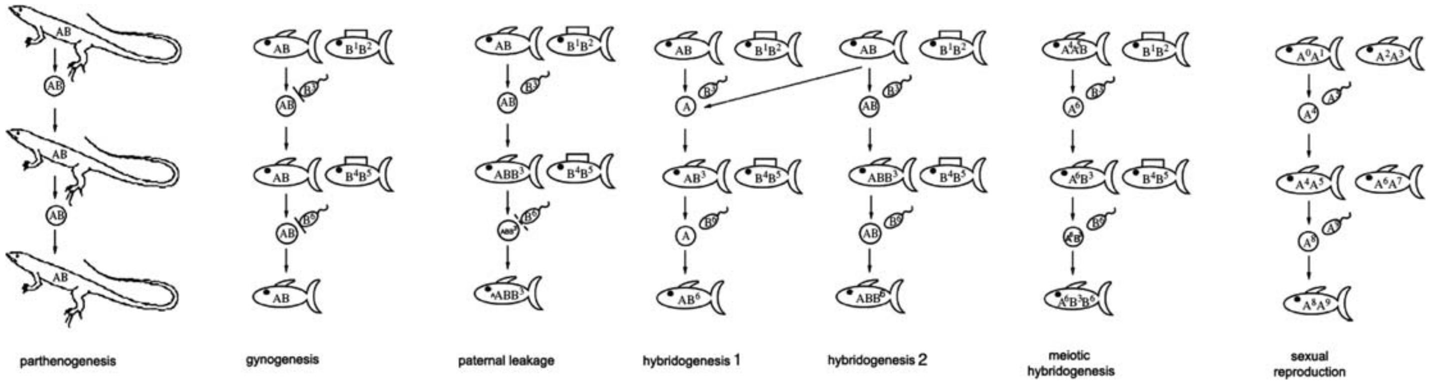

Fig. 19.1 Principles of major reproductive modes in teleost fishes with increasing quantities of sexual elements (expanded from Lampert and Schartl 2008). Capitals illustrate genomes, different letter codes indicate different species. Numbers (0–9) differentiate individual genomes derived from recombination. *Parthenogenesis* (restricted to reptiles): hybrid females produce unreduced ova (AB) that develop into all-female offspring, genetically identical to their mother (AB); *Gynogenesis* (sperm-dependent parthenogenesis): hybrid females (AB) produce unreduced ova (AB) that develop into all-female offspring (AB); sperm are, however, needed from a closely related sexual species to trigger embryonic development without contributing to the offspring's genotypes; reproduction in gynogenesis is truly clonal; *Paternal leakage*: Instead of only triggering the embryo's development, the sperm and egg pronucleus fuse and the offspring show paternal genetic contribution in form of an additional chromosome set resulting in triploids (1), or in form of microchromosomes (2); *Hybridogenesis 1*: the hybrid female's genome (A) is passed on clonally to the offspring while the other genome is substituted every generation (B, B^3, B^6) (hemiclonal); *Hybridogenesis 2*: the hybrid female's genome (AB) is passed on clonally to the offspring while the sexual species' genome (B^3) first elevates the ploidy level and then is substituted every generation (B^6); *Meiotic hybridogenesis*: After pairing of homologous chromosomes (A^4A^5), the third set of unmatched chromosomes (B) is eliminated, and the remaining ones form bivalents and undergo normal meiosis. The recombined haploid eggs (A^6) are fertilized by haploid sperm of the host resulting in diploid females which produce unreduced diploid eggs (A^6B^3). The allotriploidy is restored by fertilization ($A^6B^3B^6$); *Sexual reproduction*: both mating partners produce haploid germ cells that are unique due to recombination, and result in highly variable individual offspring

It has been a longstanding enigma why so few organisms exist that combine parthenogenetic and sexual cycles of reproduction (Green and Noakes 1995). A number of recent studies have shown that parthenogens can have cryptic sex (e.g., D′Souza et al. 2006 and Chapter 18; Omilian et al. 2006) and suggest that rare sexual processes may be more common than previously thought (Beukeboom 2007). In the following sections, we will provide an overview on gynogenetic and hybridogenetic teleost fishes that apparently show amazingly complex reproductive modes (see also Table 19.1). However, deeper insight suggests they may well be efficiently exploiting both, the benefits of sexual and asexual reproduction.

19.2 Poeciliidae (Livebearing Toothcarps)

Members of this family have internal fertilization using an insemination apparatus (gonopodium), with females giving birth to broods of live young. More than 200 species in approximately 30 genera inhabit freshwater and brackish environments of North and South America. Two genera (*Poecilia* and *Poeciliopsis*) contain unisexual representatives; in each case they include both diploid and triploid unisexual forms.

19.2.1 Poecilia formosa

The Amazon Molly is a diploid gynogen, native to freshwater habitats in northeastern Mexico and southeastern Texas (Kallman 1962b; Turner et al. 1983; Schlupp et al. 2002;). When discovered by Hubbs and Hubbs (1932), *Poecilia formosa* was the first vertebrate conclusively demonstrated to be clonal using tissue transplantation experiments (Kallman 1962a). Molecular genetic data revealed that this all-female species arose via hybridization approximately 81,000–280,000 years ago (Schartl et al. 1995b; Lampert and Schartl 2008; Loewe and Lamatsch 2008) between two sexual species: the shortfin molly, *Poecilia mexicana,* as the ancestral female parent and an unidentified ancestor of the sailfin molly, *Poecilia latipinna,* as the male parent (Avise et al. 1991; Schartl et al. 1995b) (Fig. 19.2). For reproduction, the gynogens depend on either of these parental species, or on the broadspotted molly, *P. latipunctata*, as their sexual hosts (Niemeitz et al. 2002; Schlupp et al. 2002). Two forms of paternal leakage have been shown so far: polyploidy results if the whole sperm genome remains in the ovum, and supernumerary microchromosomes can be observed if only small quantities of the sperm's chromosome set fail to be eliminated (as usual in gynogenesis) and remain in the ovum (for reviews, see Lampert and Schartl 2008; Schlupp et al. 1998). Polyploids occur in natural habitats as triploid biotypes, mostly where diploids are sympatric with a subspecies of *P. mexicana* (mlm), but only rarely where diploids occur sympatrically with *P. latipinna* (mll) (Schultz and Kallman 1968; Rasch and Balsano 1989; Lampert

Table 19.1 List of the known, so-called "unisexual" teleost fishes

Family _Genus/biotype_	Ploidy	Genome composition (*original maternal genome if known)	Karyotype	Reprod. mode	Host switch (if known)	References
Poeciliidae _Poecilia_						
P. formosa (mexicana-latipinna)	2n	_m*l_	2n = 46	G	_P. latipunctata_	Hubbs and Hubbs (1932); Lampert and Schartl (2008); Loewe and Lamatsch (2008); Schlupp et al. (2002)
mexicana-2latipinna	3n	_m*ll_	3n = 69	G		Schultz and Kallman (1968)
2mexicana-latipinna	3n	_m*lm_	3n = 69	G		Lampert et al. (2005); Schories et al. (2007)
Poeciliopsis						
monacha-lucida	2n	_M*L_	2n = 48	H		Angers and Schlosser (2007); Schultz (1969, 1977)
monacha-latidens	2n	_M*Lat_	2n = 48	H		Schultz (1977)
monacha-occidentalis	2n	_M*O_	2n = 48	H		Schultz (1977)
monacha/ viriosa x _lucida_	2n	_(M*xV)L_	2n = 48	H		Schultz (1977); Vrijenhoek and Schultz (1974); Mateos and Vrijenhoek (2002)
2_monacha-lucida_	3n	_M*ML_	3n = 72	G	_Poeciliopsis ssp._	Schultz (1967, 1969, 1977)
monacha-2lucida	3n	_M*LL_	3n = 72	G	_Poeciliopsis ssp._	Schultz (1967, 1969, 1977)
monacha-lucida-viriosa	3n	_M*LV_	3n = 72	G	_Poeciliopsis ssp._	Schultz (1967, 1977); Mateos and Vrijenhoek (2005)
Cyprinodontidae _fundulus_						
diaphanus-heteroclitus	2n	_d*h_	2n = 48	G		Fritz and Garside (1974); Dawley (1992)
2diaphanus-heteroclitus	3n	_d*hd_	3n = 72	?		Dawley (1992)

Table 19.1 (continued)

Family *Genus/biotype*	Ploidy	Genome composition (∗original maternal genome if known)	Karyotype	Reprod. mode	Host switch (if known)	References
Atherninidae						
Menidia clarkhubbsi						
(beryllina x peninsulae-“like”*)*	2n	*B∗P*	?	G		Echelle and Moisier (1982); Echelle and Echelle (1997)
2beryllina-peninsulae	3n	*B∗BP*	?	?		Echelle et al. (1988, 1989)
beryllina-2peninsuae	3n	*B∗PB*	?	?		Echelle et al. (1988, 1989)
Cyprinidae						
Carassius						
gibelio	3n	*Carassius auratus* x *Cyprinus carpio*	3n = 156–162	G		Wei et al. (2003)
gibelio	4n	3n *gibelio* x *C. carpio*	4n = 212	G		Zhu and Gui (2007)
langsdorfii	3n	*langsdorfii* 2n x *C.a. auratus*	3n = 150–156	G		Murayama et al. (1986); Murakami and Fujitani (1997)
langsdorfii	4n	?	4n = 206	G		Murayama et al. (1986)
Phoxinus						
eos-neogaeus	2n	*e∗n*	2n = 50	G		Goddard et al. (1989); Goddard and Dawley (1990)
2eos-neogaeus	3n	*e∗ne*	3n = 75	“triploid hybridogenesis”		Goddard et al. (1989); Goddard and Dawley (1990)
eos- 2neogaeus	3n	*e∗nn*	3n = 75	G		Goddard et al. (1989); Goddard and Dawley (1990)
eos-neogaeus / 2eos-neogaeus	2n/3n mosaic	*e∗n/e∗en*	2n = 50 / 3n = 75	G		Goddard et al. (1989); Goddard and Dawley (1990)
eos-neogaeus /eos-2neogaeus	2n/3n mosaic	*e∗n/e∗ne*	2n = 50/3n=75	G		Goddard et al. (1989)
Squalius						
alburnoides						
(*pyrenaicus*-unknown ancestor)	2n	P∗A	2n = 50	clonal eggs+fertilization	*S. carolitertii*	Alves et al. (2001)

Table 19.1 (continued)

Family *Genus/biotype*	Ploidy	Genome composition (∗original maternal genome if known)	Karyotype	Reprod. mode	Host switch (if known)	References
carolitertii-unknown ancestor	2n	C∗A	2n = 50	H		Alves et al. (2001)
all-male lineage	2n	AA	2n = 50	Meiotic		Alves et al. (2001)
triploid hybrids	3n	P∗AA	3n = 75	Meiotic hybridogenesis		Alves et al. (2001)
	3n	P∗PA	3n = 75	Meiotic hybridogenesis		Alves et al. (2001)
	3n	C∗AA	3n = 75	Meiotic hybridogenesis ?		Alves et al. (2001) ;
	3n	C∗CA	3n = 75	Meiotic hybridogenesis		Alves et al. (2001)
Cobitidae						
Misgurnus anguillicaudatus	2n	*?*	2n = 50	G		Morishima et al. (2002, 2008a)
Misgurnus anguillicaudatus	3n	*?*	3n = 75	Meiotic hybridogenesis		Zhang and Arai (1999); Morishima et al. (2002, 2008a,b); Itono et al. (2007); Oshima et al. (2005); Arai (2003)
Misgurnus anguillicaudatus	4n	*?*	4n = 100	?		Arai et al. (1991, 1993)
Misgurnus anguillicaudatus	2n/3n mosaic	*?*	2n = 50/ 3n = 75	Meiotic hybridogenesis, clonal eggs+fertilization		Morishima et al. (2004)
Cobitis taenia complex						
elongatoides-taenia	2n	*ET*	2n = 49	G		Janko et al. (2007)
elongatoides-taenia / 2 elongatoides-taenia	2n/3n	ET/EET mosaic	2n = 49/ 3n = 74	G		Janko et al. (2007)
2 *elongatoides-taenia*	3n	*EET*	3n = 74	G		Janko et al. (2007)
elongatoides-2taenia	3n	*ETT*	3n = 73	G		Janko et al. (2007)
3*elongatoides-taenia*	4n	*EEET*	4n = 99	G?		Janko et al. (2007)
2*elongatoides-2taenia*	4n	*EETT*	4n = 98	G?		Janko et al. (2007)

Table 19.1 (continued)

Family *Genus/biotype*	Ploidy	Genome composition (∗original maternal genome if known)	Karyotype	Reprod. mode	Host switch (if known)	References
elongatoides-3*taenia*	4n	*ETTT*	4n = 97	G?		Janko et al. (2007)
elongatoides-tanaitica	2n	*ETa*	2n = 50	G?		Janko et al. (2007)
2*elongatoides-tanaitica*	3n	*ETaE*	3n = 75	G?		Janko et al. (2007)
elongatoides-2*tanaitica*	3n	*ETaTa*	3n = 75	G?		Janko et al. (2007)
elongatoides-tanaitica-taenia	3n	*ETaT*	3n = 74	G?		Janko et al. (2007)
3*elongatoides-tanaitica*	4n	*EEETa*	4n = 100	G?		Janko et al. (2007)
Triploid biotype	3n	*C. elongatoides-taurica-taenia*	3n = 75	G?		Janko et al. (2007)
Diploid biotype	2n	E-*albicolaris(=strumicae)*	2n = 50	G?		Janko et al. (2007)
Triploid biotype	3n	*C. elongatoides-tanaitica-albicolaris(=strumicae)*	3n = 75	G?		Janko et al. (2007)
Tetraploid biotype	4n	C. 2*elongatoides-taenia-melanoleuca*	4n = 98?	G?		Janko et al. (2007); Vasil’ev et al. (2007)
Tetraploid form (3n + 24)	4n	*taenia* (2) x unknown sp.[a] (2)	4n = 98	G?		Vasil’ev et al. (1989)
Tetraploid form (3n + 25)	4n	*taenia* x unknown sp.[a] (2) x *granoei*	4n = 99	G?		Vasil’ev et al. (1989)
Tetraploid form (3n + 24)	4n	*taenia* (2) x unknown sp.[a] (2)	4n = 98	G?		Vasil’ev et al. (1989)
Tetraploid form (3n + 25)	4n	*taenia* x unknown sp.[a] (2) x *“granoei”(=melanoleuca)*	4n = 99	G?		Vasil’ev et al. (1989)
C. 2hankugensis-longicorpus	3n	*Hlh*	3n = 73	Meiotic hybridogenesis		Kim and Lee (1990, 2000)
C. hankugensis-2longicorpus	3n	*hll*	3n = 74	Meiotic hybridogenesis?		Kim and Lee (1990, 2000)

[a] Unknown sp. of Vasil’ev et al. (1989) is probably *C. elongatoides* (Janko, personal communication).

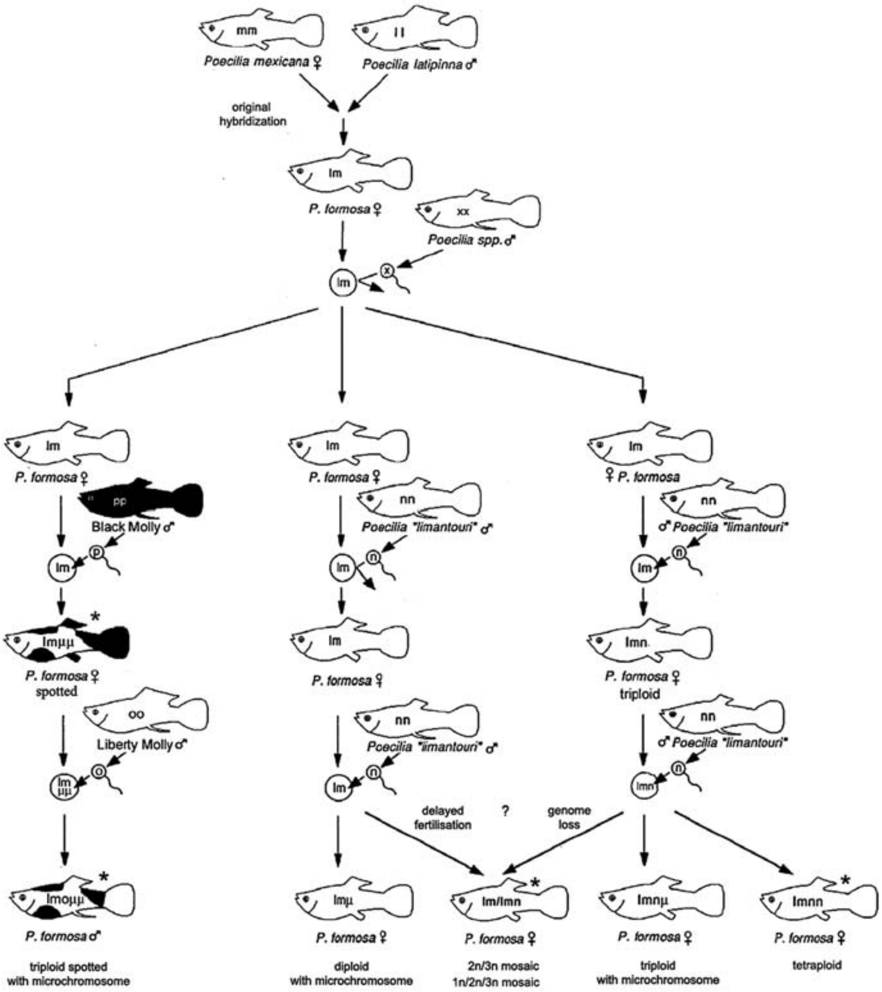

Fig. 19.2 Graphic summary of the breeding complex of *Poecilia formosa*, updated after Dawley (1989). ∗ Only observed under laboratory conditions

et al. 2005). Under laboratory conditions, tetraploids may also occur (Lampert et al. 2008) and even somatic mosaics (Lamatsch et al. 2002; Lampert et al. 2007a), in which the reproductive mode is also gynogenetic. Microchromosomes are found in nature (Sola et al. 1993; Lamatsch et al. 2004) as well as under laboratory conditions (Schartl et al. 1995a; Nanda et al. 2007) and show that microchromosomes are not necessarily as genetically inert as widely assumed (Camacho et al. 2000). A combination of these two types of introgression events in the laboratory has been found to result in unusual triploid males (Lamatsch et al. 2000; and Lamatsch, unpublished data). Additional genetic material derived from paternal introgression events might possibly ensure the reproductive success and evolutionary longevity of *Poecilia formosa* (Loewe and Lamatsch 2008).

19.2.2 Poeciliopsis

Unisexual species of *Poeciliopsis* are native to desert arroyos in northwestern Mexico. Diploid forms originate through crosses between the sexual Headwater livebearer (*Poeciliopsis monacha*; *M*, as the female parent) and the Clearfin livebearer (*Poeciliopsis lucida*; *L*) (Schultz 1973), Gila topminnow (*P. occidentalis*; *O*) or the Lowland livebearer (*P. latidens*; *lat*), as the male parent, respectively (Fig. 19.3; for historical biogeography of the genus, see Mateos et al. 2002). These

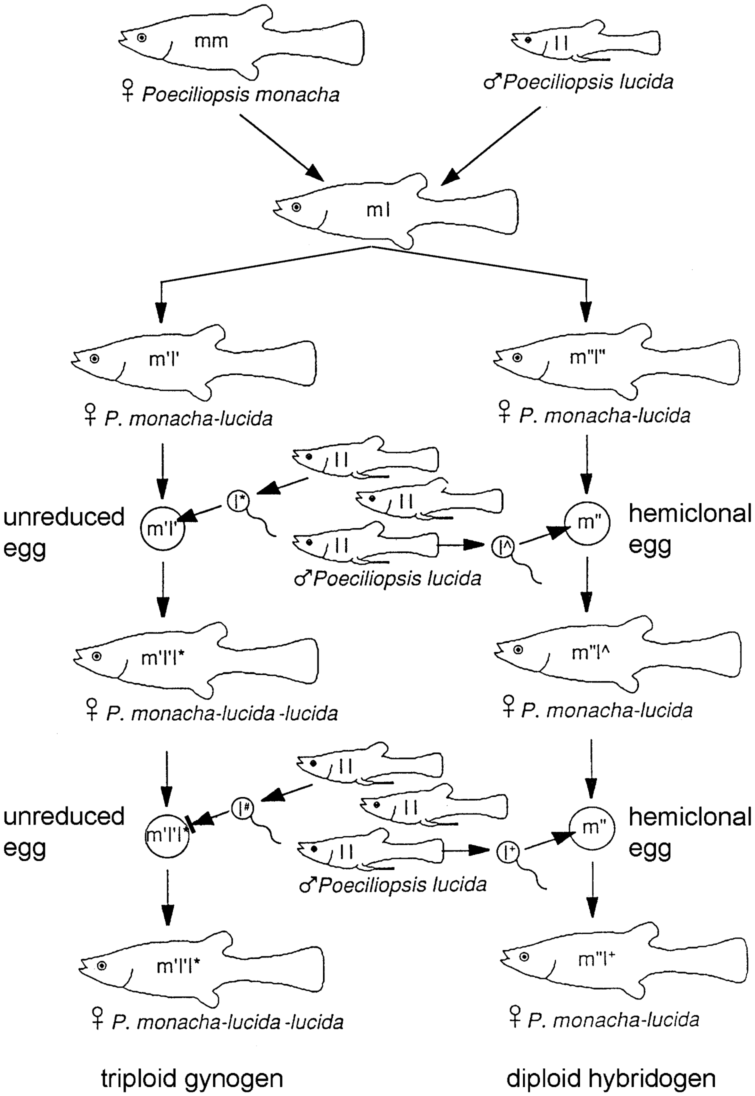

Fig. 19.3 Example of the breeding complex of *Poeciliopsis*, after Dawley (1989) that shows *P. lucida* as sperm-donor but see Table 19.1 for all possible combinations

diploid hybrids reproduce via hybridogenesis, in which the haploid *M* genome is transmitted clonally to eggs, whereas the paternal genome is excluded and replaced in each generation by insemination from the respective *Poeciliopsis* males (Schultz 1967; Mateos and Vrijenhoek 2005). Triploid forms, however, reproduce gynogenetically (Schultz 1967). Three different triploid biotypes are known: *P. 2monacha-lucida* (*MML*), *P monacha-2lucida* (*MLL*) and *P. monacha-lucida-viriosa* (*MLV*, a "trihybrid") that originated by the fertilization of an unreduced *ML*-egg by a *V*-sperm. Although in nature *MML*, *MLL* and *MLV* appear to use sperm from sympatric *P. monacha, P. lucida* and *P. viriosa*, respectively, these triploids can alternatively use sperm from different *Poeciliopsis* species to activate development of the clonal egg (Schultz 1967). In contrast to allodiploidy, allotriploidy has arisen only a few times in *Poeciliopsis* and is of rather recent origin (Mateos and Vrijenhoek 2005; Quattro et al. 1991, 1992). Further ploidy elevation (e.g., tetraploidy) has not been found so far.

19.3 Cyprinodontidae (Pupfishes)

The family Cyprinodontidae contains more than 100 species distributed mostly in fresh and brackish waters throughout the Americas, Africa and Eurasia. Like all killifishes, pupfishes have external fertilization, are egg-layers and mostly reproduce bisexually.

19.3.1 Fundulus diaphanus-heteroclitus

One of the few departures from bisexual reproduction involves an all-female clonal biotype within the otherwise sexual species of *Fundulus*. This biotype, known from two sites in Nova Scotia, Canada, is probably gynogenetic (Dawley 1992) and arose through hybridization between sexual *F. diaphanus* (banded killifish) and *F. heteroclitus* (mummichog) (Dawley et al. 1999, 2000; Hernandez Chavez and Tuergeon 2007). Interspecies hybridization between *F. heteroclitus* and *F. diaphanus* occurs over a wide geographic range (Hernandez Chavez and Tuergeon 2007), but gynogens were only observed at two sites, indicating that only specific crosses between parental species result in clonal reproduction. With all clones bearing the same *F. diaphanus* mtDNA-haplotype, its origin probably goes back to a few rather recent hybridization events (Dawley 1992; Hernandez Chavez and Tuergeon 2007) while microsatellite data raise the possibility of several independent origins of asexuality (Hernandez Chavez and Tuergeon 2007). The unisexual hybrids are mostly diploid and only rarely triploid. Triploids show DNA contents close to what would be expected for a hybrid with a double dose of the *F. diaphanus* genome and a single dose of the *F. heteroclitus* genome (*DDH*) (Dawley 1992), indicating paternal leakage as source of triploidy. However, the potential role of paternal introgression, sexual reproduction, occasional recombination and mutational events has not been conclusively addressed to date.

19.4 Atherinopsidae (Neotropical Silversides)

This family contains approximately 104 species in 13 genera that are distributed throughout the tropical and temperate waters of the Americas, including both marine and freshwater habitats. So far, unisexuality has only been discovered in one species.

19.4.1 Menidia clarkhubbsi

Species of *Menidia* are small, silvery-sided, planktivorous fishes that form dense, highly mobile foraging schools along the Texas coast and eastward at sites on the northern Gulf of Mexico. They are egg-layers with external fertilization and have no striking sexual dimorphism. The unisexual-bisexual complex comprises the bisexual Inland silverside (*M. beryllina*) and the Tidewater silverside (*M. peninsulae*), F_1 hybrids between the two species and several different all-female clones (*M. clarkhubbsi*, Texas silverside) that arose through multiple hybridizations between males of *M. beryllina* and females of an extinct or as-yet undetected species genetically similar to *M. peninsula* (Echelle and Moisier 1982; Echelle et al. 1983; Echelle and Echelle 1997). The complex consists mainly of diploid females and there is no compelling evidence of persistent polyploid clones, although rare occurrence of wild-caught triploids has been stated (Echelle et al. 1988, 1989). It is not yet resolved whether these triploids occur by paternal leakage involving a diploid egg from *M. clarkhubbsi* (*BP*) and a haploid sperm of *M. peninsulae* (*P*) (Echelle et al. 1989) or by back-crosses of F_1 hybrids, producing unreduced gametes, to the parental species (*BPP*, *BBP*) (Echelle et al. 1988), and how they reproduce. Low abundance seems characteristic of this unisexual complex: Despite intense research, no environmental situations were found where unisexual *Menidia* are predictably more abundant than their bisexual relatives (Echelle et al. 1989; Echelle and Echelle 1997). This may reflect competition with the diversity of other forms of *Menidia* (two bisexual species, their hybrids and backcross progeny) as well as the lack of opportunity for origins of new unisexual species due to the absence of one of its bisexual progenitors (the missing *M. peninsulae*-like form). Thus, the existing *M. clarkhubbsi* species complex may be a relict of a once more diverse, and therefore more abundant assemblage of clones.

19.5 Cyprinidae (Minnows and Allies)

Members of this huge taxonomic assemblage are native to North America, Eurasia and Africa. With more than 1,600 species in nearly 300 genera, this is the most species-rich family of fishes. However, only a few clonal or hemiclonal biotypes are known.

The Japanese crucian carp (*Carassius auratus*) was morphologically classified into several subspecies: kinbuna (*C. a. ssp.*), nagabuna (*C. a. burgeri*), nigorobuna (*C. a. grandoculis*), gengorobuna (*C. a. cuvieri*), ginbuna (*C. a. langsdorfii*) and Prussian carp (*C. a. gibelio*). Recent research based on mitochondrial *Cytochrome b* sequences (Kalous et al. 2007), however, has characterized *C. a. cuvieri*, *langsdorfii* and *gibelio* as distinct species, leaving only *grandoculis*, *burgeri* and the kinbuna as subspecies of *C. auratus.*

19.5.1 Carassius gibelio

The gynogenetic Prussian carp was originally described in a population from the Shuangfeng Reservoir in northern China and now invasively spreads in freshwater streams, ponds and lakes over a wide geographic range from northern Europe to Asia (e.g., Veetema et al. 2005; Flajshans et al. 2007; Verreycken et al. 2007; Leonardos et al. 2008). The triploid gynogen originated from an ancient hybridization event, with *Carassius auratus* being the maternal and *Cyprinus carpio* being the paternal ancestor (Chun et al. 2001). Seven different clones have been identified, differing significantly in body shape, growth rate, spawning time, serum protein phenotype, karyotype etc. (Zhou et al. 2000; Yi et al. 2003). This species shows two unusual characteristics for unisexual breeding complexes: (1) populations contain up to 20% males in natural habitats (Abramenko et al. 1998); (2) two reproductive modes exist: gynogenesis and gonochoristic reproduction; besides its role in activating the egg, sperm contribute to the progeny in a high percentage of cases. Cytological observations have revealed two different patterns of sperm development: if eggs are inseminated with heterologous sperm (i.e., from other species), the entered sperm does not decondense and is then eliminated from the zygote. This is the normal process of gynogenesis and gives rise to all-female progeny. However, when the eggs are inseminated with homologous sperm of silver crucian carp males, the sperm undergoes normal decondensation and pronucleus formation and fuses with the female pronucleus. The fused nucleus of the zygote undergoes recombination and extra chromosomes (about half of the maternal chromosomes) are eliminated from the egg. In this case, genetically diverse offspring are produced (including males), similarly as in gonochoristic reproduction. This form of gynogenesis has been referred to as "allogynogenesis" (Jiang et al. 1983). As a consequence, the complex consists of diploid individuals (males and some of the females), mostly triploid individuals (almost exclusively females) and rare tetraploid females (Fan and Liu 1990; Zhu and Gui 2007). In one clone, paternal leakage of subgenomic amounts of genetic material has been detected in the offspring. Phenotype similarity with the sperm-donor implied that these microchromosomes might carry genes that are expressed in the foreign genetic background (Yi et al. 2003). Although this is an unusual situation since supernumerary chromosomes are often found to be genetically inert (Camacho et al. 2000), it has also been demonstrated in microchromosomes of *Poecilia formosa* expressing the macromelanophore locus (Schartl et al. 1997).

19.5.2 *Carassius langsdorfii*

The Japanese silver crucian carp, ginbuna, is widely distributed in Japan. There are three forms of females in this complex: a bisexual diploid form as well as a gynogenetic triploid and tetraploid form (Kobayashi et al. 1970, 1977). Although the hybrid origin of polyploid ginbuna has been revealed with nuclear markers (Murakami and Fujitani 1997), its ancestral parents have not yet been identified. According to Murakami et al. (2001), triploid ginbuna have been derived from two different maternal lineages approximately 70,000–160,000 years ago and it seems likely that the goldfish, *C. a. auratus,* contributed to the ploidy elevation from diploid hybrid ginbuna (Murakami et al. 2002). In mating experiments of triploid ginbuna with male goldfish (*C. a. auratus*) diploid-triploid and diploid-triploid-tetraploid mosaic offspring were obtained, most of which turned out to be males (Murayama et al. 1986). Since no paternal contribution could be detected, the exact mechanism remains unclear. Other authors have discovered rare triploid (Muramoto 1975) and tetraploid males (Murakami and Fujitani 1997) from natural populations. Despite warnings based on experiences with other species of *Carassius* invading native fish communities (Crivelli 1995; Fraser and Adams 1997), individuals of *C. langsdorfii* have lately been found in Europe in the River Elbe basin (Czech Republic) (Kalous et al. 2007), probably accidentally being introduced along with imports of commercially important fishes like Koi carps (*Cyprinus carpio*). Their impact on endemic fish fauna needs to be assessed urgently.

19.5.3 *Phoxinus eos-neogaeus*

The *Phoxinus eos-neogaeus* complex is widely distributed in north-eastern America and occupies very heterogeneous habitats (Angers and Schlosser 2007). It originated by multiple hybridization between males of the northern redbelly dace (*P. eos*) and females of the finescale dace (*P. neogaeus*) (Dawley et al. 1987; Goddard et al. 1989; Angers and Schlosser 2007). Altough these diploid hybrids reproduce by sperm-dependent parthenogenesis (Goddard et al. 1998), the exclusion mechanism, which normally clears the egg from the sperm in gynogenesis, often fails in this hybrid complex, leading to an unusually high level of sperm incorporation (Fig. 19.4). As a consequence, five different hybrid biotypes are found in the complex: (1) the strictly clonal, all-female diploid *P. eos-neogaeus* lineage (*en*), (2) triploid *P. 2eos-neogaeus (ene)*, (3) triploid *P. eos-2neogaeus (enn)*, (4) mosaic *P. eos-neogaeus / 2eos-neogaeus (en/ene)* and (5) mosaic *P. eos- neogaeus / eos-2neogaeus* (*en/enn*) (Goddard et al. 1989; Goddard and Dawley 1990). The first four of these hybrid biotypes have been found in natural populations (Doeringsfeld et al. 2004), whereas the latter mosaic biotype with an additional *neogaeus* genome has only been reported from a laboratory mating (Goddard et al. 1989). Where the diploid clonal hybrid occurs sympatrically with only one parental species (typically *P. eos*), the complex is comprised of three biotypes: the diploid clone augmented with triploids and mosaics carrying an additional genome from that species (Dawley

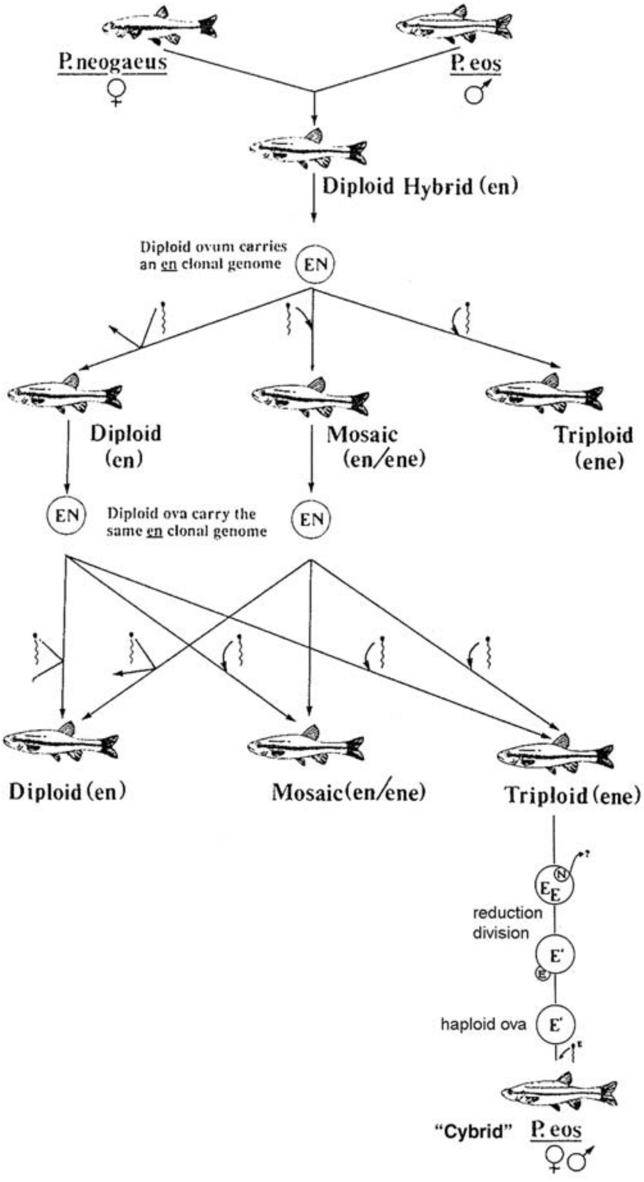

Fig. 19.4 Graphic example of the breeding complex of *P. eos-neogaeus* showing diploid, triploid and diploid-triploid mosaic hybrids when mating with *P. eos* (modified from Goddard and Dawley (1990) and Goddard and Schultz (1993)

et al. 1987). Where it occurs in sympatry with both parental species, all five biotypes may be present (Goddard et al. 1989; Doeringsfeld et al. 2004). Mosaic individuals (*en/een*) produce diploid eggs (*en*), which may develop gynogenetically or incorporate sperm. Triploids (*een*), however, exclude the *neogaeus* genome before they undergo reduction division, resulting in haploid (*e*) ova, a process comparable to meiotic hybridogenesis (see *Squalius, Misgurnus*). When fertilized by *P. eos* sperm, the resultant offspring are indistinguishable from males and females of *P. eos* (Goddard and Schultz 1993) although they carry the mitochondrial DNA of *P. neogaeus*. These are called "cybrids" in contrast to nuclear hybrids (gynogens, triploids and mosaics). Doeringsfeld et al. (2004) conclude that the distribution and ecological success of the hybrid complex is a function of both, an apparently broadly adapted clonal lineage and additional genetic and phenotypic variation expressed by various polyploid biotypes.

19.5.4 Squalius alburnoides

The Iberian fishes of the *Squalius* (previously *Leuciscus*, *Tropidophoxinellus* or *Rutilus*) *alburnoides* complex (for nomenclature, see Kottelat 1997 and Collares-Pereira et al. 1999) contain mixed reproductive systems of diploid, triploid and tetraploid forms with highly female biased sex ratios. The complex arose through interspecific crosses between *S. pyrenaicus* (*P*-genome) and males of an apparently extinct species (*A*-genome) (Alves et al. 2001). The most common form of the complex includes hybrid females and males with diploid (*PA*), triploid (*PAA* and *PPA*) and tetraploid (*PPAA*, *PAAA* and *PPPA*) genomes (Gromicho et al. 2006) (see Fig. 19.5). The second form of the complex comprises a diploid nuclear non-hybrid but all-male lineage (*AA*) with *pyrenaicus*-mtDNA that is hypothesized to have been reconstituted within the complex by triploid *PAA*-females (Alves et al. 2002) (Fig. 19.6). Recent research revealed that the paternal ancestor of the complex was an *Anaecypris hispanica*-like species according to evidence from cytogenetics (Gromicho et al. 2006), microsatellites (Crespo-Lopez et al. 2007) and nuclear sequence data (Robalo et al. 2006). While this ancestral species seems to be extinct in most or all relevant river basins, its nuclear genome is preserved in the all-male form (with *pyrenaicus*-mtDNA). The oogenesis of triploid *PAA*-females is mostly achieved by "meiotic hybridogenesis" (Alves et al. 1998), which involves elimination of the *P* (*Squalius*) genome, followed by random segregation and recombination between the two remaining genomes, generating *A*-ova. This mechanism has been first assumed to be operating in certain triploid hybrid *Rana esculenta* frogs (Günther et al. 1979) and seems to be similar to males of all-triploid Batura-toads (Stöck et al. 2002). When these recombined *A*-ova are fertilized with recombined haploid *A*-sperm of *AA*-males, new *AA*-all-male (with *pyrenaicus*-mtDNA) progeny is restored. Alves et al. (2004), however, reported a triploid female that generated both large triploid and small haploid eggs resulting in all-female progeny. In diploid hybrid *PA*-females, few eggs (< 3%) develop by gynogenesis. The majority of *PA*-females transmits the complete hybrid genome to the egg and fertilization results in triploid progeny (Alves et al. 1998). In all other forms of the complex, reproductive modes include syngamy (Alves et al. 2001). Males in the *S. alburnoides* complex are fertile and play a role in the dynamics of the complex: diploid hybrids (*PA*) produce unreduced sperm, while others designated as diploid "nuclear non-hybrid males" (*AA*) produce reduced sperm (Alves et al. 1999).

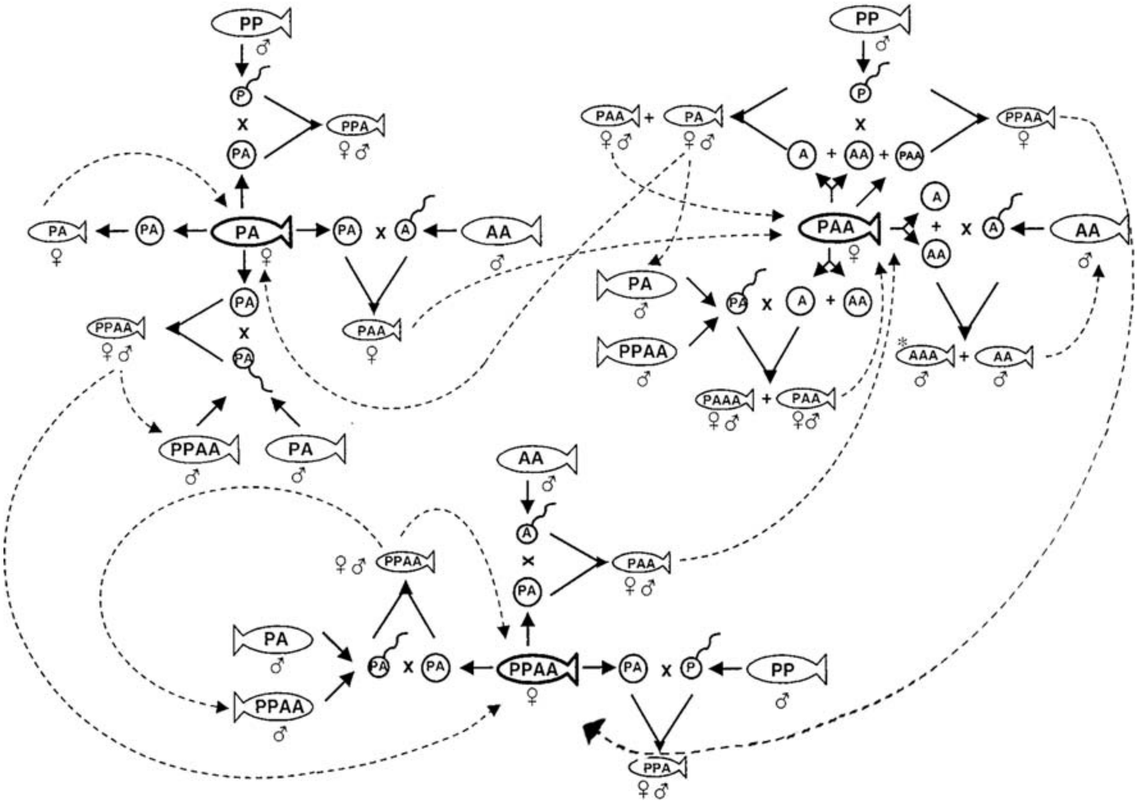

Fig. 19.5 Putative relationships between the various forms of the *S. alburnoides* complex modified from Alves et al. (2001). ∗ Never observed in natural populations, but in breeding experiments

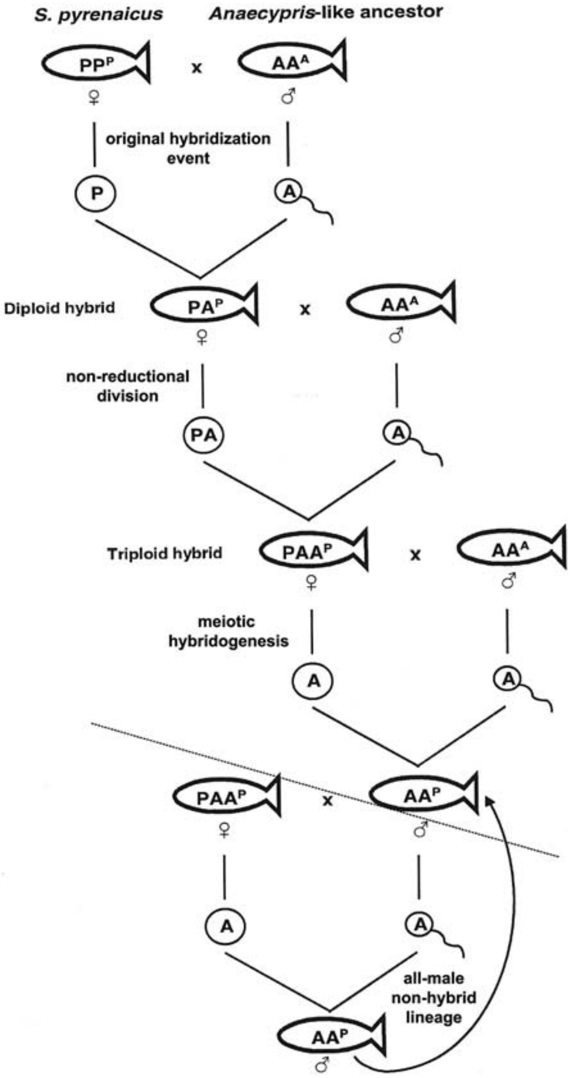

Fig. 19.6 Hypothetical evolutionary trajectory of the all-male non-hybrid lineage within the hybrid *S. alburnoides* complex. Above dashed line, mechanism of the origin of the hybrid complex; below dashed line, perpetuating mechanism of the all-male non-hybrid lineage (modified from Gromicho et al. 2006)

In the absence of *S. pyrenaicus* (e.g., N-Portugal), the complex seems to be maintained by crosses with males of *S. carolitertii* (*CC*) and by diploid hybrid males (*CA*), although the mtDNA found in *S. alburnoides* is *S. pyrenaicus*-like (Cunha et al. 2004; Pala and Coelho 2005; Sousa Santos et al. 2006). Recently, also extensive mtDNA-introgression from a related species (*S. aradensis*) into the *S. alburnoides* has been detected (Sousa Santos et al. 2006). A spectacular novel finding has been added to the knowledge about the evolutionary dynamics of the complex: In two populations from the NW-Iberian Douro drainage, tetraploid individuals represent 85.6–97.5% of the population, with no observed sex ratio bias.

Using flow cytometry of blood and sperm, microsatellite data and experimental crosses, Cunha et al. (2008) describe two gonochristic allotetraploid populations (*CCAA*) with normal meiosis. This illustrates how the evolutionary dynamics of a hybrid complex may contribute to polyploid speciation. Such processes have been predicted for polyploid fishes at least as early as 30 years ago by Schultz (1979).

Studying gene expression, Pala et al. (2008) found in some triploid forms of the *S. alburnoides* complex "that a compensation mechanism exists, reducing transcript levels to the diploid state". Their data suggest a silencing of one of the three alleles, although unexpectedly, it is not a whole haplome that is inactivated. The allelic expression patterns differ between genes and between different tissues for one and the same gene.

19.6 Cobitidae (Loach Fishes)

This family comprises about 150 extant freshwater species that inhabit Eurasia, with species diversity being the highest in southern Asia. These fish are typically bottom-dwellers with downward facing mouths and "wormlike" or fusiform (spindle-shaped) bodies. A recent molecular phylogeny was proposed by Slechtova et al. (2008).

19.6.1 Cobitis

Spined loaches form a monophyletic group within the cypriniforms, which contains more than 42 species of freshwater fishes (Sawada 1982). Apparently, nothing is known about the occurrence of hybrid complexes among the Iberian species. In Central and Eastern Europe, at least seven diploid gonochoristic spined loach species are found (Vasil'ev et al. 2007). In addition, since the beginning of the 1980's, diploid, triploid and tetraploid all-female spined loach forms were discovered (Vasil'ev and Vasil'eva 1982; Vasil'ev et al. 1989; Bohlen 2000; Rab et al. 2000; Bohlen and Rab 2001; Boron et al. 2003; Janko et al. 2003) (Fig. 19.7). The

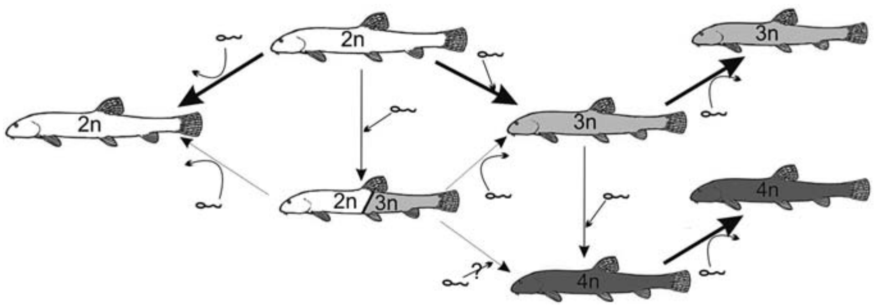

Fig. 19.7 Representation of the European *Cobitis* breeding complex (kindly provided by K. Janko; with modifications)

C. taenia complex sensu Janko comprises six parapatric species known to hybridize (*C. elongatoides, C. taenia, Cobitis tanaitica, Cobitis taurica, Cobitis strumicae and Cobitis melanoleuca*) (Choleva et al. 2008).

These asexual lineages arose by hybridization between three species: *Cobitis taenia*, *C. elongatoides* and *C. tanaitica.* Diploid, triploid and tetraploid all-female hybrids between *C. elongatoides* and *C. taenia* are found, as well as triploid and tetraploid all-female hybrids between *C. elongatoides* and *C. tanaitica*, co-occurring over a large range with their parental species (Slechtova et al. 2000; Bohlen and Rab 2001). Triploids predominate in most populations, but diploid hybrids are common at some localities in the Oder and Elbe river basins (Bohlen et al. 2002; Slechtova et al. 2000). For the *C. elongatoides-C.taenia* hybrid complex (Vasil'ev et al. 1989; Saat 1991), gynogenetic reproduction has been reported. This is also presumed for the *C. elongatoides-tanaitica* hybrids as they are always associated with the parental species (Bohlen and Rab 2001). The latter form all-female populations with mostly triploids, but without any evidence for hybridogenesis. MtDNA analyses of both hybrid complexes showed that hybridization resulting in *C. elongatoides-taenia* asexuals was reciprocal, and that the asexual lineages are of recent polyphyletic origin. Taking advantage of previous knowledge of the genome composition of polyploids, Janko et al. (2003) further concluded that polyploidy has been achieved by backcrosses of diploid F_1-hybrids to both parental species. Mezhzherin and Chudakorova (2002) analysed the Dnieper river *C. taenia* hybrid complex using allozymes and found it to consist of 87% polyploid females triploids, tetraploids and "possibly a few pentaploids". The tetraploid hybrids in the Moscow River comprise some males without normal spermatozoa. However, experimental crosses between gynogenetic triploid females and tetraploid males revealed that these males could trigger gynogenesis of clonal forms in a few cases (Vasil'ev et al. 2003). Using multilocus fingerprinting, Vasil'ev et al. (2007) found tetraploids in the Don Basin to exhibit clonal inheritance, while female tetraploids in the Moscow River may also have arisen de novo, i.e., by fertilization of clonal triploid eggs. Some tetraploids are supposedly trihybrids (Vasil'ev et al. 2007), as also described by Choleva et al. (2008).

A comprehensive review of diversity and systematics of the Central and Eastern European *Cobitis taenia* complex has been published by Janko et al. (2007). Janko et al. (2005) and Culling et al. (2006) reconstructed the Quaternary biogeography of the sexual parental and clonal hybrid lineages of European *Cobitis* from two separate refuges. The authors found multiple Prae-Würmian and Holocene origins of asexuality, irrespective of the parental populations involved and similar dispersal potential of diploid and triploid lineages.

Another hybridogenetic complex of *Cobitis* occurs in East Asia. In the Korean *C. hankugensis* (previously *sinensis*: *S*) – *C. longicorpus* (*O*) complex, diploid *SO*-hybrids produce diploid ova, whose fertilization leads to triploids, which produce haploid ova (Kim and Lee 1990, 2000; Saitoh et al. 2004). According to Kim and Lee (2000), *SSO*-triploids eliminate the *O*-genome and perform meiosis with the remaining *S*-genomes (i.e., meiotic hybridogenesis). When these haploid *S*-eggs are fertilized by normal *C. hankugensis* males, diploid *C. hankugensis* are regenerated but carry foreign *C. longicorpus* mtDNA (nucleo-cytoplasmic hybrids; Saitoh et al. 2004). Kim and Lee (2000) present a hypothetical scheme on the interactions in the breeding complex (not shown).

19.6.2 Misgurnus anguillicaudatus

The oriental weather loach, *Misgurnus anguillicaudatus*, is a common freshwater fish that inhabits shallow ponds and paddy fields all over Japan, Korea, China, Vietnam and other Asian regions (Arai 2003). Although bisexually reproducing, diploid individuals are most common in the wild populations of Japan, a relatively small number of triploid individuals have also been discovered (Morishima et al. 2002; Arai 2003; Oshima et al. 2005). In wild populations of Japan, no natural tetraploids have been found despite intense screening (Arai, personal communication). Ojima and Takai (1979) and Arai et al. (1991) found tetraploids; however, all of these were of "commercial" origin; possibly from the Yang-tze (= Chiangjiang) River basin of China, where natural tetraploids and diploids occur sympatrically (Li et al. 2008). In 2002, Morishima and colleagues discovered a diploid clonal lineage within the wild population of the northern area of Hokkaido, although its origin remains unclear (Morishima et al. 2008a) (Fig. 19.8). These females generate unreduced diploid eggs by premeiotic endomitosis (Itono et al. 2006), which are activated by sperm from bisexually reproducing diploid loaches and develop gynogenetically into clonal offspring (Itono et al. 2007). Paternal leakage into unreduced eggs may lead to triploid individuals (Morishima et al. 2002; Oshima et al. 2005) or diploid-triploid mosaics (Morishima et al. 2004). Triploid females have been reported to produce mainly haploid eggs by meiotic hybridogenesis (Morishima et al. 2008b; see above): After pairing of homologous chromosomes, the third set of unmatched chromosomes is eliminated and the remaining bivalents undergo normal meiosis, resulting in haploid eggs. Therefore, diploid gynogenetic progeny can be produced from haploid eggs of these triploid loaches after normal fertilization with the sperm of bisexual diploids. Small numbers of diploid, triploid and aneuploid eggs have also been reported from triploid loaches (Oshima et al. 2005). Triploid males, however, appear to be sterile (Itono et al. 2006). In contrast, diploid-triploid mosaic males produce fertile diploid sperm (Morishima et al. 2004), whereas diploid-triploid mosaic females have been found to lay haploid, diploid and triploid eggs simultaneously (Yoshikawa et al. 2007). The occurrence of meiotic hybridogenesis in triploids derived from clonal gynogenetic *M. anguillicaudatus* suggests the presence of two distinct genomes in the clone. Arias-Rodriguez et al. (in press) found that inter-populational hybrid loaches produced unreduced or other unusual eggs. Taken together, these data suggest a hybrid origin of *M. anguillicaudatus*, but its exact origin and ancestors are still not clarified.

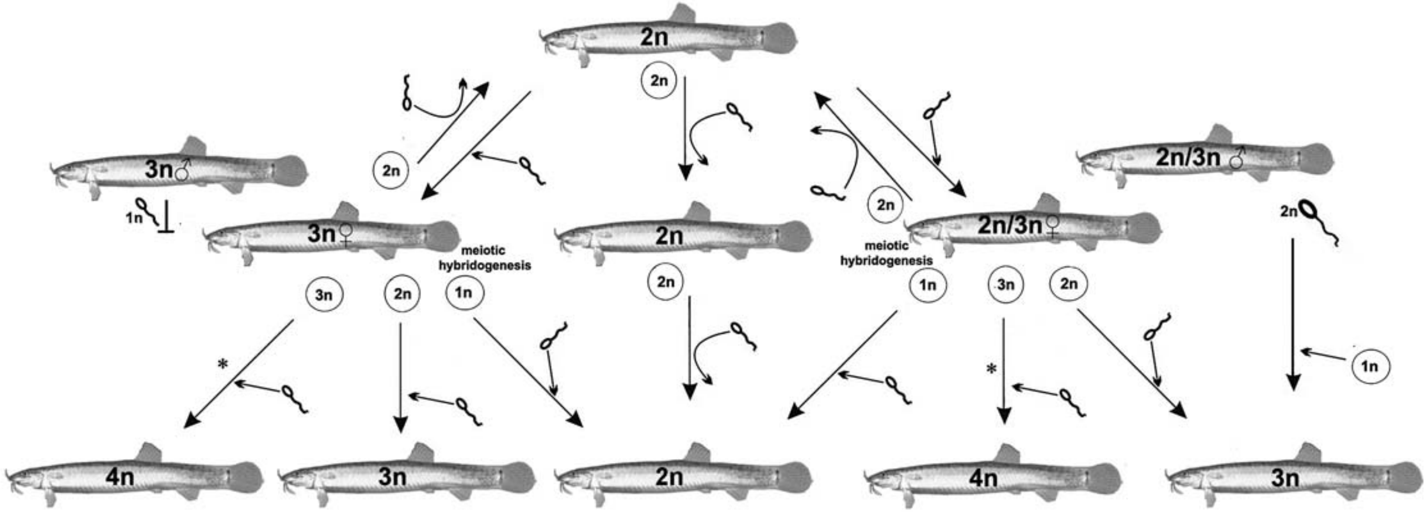

Fig. 19.8 Putative representation of the cryptic clonal lineages in the loach *Misgurnus anguillicaudatus* from Japan. ∗ Never observed in natural populations, tetraploid offspring only observed in breeding experiments!

19.7 Conclusions

As we have outlined above, teleost fishes have not completely "lost sex". In contrast, the entire range of imaginable stages between sperm-dependent parthenogenesis without or with different amounts of paternal leakage (e.g., *Poecilia, Carassius gibelio*) to hybridogenesis with hemiclonal diploid gametogenesis (e.g., *Poeciliopsis*) or genome elimination, followed by a normal meiosis (e.g., *Squalius, Misgurnus*, *Phoxinus*), has evolved in this group (Fig. 19.1).

Moreover, some systems include a combination of these phenomena and form species complexes that are governed by the coexistence of several reproductive modes in addition to normal sexual reproduction (e.g., *Squalius, Cobitis, Phoxinus*) and/or may even lead back to the evolution of sexuality in allotetraploids via triploids (e.g., *Squalius*).

True parthenogenesis (i.e., sperm-independent reproduction) has not been found in fishes; in vertebrates, it seems restricted to reptiles (see also Chapter 21). All described teleost complexes are of hybrid origin (even the so far mysterious *Misgurnus anguillicaudatus* seems to be of hybrid origin, see Morishima et al. 2008b) and the resulting clonal or hemiclonal reproduction can be viewed as a serendipitous effect of miscegenation between two species, which are sufficiently closely related to form viable hybrids and too distantly related for their genomes to execute normal meiosis (Moritz et al. 1989). However, between sperm-dependent parthenogenesis and true sexual reproduction, all kinds of intermediate states have evolved in teleosts. Nevertheless, all of these are sperm-dependent. This dependence may be explained by the need for a mechanical and/or chemical trigger to initiate embryogenesis in teleost ova (cf. Dawley et al. 1987; Pandian and Koteeswaran 1998). Once gynogenesis is established in a system as insemination without paternal genetic input, rare failure of the normal sperm-exclusion might lead to paternal leakage: a tiny genetic contribution of these "pseudo-fathers" that may or may not be phenotypically expressed. A higher amount of paternal leakage may comprise the complete incorporation of the foreign sperm nucleus and, as a consequence, ploidy elevation in a usually still all-female situation.

As opposed to the relatively rare paternal leakage, regular fertilization and biparental gene expression in the offspring has evolved in hybridogenesis, but one genome is pre-meiotically eliminated. Here, we find either hemiclonal transmission of one genome without recombination (as in diploid hybridogens) and replacement of the other genome from a normal sexually recombining species. Alternatively, after the elimination of one complete chromosome set, some triploids may produce clonal diploid eggs, while others even enter a normal meiosis and produce recombined gametes (meiotic hybridogenesis).

Regarding the observed stages of complete or partial asexuality, two important aspects should be addressed by future research: (I) a hybrid may have a given reproductive mechanism immediately after it has formed (e.g., caused by the alteration of normal meiosis to automixis; Lampert et al. 2007b), just as a result of the genomic distance between the parental forms (e.g., for true gynogens, the "balance hypothesis" according to Moritz et al. 1989). (II) Ongoing interactions between sexual progenitors and hybrids (genome shuffling, addition of a foreign genome, intergenomic recombination and exchange, introgression) enabled by paternal leakage, genome addition, and various forms of hybridogenesis may, over time, lead to a higher compatibility and co-evolution of the hybridizing genomes with the result that these interspecies interactions may experience a true evolutionary transition from one reproductive mode to another. Indications of such "evolution in action" have been observed in *Squalius alburnoides*, for example (Alves et al. 2001, Cunha et al. 2008). We encounter these complexes in a certain stage but we need to understand if and how ongoing interspecies and intergenomic interactions may change in evolutionary time.

Interestingly, the speculative evolutionary (re-) transition from asexuality to sexuality (gynogenesis → paternal leakage → hybridogenesis → meiotic hybridogenesis → meiotic allotetraploids) in teleosts seems to be accompanied by an increasing number of males (e.g., *Squalius*, *Cobitis*) shifting the all-female (e.g., *Poecilia*) to a female-biased situation or perhaps again to a balanced situation as in bisexual allotetraploids (*Squalius*). This effect, however, seems rather a bi-product of introgression of genetic material (microchromosomes, entire chromosome sets) than a directed evolutionary tendency.

Another question that should be addressed by future research is the reason for the occurrence or absence of "meiotic hybridogenesis" in some triploids. Similarly to triploid *Rana esculenta* frogs (Günther et al. 1979) and triploid *Bufo baturae* toads (Stöck et al. 2002), triploids in *Squalius, Cobitis*, *Misgurnus* and *Phoxinus* have developed mechanisms that can exclude one entire chromosome set (the one that is "minority" in such 2n + 1n triploids) before they enter an apparently normal diploid meiosis. In contrast, hybrids in *Poeciliopis* (which are hybridogenetic in their diploid forms) become gynogenetic as triploids. We speculate if the reason could be some kind of trihybridity (the involvement of a third, slightly different genome) in the latter form.

Sperm-dependent unisexuals (gynogens and hybridogens) are normally primarily restricted to the range(s) of the one (or more) bisexual species on which they depend, the parental forms in most cases. It has been documented, however, that asexual lineages may rarely use sperm from a non-parental species or even switch their host (Choleva et al. 2008). This latter phenomenon has been discussed by Choleva et al. (2008) who found it in four genera of (partly) "asexual" lineages of fish and two amphibians.

Recent research (e.g., Ogielska et al. 2004 in hybridogenetic frogs; and Bi and Bogart 2006 and Bi et al. 2007 in gynogenetic salamanders) shows that once the genetic material of two species is present in the same nucleus (as in gynogens or hybridogens), a variety of intergenomic exchange events ("intergenomic (re)combination") can be expected to occur (Mable 2007). These phenomena remain to be investigated in several of the described teleost complexes with adequate molecular-cytogenetic methods. Such techniques might reveal that the intergenomic barriers between the genomes of the parental species are likely much more "porous" than assumed in the "classical" concepts of gynogenesis and hybridogenesis. One of the challenges for future research, using the technological advances that provide access to genomic information, is to address how frequently inter-species interactions result in intergenomic gene transfer and recombination. These phenomena may be the key points to understand the nature of these outstanding hybrid breeding-complexes. By broadening the view of Moritz et al. (1989), we regard them as the result of interactions between lineages reflecting a wide spectrum of interspecies genomic exchange: Thus these viable, often female-biased hybrids with ameiotic or partly meiotic gametogenesis may be viewed as "genome-shuttles" that shuffle genetic material between parental lineages over long evolutionary periods in a framework that can be considered to be a "mobile hybrid zone". "Homologous recombination, as in sex, is important for population genetics – shuffling of minor variants, but relatively insignificant for large-scale evolution. Evolutionary innovations depend much more on illegitimate recombination, which makes novel genes by gene duplication and by gene chimaerism – essentially mutational forces" (Cavallier-Smith 2002).

Acknowledgements We thank Robert B. Vrijenhoek, Karel Janko, Kathryn Goddard-Doms, Katsutoshi Arai, Robert M. Dawley, Maria Collares-Pereira and Manuela Coelho for valuable comments on earlier versions of the manuscript as well as for the permission to develop some of our figures based on their previous versions, and Manfred Schartl for discussion on some aspects. The English has kindly been improved by Duncan Cameron.

Glossary (for details: see also Fig. 19.1)

Gynogenesis: sperm-dependent parthenogenesis. Hybrid females produce unreduced ova that develop into all-female offspring; sperm are needed from a closely related sexual species to trigger embryonic development without genetic contribution to the offspring.

Hemiclonal reproduction, Hybridogenesis: the hybrid female's genome is passed on clonally to the offspring while the other genome is substituted every generation (hemiclonal). It is also possible that the genome of the sexual species first elevates the ploidy level before it gets substituted.

Parthenogenesis: Hybrid females produce unreduced ova that develop into all-female offspring being genetically identical to their mother.

Paternal leakage: Instead of only triggering embryogenesis (Gynogenesis), the offspring show paternal genetic contribution in form of an additional chromosome set (triploids) or microchromosomes.

References

Abramenko MI, Poltavseva TG, Vasetskii SG (1998) Discovery of triploid males in lower Don populations of the crucian carp *Carassius auratus gibelio* (Bloch). Doklady Akademii Nauk 363: 415–418

Alves MJ, Coelho MM, Collares-Pereira MJ (1998) Diversity in the reproductive modes of females of the *Rutilus alburnoides* complex (Teleostei, Cyprinidae): a way to avoid the genetic constraints of uniparentalism. Mol Biol Evol 15: 1233–1242

Alves MJ, Coelho MM, Collares-Pereira MJ (2001) Evolution in action through hybridisation and polyploidy in an Iberian freshwater fish: a genetic review. Genetica 111: 375–385

Alves MJ, Coelho MM, Prospero MI, Collares-Pereira MJ (1999) Production of fertile unreduced sperm by hybrid males of the *Rutilus alburnoides* complex (Teleostei, cyprinidae). An alternative route to genome tetraploidization in unisexuals. Genetics 151: 277–283

Alves MJ, Collares-Pereira MJ, Dowling TE, Coelho MM (2002) The genetics of maintenance of an all-male lineage in the *Squalius alburnoides* complex. J Fish Biol 60: 649–662

Alves MJ, Gromicho M, Collares-Pereira MJ, Crespo-Lopez ME, Coelho MM (2004) Simultaneous production of triploid and haploid eggs by triploid *Squalius alburnoides* (Teleostei: Cyprinidae). J Exp Zool 301A: 552–558

Angers B, Schlosser IJ (2007) The origin of *Phoxinus eos-neogaeus* unisexual hybrids. Mol Ecol 16: 4562–4571

Arai K (2003) Genetics of the loach, *Misgurnus anguillicaudatus*: recent progress and perspective. Folia Biol (Krakow) 51: 107–117

Arai K, Matsubara K, Suzuki R (1991) Chromosomes and developmental potential of progeny of spontaneous tetraploid loach *Misgurnus anguillicaudatus*. Nippon Suisan Gakkaishi 57: 2173–2178

Arai K, Matsubara K, Suzuki R (1993) Production of polyploids and viable gynogens using spontaneously occurring tetraploid loach, *Misgurnus anguillicaudatus*. Aquaculture 117: 227–235

Arias-Rodriguez L, Yasui GS, Katsutoshi Arai (in press) Disruption of normal meiosis in artificial inter-populational hybrid females of *Misgurnus* loach. Genetica 136: 49–52

Avise JC, Trexler JC, Travis J, Nelson WS (1991) *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. Evolution 45: 1530–1533

Beukeboom LW (2007) Sex to some degree. Heredity 98: 123–124

Beukeboom LW, Batenburg FHD (1999) The effect of paternal leakage on the rate of Muller's ratchet. 7th Conference of ESEB, Barcelona, Spain 1999, poster presentation

Beukeboom LW, Vrijenhoek RC (1998) Evolutionary genetics and ecology of sperm-dependent parthenogenesis. J Evol Biol 11: 755–782

Beukeboom LW, Weinzierl RP, Michiels NK (1995) Amazon molly and Muller's ratchet. Nature 375: 111–112

Bi K, Bogart JP (2006) Identification of intergenomic recombinations in unisexual salamanders of the genus *Ambystoma* by genomic in situ hybridization (GISH). Cytogenet Genome Res 112: 307–312

Bi K, Bogart JP, Fu J (2007) Intergenomic translocations in unisexual salamanders of the genus *Ambystoma* (Amphibia, Caudata). Cytogenet Genome Res 116: 289–297

Bohlen J (2000) Similarities and differences in the reproductive biology of loaches (*Cobitis* and *Sabanejewia*) under laboratory conditions. Folia Zoologica 49: 179–186

Bohlen J, Rab P (2001) Species and hybrid richness in spined loaches of the genus *Cobitis* (Teleostei: Cobitidae), with a checklist of European forms and suggestions for conservation. J Fish Biol 59: 75–89

Bohlen, Rab P, Slechtova V, Rabova M, Ritterbusch D, Freyhof J (2002) Hybridogenous biotypes in spined loaches (genus *Cobitis*) in Germany with implications for conservation. In: Collares-Pereira MJ, Coelho MM, Cowx IG (eds) Conservation of freshwater fishes: options for the future. Blackwell Science, Oxford, pp. 311–321

Boron A, Culling M, Pulym A (2003) Cytogenetic characteristics of the fish genus *Cobitis* from England. Folia Biol (Krakow) 51 Suppl: 13–16

Camacho JP, Sharbel TF, Beukeboom LW (2000) B-chromosome evolution. Philos Trans R Soc Lond B 355: 163–178

Campton (2008) Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. In: Ryman N, Utter F (eds) Population genetics and fishery management. University of Washington Press, Seattle, pp. 161–192

Cavallier-Smith T (2002) Origins of the machinery of recombination and sex. Heredity 88: 125–141

Choleva L, Apostolou A, Rab P, Janko K (2008) Making it on their own: sperm-dependent hybrid fishes (*Cobitis*) switch the sexual hosts and expand beyond the ranges of their original sperm donors. Philos Trans R Soc B 363: 2911–2919

Chun FL, Yang ST, Gui JF (2001) Differential screening and characterization analysis of the egg envelope glycoprotein ZP3 cDNAs between gynogenetic and gonochoristic crucian carp. Cell Res 11: 17–27

Collares-Pereira MJ, Alves MJ, Coelho MM (1999) Reassessment of the generic position of the Iberian cyprinid *alburnoides* complex: its return to the genus *Leuciscus*. J Fish Biol 54: 465–468

Crespo-Lopez ME, Pala I, Duarte TE, Dowling TE, Coelho MM (2007) Genetic structure of the diploid–polyploid fish *Squalius alburnoides* in southern Iberian basins Tejo and Guadiana, based on microsatellites. J Fish Biol 71: 423–436

Crivelli AJ (1995) Are fish introductions a threat to endemic freshwater fishes in the northern Mediterranean region? Biol Conserv 72: 311–319

Culling MA, Janko K, Boron A, Vasil'ev VP, Cote IM, Hewitt GM (2006) European colonization by the spined loach (*Cobitis taenia*) from Ponto-Caspian refugia based on mitochondrial DNA variation. Mol Ecol 15: 173–190

Cunha C, Coelho MM, Carmona JA, Doadrio I (2004) Phylogeographical insights into the origins of the *Squalius alburnoides* complex via multiple hybridization events. Mol Ecol 13: 2807–2817

Cunha C, Doadrio I, Coelho MM (2008) Speciation towards tetraploidization after intermediate processes of non-sexual reproduction. Philos Trans R Soc B 363: 2921–2929

Dawley (1989) An introduction to unisexual vertebrates. In: Dawley RM, Bogart JP (eds) Evolution and ecology of unisexual vertebrates, Bulletin 466. New York State Museum, Albany, New York, pp. 1–18

Dawley RM (1992) Clonal hybrids of the common laboratory fish *Fundulus heteroclitus*. Proc Natl Acad Sci USA 89: 2485–2488

Dawley RM, Chrzanowski D, Phiel KL, Beaulieu K, Goddard KA (1999) Allozyme analysis of clonal diversity among unisexual hybrids of the killifishes *Fundulus diaphanus* and *F. heteroclitus*. J Hered 90: 607–612

Dawley RM, Schultz RJ, Goddard KA (1987) Clonal reproduction and polyploidy in unisexual hybrids of *Phoxinus eos* and *Phoxinus neogaeus* (Pisces:Cyprinidae). Copeia 1987: 275–283

Dawley RM, Yeakel AM, Beaulieu KA, Phiel KL (2000) Histocompatibility analysis of clonal diversity in unisexual hybrids of the killifishes *Fundulus heteroclitus* and *Fundulus diaphanus*. Can J Zool 78: 923–930

Doeringsfeld MR, Schlosser IJ, Elder JF, Evenson DP (2004) Phenotypic consequences of genetic variation in a gynogenetic complex of *Phoxiuns eos-neogaeus* clonal fish (Pisces: Cyprinidae) inhabiting a heterogeneous environment. Evolution 58: 1261–1273

D′Souza TG, Schulte RD, Schulenburg H, Michiels NK (2006) Paternal inheritance in parthenogenetic forms of the planarian *Schmidtea polychroa*. Heredity 97: 97–101

Dubois A (1990) Nomenclature of parthenogenetic, gynogenetic and "hybridogenetic" vertebrate taxons: new proposals. Alytes (Paris) 8: 61–74

Echelle AA, Echelle AF (1997) Patterns of abundance and distribution among members of a unisexual-bisexual complex of fishes (Atherinidae: Menidia). Copeia 1997: 249–259

Echelle AA, Echelle AF, Crozier CD (1983) Evolution of an all-female fish, *Menidia clarkhubbsi* (Atherinidae). Evolution 37: 772–784

Echelle AA, Echelle AF, DeBault LE, Durham DW (1988) Ploidy levels in silverside fishes (Atherinidae, Menidia) on the Texas coast: flow-cytometric analysis of the occurrence of allotriploidy. J Fish Biol 32: 835–844

Echelle, Echelle AF, Middaugh DP (1989) Evolutionary biology of the *Menidia clarkhubbsi* complex of unisexual fishes (Atherinidae): Origins clonal diversity, and mode of reproduction. In: Dawley RM, Bogart JP (eds) Evolution and ecology of unisexual vertebrates, Bulletin 466. New York State Museum, Albany, New York, pp. 144–152

Echelle AA, Moisier DT (1982) *Menidia clarkhubbsi*, n. sp. (Pisces: Atherinidae), an all-female species. Copeia 1982: 533–540

Fan Z, Liu G (1990) The ploidy and reproductive mechanism of crucian carp, *Carassius auratus gibelio*. J Fish Biol 36: 415–419

Flajshans M, Rodina M, Halacka K, Vetesnik L, Papousek I, Gela D, Luskova V, Lusk S (2007) Ploidy levels and reproductive patterns of *Carassius auratus gibelio* as revealed by means of artificial hybridization and flow cytometry. Aquaculture 272: S256

Fraser D, Adams CE (1997) A crucian carp *Carassius carassius* (L.) in Loch Rannoch, Scotland: further evidence of the threat posed to unique fish communities by introduction of alien fish species. Aquatic Conserv 7: 323–326

Fritz ES, Garside ET (1974) Identification and description of hybrids of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae) from Porters Lake, Nova Scotia, with evidence for absence of backcrossing. Can J Zool 52: 1433–1442

Georgiades P, Watkins M, Burton GJ, Ferguson-Smith AC (2001) Roles for genomic imprinting and the zygotic genome in placental development. Proc Natl Acad Sci USA 98: 4522–4527

Goddard KA, Dawley RM (1990) Clonal inheritance of a diploid nuclear genome by a hybrid freshwater minnow (*Phoxinus eos-neogaeus*, Pisces: Cyprinidae). Evolution 44: 1052–1065

Goddard, Dawley RM, Dowling TE (1989) Origin and genetic relationships of diploid, triploid and diploid-triploid mosaic biotypes in the *Phoxinus eos-neogaeus* unisexual complex. In: Dawley RM, Bogart JP (eds) Evolution and ecology of unisexual vertebrates, Bulletin 466. New York State Museum, Albany, New York, pp. 268–280

Goddard KA, Megwinoff O, Wessner LL, Giaimo F (1998) Confirmation of gynogenesis in *Phoxinus eos-neogaeus* (Pisces: Cyprinidae). J Hered 89: 151–157

Goddard KA, Schultz RJ (1993) Aclonal reproduction by polyploid members of the clonal hybrid species *Phoxinus eos-neogaeus* (Cyprinidae). Copeia 3: 650–660

Green RF, Noakes DLG (1995) Is a little bit of sex as good as a lot? J Theor Biol 174: 87–96

Gromicho M, Coelho MM, Alves MJ, Collares-Pereira MJ (2006) Cytogenetic analysis of *Anaecypris hispanica* and its relationship with the paternal ancestor of the diploid-polyploid *Squalius alburnoides* complex. Genome 49: 1621–1627

Günther R, Uzzel T, Berger L (1979) Inheritance patterns in triploid *Rana* "*esculenta*" (Amphibia, Salientia). Mitt Zool Mus Berlin 55: 35–37

Hernandez Chavez C, Tuergeon J (2007) Asexual and sexual hybrids between *Fundulus diaphanus* and *F. heteroclitus* in the Canadian Atlantic region. Mol Ecol 16: 1467–1480

Hubbs C (1964) Interactions between a bisexual fish species and its gynogenetic sexual parasite. Bull Texas Memorial Mus 8: 1–72

Hubbs CL, Hubbs LC (1932) Apparent parthenogenesis in nature, in a form of fish of hybrid origin. Science 76: 628–630

Itono M, Morishima K, Fujimoto T, Bando E, Yamaha E, Arai K (2006) Premeiotic endomitosis produces diploid eggs in the natural clone loach, *Misgurnus anguillicaudatus* (Teleostei: Cobitidae). J Exp Zool 305A: 513–523

Itono M, Okabayashi N, Morishima K, Fujimoto T, Yoshikawa H, Yamaha E, Arai K (2007) Cytological mechanisms of gynogenesis and sperm incorporation in unreduced diploid eggs of the clonal loach, *Misgurnus anguillicaudatus* (Teleostei: Cobitidae). J Exp Zool 307A: 35–50

Janko K, Flajshhans M, Choleva L, Bohlen J, Slechtova V, Rabova M, Lajbner Z, Slechta V, Ivanovak P, Dobrovolok I, Culling M, Persat H, Kotusz J and Rab P (2007) Diversity of European spined loaches (genus *Cobitis* L.): an update of the geographic distribution of the *Cobitis taenia* hybrid complex with a description of new molecular tools for species and hybrid determination. J. Fish Biol. 71 Suppl C: 387–408

Janko K, Culling MA, Rab P, Kotlik P (2005) Ice age cloning – comparison of the Quaternary evolutionary histories of sexual and clonal forms of spiny loaches (*Cobitis*: Teleostei) using the analysis of mitochondrial DNA variation. Mol Ecol 14: 2991–3004

Janko K, Kotlik P, Rab P (2003) Evolutionary history of asexual hybrid loaches (*Cobitis*: Teleostei) inferred from phylogenetic analysis of mitochondrial DNA variation. J Evol Biol 16: 1280–1287

Jiang JG, Yu HX, Chen BD, Liang SC, Shan SX, Yang DL, Lin SE, Shen GQ (1983) Biological effect of heterologous sperm on gynogenetic offspring in *Carassius auratus gibelio*. Acta Hydrobiol Sin 8: 1–13

Kallman KD (1962a) Gynogenesis in the teleost, *M. formosa*, with discussion of the detection of parthenogenesis in vertebrates by tissue transplantation. J Genet 58: 7–21

Kallman KD (1962b) Population genetics of the gynogenetic teleost *Mollienesia formosa* (Girard). Evolution 16: 497–504

Kalous L, Slechtova V, Bohlen J, Petrtyl M, Svatora M (2007) First European record of *Carassius langsdorfii* from the Elbe basin. J Fish Biol 70 Suppl A: 132–138

Kim IS, Lee EH (2000) Hybridization experiment of diploid-triploid cobitid fishes, *Cobitis sinensis-longicorpus* complex (Pisces: Cobitidae). Folia Zool 49: 17–22

Kim IS, Lee JH (1990) Diploid-triploid hybrid complex of the Spined loach *Cobitis sinensis* and *C. longicorpus* (Pisces, Cobitidae). Korean J Ichthyol 2: 203–210

Kobayashi H, Kawashima Y, Takeuchi N (1970) Comparative chromosome studies in the genus *Carassius*, especially with a finding of polyploidy in the ginbuna (*C. auratus langsdorfii*). Japanese J Ichthyol 17: 153–160

Kobayashi H, Nakano K, Nakamura M (1977) On the hybrids, 4n ginbuna (*Carassius auratus langsdorfii*) x kinbuna (*Carassius auratus* subsp.), and their chromosomes. Bull Jap Soc Sci Fisheries 46: 413–418

Kottelat M (1997) Environmental biology of fishes. An heuristic checklist of the freshwater fishes of Europe (exclusive of former USSR), with an introduction for non-systematists and comments on nomenclature and conservation. Biologia (Bratislava) Sect Zool 52 Suppl: 1–271

Lamatsch DK, Nanda I, Epplen JT, Schmid M, Schartl M (2000) Unusual triploid males in a microchromosome-carrying clone of the Amazon molly, *Poecilia formosa*. Cytogenet Cell Genet 91: 148–156

Lamatsch DK, Nanda I, Schlupp I, Epplen JT, Schmid M, Schartl M (2004) Distribution and stability of supernumerary microchromosomes in natural populations of the Amazon molly, *Poecilia formosa*. Cytogenet Genome Res 106: 189–194

Lamatsch DK, Schmid M, Schartl M (2002) A somatic mosaic of the gynogenetic Amazon molly. J Fish Biol 60: 1417–1422

Lampert KP, Lamatsch DK, Epplen JT, Schartl M (2005) Evidence for a monophyletic origin of triploid clones of the Amazon molly, *Poecilia formosa*. Evolution 59: 881–889

Lampert KP, Lamatsch DK, Fischer P, Schartl M (2008) A tetraploid Amazon molly, *Poecilia formosa*. J Hered 99: 223–226

Lampert KP, Schartl M (2008) The origin and evolution of a unisexual hybrid: *Poecilia formosa*. Philos Trans R Soc Lond B 363: 2901–2909

Lampert KP, Steinlein C, Schmid M, Fischer P, Schartl M (2007a) A haploid-diploid-triploid mosaic of the Amazon molly, *Poecilia formosa*. Cytogenet Genome Res 119: 131–134

Lampert KP, Lamatsch DK, Fischer P, Epplen JT, Nanda I, Schmid M, Schartl M. (2007b) Automictic reproduction in interspecific hybrids of poeciliid fish. Curr Biol 17: 1948–1953

Leonardos ID, Tsikliras AC, Eleftheriou V, Cladas Y, Kagalou I, Chortatou R, Papigioti O (2008) Life history characteristics of an invasive cyprinid fish (*Carassius gibelio*) in Chimaditis Lake (northern Greece). J Appl Ichthyol 24: 213–217

Li Y, Yin J, Wang J, Yuan X, Wei J, Sun X, Arai K (2008) A study on the distribution of polyploid loaches in China. Nippon Suisan Gakkaishi 74: 177–182

Loewe L, Lamatsch DK (2008) Quantifying the threat of extinction from Muller's ratchet in the diploid Amazon molly (*Poecilia formosa*). BMC Evol Biol 8: 88–108

Lynch M, Burger R, Butcher D, Gabriel W (1993) The mutational meltdown in asexual populations. J Hered 84: 339–344

Mable BK (2007) Sex in the postgenomic era. Trends Ecol Evol 22: 559–561

Mateos M, Vrijenhoek RC (2002) Ancient versus reticulate origin of a hemiclonal lineage. Evolution 56: 985–992

Mateos M, Vrijenhoek RC (2005) Independent origins of allotriploidy in the fish genus *Poeciliopsis*. J Hered 96: 32–39

Mateos M, Sanyur OI, Vrijenhoek RC (2002) Historical biogeography of the livebearing fish genus *Poeciliopsis* (Poeciliidae: Cyprinodontiformes). Evolution 56: 972–984

Mezhzherin SV, Chudakorova TY (2002) The genetic structure of the diploid-polyploid complex of the spined loach *Cobitis taenia* (Cypriniformes: Cobitidae) from the middle Dnieper basin. Russ J Genet 38: 70–75

Morishima K, Nakamura-Shiokawa Y, Bando E, Li YJ, Boron A, Khan MMR, Arai K (2008a) Cryptic clonal lineages and genetic diversity in the loach *Misgurnus anguillicaudatus* (Teleostei: Cobitidae) inferred from nuclear and mitochondrial DNA analyses. Genetica 132: 159–171

Morishima K, Yoshikawa H, Arai K (2008b) Meiotic hybridogenesis in triploid *Misgurnus* loach derived from a clonal lineage. Heredity 100: 581–586

Morishima K, Oshima K, Horie S, Fujimoto T, Yamaha E, Arai K (2004) Clonal diploid sperm of the diploid-triploid mosaic loach, *Misgurnus anguillicaudatus* (Teleostei:Cobitidae). J Exp Zool 301A: 502–511

Morishima K, Horie SYE, Arai K (2002) A cryptic clonal line of the loach *Misgurnus anguillicaudatus* (Teleostei: Cobitidae) evidenced by induced gynogenesis, interspecific hybridization, microsatellite genotyping and multilocus DNA fingerprinting. Zool Sci 19: 565–575

Moritz C, Brown WM, Densmore LD, Wright JW, Vyas D, Donnellan S, Adams M,Braverstock, P (1989). Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekkonidae). In: RM Dawley and JB Bogart (eds) Evolution and ecology of unisexual vertebrates. Bulletin 466, New York State Museum, Albany, New York, pp. 87–112

Muller HJ (1964) The relation of recombination to mutational advance. Mutat Res 1: 2–9

Murakami M, Fujitani H (1997) Polyploid-specific repetitive DNA sequences from triploid ginbuna (Japanese silver crucian carp, *Carassius auratus langsdorfi*). Genes Genet Syst 72: 107–113

Murakami M, Matsuba C, Fujitani H (2001) The maternal origins of the triploid ginbuna (*Carassius auratus langsdorfi*): phylogenetic relationships within the *C. auratus* taxa by partial mitochondrial D-loop sequencing. Genes Genet Syst 76: 25–32

Murakami M, Matsuba C, Fujitani H (2002) Characterization of DNA markers isolated from the gynogenetic triploid ginbuna (*Carassius auratus langsdorfi*) by representational difference analysis. Aquaculture 208: 59–68

Muramoto J (1975) A note on triploidy of the *Funa* (Cyprinidae, Pisces). Proc Japan Acad 51: 583–587

Murayama Y, Hijikata M, Kojima M, Noda M, Kajishima T (1986) The appearance of diploid-triploid and diploid-triploid-tetraploid mosaic individuals in polyploid fish, ginbuna (*Carassius auratus langsdorfii*). Experientia 42: 187–188

Nanda I, Schlupp I, Lamatsch DK, Lampert KP, Schmid M, Schartl M (2007) Stable inheritance of host species-derived microchromosomes in the gynogenetic fish, *Poecilia formosa*. Genetics 177: 917–926

Niemeitz A, Kreutzfeld R, Schartl M, Parzefall J, Schlupp I (2002) Male mating behaviour of a molly, *Poecilia latipunctata*: a third host for the sperm-dependent Amazon molly, *Poecilia formosa*. Acta Ethologica 5: 45–49

Ogielska M, Kierzkowski P, Rybacki M (2004) DNA content and genome composition of diploid and triploid water frogs belonging to the *Rana esculenta* complex (Amphibia, Anura). Can J Zool 82: 1894–1901

Omilian AR, Cristescu MEA, Dudycha JL, Lynch M (2006) Ameiotic recombination in asexual lineages of *Daphnia*. Proc Natl Acad Sci USA 103: 18638–18643

Ojima Y, Takai A (1979) The occurrence of spontaneous polyploid in the Japanese common loach, *Misgurnus anguillicaudatus*. Proc Japan Acad 55: 487–491

Oshima K, Morishima K, Yamaha E, Arai K (2005) Reproductive capacity of triploid loaches obtained from Hokkaido Island, Japan. Ichthyol Res 52: 1–8

Pala I, Coelho MM (2005) Contrasting views over a hybrid complex: between speciation and evolutionary "dead-end". Gene 347: 283–294

Pala I, Coelho MM, Schartl M. (2008) Dosage compensation by gene-copy silencing in a triploid hybrid fish. Curr Biol 18: 1344–1348

Pandian TJ, Koteeswaran R (1998) Ploidy induction and sex control in fish. Hydrobiologia 384: 167–243

Quattro JM, Avise JC, Vrijenhoek RC (1991) Molecular evidence for multiple origins of hybridogenetic fish clones (Poeciliidae: *Poeciliopsis*). Genetics 127: 391–398

Quattro JM, Avise JC, Vrijenhoek RC (1992) Mode of origin and sources of genotypic diversity in triploid gynogenetic fish clones (*Poeciliopsis*: Poeciliidae). Genetics 130: 621–628

Rab P, Rabova M, Bohlen J, Lusk S (2000) Genetic differentiation of the two hybrid diploid-polyploid complexes of loaches, genus *Cobitis* (Cobitidae), involving *C. taenia*, *C. elongatiodes* and *C. spp.* in the Czech Republic. Folia Zoologica 49 Suppl1: 55–66

Rasch, Balsano JS (1989) Trihybrids related to the unisexual molly fish, *Poecilia formosa*. In: Dawley RM, Bogart JP (eds) Evolution and ecology of unisexual vertebrates, Bulletin 466. New York State Museum, Albany, New York, pp. 252–267

Robalo JI, Sousa Santos C, Levy A, Almada VC (2006) Molecular insights on the taxonomic position of the paternal ancestor of the *Squalius alburnoides* hybridogenetic complex. Mol Phyl Evol 39: 276–281

Rougier N, Werb Z (2001) Minireview: parthenogenesis in mammals. Mol Reprod Dev 59: 468–474

Saat TV (1991) Reproduction of the diploid and polyploid spinous loach (*Cobitis*, Teleostei). Oocyte maturation and fertilization in the triploid form. Soviet J Dev Biol 22: 332–338

Saitoh K, Kim IS, Lee EH (2004) Mitochondrial gene introgression between spined loaches via hybridogenesis. Zool Sci 21: 795–798

Sawada Y (1982) Phylogeny and zoogeography of the superfamily Cobitoidea (Cyprinoidei, Cypriniformes). Mem Fac Fisher Hokkaido Univ 28: 65–223

Schartl A, Hornung U, Nanda I, Wacker R, Muller-Hermelink HK, Schlupp I, Parzefall J, Schmid M, Schartl M (1997) Susceptibility to the development of pigment cell tumors in a clone of the Amazon molly, *Poecilia formosa*, introduced through a microchromosome. Cancer Res 57: 2993–3000

Schartl M, Nanda I, Schlupp I, Wilde B, Epplen JT, Schmid M, Parzefall J (1995a) Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. Nature 373: 68–71

Schartl M, Wilde B, Schlupp I, Parzefall J (1995b) Evolutionary origin of a parthenoform, the Amazon molly *Poecilia formosa*, on the basis of a molecular genealogy. Evolution 49: 827–835

Schlupp I (2005) The evolutionary ecology of gynogenesis. Annu Rev Ecol Evol Syst 36: 399–417

Schlupp I, Nanda I, Dobler M, Lamatsch DK, Epplen JT, Parzefall J, Schmid M, Schartl M (1998) Dispensable and indispensable genes in an ameiotic fish, the Amazon molly *Poecilia formosa*. Cytogenet Cell Genet 80: 193–198

Schlupp I, Parzefall J, Schartl M (2002) Biogeography of the Amazon molly, *Poecilia formosa*. J Biogeogr 29: 1–6

Schories S, Lampert KP, Lamatsch DK, Garcia de Leon FJ, Schartl M (2007) Analysis of a possible independent origin of triploid *P. formosa* outside of the Río Purificación river system. Frontiers Zool 4:13

Schultz RJ (1967) Gynogenesis and triploidy in the viviparous fish *Poeciliopsis*. Science 157: 1564–1567

Schultz RJ (1969) Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am Nat 103: 605–661

Schultz RJ (1973) Unisexual fish: laboratory synthesis of a "species". Science 179: 180–181

Schultz RJ (1977) Evolution and ecology of unisexual fishes. Evol Biol 10: 277–331

Schultz RJ, Kallman KD (1968) Triploid hybrids between the all-female teleost *Poecilia formosa* and *Poecilia sphenops*. Nature 219: 280–282

Schultz RJ (1979) Role of polyploidy in the evolution of fishes. Basic Life Sci 13: 313–340

Scott RJ, Spielman M (2006) Genomic imprinting in plants and mammals: how life history constrains convergence. Cytogenet Genome Res 113: 53–67

Scribner KT, Page KS, Bartron ML (2000) Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. Rev Fish Biol Fisheries 10: 293–323

Slechtova V, Bohlen J, Perdices A (2008) Molecular phylogeny of the freshwater fish family Cobitidae (Cypriniformes : Teleostei): Delimitation of genera, mitochondrial introgression and evolution of sexual dimorphism. Mol Phyl Evol 47: 812–831

Slechtova V, Luskova V, Slechta V, Lusk S, Halacka K, Bohlen J (2000) Genetic differentiation of two diploid-polyploid complexes of spined leach, genus *Cobitis* (Cobitidae), in the Czech Republic, involving *C. taenia*, *C. elongatoides,* and *C.* spp.: Allozyme interpopulation and interspecific differences. Folia Zoologica 49: 67–78

Sola L, Rossi AR, Bressanello S, Rasch EM, Monaco PJ (1993) Cytogenetics of bisexual/unisexual species of *Poecilia*. V. Unisexual poeciliids with anomalous karyotypes from northeastern Mexico. Cytogenet Cell Genet 63: 189–191

Sousa Santos C, Collares-Pereira MJ, Almada VC (2006) Evidence of extensive mitochondrial introgression with nearly complete substitution of the typical *Squalius pyrenaicus*-like mtDNA of the *Squalius alburnoides* complex (Cyprinidae) in an independent Iberian drainage. J Fish Biol 68 Suppl B: 292–301

Stöck M, Lamatsch DK, Steinlein C, Epplen JT, Grosse WR, Hock R, Klapperstuck T, Lampert KP, Scheer U, Schmid M, Schartl M (2002) A bisexually reproducing all-triploid vertebrate. Nat Genet 30: 325–328

Turner BJ, Balsano JS, Monaco PJ, Rasch EM (1983) Clonal diversity and evolutionary dynamics in a diploid-triploid breeding complex of unisexual fishes (*Poecilia*). Evolution 37: 798–809

Vasil'ev VP, Akimova NV, Emel′yanova NG, Pavlov DA, Vasil'eva ED (2003) Reproductive capacities in the polyploid males of spined loaches from the unisexual-bisexual complex, occurred in the Moscow River. Folia Biol (Krakow) 51 Suppl: 67–73

Vasil'ev VP, Vasil'eva KD (1982) A new diploid-polyploid complex in fishes. Dokl Akad Nauk SSSR 266: 250–252

Vasil'ev VP, Vasil'eva KD, Levenkova ES (2007) Trihybrid origin of an all-female tetraploid form of fish from the genus *Cobitis* (Pisces, Cobitidae). Doklady Biol Sci 413: 107–110

Vasil'ev, Vasil'eva KD, Osinov AG (1989) Evolution of a diploid-triploid-tetraploid complex in fishes of the genus *Cobitis* (Pisces, Cobitidae). In: Dawley RM, Bogart JP (eds) Evolution and ecology of unisexual vertebrates, Bulletin 466. New York State Museum, Albany, New York, pp. 153–169

Veetema M, Eschbaum R, Albert A, Saat T (2005) Distribution, sex ratio and growth of *Carassius gibelio* (Bloch) in coastal and inland waters of Estonia (north-eastern Baltic Sea). J Appl Ichthyol 21: 287–291

Verreycken H, Anseeuw D, Van Thuyne G, Quataert P, Belpaire C (2007) The non-indigenous freshwater fishes of Flanders (Belgium): review, status and trends over the last decade. J Fish Biol 71: 160–172

Vrijenhoek RC (1994) Unisexual fish: model systems for studying ecology and evolution. Annu Rev Ecol Syst 25: 71–96

Vrijenhoek RC, Angus RA, Schultz RJ (1977) Variation and heterozygosity in sexually vs. clonally reproducing populations of *Poeciliopsis*. Evolution 31: 767–781

Vrijenhoek RC, Schultz RJ (1974) Evolution of a trihybrid unisexual fish (*Poeciliopsis*, Poeciliidae). Evolution 28: 306–319

Wei WH, Zhang J, Zhang YB, Zhou L, Gui JF (2003) Genetic heterogeneity and ploidy level analysis among different gynogenetic clones of the polyploid gibel carp. Cytometry Part A 56A: 46–52

Wetherington JD, Kotora KE, Vrijenhoek RC (1987) A test of the spontaneous heterosis hypothesis for unisexual vertebrates. Evolution 41: 721–723

Yi MS, Li YQ, Liu JD, Zhou L, Yu QX, Gui JF (2003) Molecular cytogenetic detection of paternal chromosome fragments in allogynogenetic gibel carp, *Carassius auratus gibelio* Bloch. Chromosome Res 11: 665–671

Yoshikawa H, Morishima K, Fujimoto T, Yamaha E, Arai K (2007) Simultaneous formation of haploid, diploid and triploid eggs in diploid–triploid mosaic loaches. J Fish Biol 71: 250–263

Zhang Q, Arai K (1999) Aberrant meioses and viable aneuploid progeny of induced triploid loach (*Misgurnus anguillicaudatus*) when crossed to natural tetraploids. Aquaculture 175: 63–76

Zhou L, Wang Y, Gui JF (2000) Analysis of genetic heterogeneity among five gynogenetic clones of silver crucian carp, *Carassius auratus gibelio* Bloch, based on detection of RAPD molecular markers. Cytogenet Cell Genet 88: 133–139

Zhu HP, Gui JF (2007) Identification of genome organization in the unusual allotetraploid form of *Carassius auratus gibelio*. Aquaculture 265: 109–117