Chapter 3

Molecular Systematics of *Sander*, and Hybridization between Walleye and Sauger

Neil Billington and Brian L. Sloss

3.1 INTRODUCTION

Sander and its percid relatives have been the subject of several phylogenetic inquiries. The purpose of this chapter is to focus on efforts to elucidate the intragenus and family-wide relationships using molecular genetic techniques. In addition to the currently recognized phylogenetic relations among *Sander* and other Percidae, a review of previous research is presented outlining the various methods and findings from key research into their evolution. A preview of future techniques and efforts is provided. The last half of this chapter is focused on hybridization between walleye and sauger and includes the occurrence of natural hybrids and methods used to detect the hybrids effectively, as well as brief reference to other *Sander* species hybrids.

3.2 MOLECULAR SYSTEMATICS

The inherent value in understanding the phylogenetic relationships for a group of organisms is not always self-evident. Mayden and Wood (1995) suggested three primary roles of modern systematic biology: discovering natural diversity, determining patterns of natural order, and discerning relationships among the products of evolutionary descent. The analysis and discovery of phylogenetic histories enable us to investigate the evolutionary past of a group of organisms at many different levels (Maddison 1996). Understanding the evolution of specific organisms or groups of organisms mandates an accurate estimation of the relationships among these taxa. Without accurate sister-group relationships, studies attempting to partition characteristics such as behavior, morphology, ecology, physiology, and many other attributes of organisms as the result of historical constraint or as the result of independent divergence are speculative (Mayden and Wood 1995). The accurate construction of historical biogeographical distributions of taxa is reliant on an accurate phylogeny of the group of taxa being studied. Furthermore, hypotheses of phylogenetic relationships and biogeographical distribution allow researchers to examine the evolution of groups of organisms in relation to their origin, age of divergence, and probable patterns of dispersal (Mayden and Wood 1995). A comprehensive understanding of the evolution and diversification of taxonomic lineages

can provide valuable insight into life history strategies, adaptability, and resilience to future perturbations.

Multiple molecular genetic techniques have been used to study percid phylogenetic relationships. These have included allozyme markers (e.g., Page and Whitt 1973a, 1973b; Wood and Mayden 1997), a combination of allozyme and mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis (e.g., Billington et al. 1990, 1991), DNA sequencing of portions of the mtDNA control region (e.g., Faber and Stepien 1997, 1998; Turner 1997), the mtDNA cytochrome *b* gene (e.g., Song et al. 1998; Near 2002), multiple mtDNA genes (e.g., Sloss and Billington 2004; Sloss et al. 2004), and a combination of mitochondrial and nuclear DNA genes (Keck and Near 2008). These studies have focused on relations within *Sander*, between *Sander* and other percid species, or the relations within Percidae as a whole.

3.2.1 Relationship among *Sander* species

The genus *Sander*, the pikeperches, is composed of five species: the North American endemics, sauger (Griffith and Smith, 1834) and walleye (Mitchill, 1818), and three species of European endemics, the pikeperch or zander (Linnaeus, 1758), estuarine perch or sea pikeperch (Cuvier, 1828), and Volga pikeperch (Gmelin, 1789) (Collette and Bănărescu 1977; Craig 2000). The blue pike (Hubbs, 1926) was a putative subspecies of walleye once endemic to Lake Erie, Lake Ontario, and the upper Niagara River that became extinct by the late 1960s (McAllister et al. 1985; Campbell 1987). The biology of these species was reviewed by Craig (2000) and their distributions described and mapped by Collette and Bănărescu (1977).

A comprehensive intrageneric phylogeny was proposed by Billington et al. (1990) who used both allozymes and whole-molecule mtDNA RFLP analysis of three *Sander* species: walleye, sauger, and zander. Billington et al. (1990) used published molecular clock calibrations for allozymes and mtDNA RFLP data to estimate divergence times of the species that were compared. Walleye and sauger were resolved as each other's closest relatives with divergence times estimated as 3.12 ± 1.33 (±SE) million years before present (MYBP) from allozyme data and 4.06 ± 0.73 MYBP for mtDNA data. Estimated divergence times between the North American species and the European zander were 10.59 ± 2.74 MYBP from allozyme data and 7.86 ± 1.18 MYBP for mtDNA data. Subsequently, Billington et al. (1991) added a fourth species, the European Volga pikeperch, and found that the two European species clustered together with an estimated divergence time of 6.02 MYBP from allozyme data and 1.98 ± 0.47 MYBP for mtDNA data. Estimated divergence times between the North American species and the European pikeperches in this second study were 13.28 ± 3.78 MYBP from allozyme data and 7.42 ± 1.01 MYBP for mtDNA data (Billington et al. 1991). These estimated divergence times between North American and European species were consistent with the hypothesis that *Sander* colonized North America via Beringia about 10 million years ago during the Pliocene (Stetovidov and Dorofeeva 1963; Collette and Bănărescu 1977) and discounted hypotheses of an earlier Miocene Atlantic colonization route (Lindberg 1961, 1962, cited in Stetovidov and Dorofeeva 1963) or a more recent Pleistocene Atlantic colonization route (Čihař 1975).

Walleye and sauger were again shown to be more closely related to each other than to zander based upon sequencing of the mtDNA control region (D-loop) (Faber and Stepien 1997). Interestingly, *Sander* appeared to be paraphyletic (not all descended from a common ancestor) in that it formed a sister group with yellow perch. Faber and Stepien (1997) esti-

mated a divergence time of 3.85 ± 0.90 MYBP between walleye and sauger, values similar to those obtained by Billington et al. (1990) and 4.75 ± 1.45 MYBP between the North American species and the European zander, which was about half of the values reported by Billington et al. (1990, 1991), but still more compatible with a Beringian colonization route than alternative hypotheses.

Faber and Stepien (1998) added Volga pikeperch to the three other *Sander* species studied by Faber and Stepien (1997) and found divergence time estimates of 4.05 ± 0.50 MYBP between the North American and European *Sander* species, similar to their previous values. Faber and Stepien (1998) found that the two European species diverged 1.80 ± 0.30 MYBP, similar to the mtDNA divergence time of 1.98 ± 0.47 MYBP for mtDNA (Billington et al. 1991), whereas the allozyme data suggested a longer divergence time of approximately 6 MYBP (Billington et al. 1991). Differences in the divergence dates for the comparison of the two North American *Sander* species and the two European *Sander* species that were examined by the two mtDNA data sets were probably due to the different approaches used. The studies conducted by Billington et al. (1990, 1991) were based upon whole-molecule mtDNA RFLP analysis where a mixture of rapidly evolving and more slowly evolving genes were surveyed, whereas the Faber and Stepien (1997, 1998) studies involved sequencing the mtDNA D-loop region, which is one of the more rapidly evolving regions of the molecule.

A recent paper by Murray et al. (2009) reported that fossil remains of a *Sander* species were found in the Canadian Arctic on Ellesmere Island in deposits that date to approximately 4.5–5 MYBP; the new species was named *Sander teneri*. This fossil evidence is important because it provided the first prePleistocene fossil for a *Sander* species in North America and further evidence for a Pliocene colonization of North America from Eurasia via Beringia.

3.2.2 Relationship to Other Members of the Percidae

The family Percidae (Order Perciformes) consists of more than 190 species (>178 species in North America, 14 species in Eurasia) in 10 genera (Bart and Page 1992; Page 2000; see also Chapter 2). Percids are found in freshwater habitats of temperate and subarctic regions of the northern hemisphere (Craig 2000). In North America, percid importance lies in their great diversity, their propensity to exist in large populations, their influences on the ecology of streams and rivers (Bart and Page 1992), and for the larger species their value as targets of sport and commercial fisheries. The Eurasian diversity of percids is much less with only 14 species placed in six genera, although nearly half of these are valued species in the commercial fishing industry. The perches (*Perca*) and pikeperches (*Sander*) are the only percids to occur natively in both North American and Eurasian waters. The recent invasion of the Great Lakes by the ruffe (Pratt et al. 1992), an exotic from Europe, has raised the number of percid genera shared between the two continents to three.

The phylogenetic relationships within Percidae have been the focus of numerous scientific inquiries. Attempts at elucidating a large-scale, family-wide phylogenetic hypothesis have been restricted to nine primary studies (Collette 1963; Hubbs 1971; Collette and Bănărescu 1977; Page 1985; Coburn and Gaglione 1992; Wiley 1992; Song et al. 1998; Sloss et al. 2004; J. C. Bruner, Chapter 2, this volume). Of these studies, only two, Song et al. (1998) and Sloss et al. (2004), have explicitly focused on the molecular systematics of the entire Percidae based on DNA sequence data. The remaining works focused on various combinations of morphological, osteological, reproductive, and behavioral characteristics (see Chapter 2).

Song et al. (1998) were the first to employ DNA sequence data in an effort to elucidate phylogenetic relationships among Percidae. This study was also the first to use multiple algorithms, neighbor-joining (Saitou and Nei 1987) and stepwise heuristic searches with branch-swapping (Swofford et al. 1996), and optimality criteria, Fitch parsimony (Fitch 1971), and minimum evolution (Kidd and Sgaramella-Zonta 1971; Rzhetsky and Nei 1993), coupled with statistical measures of support for the resulting relationships, including bootstrap (Felsenstein 1985) and decay indices (Bremer 1988). Cytochrome *b* sequence data were acquired for 21 percid species representing all genera except *Percarina* (*demidoffi*) and numerous potential outgroup taxa from within the Perciformes. Their analyses resulted in a final hypothesis that supported a three-subfamily classification within the Percidae: the Percinae (*Perca* and *Gymnocephalus*), Luciopercinae (*Stizostedion* and Romanichthyini), and Etheostomatinae (North American darter genera). Throughout their parsimony-based analysis and paralinear (Saitou and Nei 1987) and stepwise heuristic searches with branch-swapping (Swofford et al. 1996), and optimality criteria, Fitch parsimony (Fitch 1971), and minimum evolution (Kidd and Sgaramella-Zonta 1971; Rzhetsky and Nei 1993), coupled with a distance-based statistical measure of support for the resulting relationships, the bootstrap (Felsenstein 1985), the topology of their trees consistently recovered a two-clade scenario with Percinae and Luciopercinae in one clade and Etheostomatinae representing the other clade. This topology was consistent with the zoogeographical discussion of Collette and Bănărescu (1977) in that the subfamily Etheostomatinae has been separated from the other percids for a long evolutionary period.

The most recent attempt to use molecular genetic data to determine the relations among the percids was that of Sloss et al. (2004), who used combined mtDNA data of cytochrome *b* (as in Song et al. 1998) and the 12S rRNA gene for 54 species of percids from 9 of the 10 recognized genera (missing *Percarina*) and including all but one recognized subgenus of darters. Sloss et al. (2004) used maximum parsimony, maximum likelihood, and Bayesian analyses to resolve a family-wide phylogeny (Figure 3.1). Key facets of the phylogeny included monophyly of all genera (where multiple representatives occurred) except for *Etheostoma* and *Zingel*. The trees depicted *Sander* as monophyletic and supported the same two highly resolved clades as did Song et al. (1998): North American *Sander* and European *Sander*. *Sander* was resolved in a clade with *Zingel* and *Romanichthys*, two genera of percids found in Eurasian waters and thought to have diverged (probably from a pikeperch-type ancestor based on this phylogeny) in response to similar environmental conditions (Collette and Bănărescu 1977) and thus show many similarities in morphology and behavior to the darters (Song et al. 1998). Sloss et al. (2004) found moderate evidence for *Perca* being the basal lineage of Percidae, with *Gymnocephalus* (the ruffe) being more closely related to a clade of *Sander*, *Zingel*, and *Romanichthys* than to *Perca*. Nevertheless, the authors concluded that a basal trichotomy existed between the three subfamilial groups with *Gymnocephalus* moving from the Percinae to the Luciopercinae (Figure 3.2). Therefore, the current molecular-based subfamilial classification is: Etheostomatinae (*Etheostoma*, *Percina*, *Ammocrypta*, *Crystallaria*), Luciopercinae (*Sander*, *Zingel*, *Romanichthys*, *Gymnocephalus*), and Percinae (*Perca*).

3.2.3 Future Directions in Percid Molecular Systematics

The pursuit of phylogenetic relationships among species is sometimes looked at by applied management professionals as an esoteric effort. As stated previously in this chapter, understanding the evolutionary and phylogeographical patterns that have resulted in

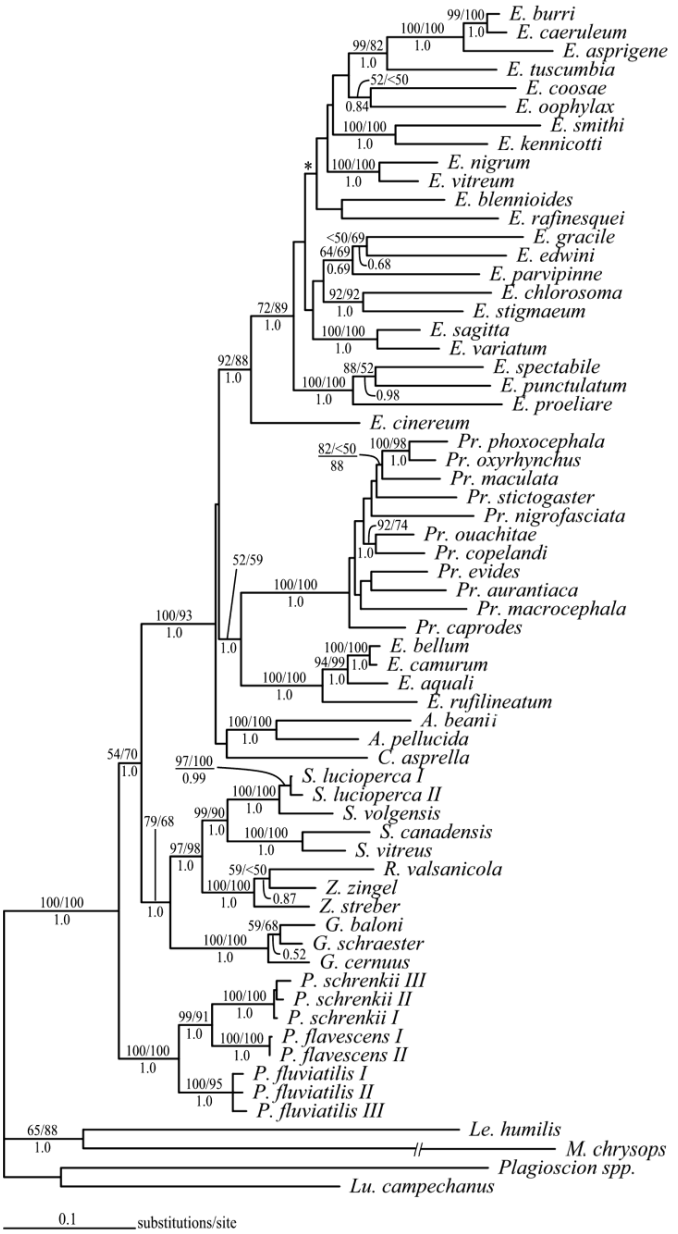

Figure 3.1. Maximum likelihood estimated tree topology for the combined cytochrome *b* and 12S mtDNA sequence data from Sloss et al. (2004). Numbers above the nodes represent maximum likelihood bootstrap support–maximum parsimony bootstrap support and the number below the nodes are Bayesian posterior probabilities. Asterisk (*) indicates node resolved in all analyses with a BAY posterior probability of 96%, but bootstrap values less than 50%. (Reprinted with permission from Elsevier Publishing from the original publication of Sloss et al. 2004.)

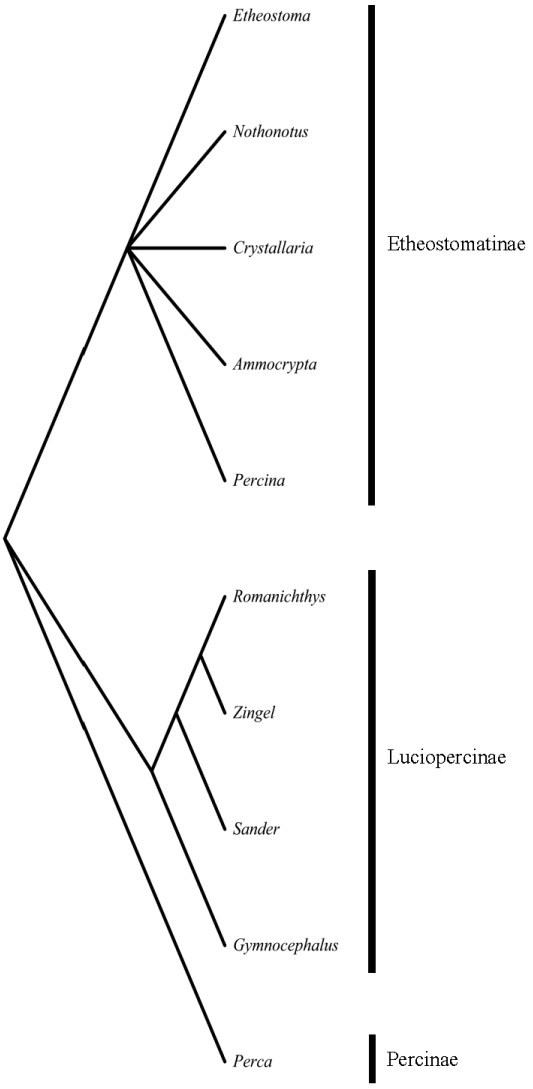

Figure 3.2. Simplified overview of the phylogenetic relationships of percid genera resolved in Sloss et al. (2004) with subfamilial designations. Topology is based on a combination of consistent resolution with moderate to high support in a majority of the inferences. (Modified and reprinted with permission from Elsevier Publishing from the original publication of Sloss et al. 2004.)

our current biodiversity provides the clearest foundation upon which to base conservation of genetic and ecological resources in the future. Recent advances in phylogenetic inference and genetic data acquisition have allowed new and improved resolution to challenges in the field of molecular systematics and conservation (see Avise 2010). Key among these advances is the use of multilocus–multigenome sequence data sets where DNA sequence data from numerous genes are used to converge on a species tree hypothesis versus single

gene tree topologies. Advances in sequencing technology have allowed for more cost-effective and time-efficient sampling of multiple gene regions. Further advances (i.e., next generation sequencing) that are capable of generating megabases of DNA data in a short period of time have the potential to reinvigorate issues of phylogeography and phylogenetic relationships.

The molecular systematics of Percidae, despite the resolution of numerous internal relationships, is far from resolved. Within Percidae, several key issues remain. First, the overall relations among the subfamilies is important in understanding the overall evolution of the family. Understanding the patterns of colonization of North America from Eurasian percid ancestors provides key findings not just to percid systematics but to numerous phylogeographically relevant questions of colonization. Second, further phylogenetic resolution within the family will probably focus on the relations among and within the various genera, subgenera, and species groups of darters in Etheostomatinae. This group is second only to the minnows (Cyprinidae) in terms of diversity in North America and a majority of the species occur in sensitive conservation areas. Finally, the level of variation within species and overall conservation of genetic diversity is part of a continuum of genetic diversity from family-level relations to stock-based differences. Understanding the higher-order relations provides a frame of reference for specific, population- or stock-based studies of diversity and conservation of diversity. We suggest future studies of percid phylogenetic relations using molecular data focus on the inclusion of multiple nuclear loci, rely on geographical representatives in the data set (versus single exemplars), and strive to complete species sampling of *Sander* to include estuarine perch *S. marinus*, or sea pikeperch, and include the species in the one genus missing in major work to date, *Percarina demidoffi*.

3.3 HYBRIDIZATION BETWEEN WALLEYE AND SAUGER

3.3.1 Method of Hybridization and Types of Hybrids

Walleye and sauger are externally fertilizing, broadcast-spawning fish that release eggs and sperm at night on gravel, rubble, or riprap substrate in early spring. Walleye may spawn slightly before sauger, but their spawning periods can overlap in some systems (see Chapter 7). In turbid conditions, hybridization can occur presumably due to a failure to recognize a conspecific mate (Billington et al. 1997). Walleye × sauger F_1 hybrids are fertile (Hearn 1986), and the F_1 and subsequent hybrids can backcross with either parental species leading to introgression: the movement of genes of one species into another (Campton 1987, 1990).

Typically, any walleye × sauger hybrid has been referred to as a saugeye. However, saugeyes are technically the result of the directional cross of a male sauger and a female walleye. This cross is commonly used in both aquaculture and propagation programs in various states (see Chapter 13). The reciprocal cross, a male walleye × a female sauger, usually performs less well in culture (Malison et al. 1990), and no formal name has been assigned to this cross. If the same procedure that was used to come up with the name saugeye (first four letters of the male parent and last three letters of the female parent) is used, the name for the reciprocal cross presumably would be "wallger," but this term has not been used to date in the literature.

3.3.2 Morphological Comparison of Walleye, Sauger, and their F_1 Hybrid

Several external morphological characteristics distinguish walleye from sauger (Scott and Crossman 1973; Trautman 1981; Page and Burr 1991). Typically, (1) walleyes (Figure 3.3A and cover) have a lighter skin pigmentation (light-yellow to green) than do saugers (dark-yellow to brown) (Figure 3.3B and book cover); (2) walleyes have unscaled cheeks, whereas saugers have scaled cheeks; (3) walleyes have up to 13 short, lightly colored saddles that reach less than one-fifth of the way down the side of the body, whereas saugers have three dark saddles that reach all the way down the sides of their bodies; (4) walleyes have just one large dark blotch at the posterior end of the first dorsal fin, while saugers have a series of dark speckles arranged in a number of lines across their first dorsal fin; (5) the lower lobe of the caudal fin of walleyes have a white tip, but that of saugers do not. Within the abdominal cavity there are differences in the number and length of the pyloric caecae: walleyes have three pyloric caecae that are as long or longer than the stomach, while saugers have four or more pyloric caecae that are shorter than the stomach (Scott and Crossman 1973; Trautman 1981).

First generation (F_1) hybrids tend to be intermediate for these characteristics, but often express features of both parental species (Scott and Crossman 1973; Trautman 1981). External characteristics typically found in saugeyes (Figure 3.3C) focus on several pigment-related characteristics including dark mottled "saddles" that extend below the lateral line (saddles extend below the lateral line in saugeyes, but the body color and the saddles can often be lighter than seen in saugers) and fin pigmentation (saugeye dorsal fins are generally darker than either parent and possess a dark blotch at the base of the first dorsal fin that is smaller and less pronounced than that found in walleyes, while the speckles on the first dorsal fin tend to be smaller than those seen in saugers) (Stroud 1948; Nelson 1968; Flammang and Willis 1993). The white tip on the bottom of the lower caudal fin lobe, when present, is smaller in saugeyes than in walleyes, and saugeye cheeks tend to be partially scaled. Saugeyes tend to have more than three pyloric caecae, as in saugers, but they are as long as the stomach, as in walleyes. In addition, Nelson (1968) reported that the embryo and larval stages of the F_1 hybrids tend to more closely resemble their female parents.

Backcrosses of F_1 hybrids to either of the parental species are often more difficult to detect by morphological criteria, because they often tend to resemble one of the parental species. For example, when a sauger–walleye F_1 hybrid backcrosses with a pure sauger, their offspring often tend to resemble a sauger. Therefore, it can be difficult to separate hybrids from their parental species by morphological criteria, especially if backcrossing has occurred.

3.3.3 Genetic Markers for Detecting Hybridization

3.3.3.1 Allozyme Markers

Protein electrophoresis has been used to examine genetic variation within and among species, and was the most popular method before the advent of DNA sequencing and microsatellites (i.e., from 1960 to about 1985). Allozymes are the different variant forms of an enzyme that are coded by different alleles at the same locus. Protein molecules can have different net charges because some of the amino acids have positive and negative charges. The net charge of a protein molecule can change when allelic differences occur at a protein coding locus. However, allozymes have the disadvantage that the net charge may be unchanged by

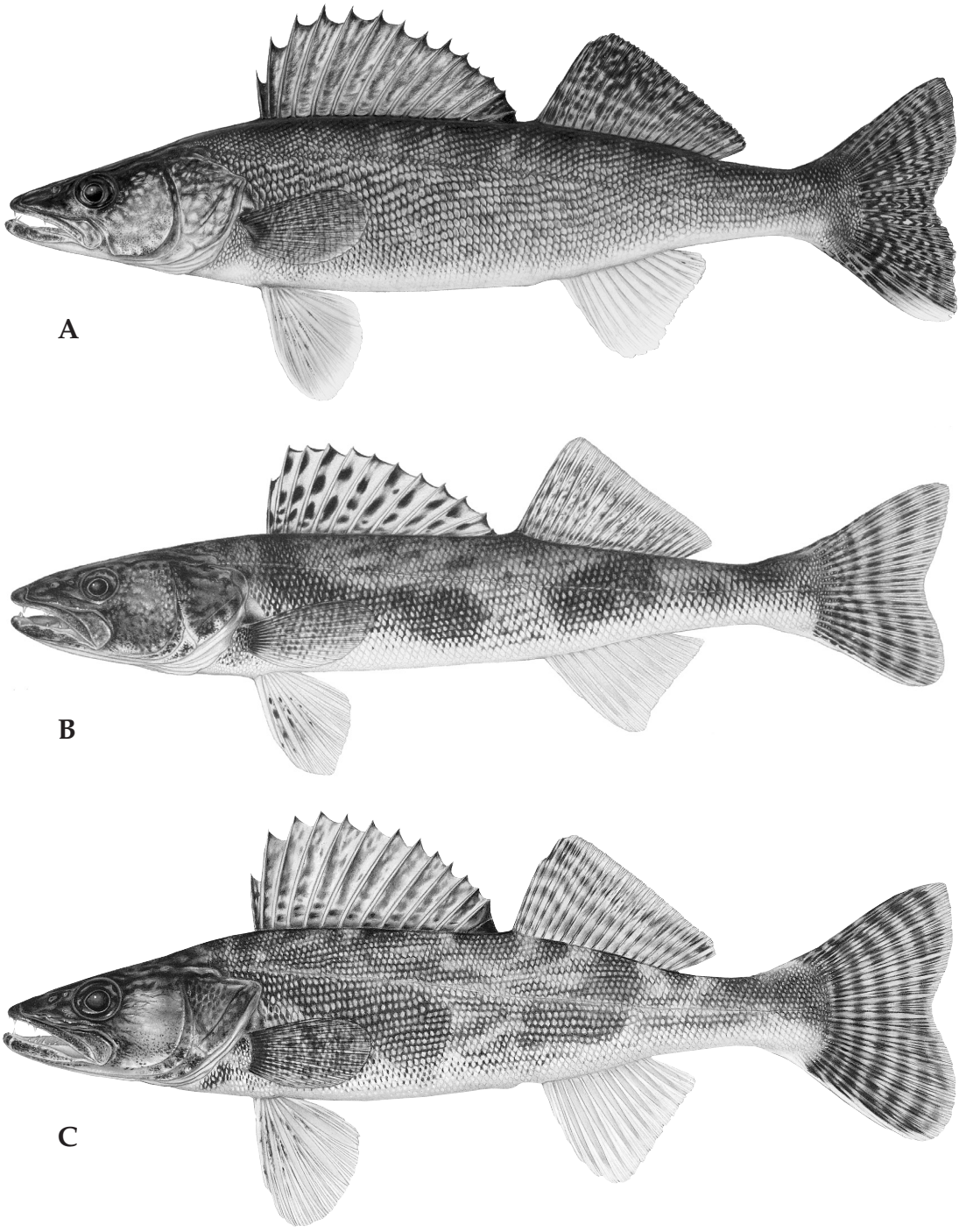

Figure 3.3. (A) Adult male walleye, (B) adult sauger, and (C) adult saugeye, the F_1 hybrid cross between a female walleye and a male sauger, showing shading patterns and external markings (illustrations from J. R. Tomelleri with permission).

mutation, sometimes leading to misleading results. Mixtures of proteins can be separated by electrophoresis in a gel medium by passing a direct electric current through the gel and the migration of the proteins then revealed by staining. The chemical composition, ionic strength, and pH of the buffer solution, and the time needed for electrophoresis can affect the resolution of the protein bands. Most proteins studied are enzymes that can be revealed by histochemical staining with a mixture of the substrate, cofactors, and stains or other reactants that precipitate a colored band on the gel. A recent review of the techniques used and interpretation of the results is provided by May (2003).

One method for detecting hybrid fishes is by genetic screening if diagnostic loci have been identified between the species involved (Campton 1987, 1990). Saugers and walleyes show fixed allelic differences at four protein coding loci: *mMDH-1** for malate dehydrogenase (E.C. 1.1.1.37) and *PGM-1** for phosphoglucomutase (E.C. 5.4.2.2) from muscle, and *ALAT** for alanine aminotransferase (E.C. 2.6.1.2) and *IDDH** for L-iditol 2-dehydrogenase (E.C. 1.1.1.14) from liver (Billington et al. 1990; Van Zee et al. 1996; Fiss et al. 1997). It is important to note that with four diagnostic loci, there is still a 6.25% chance that some introgressed alleles would be missed, because the probability of misidentifying a backcross as a parental is $([1/2])^n$, where n is the number of diagnostic loci between the two species being examined (Campton 1990). Two additional loci, *sMDH-3** (also known as *sMDH-B**, for malate dehydrogenase E.C. 1.1.1.37) and *PROT-3** (general muscle protein, which has no E.C. number), are informative in saugers (polymorphic in walleyes, but fixed for one allele in saugers) (Billington et al. 1990, 2004). One additional locus, *SOD** (superoxide dismutase) (E.C. 1.15.1.1), is informative in walleyes (polymorphic in saugers, but fixed for one allele in walleyes) (Billington et al. 2004). Enzyme numbers are those recommended by the International Union of Biochemistry and Molecular Biology, Nomenclature Committee (IUBMBNC 1992), and genetic nomenclature follows that recommended by Shaklee et al. (1990). By using these loci, it is possible to screen North American *Sander* samples by protein electrophoresis to confirm species identification, detect F_1 hybrids (which will be heterozygous at all of the diagnostic loci), or second-generation (F_2) hybrids or backcrossed individuals (often collectively referred to as F_x hybrids). Backcrossed individuals will be heterozygous at a portion of the diagnostic loci, with the remaining loci being homozygous; the direction of backcrossing can be inferred by the alleles that are homozygous. Occasionally, multigenerational hybrids will also appear. These include F_2 hybrids, repeated backcrosses, or crosses between F_1 and backcrossed individuals. These individuals can have two copies of the diagnostic alleles for one species at one locus, but two copies of the diagnostic alleles of the other species at another locus. They can also be homozygous or heterozygous at other loci.

Numerous studies have now reported the occurrence of natural sauger–walleye hybrids (Ward and Berry 1995; Billington et al. 1996, 1997, 2004, 2006; Van Zee et al. 1996; White et al. 2005), introgression between the two species (Billington et al. 1988, 2004), and difficulties in using morphological characters to discriminate walleye, sauger, and their hybrids compared with protein electrophoresis (Flammang and Willis 1993; Ward and Berry 1995; Billington et al. 1996, 1997, 2004; Van Zee et al. 1996; White et al. 2005). Given the number of reported cases of natural hybridization between saugers and walleyes with the further complication of extensive stocking of saugeyes, Billington et al. (1997) recommended genetic screening of both *Sander* species before their use as broodstock in order to maintain the genetic integrity of sauger and walleye populations.

3.3.3.2 Mitochondrial DNA (mtDNA) Markers

There are three ways in which mtDNA markers can be screened: whole-molecule mtDNA RFLP analysis, PCR-RFLP analysis, and DNA sequencing (Billington 2003). In whole-molecule mtDNA analysis, pure mtDNA can be obtained and then digested with restriction endonucleases, the fragments separated by gel electrophoresis, and the results visualized by staining the DNA or by radiolabeling DNA and visualizing by autoradiography. It is also possible to extract total DNA, digest it with restriction endonucleases, and visualize the mtDNA fragments with a labeled pure mtDNA probe (Billington and Hebert 1990). In PCR-RFLP analysis, primers are made for conserved regions of the mtDNA molecule and the region between these primers is amplified by the polymerase chain reaction (PCR). Then the amplified DNA fragment is digested with restriction endonucleases and the fragments visualized by staining after separation by means of gel electrophresis. Finally, sections of mtDNA amplified by PCR can be sequenced and differences among the sequences examined. See Billington (2003) for a detailed account of the different methods for examining mtDNA markers.

Mitochondrial DNA markers can provide valuable information on the direction of hybridization because they identify the female parent (walleye or sauger) of a naturally produced F_1 hybrid owing to the maternal inheritance of mtDNA (Brown 1983; Campton 1987, 1990). Knowing the female parent of an F_1 hybrid allows the identity of the paternal parent to be inferred. This can be determined by whole-molecule mtDNA analysis (Billington et al. 1988, 1990) or by mtDNA PCR-RFLP analysis. For PCR-RFLP analysis, walleye markers have already been developed by Merker and Woodruff (1996) and markers to differentiate walleyes and saugers by Kassler and Philipp (2001). Kassler and Philipp (2001) found that two restriction endonucleases (*Bst*U I and *Msp* I) gave restriction patterns that were diagnostic between walleyes and saugers from the Ohio River in the mtDNA Dloop/12S RNA mtDNA region. In addition, Kyle and Wilson (2007a, 2007b) found differences in a 500-base pair (bp) fragment of the cytochrome *b* gene sequence of walleye and sauger that could be used in forensic analysis to discriminate their fillets.

3.3.3.3 Potential Microsatellite DNA Markers

Molecular methods of hybrid detection that do not rely on lethal sampling or cryogenic or frozen tissue preservation are favored for their relatively easy demands on field crews. A primary genetic marker of great potential for these studies is microsatellite DNA loci. Microsatellites are tandemly repeated DNA sequences (e.g., AG, CT, ACGT, GTT) that occur more or less randomly throughout the nuclear genome of most eukaryotes. Variability at microsatellite loci is observed in the number of tandem repeats present (e.g., AG_{12}, AG_{15}; Wright and Bentzen 1994). In general, microsatellites are thought to be located in noncoding regions of the genome and, as such, the selective pressures usually limiting the rapid mutation of coding regions of the genome are relaxed for microsatellite regions. In fact, microsatellites are particularly useful due to their high rate of mutation and, subsequently, high levels of polymorphism (Litt and Luty 1989; Tautz 1989; Weber and May 1989; Goldstein and Pollock 1997). Numerous studies have indicated that microsatellites are often more efficient at resolving population structure than are allozymes (Bowcock et al. 1994; Estoup et al. 1995; Blouin et al. 1996; Jarne and Lagoda 1996) or DNA sequencing (Bowcock et al. 1994; Angers and Bernatchez 1997; Brünner et al. 1998). Several recent studies have shown microsatellites

to contain diagnostic alleles for hybridization studies in fishes such as Asian carp (Mia et al. 2005), cutthroat trout (Peacock and Kirchoff 2004), and muskellunge and northern pike (Sloss et al. 2008).

The use of microsatellite loci is dependent on the identification of specific microsatellites and the DNA sequence surrounding them (i.e., the flanking sequence). The advent of the PCR, whereby single copies of DNA can be amplified exponentially, has permitted the widespread employment of microsatellite identification and analysis. The PCR requires small pieces of single-stranded DNA (~18–25 bp) called primers, which are complementary to portions of DNA that flank a target sequence (e.g., a microsatellite or gene). Through the combination of these primers (for walleye primers see Borer et al. 1999; Wirth et al. 1999; Eldridge et al. 2002; Cena et al. 2006), a DNA template (e.g., walleye or sauger DNA), a DNA polymerase (an enzyme that synthesizes double-stranded DNA from single-stranded DNA), other chemical components, and a series of specific temperature fluctuations, the target DNA is amplified exponentially. The result is a section of DNA that is present in sufficient amounts and purity to conduct analyses on either its size (e.g., microsatellite length variation) or its base-pair sequence (a gene's DNA sequence).

Based on the distribution of allele frequencies at eight walleye-based microsatellites (B. L. Sloss, unpublished data), future analysis of walleye–sauger hybridization may be aided by the use of microsatellite genotyping. Several loci (*Svi-2*, *Svi-7*, and *Svi-17*) show allele frequency distributions in Wisconsin walleyes (Franckowiak et al. 2009) that overlap only at the extremes with the allele distributions observed in Montana saugers (Billington et al. 2006). In particular, *Svi-2* shows two alleles in sauger (205 and 195) that were observed in only one fish each and are 36+ bp different in size than the smallest sauger allele (241) observed for several fish. When compared with walleye allele ranges, these two alleles fall within the middle of those observed for Wisconsin walleyes (189–219). Several other analyses will need to be conducted to further examine the utility of these markers in identifying F_x hybrids; a difficult identification based on allozymes alone due to the small number of polymorphic and diagnostic loci available. Furthermore, the collection of highly variable genetic data such as microsatellites can provide for robust admixture detection including backcrosses and post F_1 hybrids through Bayesian-based analyses (Pritchard et al. 2000; see Miller et al. 2009 for an example in muskellunge).

3.3.4 Frequency of Hybridization

In most natural populations of walleye and sauger, hybridization is rare. The incidence of hybridization tends to increase if the two species have not evolved together in the same system (for example, as a result of stocking or diversion of rivers), or if natural systems are altered, for example, by impoundment. The percentage of hybrid and introgressed (F_x) fishes reported in populations ranges from 1% to 39% (Table 3.1). All of these water bodies have been impounded with the exception of Lake Simcoe, Ontario, and the section of the Yellowstone River in Montana that was surveyed. In the case of Normandy Reservoir, Tennessee, the walleye population was thought to have failed and saugeyes were stocked, but walleyes, saugeyes, and F_x hybrids between walleyes and saugeyes were found (Fiss et al. 1997). In all cases, except Lake Simcoe, when morphological and protein electrophoretic studies were compared in the same study, the hybridization percentage was always underestimated by morphological examination. In Lake Simcoe, all of the fish examined appeared to be walleyes by both

Table 3.1. Frequency of hybridization between walleye and sauger in various North American water bodies reported from the scientific literature. For the type of study, electrophoresis refers specifically to protein electrophoresis and mtDNA RFLP means mitochondrial DNA restriction fragment length polymorphism analysis.

Water body	Percent hybrids	Type of study	Reference
Lake Sharpe, South Dakota	1%	Morphology	Nelson and Walburg (1977)
Lake Sharpe, South Dakota	4%	Electrophoresis	Graeb et al. (2010)
Lake Francis Case, South Dakota	1%	Morphology	Nelson and Walburg (1977)
Lake Francis Case, South Dakota	4%	Electrophoresis	Graeb et al. (2010)
Lewis and Clark Lake, South Dakota	10%	Morphology	Nelson and Walburg (1977)
Lewis and Clark Lake, South Dakota	10%	Morphology Electrophoresis	Van Zee et al. (1996)
Lewis and Clark Lake, South Dakota	9–39%	Morphology Electrophoresis	Billington et al. (2004)
Lewis and Clark Lake, South Dakota	21%	Electrophoresis	Graeb et al. (2010)
Lake Sakakawea, North Dakota	10%	Morphology Electrophoresis	Ward and Berry (1995)
Lake Sakakawea, North Dakota	20%	Morphology Electrophoresis	Billington et al. (2006)
Peoria Pool, Illinois River, Illinois	2–4%	Morphology Electrophoresis	Billington et al. (1996)
Peoria Pool, Illinois River, Illinois	2–14%	Morphology Electrophoresis	Billington et al. (1997)
Missouri River, Montana	5–10%	Electrophoresis	McMahon and Gardner (2001)
Missouri River, Montana	0–22%	Morphology Electrophoresis	Koigi (2004); Billington et al. (2006)
Yellowstone River, Montana	0–4%	Morphology Electrophoresis	Koigi (2004); Billington et al. (2006)
Yellowstone River, Montana	15%	Electrophoresis	McMahon and Gardner (2001)
Lake Simcoe, Ontario	17%	Morphology Electrophoresis mtDNA RFLP	Billington et al. (1998)
Ohio River, Ohio	21%	Morphology Electrophoresis mtDNA RFLP	White et al. (2005)
Normandy Reservoir, Tennessee	25%	Morphology Electrophoresis	Fiss et al. (1997)

morphological examination and by protein electrophoresis, but some individuals contained sauger mtDNA due to introgression (Billington et al. 1988). The inability to adequately detect hybrids based upon morphology, especially for F_x hybrids, has serious fisheries management implications if wild fish are to be used as broodstock for supplemental stocking programs and if fish of hybrid origin are inadvertently propagated.

3.3.5 Potential and Actual Impact of Stocked Saugeyes

Saugeyes have been stocked extensively in the central United States because they can withstand warm, eutrophic waters with high flushing rates better than can walleyes, and they have faster growth rates than either of the parental species (Lynch et al. 1982; Pyle et al. 1997; see Chapters 12 and 13). Saugeyes have also been stocked into waters to prevent stunting of crappie populations. Improved growth of both white crappie (Boxrucker 2002) and black crappie (Galinat et al. 2002) populations has been observed following the stocking of saugeyes.

Saugeyes stocked into water bodies that contain walleyes and saugers have the potential to cross with both species, thereby breaking down the coadapted gene complexes and causing introgression (Campton 1987, 1990; Leary et al. 1995). Even if saugeyes are stocked into isolated water bodies there is the potential for them to be moved illegally by anglers, or washed into rivers during flooding, allowing them to hybridize with walleyes and saugers. Similarly, saugeyes stocked into reservoirs may be washed over the dam during periods of high water into the rivers below, once again exposing them to potential hybridization with walleyes and saugers.

White and Schell (1995) used protein electrophoresis to observe recombinant genotypes between walleyes and saugers in three Ohio River pools and confirmed that hybrid reproduction had occurred there. They noted that these three pools are affected by watersheds that had received large numbers of saugeye stockings. White and Schell (1995) suggested that saugeyes should not be stocked where self-sustaining parental walleye and sauger populations occur. A later study by White et al. (2005) on walleye × sauger hybrids in the Ohio River showed that 27% of the fish identified as walleyes contained sauger alleles and 63% of these were F_1 hybrids that had a walleye female parent and a sauger male parent, i.e., they were saugeyes. Fiss et al. (1997) reported saugeyes hybridizing with each other and with walleyes in Normandy Reservoir, Tennessee, leading to widespread introgression. They recommended that saugeyes should not be stocked where there is the likelihood of an interaction with walleyes or saugers.

3.3.6 Hybrids between Other *Sander* Species

No natural hybrids have been reported between any of the three European *Sander* species. However, artificial hybrids between female zander and male Volga pikeperch have been reported and their hybrid status confirmed by random amplified polymorphic DNA (RAPD) analysis (Müller et al. 2004). These hybrids were made to study their potential for aquaculture, and their oxygen tolerance was found to be intermediate to that of their parental species (Müller et al. 2006). A detailed examination of the morphological and meristic characteristics of the F_1 hybrids compared with their parental species showed that the F_1 hybrids can be differentiated from Volga pikeperch and zander based upon multivariate analyses of morphometric and meristic characters (Specziár et al. 2009).

3.4 REFERENCES

Angers, B., and L. Bernatchez. 1997. Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. Molecular Biology and Evolution 14:230–238.

Avise, J. C. 2010. Perspective: conservation genetics enters the genomics era. Conservation Genetics 11:665–669.

Bart, H. L., and L. M. Page. 1992. The influence of size and phylogeny on life history variation in North American percids. Pages 553–572 *in* R. L. Mayden, editor. Systematics, historical ecology, and North American freshwater fishes. Stanford University Press, Stanford, California.

Billington, N. 2003. Mitochondrial DNA. Pages 59–100 *in* E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Billington, N., R. C. Brooks, and R. C. Heidinger. 1996. Use of cellulose acetate electrophoresis to rapidly screen sauger broodstock for sauger–walleye hybrids. Progressive Fish-Culturist 58:248–252.

Billington, N., R. C. Brooks, and R. C. Heidinger. 1997. Frequency of natural hybridization between saugers and walleyes in the Peoria Pool of the Illinois River determined by morphological and electrophoretic criteria. North American Journal of Fisheries Management 17:220–224.

Billington, N., R. G. Danzmann, P. D. N. Hebert, and R. D. Ward. 1991. Phylogenetic relationships among four members of the genus *Stizostedion* determined by mitochondrial DNA and allozyme analyses. Journal of Fish Biology 39 (Supplement A):251–258.

Billington, N., and P. D. N. Hebert. 1990. Technique for determining mitochondrial DNA markers in blood samples from walleyes. Pages 492–498 *in* N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. Fish marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.

Billington, N., P. D. N. Hebert, and R. D. Ward. 1988. Evidence of introgressive hybridization in the genus *Stizostedion*: interspecific transfer of mitochondrial DNA between sauger and walleye. Canadian Journal of Fisheries and Aquatic Sciences 45:2035–2041.

Billington, N., P. D. N. Hebert, and R. D. Ward. 1990. Allozyme and mitochondrial DNA variation among three species of *Stizostedion* (Percidae): phylogenetic and zoogeographical implications. Canadian Journal of Fisheries and Aquatic Sciences 47:1093–1102.

Billington, N., R. N. Koigi, B. D. S. Graeb, and D. W. Willis. 2004. Hybridization between sauger and walleye in Lewis and Clark Lake, South Dakota, determined by protein electrophoresis. Pages 115–116 *in* T. P. Barry and J. A. Malison, editors. Proceedings of PERCIS III, the third international percid fish symposium, University of Wisconsin, Madison, Wisconsin, USA, July 20–24, 2003. University of Wisconsin Sea Grant Institute, Madison.

Billington, N., R. N. Koigi, B. L. Sloss, R. P. Franckowiak, and J. Xiong. 2006. Genetic variation and hybridization with walleye in Montana sauger populations determined by protein electrophoresis and microsatellite analysis. Report of the Department of Biological and Environmental Sciences, Troy University, and Molecular Conservation Genetics Laboratory, University of Wisconsin–Stevens Point, to Montana Department of Fish, Wildlife and Parks. Available: http://fwpiis.mt.gov/content/getItem.aspx?id=25583 (January 2011).

Blouin, M. S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. Molecular Ecology 5:393–401.

Borer, S., L. M. Miller, and A. R. Kapuscinski. 1999. Microsatellites in walleye *Stizostedion vitreum*. Molecular Ecology 8:336–338.

Bowcock, A. M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J. R. Kidd, and L. L. Cavalli-Sforza. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455–457.

Boxrucker, J. 2002. Improved growth of a white crappie population following stocking of saugeyes (sauger × walleye): a top-down, density-dependent growth response. North American Journal of Fisheries Management 22:1425–1437.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42:795–803.

Brown, W. M. 1983. Evolution of animal mitochondrial DNAs. Pages 62–88 *in* M. Nei and R. K. Koehn, editors. Evolution of genes and proteins. Sinauer, Sunderland, Massachusetts.

Brünner, P. C., M. R. Douglas, and L. Bernatchez. 1998. Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic char *Salvelinus alpinus* (Teleostei: Salmondiae) from central alpine lakes. Molecular Ecology 7:209–223.

Campbell, R. R.1987. Status of the blue walleye, *Stizostedion vitreum glaucum*, in Canada. Canadian Field-Naturalist 101:245–252.

Campton, D. E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. Pages 161–192 *in* N. Ryman and F. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle.

Campton, D. E. 1990. Application of biochemical and molecular genetic markers to analysis of hybridization. Pages 241–263 *in* D. H. Whitmore, editor. Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton, Florida.

Cena, C. J., G. E. Morgan, M. D. Malette, and D. D. Heath. 2006. Inbreeding, outbreeding and environmental effects on genetic diversity in 46 walleye (*Sander vitreus*) populations. Molecular Ecology 15:303–320.

Čihař, J. 1975. Geographic and ecological variability of perch [*Perca fluviatilis* (Linnaeus)] and its distribution from Eurasia to North America. Acta Musei Nationalis Praegae 31B:57–89.

Coburn, M. M., and J. I. Gaglione. 1992. A comparative study of percid scales (Teleostei: Perciformes). Copeia 1992:986–1001.

Collette, B. B. 1963. The subfamilies, tribes and genera of the Percidae (Teleostei). Copeia 1963:615–623.

Collette, B. B., and P. Bănărescu. 1977. Systematics of the fishes of the family Percidae. Journal of the Fisheries Research Board of Canada 34:1450–1463.

Craig, J. F. 2000. Percid fishes: systematics, ecology and exploitation. Blackwell Science, Oxford, UK.

Eldridge, W. H., M. D. Bacigalupi, I. R. Adelman, L. M. Miller, and A. R. Kapuscinski. 2002. Determination of relative survival of two stocked walleye populations and resident natural-origin fish by microsatellite DNA parentage assignment. Canadian Journal of Fisheries and Aquatic Sciences 59:282–290.

Estoup, A., C. Tailliez, J. M. Cornuet, and M. Solignac. 1995. Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). Molecular Biology and Evolution 12:1074–1084.

Faber, J. E., and C. A. Stepien. 1997. The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species and genera of the Percidae. Pages 129–143 *in* T. D. Kocher and C. A. Stepien, editors. Molecular systematics of fishes. Academic Press, San Diego, California.

Faber, J. E., and C. A. Stepien. 1998. Tandemly repeated sequences in the mitochondrial DNA control region and phylogeography of the pike-perches *Stizostedion*. Molecular Phylogenetics and Evolution 10:310–322.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 13:93–104.

Fiss, F. C., S. M. Sammons, P. W. Bettoli, and N. Billington. 1997. Reproduction among saugeyes (F_x hybrids) and walleyes in Normandy Reservoir, Tennessee. North American Journal of Fisheries Management 17:215–219.

Fitch, W. M. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. Systematic Zoology 20:406–416.

Flammang, M. K., and D. W. Willis. 1993. Comparison of electropherograms and external characteristics for distinguishing juvenile walleyes and saugeyes. Prairie Naturalist 25:255–260.

Franckowiak, R. P., B. L. Sloss, M. A. Bozek, and S. P. Newman. 2009. Temporal effective size estimates of a managed walleye *Sander vitreus* population and implications for genetic-based management. Journal of Fish Biology 74:1086–1103.

Galinat, G. F., D. W. Willis, B. G. Blackwell, and M. J. Hubers. 2002. Influence of a saugeye (sauger × walleye) introduction program on the black crappie population in Richmond Lake, South Dakota. North American Journal of Fisheries Management 22:1416–1424.

Goldstein, D. B., and D. D. Pollock. 1997. Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. Journal of Heredity 88:335–342.

Graeb, B. D. S., D. W. Willis, N. Billington, R. N. Koigi, and J. A. VanDeHey. 2010. Age-structured assessment of walleye, sauger, and naturally produced hybrids in Missouri River reservoirs. North American Journal of Fisheries Management 30:887–897.

Hearn, M. C. 1986. Reproductive viability of sauger–walleye hybrids. Progressive Fish-Culturist 48:149–150.

Hubbs, C. 1971. Survival of intergroup percid hybrids. Japanese Journal of Ichthyology 18:65–75.

IUBMBNC (International Union of Biochemistry and Molecular Biology, Nomenclature Committee). 1992. Enzyme nomenclature, 1992. Academic Press, San Diego, California.

Jarne, P., and P. J. L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology and Evolution 11:424–429.

Kassler, T. W., and D. P. Philipp. 2001. Genetic assessment of two *Stizostedion* species and their hybrid in the Ohio River. Report of the Illinois Natural History Survey Center for Aquatic Ecology to the Ohio Department of Natural Resources, Division of Wildlife, Athens.

Keck, B. P., and T. J. Near. 2008. Assessing phylogenetic resolution among mitochondrial, nuclear, and morphological datasets in *Nothonotus* darters (Teleostei: Percidae). Molecular Phylogenetics and Evolution 46:708–720

Kidd, K. K., and L. A. Sgaramella-Zonta. 1971. Phylogenetic analysis: concepts and methods. American Journal of Human Genetics 23:235–252.

Koigi, R. N. 2004. Genetic variation, hybridization and introgression in Montana sauger populations. Master's thesis. Troy State University, Troy, Alabama.

Kyle, C. J., and C. C. Wilson. 2007a. Mitochondrial DNA identification of game and harvested freshwater fish species. Forensic Science International 166:68–76.

Kyle, C. J., and C. C. Wilson. 2007b. Erratum to "Mitochondrial DNA identification of game and harvested freshwater fish species." Forensic Science International 180:59

Leary, R. F., F. W. Allendorf, and G. K. Sage. 1995. Hybridization and introgression between introduced and native fish. Pages 91–101 *in* H. L. Schramm, Jr. and R. G. Piper, editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Litt, M., and J. A. Luty. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat with the cardiac muscle actin gene. American Journal of Human Genetics 44:397–430.

Lynch, W. E., Jr., D. L. Johnson, and S. A. Schell. 1982. Survival, growth, and food habits of walleye × sauger hybrids (saugeye) in ponds. North American Journal of Fisheries Management 4:381–387.

Maddison, W. P. 1996. Molecular approaches and the growth of phylogenetic biology. Pages 47–63 *in* J. D. Ferraris and S. R. Palumbi, editors. Molecular zoology: advances, strategies, and protocols. Wiley, New York.

Malison, J. A., T. B. Kayes, J. A. Held, and C. S. Amundson. 1990. Comparative survival and growth

and reproductive development of juvenile walleye and sauger and their hybrids reared under intensive culture conditions. Progressive Fish-Culturist 52:73–82.

May, B. 2003. Allozyme variation. Pages 23–36 *in* E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Mayden, R. L., and R. M. Wood. 1995. Systematics, species concepts, and the evolutionarily significant unit in biodiversity and conservation biology. Pages 58–113 *in* J. L. Nielsen and D. A. Powers, editors. Evolution and the aquatic ecosystem: defining unique units in population conservation. American Fisheries Society, Symposium 17, Bethesda, Maryland.

McAllister, D. E., B. J. Parker, and P. M. McKee. 1985. Rare, endangered, and extinct fishes in Canada. National Museum of Canada, National Museum of Natural Sciences, Syllogeus 54, Ottawa.

McMahon, T. E., and W. M. Gardner. 2001. Status of sauger in Montana. Intermountain Journal of Sciences 7:1–21.

Merker, R. J., and R. C. Woodruff. 1996. Molecular evidence for divergent breeding groups of walleye (*Stizostedion vitreum*) in tributaries to western Lake Erie. Journal of Great Lakes Research 22:280–288.

Mia, M. Y., J. B. Taggart, A. E. Gilmour, A. A. Gheyas, T. K. Das, A. H. M. Kohinoor, M. A. Rahman, M. A. Sattar, M. G. Hussain, M. A. Mazid, D. J. Penman, and B. J. McAndrew. 2005. Detection of hybridization between Chinese carp species (*Hypophthalmichthys molitrix* and *Aristichthys nobilis*) in hatchery broodstock in Bangladesh, using DNA microsatellite loci. Aquaculture 247:267–273.

Miller, L. M., S. W. Mero, and J. A. Younk. 2009. The genetic legacy of stocking muskellunge in a northern Minnesota lake. Transactions of the American Fisheries Society 138:602–615.

Müller, T., M. Bódis, and M. Bercsényi. 2006. Comparative oxygen tolerance of pikeperch *Sander lucioperca*, Volga pikeperch *S. volgensis* and their hybrids *S. lucioperca* × *S. volgensis*. Aquaculture Research 37:1262–1264.

Müller, T., J. Taller, G. Nyitrai, B. Kucska, I. Cernák, and M. Bercsényi. 2004. Hybrid of pikeperch, *Sander lucioperca* L. and Volga perch, *S. volgense* (Gmelin). Aquaculture Research 35:915–916.

Murray, A. M., S. L. Cumbaa, C. R. Harington, G. R. Smith, and N. Rybczynski. 2009. Early Pliocene fish remains from Arctic Canada support a prePleistocene Beringian dispersal of percids (Teleostei: Perciformes). Canadian Journal of Earth Sciences 46:557–570.

Near, T. J. 2002. Phylogenetic relationships of *Percina* (Percidae: Etheostomatinae). Copeia 2002:1–14.

Nelson, W. R. 1968. Embryo and larval characteristics of sauger, walleye, and their reciprocal hybrids. Transactions of the American Fisheries Society 97:167–174.

Nelson, W. R., and C. H. Walburg. 1977. Population dynamics of yellow perch (*Perca flavescens*), sauger (*Stizostedion canadense*) and walleye (*S. vitreum vitreum*) in four main stem Missouri River reservoirs. Journal of the Fisheries Research Board of Canada 34:1748–1763.

Page, L. M. 1985. Evolution of reproductive behaviors in percid fishes. Illinois Natural History Survey Bulletin 33:275–295.

Page, L. M. 2000. Etheostomatinae. Pages 225–253 *in* J. F. Craig, editor. Percid fishes: systematics, ecology, and exploitation. Blackwell Science, Oxford, UK.

Page, L. M., and B. M. Burr. 1991. A field guide to freshwater fishes: North America north of Mexico. Peterson Field Guide Series, Houghton Mifflin Company, Boston.

Page, L. M., and G. S. Whitt. 1973a. Lactate dehydrogenase isozymes, malate dehydrogenase isozymes and tetrazolium oxidase mobilities of darters (Etheostomatini). Comparative Biochemistry and Physiology 44B:611–623.

Page, L. M., and G. S. Whitt. 1973b. Lactate dehydrogenase isozymes of darters and the inclusiveness of the genus *Percina*. Illinois Natural History Survey Bulletin Notes 82:1–7.

Peacock, M. M., and V. Kirchoff. 2004. Assessing the conservation value of hybridized cutthroat trout

populations in the Quinn River Drainage, Nevada. Transactions of the American Fisheries Society 133:309–325.

Pratt, D. M., W. H. Blust, and J. H. Selgeby. 1992. Ruffe, *Gymnocephalus cernuus*: newly introduced to North America. Canadian Journal of Fisheries and Aquatic Sciences 49:1616–1618.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Pyle, C. R., S. J. Fisher, and D. W. Willis. 1997. Seasonal diets and growth of saugeye in Lake Goldsmith, South Dakota. Prairie Naturalist 29:119–128.

Rzhetsky, A., and M. Nei. 1993. Theoretical foundation of the minimum-evolution method of phylogenetic inference. Molecular Biology and Evolution 10:1073–1095.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184, Ottawa.

Shaklee, J. B., F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 119:2–15.

Sloss, B. L., and N. Billington. 2004. Molecular phylogenetics of Etheostomatinae (Teleostei, Percidae): insights from combining cytochrome *b* and 12S ribosomal RNA mitochondrial DNA variation. Pages 59–60 *in* T. P. Barry and J. A. Malison, editors. Proceedings of PERCIS III, the third international percid fish symposium, University of Wisconsin, Madison, Wisconsin, USA, July 20–24, 2003. University of Wisconsin Sea Grant Institute, Madison.

Sloss, B. L., N. Billington, and B. M. Burr. 2004. A molecular phylogeny of the Percidae (Teleostei, Perciformes) based on mitochondrial DNA sequence. Molecular Phylogenetics and Evolution 32:545–562.

Sloss, B. L., R. P. Franckowiak, and E. L. Murphy. 2008. Development of new microsatellite loci and multiplex reactions for muskellunge (*Esox masquinongy*). Molecular Ecology Notes 8:811–813.

Song, C. B., T. J. Near, and L. M. Page. 1998. Phylogenetic relationships among percid fishes as inferred from mitochondrial cytochrome *b* DNA sequence data. Molecular Phylogenetics and Evolution. 10:343–353.

Specziár, A., M. Bercsényi, and T. Müller. 2009. Morphological characteristics of hybrid pikeperch (*Sander lucioperca* ♀ × *Sander volgensis* ♂) (Osteichthyes, Percidae). Acta Zoologica Academiae Scientiarum Hungaricae 55:39–54.

Stetovidov, A. N., and E. A. Dorofeeva. 1963. Systematics, origin, and history of the distribution of the Eurasian and North American perches and pikeperches (genera *Perca*, *Lucioperca* and *Stizostedion*). Voprosy Ikhtiologii 3: 625–651 (Translated from Russian by NMFS Systematics Laboratory 28).

Stroud, R. H. 1948. Notes on growth of hybrids between the sauger and the walleye (*Stizostedion canadense* × *S. vitreum vitreum*) in Norris Reservoir, Tennessee. Copeia 1948:297–298.

Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pages 407–514 *in* D. M. Hillis, C. Moritz, and B. K. Mabel, editors. Molecular systematics, 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acid Research 14:6463–6471.

Trautman, M. B. 1981. The fishes of Ohio (revised edition). The Ohio State University Press, Columbus.

Turner, T. F. 1997. Mitochondrial control region sequences and phylogenetic systematics of darters (Teleostei: Percidae). Copeia 1997:319–338.

Van Zee, B. E., N. Billington, and D. W. Willis. 1996. Morphological and electrophoretic examination of *Stizostedion* samples from Lewis and Clark Lake, South Dakota. Journal of Freshwater Ecology 11:339–344.

Ward, N. E., III, and C. R. Berry, Jr. 1995. Evaluation of skin pigmentation for identifying adult sauger and walleye–sauger F_1 hybrids collected from Lake Sakakawea, North Dakota. Progressive Fish-Culturist 57:302–304.

Weber, J. L., and P. E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American Journal of Human Genetics 44:388–396.

White, M. M., T. W. Kassler, D. P. Philipp, and S. A. Schell. 2005. A genetic assessment of Ohio River walleyes. Transactions of the American Fisheries Society 134:661–675.

White, M. M., and S. Schell. 1995. An evaluation of the genetic integrity of Ohio River walleye and sauger stocks. Pages 52–60 *in* H. L. Schramm, Jr. and R. G. Piper, editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Wiley, E. O. 1992. Phylogenetic relationships of the Percidae (Teleostei: Perciformes): a preliminary hypothesis. Pages 247–267 *in* R. L. Mayden, editor. Systematics, historical ecology, and North American freshwater fishes. Stanford University Press, Stanford, California.

Wirth, T., R. Saint-Laurent, and L. Bernatchez. 1999. Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*), and cross-species amplification within the family Percidae. Molecular Ecology 8:1960–1962.

Wood, R. M., and R. L. Mayden. 1997. Phylogenetic relationships of selected darter subgenera (Teleostei: Percidae) as inferred from analysis of allozymes. Copeia 1997:265–274.

Wright, J. M., and P. Bentzen. 1994. Microsatellites: genetic markers for the future. Reviews in Fish Biology and Fisheries 4:384–388.