Evolution in Mixed Company
Evolutionary Inferences from Studies of Natural Hybridization in Salmonidae

Spawning bull trout
If the intellectual vigor of a field of study is reflected by the number and intensity of scientific controversies within that field, then evolutionary biology remains a vital discipline. Controversial issues have included, and in many cases continue to include: the nature and origin of species, the basis and mechanisms of inheritance, the extent and nature of genetic variation within populations, and the role of hybridization in evolution (Mayr 1982). In the latter case, I refer to successful mating between individuals from two populations that are genetically distinct in at least one measurable trait. This definition (cf. Harrison 1990; Arnold 1997) emphasizes that hybridization includes mating between conspecifics (from genetically distinct populations). Although Arnold’s (1997) definition includes the additional point that at least some of the F1 progeny are viable and fertile, I will consider “successful” hybridization as not necessarily requiring fertility because even infertile (but viable) hybrids can have evolutionary consequences. For instance, reinforcement, or selection for pre-mating isolation as the result of selection against hybrids, is an important model of speciation (Dobzhansky 1940). Introgressive hybridization, however, clearly requires that hybrids be both viable and fertile as it represents the movement of alleles from one (genetically distinct) population to another by hybridization and backcrossing. Within this context, a hybrid zone is a geographic area where genetically distinct populations overlap spatially and temporally such that hybridization between them occurs (cf. Arnold 1997).

Within the general field of hybridization, controversial issues have included: (1) the meaning of hybridization to the recognition of “good” species (i.e., can taxa that hybridize still be considered distinct species?), (2) the role of hybridization in creating evolutionary novelty and perhaps new species (i.e., is hybridization an evolutionary dead-end or a source of novel diversity?), and (3) the roles of endogenous (internal genetic mechanisms) versus exogenous (environmentally dependent) selection in structuring hybrid zones (Arnold 1997; Barton 2001). As Arnold (1997) has detailed, alliance to opposing sides of these issues tended to fall along taxonomic lines. In particular, zoologists, beginning at least with Darwin, tended to regard hybridization as a problem for recognizing hybridizing populations as distinct species. In addition, zoologists have tended to study hybridization with the view that it can reveal processes that influence gene flow and genetic divergence between species. By contrast, botanists, beginning with Linnaeus and the plant “hybridizers” regarded hybridization as an important creative process to generate evolutionary novelty including the emergence of “recombinant” species (Barton 2001). This taxonomic division regarding the role of hybridization in evolution has become increasingly blurred in recent years with the recognition that hybridization can act in both restrictive (but illuminating) and creative ways (Arnold 1997; Barton 2001). Despite this encouraging development, important uncertainties in the study of hybridization include the form of selection structuring hybrid zones, the origin (primary or secondary intergradation) of hybrid zones, and the role of hybridization in adaptation and speciation.

Within these theoretical contexts, this chapter reviews studies of hybridization in salmonid fishes and their contributions to resolving contentious issues in
evolution. I begin by reviewing the taxonomic and geographic distribution of hybridization in salmonids, the environmental and ecological factors that appear to influence patterns of hybridization, and evolutionary inferences that have been made based on salmonids. I end by highlighting the major uncertainties that remain and the outstanding features of salmonid biology that could be used to address these uncertainties. Hybridization is increasingly viewed as important to biological conservation as one of the major causes of loss of biodiversity, along with overexploitation, habitat degradation, exclusion by exotics, and chains of extinction (Rhymer and Simberloff 1996). I do not, however, treat the significance of hybridization to salmonid conservation because this was recently reviewed by Allendorf et al. (2001). In addition, I do not review methods of detection of hybridization because this subject is well covered in other reviews (e.g., Campton 1987; Scribner et al. 2000).

1. Taxonomic Distribution of Hybridization in Salmonids

Mayr (1963) discussed five categories of hybridization: (a) “occasional” hybridization between sympatric species where hybrids were inviable or sterile, (b) hybrids with at least some fertility such that occasional backcrossing occurred, (c) formation of zones of secondary contact where partial interbreeding takes place, (d) complete breakdown of reproductive isolation with the production of hybrid swarms, and (e) production of a “new specific entity” via hybridization and polyploidy (allopolyploidy). The taxonomic and geographic distribution of hybridization in salmonid fishes provides ample demonstration of classes (a)–(d), while the possibility of allopolyploidy as a speciation mechanism in salmonids is intriguing, but inconclusive (Mina 1992; Svardson 1998).

Avise (1994) demonstrated that hybridization tends to be more common in the “lower” vertebrates relative to birds and mammals, and Hubbs (1955) provided the first summary to indicate that hybridization and introgression are widespread in fishes. In his review, Hubbs (1955) gave anecdotal accounts of hybridization between species in Oncorhynchus, Salvelinus, and Coregonus, and even an intergeneric mating in nature between brown trout and brook charr (see also Brown 1966). Aside from basic listings (see also Schwartz 1982; Chevassus 1979; Scribner et al. 2000), there has been little further discussion of the evolutionary implications of hybridization in the salmonids. A summary of existing information compiled 42 years later (Arnold 1997) provided virtually no information on hybridization in salmonid fishes. Perhaps the most thorough work on hybridization in salmonid fishes was that of Svardson (1998) who reviewed data on inherited variation in morphological and meristic traits used to investigate hybridization and introgression in Scandinavian Coregonus. Because of the limitations of morphological variation for studying hybridization (see Campton 1987), studies of hybridization in salmonids before the 1970s were limited to putative identification of hybrids in nature or to the morphological description of hybrids produced artificially in the laboratory. It was not until the development of protein electrophoresis in the mid-1960s and DNA-based technology in the
late 1970s that the true taxonomic, temporal, and geographic extent of hybridization in salmonids became clear (Verspoor and Hammar 1991).

The first recorded case where biochemical markers were used to document interspecific hybridization in salmonids was that between Atlantic salmon and brown trout (Payne et al. 1972). The development of genetic markers for specific and hybrid diagnoses was clearly a crucial development for the study of hybridization. For example, despite being naturally sympatric in Europe and of intense fishery interest, these two species were not suspected or documented to produce hybrids in nature until the use of allozyme assays became common.

It is clear that from a summary of documented cases of hybridization using diagnostic molecular markers, that hybridization is common to all major lineages of salmonids (Figure 8.1). Certainly, hybridization and introgression have been recorded in *Salmo, Salvelinus, Coregonus*, some members of *Oncorhynchus*, and even one of the most basal lineages (*Brachymystax*). Because of the cursory nature of most studies of hybridization in salmonids, identifying trends in the taxonomic distribution of hybridization is difficult. It does appear, however, that hybridization is much less common within the Pacific coast genus *Oncorhynchus* than within other salmonid genera. All but one incidence of natural hybridization within this genus are limited to hybridization between rainbow trout and various subspecies of cutthroat trout (Verspoor and Hammar 1991). What might be the reason(s) for apparently reduced incidence of hybridization in *Oncorhynchus*?

First, the age of the species complex may be related to propensity for hybridization. More distantly related species, by definition, show greater levels of genomic divergence, which would limit the opportunities for hybridization, owing to major ecological differences, as well as the outcomes of hybridization owing to low hybrid viability (May et al. 1975; Verspoor and Hammar 1991; Avise 1994). For instance, estimates of average pairwise Nei’s *D* in *Oncorhynchus* (0.55, Utter et al. 1973) are about twice that reported between Atlantic salmon and brown trout (about 0.3) and among various members of *Salvelinus* (about 0.2–0.3), and natural hybridization appears much more common in the latter two genera than in Pacific salmon. Within genera, however, there appears to be little clear correspondence between measures of genomic divergence and intrinsic isolation (Verspoor and Hammar 1991; see Section 4.3 below). Similarly, despite fewer records of natural hybridization among the five species of Pacific salmon *Oncorhynchus*, they exhibit similar or lower divergences (*d* = 1.68) as measured by rDNA ITS sequences than do Atlantic salmon and brown trout (*d* = 1.71) or various species of char (*d* = 2.10, Phillips et al. 1992).

A second argument that may explain variation in extent of hybridization is the stability of watersheds. Habitat disturbances from climate change, glaciation, sea level changes, and tectonic activity may promote secondary contact and the formation of hybrid zones between previously allopatric populations (e.g., Hewitt 2000). This hypothesis, however, probably does not explain the lower incidence of hybridization within *Oncorhynchus* because if anything, the range of Pacific salmon has probably undergone more dramatic geological changes over the last 10 million years than the range of *Salmo* (Montgomery 2000).
Figure 8.1. Geographic localities of hybridization in salmonids. Hybrids were detected using biochemical/molecular assays and, in some cases, morphological analyses. Closed symbols represent hybridization within the natural range of both species. Open circles represent examples of hybridization in nature, but involving one or both species introduced outside their native range(s). 1 = Dolly Varden (Salvelinus malma) and bull trout (S. confluentus), 2 = bull trout and brook trout (S. fontinalis), 3 = westslope cutthroat trout and Yellowstone cutthroat trout (O. c. bouvieri), 4 = rainbow trout and Yellowstone cutthroat trout, 5 = rainbow trout (Oncorhynchus mykiss) and coastal cutthroat trout (O. clarki clarki), 6 = rainbow trout and westslope cutthroat trout (O. c. lewisi), 7 = chinook salmon (O. tshawytscha) and coho salmon (O. kisutch), 8 = inconnu (Stenodus leucichthys) and lake whitefish (Coregonus clupeaformis), 9 = lake whitefish and least cisco (C. sardinella), 10 = lake whitefish and Arctic cisco (C. autumnalis), 11 = Arctic cisco and least cisco (Reist et al. 1992, pp. 8–11), 12 = chinook salmon and pink salmon (O. gorbuscha), 13 = brook trout and Arctic char (S. alpinus), 14 = Arctic char and lake trout (S. namaycush), 15 = Atlantic salmon (Salmo salar) and brown trout, 16 = brown trout (Salmo trutta) and brook trout, 17 = European whitefish (Coregonus lavaretus) and vendace (C. albula) (Pethon 1974), 18 = incipient species of brown trout (Salmo marmoratus) and (S. trutta fario) (Giuffra et al. 1996), 19 = European grayling (Thymallus thymallus) and Arctic grayling (T. arcticus) (Shubin and Zakharov 1984), 20 = “sharp-” and “blunt-nosed” forms of lenox (Brachymystax lenox). Example authorities for each case, except where noted, are reported in the text. The dashed line represents the approximate southward limit of ice sheets in the Northern Hemisphere during the most recent glacial advance. Localities are approximate and representative only; they do not necessarily show all areas of reported hybridization.
A third hypothesis is related to the idea that Pacific salmon are the most derived members of the family (Stearley and Smith 1993; Kinnison and Hendry 2003—this volume) and that their derived status and accompanying specialized life history traits may represent stronger selection against any hybrids that are produced, or stronger selection for reproductive isolation (via reinforcement) in sympatry. For instance, Utter (2000) reviewed evidence for anthropogenic introgression in two genera of salmonids, *Oncorhynchus* and *Salmo*, and demonstrated that hybridization and introgression were much more common in freshwater-resident populations within both genera than in anadromous populations. Other work by Campton and Utter (1985), Hawkins and Quinn (1996), and Young et al. (2001) on steelhead trout and coastal cutthroat trout, and Hansen et al. (2000b) on brown trout found fewer hybrids in adults (who have made migrations between fresh water and the ocean) than in juveniles sampled in fresh water. These results suggest that there may be strong selection against hybrids when one of the parental forms is anadromous. Consequently, it is possible that variation in the extent and timing of anadromous migrations that so characterizes Pacific salmon (Hendry et al. 2003b—this volume), and their correlated effects on time and size at spawning, may more strongly limit hybridization in *Oncorhynchus* relative to *Salmo* or *Salvelinus*.

2. Geographic Distribution of Hybridization in Salmonids

The geographic distribution of natural hybridization in salmonids is strongly, but not exclusively, associated with portions of their range that were directly influenced by Pleistocene glaciation events (Figure 8.1). In large part, this association stems from fact that salmonids are north temperate in distribution and this was the major region of the Northern Hemisphere affected by glaciation (Pielou 1991). There are, however, some interesting subtleties within this general trend.

First, phylogenetic evidence has strongly indicated that hybridization between species occurs in areas where one of the two species involved no longer exists. For instance, Bernatchez et al. (1995) and Glémet et al. (1998) used combined analysis of nuclear and mitochondrial loci to demonstrate mtDNA introgression of Arctic charr mtDNA into several allopatric populations of brook charr in eastern Québec. A similar phenomenon was demonstrated in an allopatric population of lake trout that was characterized by Arctic charr mtDNA (Wilson and Bernatchez 1998). Redenbach and Taylor (2002a) observed Dolly Varden with bull trout mtDNA, and several Dolly Varden populations with introgressed bull trout mtDNA were located in areas (e.g., Vancouver Island) where bull trout are not currently found, clearly implying historical introgression. All of these instances of historical introgression in eastern and western North American charr indicate that the “footprint” of hybridization can be apparent in geographic localities where one of the participating species is no longer found, thus greatly expanding the geographic scale across which hybridization in salmonids has occurred.
Another subtlety in the geographic distribution of hybridization in salmonids is that hybridization between species may be higher in areas where salmonids have been introduced relative to where they are naturally sympatric. For instance, four species of *Oncorhynchus* have been widely introduced into the Great Lakes region of eastern North America (coho, chinook, and pink salmon, and steelhead trout). Rosenfield et al. (2000) demonstrated a low level of introgressive hybridization between pink and chinook salmon from the St. Marys River system (between lakes Superior and Huron), although hybrids between these two species have never been reported in their natural range. Further, rainbow trout and westslope cutthroat trout are largely allopatric or parapatric throughout their native ranges in western North America (Behnke 1992). Rainbow trout, however, have been widely introduced into the range of westslope cutthroat trout and introgressive hybridization between species typically results from such artificial secondary contact (Leary et al. 1984; Rubidge et al. 2001). McGowan and Davidson (1992a) reported hybridization between non-native brown trout and native Atlantic salmon in Newfoundland waters at a rate about twice that reported in Europe where both species are native. These cases of apparently greater hybridization following human-induced introductions may stem from fundamentally different causes. Hybridization between pink and chinook salmon in the Great Lakes is probably promoted, in part, by environmental differences between the Great Lakes and the Pacific basin. The two species have had a long history of sympatry in the Pacific basin where reproductive isolation is promoted, in part, by differences between species in age and size at maturity and spawning habitat distributions and preferences (e.g., Groot and Margolis 1991). Rosenfield et al. (2000) hypothesized that a lack of opportunities for spatial isolation in the St. Marys River system may promote hybridization between chinook and pink salmon in the Great Lakes. In contrast, the largely allopatric evolution of rainbow trout and westslope cutthroat trout raises the hypothesis that reinforcement may be important in salmonid speciation because pre-mating reproductive isolation between these species is incomplete, which may result from a lack of opportunities for historical interactions during mating in nature.

3. Temporal Distribution of Hybridization in Salmonids

Biochemical and molecular approaches to hybridization have been crucial for demonstrating that introgressive hybridization in salmonids is both a contemporary and an historical phenomenon. Leary et al. (1987) studied allozyme variation among seven subspecies of cutthroat trout, rainbow trout and found that westslope cutthroat trout were paraphyletic with respect to rainbow trout, and suggested that ancient hybridization explained the sharing of many alleles between these species that were not found in the other cutthroat trout subspecies. The finding of mtDNA introgression between *Salvelinus* species that are now allopatric (Bernatchez et al. 1995; Wilson and Hebert 1998) also strongly suggests historical hybridization in addition to contemporary hybridiza-
tion between sympatric populations (Hammar et al. 1989; Wilson and Hebert 1993).

Discordance in phylogenetic relationships between different classes of genetic markers and variation in sequence divergence among haplotypes has been cited as evidence of historical hybridization in a number of animal and plant taxa (Arnold 1997). Dolly Varden and bull trout are parapatric and sympatric in western North America (Baxter et al. 1997). Redenbach and Taylor (2002a) demonstrated discordance between mtDNA-based phylogenies and those based on two nuclear sequences (Figure 8.2). Although Dolly Varden and bull trout were clearly reciprocally monophyletic at two nuclear loci, the species were paraphyletic with respect to mtDNA. In particular, a “southern” clade of Dolly Varden mtDNA (from fish confirmed as Dolly Varden by morphological and nuclear DNA analyses) was more closely related to a “coastal” clade of bull trout mtDNA than to a “northern” Dolly Varden mtDNA clade (Figure 8.2). In addition, the geographic distribution of the paraphyletic Dolly Varden mtDNA clade was largely restricted to areas of current sympathy with bull trout. A notable exception, however, was the presence of paraphyletic Dolly Varden mtDNA on Vancouver Island, a region where bull trout do not currently occur. The presence of paraphyletic Dolly Varden mtDNA in otherwise “pure” Dolly Varden in currently allopatric Dolly Varden populations strongly suggests historical mtDNA introgression from bull trout to Dolly Varden (Arnold 1997; Redenbach and Taylor 2002a).

The timing of such historical introgression events is difficult to determine, but some estimates are possible. For instance, the average allozyme divergence among cutthroat trout subspecies that are not suspected of historical introgression with rainbow trout is 0.217 (Leary et al. 1987). If this value is used as a baseline to date the separation between rainbow trout and cutthroat by using a standard rate of 1 unit of Nei’s $D$ for 5 million years (Nei 1987a), then the two species diverged from a common ancestor at least 1 million years ago (see also Behnke 1992). If the present Nei’s $D$ between rainbow and westslope/coastal cutthroat of about 0.11 (Utter et al. 1973; Leary et al. 1987) is then used as an estimate of time since an ancient introgression event, this suggests that hybridization first occurred on the order of 500,000 years ago. Behnke (1992), however, suggested that rainbow trout were likely absent from the upper reaches of the Columbia River until very late in the Pleistocene and likely did not come into contact with westslope cutthroat trout until between 32,000 and 50,000 years ago, suggesting a much more recent historical hybridization. Similarly, paraphyletic Dolly Varden mtDNA is found in several lake resident populations on Vancouver Island. Because bull trout are not found on Vancouver Island presently, the mtDNA introgression between the species must have occurred at least before the island became free of ice and accessible for post-glacial colonization some 12,000 years ago (McPahil and Lindsey 1986). Furthermore, these allopatric paraphyletic Dolly Varden mtDNAs are about 0.4–0.8% divergent from their closest bull trout mtDNAs which suggests (if mtDNA mutates at approximately 1% per million years, Smith 1992) that hybridization between these species could have occurred as early as 800,000 years ago. Such ancient
Figure 8.2. (a) Phylogenetic tree of *Salvelinus* mitochondrial DNA rooted with *S. namaycush* based on neighbor-joining analysis of Kimura 2-parameter distance inferred from 503 base pairs of the tRNA-Gln (36 bp), tRNA-Ile (73 bp), and NADH-1 (453 bp) genes. Bootstrap support levels from 1000 resamplings are reported (where over 50%) at branch points. (b) Phylogenetic tree of *Salvelinus* Growth Hormone 2 sequences rooted with *S. namaycush* sequence (from McKay et al. 1996), based on neighbor-joining analysis of Kimura 2-parameter distance inferred from 484 base pairs of intron C. (c) Phylogenetic tree of *Salvelinus* ribosomal DNA internal transcribed spacer region (rDNA ITS-1) sequences using *S. namaycush* as the outgroup. The tree is derived from neighbor-joining analysis of Kimura 2-parameter distance inferred from 410 base pairs of ITS-1. In all trees, “DV” are Dolly Varden (*Salvelinus malma*) haplotypes, “BTI” and “BTC” are interior and coastal, respectively, bull trout (*S. confluentus*) haplotypes. Bootstrap support levels (where over 50%) from 1000 resamplings are marked at branch points. All haplotypes are defined in Redenbach and Taylor (2002a).
hybridization is not unreasonable. For instance, Smith (1992) reported a discordance between an mtDNA-based phylogeny of Pacific salmon that reported pink and chum salmon as sister species and one based on morphological/karyological/biochemical data where pink and sockeye salmon were sister species (see also Kinnison and Hendry 2003—this volume). He suggested that this difference in inferred relationships was caused by historical mtDNA introgression between pink and chum salmon that, from a consideration of the fossil record, occurred at least 2 million years ago.

4. Reproductive Biology, Isolation, and Hybridization in Salmonids

Aspects of reproductive biology are major factors that likely govern the incidence of interspecific hybridization, and may influence gene flow between hybrid and parental lineages. In this section, I briefly review conceptual issues of reproductive isolation and salmonid reproductive biology as it pertains to the incidence of natural hybridization. These topics bear directly on factors that may explain the genetic structure of hybrid zones in salmonids examined in the subsequent section.

4.1. Classification of Reproductive Isolation

The different aspects of genetic isolation (the prevention of hybrid formation) are most broadly categorized into extrinsic and intrinsic reproductive isolation. At this level, extrinsic isolation refers to the separation of potentially interbreeding populations by some geographic barrier such that they have no opportunity for mating. Intrinsic reproductive isolation refers to isolation imposed by properties of the organisms themselves (other than their geographic distribution) that prevent genetic exchange (see Dobzhansky 1937). Clearly, the lack of documented natural hybridization between some salmonid species results from extrinsic isolation (e.g., the masu salmon endemic to the western Pacific and cutthroat trout endemic to the eastern Pacific). The extent or breakdown of intrinsic reproductive isolation is, therefore, central to a discussion of hybridization and introgression.

Factors important to intrinsic reproductive isolation were originally classified by Dobzhansky (1937). Although he presented several different classes, they were most broadly differentiated into those that operate before mating (pre-mating isolation) and those that operate after mating (post-mating isolation). Pre-mating isolation typically involves aspects of behavior or reproductive physiology (e.g., time and place of reproduction, behavioral interactions) that restrict opportunities for interspecific pairings or actual mating (see Mayr 1963, p. 92; Palumbi 1998). Post-mating isolation encompasses phenomena that influence zygotic survival or fertility (e.g., developmental abnormalities, hybrid inviability, or infertility). More recently, it has been recognized that factors acting at the level of egg and sperm interactions (see below) may con-
stitute important processes of reproductive isolation that act after mating (i.e., post-insemination, or in the case of salmonids, post-spawning), but before the formation of zygotes. Consequently, a more inclusive characterization recognizes pre-zygotic isolation as those processes acting prior to viable zygote formation and post-zygotic processes as those acting after viable zygote formation.

A second classification of processes has been defined that is relevant to the fate of hybrids produced when pre-zygotic reproductive isolation is incomplete. This nomenclature describes the role of selection and includes: (1) endogenous, or environmentally-independent processes and (2) exogenous, or environmentally dependent processes. Endogenous selection imposes reproductive isolation regardless of the environmental context via interspecies genomic or developmental incompatibilities that directly influence hybrid viability or fertility. The work by Dobzhansky (1933) on the genetics of hybrid male sterility and its molecular dissection by Ting et al. (1998) in *Drosophila* are good examples of endogenous factors influencing selection against hybrids. Exogenous selection imposes reproductive isolation through the interaction between phenotype (as determined by genotype) and environmentally dependent natural or sexual selection against hybrid phenotypes (Rice and Hostert 1993; Rundle and Whitlock 2001; Bernatchez 2003—*this volume*). In this case, selection against (or for) hybrid genotypes is not “automatic” as in endogenous selection, but rather depends on the environmental conditions. The temporal variation in survival of hybrids between species of Darwin’s finch (*Geospiza*) and its dependence on distribution of seed sizes, which is influenced by rainfall patterns, exemplify the role of the environment on patterns of selection (see discussion summarized by Schluter 2000). Similarly, a close association between genotype survival and environmental variation within a hard-clam hybrid zone also points to the role of exogenous selection (Bert and Arnold 1995). Classification and discussion of reproductive isolation can be accomplished using a variety of nomenclature systems, but their real value lies in helping to focus research efforts on the processes involved in reproductive isolation and speciation (Berlocher 1998).

### 4.2. Pre-Zygotic Isolation in Salmonids

Some of the best studied aspects of salmonid reproductive biology (at least for salmon, trout, and char) are habitat distribution and mating behavior. Several processes are involved in reproductive isolation between sympatric species including: macro- and micro-habitat utilization for spawning, secondary sexual traits, behavioral differences, and different sizes at maturity (which may influence habitat and mate choice). For instance, Pacific salmon mature at between 2 (pink salmon) and 8 (chinook salmon) years of age, and return to spawn in streams tributary to the northeast and northwest Pacific Ocean typically from late summer to late fall. During spawning, females construct and defend gravel nest sites and are attended by one or more males that compete for access to territorial females (see Groot and Margolis 1991 and Fleming and Reynolds 2003—*this volume*, for detailed descriptions). Notwithstanding these broad similarities among species, there are consistent differences among species that limit
opportunities for hybridization (Fukushima and Smoker 1998). For instance, sockeye salmon exhibit perhaps the most specialized habitat choice in that they usually spawn in streams that are associated with lake systems (where the juveniles feed for a year or more before migrating to the ocean). In addition, sockeye salmon are the only *Oncorhynchus* that makes extensive use of submerged gravel beaches for spawning in lakes (Burgner 1991; Taylor et al. 2000). In addition, pink salmon, the smallest Pacific salmon (typically 1.5–2.5 kg) usually do not make extensive upstream migrations for spawning and spawn in small coastal rivers or close to the sea in larger systems (Heard 1991). By contrast, chinook salmon, on average the largest of all Pacific salmon (up to 45 kg, Healey 1991), may make upstream migrations of 1000 km or more and tend to spawn in larger watersheds.

Pacific salmon are also well known for the development of exaggerated secondary sexual traits and coloration (see Fig. 2 of Hendry 2001) particularly in males of each species, which likely act in mate recognition in sympatric species. The variation in size at maturity, although also extensive within species, probably constrains interspecific hybridization owing to well-developed size assortative mating and size-dependent habitat use in salmonids (Foote 1988; Beacham et al. 1988b; Hagen and Taylor 2001). Indeed, the importance of these pre-mating isolation factors in limiting opportunities for hybridization in salmonids is suggested by the relatively high incidence of hybridization in systems where such isolating factors are absent or are less well developed. For instance, reproductive isolation between species may be weakened when there are constraints on spatial segregation in introduced populations (chinook and pink salmon; Rosenfield et al. 2000), greater similarity in size at maturity (e.g., rainbow trout and westslope cutthroat trout; Rubidge et al. 2001), or less extensive secondary sexual development (various *Coregonus*; Svärds on 1998).

Although I have argued that differences in size at maturity among species may act to constrain hybridization, there is at least one circumstance where such differences may actually promote hybridization; parasitic matings by smaller “sneaker” males. Such sneaking behavior is common in intraspecific matings in salmonid fishes. Sneaking occurs when a smaller male rushes into the nest of a larger mating pair of the same species and attempts to fertilize the eggs of the female (Svedång 1992; Maekawa et al. 1993, 1994; Taborsky 1998; Fleming and Reynolds 2003—*this volume*). In cases where there are differences in size at maturity between species of salmonids, sneaking has been proposed as an explanation for interspecific hybridization (e.g., McGowan and Davidson 1992a; Kitano et al. 1994; Baxter et al. 1997; Redenbach and Taylor 2002b). Consequently, although body size differences between species are usually sufficient to prevent interspecific pairings, they may, in some cases, actually promote interspecific matings. Under such conditions complete pre-zygotic reproductive isolation may be difficult to establish because sneaker males are employing an intraspecific parasitic reproductive trait that, presumably, is relatively successful in allopatric populations (e.g., Gross 1985; Maekawa et al. 1993, 1994; Fleming and Reynolds 2003—*this volume*). In addition, the selective penalty for inter-
specific sneaking by salmonid males may be reduced because sperm is relatively abundant and energetically “cheap,” especially for iteroparous species where males have multiple opportunities to breed across years.

The extent of size differentiation between mature adults of sympatric species likely influences whether hybridization is uni- or bidirectional (Wirtz 1999). For instance, unidirectional hybridization has been reported in Salvelinus (e.g., Hammar et al. 1989; Wilson and Hebert 1993; Redenbach and Taylor 2002b), Oncorhynchus (Dowling and Childs 1992), and Salmo (e.g., McGowan and Davidson 1992a). By contrast, hybridization may occur in both directions in Oncorhynchus (e.g., Rubidge et al. 2001). Several hypotheses, encompassing both pre-zygotic and post-zygotic processes have been proposed to explain different degrees of directionality to hybridization (reviewed by Wirtz 1999). One hypothesis involves sneak spillings by males of the smaller species as outlined above. For instance, where Dolly Varden and bull trout are sympatric, males (and females) of the former mature at much smaller sizes than the latter (Figure 8.3) and hybridization, inferred from mtDNA analysis, is in the direction of male Dolly Varden × female bull trout (Figure 8.3a). This hypothesis predicts that

![Figure 8.3](image)
where sympatric species are the same or more similar in body size, hybridization should be bidirectional. Data collected from hybrid zones between sympatric rainbow trout and westslope cutthroat trout are consistent with the hypothesis; the species show greater overlap in size at maturity and hybridization is bidirectional (Figure 8.3b; Rubidge et al. 2001).

One aspect of pre-zygotic isolation that has received comparatively little attention in salmonids is gamete recognition. Considerable recent work on marine invertebrates (with external fertilization) has demonstrated the potential for physical and molecular bases of sperm-egg interactions and their influence on the probability of natural hybridization (Palumbi 1998; Eady 2001). Only a limited amount of work has been conducted on comparative analyses of gamete morphology and structure in salmonids (e.g., Groot and Alderdice 1985) and these data appear to indicate conservation of the basic structure of egg membranes. Subtle differences, however, in relative egg membrane thickness may be important in constraining hybridization, particularly under competitive conditions (Arnold 1997). This general area bears further investigation in salmonids, as experimental hybrid matings in salmonids have not been attempted under competitive conditions.

4.3. Post-Zygotic Isolation in Salmonids

Post-zygotic isolation in salmonid fishes, like other organisms, may take the form of endogenous, or environmentally independent, factors resulting from genomic or developmental incompatibilities between species that result in hybrid inviability or infertility. Alternatively, post-mating isolation can result from exogenous factors, or environmentally dependent selection against hybrids. Inferences about the importance of endogenous factors come largely from experimental crosses between species. Such inferences are limited by different procedures among experiments as well as by intraspecific variation in the response to hybridization between species (i.e., population source used in the crosses) (Chevassus 1979). A review of salmonid experimental hybridization studies (Chevassus 1979) indicated that viable and fertile hybrids were routinely produced in intrageneric crosses in Salmo, Salvelinus, and Oncorhynchus. Hybrid viability and fertility was markedly reduced in crosses between genera, but at least some crosses produced fertile hybrids (e.g., Oncorhynchus mykiss × Salmo trutta). Some quantitative data exist for percent survival to hatching for experimental matings within Oncorhynchus (R. Devlin unpublished; Alverson and Ruggerone 1997) and these data, when associated with genomic measures of similarity (either Nei’s genetic distance from allozymes, or ITS rDNA sequence divergences) show a positive, but not significant relationship (Figure 8.4).

In addition, although chromosome numbers vary from 52 to 84 among Salmo, Oncorhynchus, and Salvelinus (Allendorf and Thorgaard 1984), chromosome arm numbers are relatively conserved at about 100. Most chromosome number changes appear to be the result of centric fusions and fissions (Allendorf and Thorgaard 1984) suggesting few intrinsic chromosomal challenges to hybridization. Biases in the survival of reciprocal crosses in experimental matings
(e.g., McGowan and Davidson 1992b; R. Devlin unpublished; Alverson and Ruggerone 1997) and the general lack of extensive nuclear introgression in most salmonid systems where hybridization has been reported, however, suggest that differences in chromosome number may constrain introgression in some species through reduced fertility of hybrids. In summary, although results can be highly variable, it appears that endogenous factors operate strongly, but not necessarily completely at the intergeneric level and in many intrageneric crosses, but there is considerable potential for intrageneric gene flow. This conclusion is consistent with reports of natural introgression within genera in at least four genera: *Oncorhynchus* (Rosenfield et al. 2000), *Salvelinus* (Bernatchez et al. 1995; Baxter et al. 1997), *Salmo* (Verspoor and Hammar 1991), and *Coregonus* (Svârdson 1998).

Nevertheless, gene flow between salmonid species is likely constrained to some extent by endogenous selection against hybrids. For instance, there has been some experimental work on the performance of hybrid crosses in traits that would likely impact on their survival in nature. For instance, Lu and Bernatchez (1998) demonstrated three to four times higher embryonic mortality in hybrids between “dwarf” and “normal” lake whitefish (*Coregonus clupeaformis*) relative to parental crosses. The ecotypes of whitefish were from different

![Figure 8.4. The relationship between a measure of nuclear genomic similarity (Nei’s I) and survival to hatching in hybrid crosses within *Oncorhynchus*. Each point represents the mean of both reciprocal crosses between species. The hybridization experiments were conducted by R. Devlin (unpublished; Alverson and Ruggerone 1997) and involved two adults of each sex of each species. For each cross, approximately 500 eggs were fertilized from two females of each species. Values of Nei’s genetic identity were obtained from Utter et al. (1973) and represent variation across 18 allozyme loci. Species acronyms are: sockeye salmon (sk), chum salmon (cm), pink salmon (pk), coho salmon (co), chinook salmon (ck), and rainbow trout (rb).](image-url)
glacial phylogroups that diverged on the order of 150,000 years ago, suggesting that genetic differences driving intrinsic developmental incompatibilities can evolve within this time frame. Leary et al. (1995) reported that experimental hybrids between rainbow trout and westslope cutthroat trout had equal or higher survival to hatching, but experienced slower growth and survival to 112 days post-fertilization under laboratory conditions. Leary et al. (1993) suggested that introgression between native bull trout and brook charr introduced into parts of western North America was constrained by \( F_1 \) sterility. Experimental matings between divergent lineages of brown trout also suggest some disruptions during meiosis in hybrids (Poteaux et al. 2000), an observation that is associated with low levels of introgression where the two lineages come into artificial contact in nature (Largiander and Scholl 1996). Hybrids between “even-year” and “odd-year” populations of pink salmon from the same river showed reduced survival to maturity that were suggested to arise from the disruption of co-adapted gene complexes, one mechanism of outbreeding depression (Gharrett and Smoker 1991; Gharrett et al. 1999). Finally, it is often observed when making reciprocal hybrid matings that one type of cross displays higher survival than the reciprocal cross. For instance, R. Devlin (unpublished, summarized in Alverson and Ruggerone 1997) reported 5% survival to hatching in progeny of female Atlantic salmon \( \times \) male pink salmon crosses, but 0% survival in the reciprocal cross. Similarly, female sockeye salmon \( \times \) male coho salmon crosses had 82.9% survival, but male sockeye salmon \( \times \) female coho salmon crosses experienced 0% survival. Although the mechanism remains obscure, some of this variation may be explained by differences in egg size between species. It has been noted previously that, for instance, the cross involving females with the smaller egg size usually suffers higher mortality (e.g., Wilson and Hebert 1993; Lu and Bernatchez 1998). There may also be problems in terms of the developmental interaction between egg and sperm nuclei after fertilization that influence viability of matings in certain directions (R. Devlin, pers. comm.). It has also been suggested that in some instances, interspecific hybridization can result in skewed sex ratios in progeny (Forbes and Allendorf 1991) that can influence the extent of subsequent introgression.

One aspect of post-zygotic isolation that bears investigation in salmonids is Haldane’s rule; i.e., the tendency for sex-biased mortality or infertility of hybrids where the sex that shows the lowered viability or fertility is the heterogametic one. For instance, Coyne and Orr (1989) summarized data that supported Haldane’s rule in mammals, birds, and insects. Haldane’s rule results in incomplete post-zygotic isolation (because one sex develops normally) and this would predict higher mortality/infertility in male salmonid hybrids (the heterogametic sex). In fact, Forbes and Allendorf (1991) observed a sex ratio in favor of females in some populations of hybrids between westslope cutthroat trout and Yellowstone cutthroat trout and discussed similar results in other fishes. As stressed by Forbes and Allendorf (1991), however, the effects on hybridization on sex-dependent fertility or survival has received little detailed study in salmonids. The relatively late age of maturity, difficulty of determining sex at early ages (but see Devlin et al. 1991), and the generally limited applicability of
Haldane’s rule within the context of most situations were hybrids are produced (e.g., production facilities) probably explain the lack of attention. The relative ease of crossing and raising salmonid fishes and the broad range of divergence times of species that can be crossed (e.g., between ‘‘species pairs’’ to between genera) suggests that some insights into the role of sex-linked versus autosomal genes in reproductive isolation, for instance, could be gained by investigation of Haldane’s rule in salmonids.

Exogenous selection against hybrid progeny has been less well studied in salmonid systems, but indirect evidence suggests considerable potential for this process to constrain gene flow in nature. Experimental matings between anadromous (sockeye salmon) and freshwater-resident (kokanee) *Oncorhynchus nerka* produced hybrids that were phenotypically intermediate in traits such as developmental timing and success, salinity tolerance, and swimming performance, which may compromise survival of hybrids in nature (reviewed by Taylor 1999). Other indirect evidence for exogenous selection against salmonid hybrids comes from genetic surveys of natural populations. In at least two hybrid systems (cutthroat trout × rainbow trout and Dolly Varden × bull trout), hybrids tend to predominate in the younger age classes and adult hybrids are relatively rare (Campton and Utter 1985; Redenbach and Taylor 2002b; Rubidge et al. 2001; Young et al. 2001), which suggests that hybrid survival to maturity is rare. In these cases, the hybridizing parental species showed marked differences in life history (one is migratory and the other is not) and the complexities and rigors of migration may be an important source of selection against hybrids (cf. Hawkins and Quinn 1996; Utter 2000). By contrast, Forbes and Allendorf (1991) studied the genetic structure of different hybrid swarms between Yellowstone cutthroat trout and westslope cutthroat trout from lakes in Montana. Although taxonomically classified as subspecies, these fishes show levels of allozyme (*D* = 0.3) and mtDNA divergence (4–5%) comparable to some full taxonomic species of *Oncorhynchus* (e.g., Leary et al. 1987; Thomas and Beckenbach 1989). Forbes and Allendorf (1991) used estimates of linkage disequilibrium to test for selection against hybrids, and despite the high levels of structural gene divergence between subspecies, the authors observed stable hybrid swarms with little evidence of selection eliminating hybrids. As summarized by Utter (2000), it is perhaps the non-migratory or “simple” migratory (tributary streams to lake) life histories of these populations that provides limited opportunities for ecological selection against hybrids.

Sympatric and hybridizing populations of Dolly Varden and bull trout have also been studied to test the idea that parental species exploit alternative niches to which phenotypically intermediate hybrids may be poorly adapted. Stream-resident sympatric juvenile salmonids typically demonstrate well-marked differences in habitat use (e.g., Hartman 1965; Taylor 1991b). Juvenile Dolly Varden and bull trout coexisting in a northcentral British Columbia watershed, however, demonstrated little habitat and diet partitioning, suggesting limited potential for habitat or trophic-based ecological selection against hybrid progeny. By contrast, the two species showed marked habitat and diet partitioning when the entire life cycle was considered. Dolly Varden remain as stream-resident specialists on
stream invertebrates and mature at 15–20 cm in length, whereas bull trout migrate to an adjacent lake where they become fish-eating specialists and mature at sizes ranging from 60 to 100 cm. Ecological selection against hybrid progeny (which are intermediate in size at maturity—see Hagen and Taylor 2001) may act either through disrupted migratory behavior or via size-dependent mate or habitat selection upon maturation (see below).

Exogenous selection against hybrids may also be sexual in nature. For instance, mate selection in salmonids is often size-dependent, with fish tending to choose mates of their own size or larger (Foote 1988). In addition, for salmonids that reproduce in streams, habitat choice is also usually size-dependent with larger species using faster, deeper water with larger substrates (Wood and Foote 1996; Hagen and Taylor 2001). Consequently, if mature hybrids are intermediate in size to the parental species (e.g., Hagen and Taylor 2001), then they may be at a disadvantage during reproduction. For instance, Vamosi and Schluter (1999) demonstrated sexual selection against hybrids between benthic and limnetic species of three-spine stickleback (Gasterosteus aculeatus) in nature. The well-developed secondary sexual characteristics and behavioral repertoires of salmonids during mating suggest that such processes may also select against hybrids in salmonids.

5. Models of Hybridization Zone Structure and Evolution

One of the fundamental issues in hybridization research focuses on the evolutionary processes that determine the genetic structure of hybrid zones (Arnold 1997). All of these models incorporate dispersal and selection against hybrids, but they differ in the relative importance of these processes and in the role of the environment in selection against hybrids. These models of hybrid zone evolution were reviewed by Arnold (1997). In this section, I provide brief overviews of these models and how data on salmonids have, or could, be used to resolve remaining uncertainties.

5.1. The Tension Zone Model

The first and “simplest” model is the tension zone model (see Barton and Hewitt 1985), which posits that hybrid zones reflect a balance between dispersal of parental genotypes into the hybrid zone and endogenous (i.e., environment-independent) selection against hybrids that generates genetic clines of various widths. The mathematical theory and expectations of tension zone models described by Barton and Hewitt (1985) strongly influenced empirical studies of hybrid zones to explain the basis for variability in cline widths. Tests of tension zone models are perhaps best conducted in situations where hybridizing populations are broadly parapatric and meet as opposing “phalanxes” of genotypes. The studies of Szymura and Barton (1986, 1991) on Bombina toads and Hewitt (1993) on Chorthippus grasshoppers meeting along broad areas of sec-
ondary contact in Europe are longstanding examples of hybrid zones interpreted within the context of tension zones.

5.2. Bounded Hybrid Superiority Model

The bounded hybrid superiority model is one of the two major environment-dependent models of hybrid zones (Moore 1977) and posits that hybrids show superior fitness, relative to parental genotypes, in certain environments, and hence, that ecological factors determine the fitness of genotypes. The habitats in which hybrids show superior fitness are typically identified as transitional or intermediate ecotones between habitats in which the parental genotypes are most fit (Arnold 1997). The best examples of such hybrid zones are found in instances of “disturbed” habitats (Hubbs 1955) or where environmental change results in altered habitat characteristics through time (Arnold 1997). A potentially powerful example of such hybrid zones involves habitats that were disturbed during the many glacial and interglacial periods of the Pleistocene. The massive environmental disturbances in terms of water temperature, clarity, and drainage patterns would have provided abundant opportunities for secondary contact between divergent lineages, as well as hybridization, particularly if one species was less abundant than the other (Wilson and Bernatchez 1998; Svärdson 1998; Redenbach and Taylor 2002a). The dynamic environmental changes may have favored some hybrid genotypes and perhaps allowed these lineages to persist (e.g., mtDNA introgressed charr, Glénet et al. 1998).

Bounded superiority hybrid zones are typically suggested to be ephemeral in that they persist only as long as the intermediate environments persist either temporally or spatially (Arnold 1997). Such intermediate or disturbed habitats may also be anthropogenically derived and lead to increased rates of hybridization or increased persistence of hybrid lineages (Pethon 1974; Bartley et al. 1990: Scribner et al. 2000). The apparently long-term persistence of some salmonid hybrid zones, i.e., those that appear to have persisted since deglaciation (e.g., Baxter et al. 1997) argues that they may be stable through time and are not solely dependent on transitional environments.

5.3. Mosaic Hybrid Zone Model

A second kind of environment-dependent hybrid zone model is the mosaic model. This model, however, differs from the bounded hybrid superiority model in that rather than representing a smooth transition of genotypes through an environmental gradient or ecotone, the distribution of parental and hybrid genotypes is “patchy” and results from adaptation of parental and hybrid genotypes to alternative habitats that are themselves patchy in distribution (Howard 1986; Harrison 1990). Differential adaptations to soil types were implicated in a mosaic genetic structure in hybridizing species of crickets, Gryllus (Rand and Harrison 1989). Although in its original form, mosaic hybrid zones posited that hybrid genotypes were less fit than parentals, Arnold and Hodges (1995) and Arnold (1997) presented a review of evidence that mosaic hybrid zones may
include habitats where hybrid genotypes are more fit than one or both parental genotypes.

5.4. Evolutionary Novelty Model

Finally, Arnold (1997) proposed a new model of hybrid zones known as the "evolutionary novelty model" as a new theoretical framework to accommodate some of the empirical inconsistencies observed when adopting either environment-independent or environment-dependent models in isolation. The model essentially allows for both endogenous and exogenous selection and for the possibility that hybrid genotypes may demonstrate higher fitness than parents and, consequently, establish stable evolutionarily distinct lineages (Arnold 1997).

5.5. Salmonids and Hybrid Zone Models

In general, there have been few empirical treatments of hybridization in salmonids within the context of evaluating different models of hybrid zone evolution and structure. The shortage of such studies is likely due to the dendritic nature of stream habitats or the isolated nature of many lakes and, therefore, the low probability of forming broadly parapatric contact zones across large geographic areas. The nature of the aquatic habitats of salmonids means that hybridization zones may be limited by historical contingencies that may be quite local in effect (e.g., watershed exchanges; Duvernell and Aspinwall 1995). This leads to the hypothesis that most salmonid hybrid zones probably follow the mosaic model in structure where broadly allopatrically or parapatrically distributed taxa only come into contact and hybridize where local contingencies permitted connection of specific watersheds. This idea also suggests the importance of the spatial scale of sampling when characterizing hybrid zones. For example, hybrid zones could be mosaic in structure within individual, isolated watersheds that contain two species, but where local sympatry depends on distribution of particular habitats. Alternatively, hybrid zones could be mosaic on much broader geographic scales where sympatry may be established in some watersheds, but not others, owing to historical patterns of dispersal.

A good example of such mosaic structure involves areas of hybridization between Dolly Varden and bull trout. These two species of char are broadly parapatric in northwestern North America; Dolly Varden are coastal in distribution from western Alaska to the Olympic Peninsula, whereas bull trout are largely interior in distribution from northern California to the Yukon and Northwest Territories, that is, their ranges meet broadly along the Coastal/Cascade Mountains that separate coastal from interior drainages. Taylor et al. (2001) and Redenbach and Taylor (2002a) reported areas of sympatry and hybridization between the species in at least seven independent watersheds. These areas were found in or near tributaries of large river systems that cut through the mountain crest (e.g., the lower Fraser River) or in the headwaters of coastal (or interior) watersheds that interdigitate with interior (or coastal) watersheds.
Historical and fish distribution data that suggest that watershed exchanges between these coastal and interior watersheds have taken place post-glacially (Baxter et al. 1997). Consequently, the areas of hybridization form a mosaic pattern highly dependent on historical or contemporary opportunities for exchange between coastal and interior drainages. A further example is the mosaic distribution of hybridization between dwarf and normal lake whitefish among lakes in eastern North America (see Lu et al. 2001). On a broader geographic and temporal scale, however, hybrid zones between salmonid lineages may take the form of extensive clinal variation. For instance, Turgeon and Bernatchez (2001b) described a clinal pattern of microsatellite DNA variation along an east–west gradient in temperate North America in the cisco, *Coregonus artedi*. The clinal variation in allele frequencies in samples from populations that are currently largely isolated in distinct watersheds was apparent on a continent-wide scale. In addition, the cline was probably a signature of historical isolation between two glacial lineages of cisco that came into secondary contact via post-glacial dispersal through pro-glacial lakes (Turgeon and Bernatchez 2001b). More generally, such watershed exchanges may contribute to the formation of aquatic “suture zones”, that is, bands of geographic overlap between biotic assemblages (Remington 1968) that in turn may explain the origin of hybrid zones between previously isolated taxa.

The well-developed habitat partitioning that is often observed between salmonid species within drainages probably also promotes mosaic-like structure of salmonid hybrid zones at smaller spatial scales. For instance, Hartman and Gill (1968) studied the local distribution of coastal cutthroat trout and steelhead trout in southwestern British Columbia. Their analysis showed that cutthroat trout were found in smaller streams with lower gradient, and steelhead in larger, higher gradient streams. Where they occurred in the same streams, cutthroat tended to be found in the upper tributary reaches, whereas rainbow tended to be found in the lower reaches of the mainstem (Hartman and Gill 1968). Henderson et al. (2000) showed similar within-tributary broad-scale habitat partitioning between Yellowstone cutthroat trout and introduced rainbow trout in Idaho. Finally, Hagen and Taylor (2001) described differences in spawning habitat utilization between Dolly Varden and bull trout that were broadly sympatric within a single tributary stream. Bull trout tend to spawn in lower mainstem reaches, whereas Dolly Varden, which mature at a much smaller size than bull trout, concentrated their spawning in upper and much smaller tributary channels with groundwater seepage. These local habitat preferences should promote a mosaic pattern of hybridization within drainages, that is, hybridization will be most likely only where the distinct parental habitats are in close proximity. For example, Redenbach and Taylor (2002b) found that most 0+ (i.e., those younger than 1 year of age) Dolly Varden × bull trout hybrids were found where tributary seepage channels (Dolly Varden spawning habitats) were close to mainstem habitats (areas utilized by spawning bull trout).

Notwithstanding the constraints imposed by aquatic habitats to the structure of hybrid zones in salmonid fishes, mosaic hybrid zones could also be structured by processes inherent to tension zones (e.g., high intrinsic mortality
of hybrids; see, e.g., Lu and Bernatchez 1998). In general, however, given the evidence for relatively high survival and fertility of hybrids in the most common natural hybrid zones (i.e., those within genera), and the unique aspects of aquatic habitats it seems unlikely that salmonid hybrid zones would fall into the category of classic tension zones. Rather, intrinsic viability and fertility of many natural salmonid hybrids, and the tight linkage between salmonids and the distribution and nature of aquatic habitats, argue that environment-dependent processes generate salmonid hybrid zones that are largely mosaic-like in structure, at least at intermediate (watershed-level) geographic scales. In addition, hybrid zones within particular pairs of species may have different structures (i.e., hybrid swarms vs. relatively low numbers of hybrids relative to parentals) in different localities even within single streams (e.g., Campton and Utter 1985). This variability in structure of hybrid zones argues that local environmental conditions, either through their effects on assortative mating or selection against hybrids, are important in structuring salmonid hybrid zones.

Some recent molecular insights into salmonid hybrid zones suggest that the evolutionary novelty model may be applicable in some instances. Bernatchez et al. (1995), Glémet et al. (1998), and Wilson and Bernatchez (1998) have reported the complete introgression of mtDNA from Arctic charr into populations of brook charr and lake trout, respectively. In both instances, the authors argued that this introgression may have been promoted by positive natural selection for Arctic charr mtDNA. The mechanism of selection could have been the greater physiological performance of brook charr having mtDNA from Arctic charr, which has a more northerly latitudinal distribution in North America and may have given hybrid charr a selective advantage during cooler periglacial times (Glémet et al. 1998). In another charr hybrid system, Dolly Varden and bull trout, Redenbach and Taylor (2002a) argued that asymmetrical introgression of bull trout mtDNA into Dolly Varden was a result of the mating system, and not natural selection. In sympatry, Dolly Varden are much smaller at maturity than bull trout and the Dolly Varden males probably act as “sneak” spawners on larger bull trout pairs (see also Wirtz 1999). These same authors, however, reported introgression of type 2 growth hormone from bull trout into Dolly Varden that was significantly (three times) greater than introgression from Dolly Varden to bull trout (Redenbach and Taylor 2002b). The biased introgression of growth hormone was in marked contrast to introgression of three other nuclear loci that were roughly equal between species (i.e., bidirectional) and among themselves (Figure 8.5). Such biased introgression of a biparentally inherited locus could be a result of positive selection for bull trout growth hormone allele in Dolly Varden. Although the specific mechanism of selection is unknown, it is possible that Dolly Varden (the smaller of the two species in sympatry) obtain some advantage in terms of growth, metabolism, or even aggression (Devlin et al. 1994) from introgression of growth hormone from bull trout. Verspoor and Hammar (1991) also reported several possible cases of positive selection for allozyme alleles in brown trout and Arctic charr that resulted from introgression from Atlantic salmon and brook charr, respectively;
foreign alleles that may be maintained in the recipient species by thermal selection.

6. Hybridization and Evolution: the Window Is Still Open

6.1. Hybrid Zone Structure and Speciation

Barton and Hewitt (1985) characterized hybrid zones as "natural laboratories," while Harrison (1990) described them as "windows" through which to study the process of speciation. In essence, the observation of natural hybridization signals incomplete reproductive isolation and, depending on the level of hybridization, different stages in speciation (Jiggins and Mallet 2000). The production of hybrids, therefore, provides the opportunity to examine their evolutionary fate. If hybrids are completely viable through maturity and fertile and exhibit equal or greater reproductive success to parental genotypes, hybrid swarms will likely result and few insights into speciation will be obtained. If the performance of hybrids, however, is inferior to that of parentals such that the genetic integrity of the latter is maintained in the face of gene flow, two key inferences are possible concerning the ecology and genetics of speciation. First, the ecological and genetic processes that constrain gene flow between hybridizing species may
signal the same processes that initiate speciation. Second, if selection against hybrids results in enhanced assortative mating and pre-mating isolation, then this would constitute evidence for reinforcing selection being an agent of speciation (Butlin 1989). Below, I outline some ways in which more nascent fields of speciation research may profit from examination of salmonid hybrid zones.

Recently, Harrison and Bogdanowicz (1997) classified hybrid zones into three basic kinds based on the frequency distribution of genotypes. Hybrid zones consisting largely of a hybrid swarm of $F_1$, $F_n$, and early generation backcrosses are “unimodal,” those consisting largely of genotypes resembling parental types are “bimodal,” while those consisting of an intermediate distribution of genotypes are known as “flat” hybrid zones. Jiggins and Mallet (2000) suggested that bimodal hybrid zones were characterized by strong pre-zygotic barriers to hybridization, particularly in terms of assortative mating or fertilization. Unimodal hybrid zones, by contrast, are typified by little assortative mating (Jiggins and Mallet 2000). Salmonid hybrid zones that have been extensively studied support these generalizations. For instance, Redenbach and Taylor (2002b) reported a strongly bimodal hybrid zone between Dolly Varden and bull trout in a northcentral BC watershed (Figure 8.6a) using variation across four nuclear markers to describe the frequency distribution of genotypes. In this system, the two species differ strongly in size at maturity (see above) and size-dependent spawning habitat use (Hagen and Taylor 2001) and likely mate choice as well (e.g., Maekawa et al. 1994). First-generation hybrids made up only 3–5% of all genotypes present in the watershed, with parentals and advanced generation backcrosses or hybrids predominating (see also Bartley et al. 1990). By contrast, Campton and Utter (1985) reported a nearly unimodal distribution of genotypes in a hybrid swarm between rainbow trout and coastal cutthroat trout from a creek in western Washington (Figure 8.6b). These same authors, however, also reported other sites from within the same stream that were either characterized by genotypes of one species or the other, or both with little evidence of hybridization, indicating the importance of scale of sampling when characterizing hybrid “zones.” Nevertheless, hybrid zones within western trout are often unimodal hybrid swarms (Forbes and Allendorf 1991; Allendorf et al. 2001) as are many hybrid zones reported between species of *Coregonus* in Scandinavian lakes (e.g., Vuorinen 1988). In these later cases, the breakdown of pre-mating isolation may be an important cause of introgression and may be a result of greater similarity in body size between species (e.g., Figure 8.6; Rubidge et al. 2001), environmental changes (Seehausen et al. 1997), or recent contact in environments with limited opportunities for spawning habitat segregation (Vuorinen 1988). The implied importance of pre-mating isolation in structuring hybrid zones in salmonids is consistent with the general lack of evidence of major endogenous genetic incompatibilities between species forming natural hybrids (see above) and with the general proposition that pre-mating isolation evolves first and/or more easily than endogenous post-mating isolation (i.e., hybrid inviability or sterility, Coyne and Orr 1997).

A major uncertainty in the process of speciation concerns the relative importance of deterministic factors, such as sexual or natural selection, or random
Figure 8.6. Genetic structure in two salmonid hybrid zones. (a) Bimodal hybrid zone between Dolly Varden (Salvelinus malma) and bull trout (S. confluentus) in Kemess Creek (black bars) and from five other tributaries of the Thutade Lake watershed (white bars), British Columbia. Shown is the frequency distribution of individual juvenile char (N = 990 fish) with different numbers of bull trout alleles ranging from 0 (equivalent to homozygous for Dolly Varden alleles at all nuclear loci and exhibiting Dolly Varden mtDNA) to 9 (equivalent to homozygous for bull trout alleles and exhibiting bull trout mtDNA). Owing to small numbers of hybrid individuals, the heights of bars at 0 and 9 have been set to a maximum of 50 and actual numbers of individuals fixed for Dolly Varden or bull trout, respectively, alleles/haplotype are given above each bar. (b) Unimodal hybrid zone between coastal cutthroat trout (Oncorhynchus clarki clarki) and rainbow trout (O. mykiss) in a Washington State stream. Genotypes are designated by a hybrid score (see Campton and Utter 1985) that represents the multilocus probability that an individual fish arose from random mating within each of the two parental taxa. A score of 0 represents a high probability of cutthroat trout and 1 represents a high probability of rainbow trout. Redrawn from Campton (1987).
factors, such as genomic reorganization via random allopatric divergence or founder events, in driving the evolution of reproductive isolation (Howard and Berlocher 1998). Hybrid zones can provide insights into this question under the assumption that factors influencing the fitness and frequency of genotypes in species with incomplete reproductive isolation are also important in initiating speciation (Barton and Hewitt 1985; Jiggins and Mallet 2000; Barton 2001). This is an area where detailed study of salmonid hybrid zones can make an important contribution. In the best-studied hybrid zones in charr and western trout, there is little evidence that endogenous selection structures these hybrid zones. Rather, and as argued above, ecological factors appear to be important in constraining gene flow between species, either in terms of reproductive (e.g., Hagen and Taylor 2001; Redenbach and Taylor 2002b) or migratory ecology (Campton and Utter 1985; Young et al. 2001). Further, studies of “species pairs” (sensu Taylor 1999) of sympatric salmonids, such as “dwarf” and “normal” lake whitefish (Lu and Bernatchez 1999; Lu et al. 2001), suggest that variation in opportunities for trophic differentiation within single lakes may explain differing levels of introgression between species (see also Bernatchez 2003—this volume). This hypothesis suggests that differences in the trophic selective environments of ancestral populations may have initiated divergence between species that have come into contact secondarily. The multifarious aspects of natural selection that act against hybrids between sympatric anadromous and non-anadromous sockeye salmon, and maintain genetic isolation between them in the absence of endogenous selection (reviewed by Taylor 1999), are also consistent with the importance of exogenous selection in initiating speciation in salmonids. The importance of exogenous selection in salmonid divergence is also suggested by experimental work on conspecific populations. An excellent example involves the experiment by Bams (1976) who demonstrated reduced homing ability of hybrids between two populations of pink salmon. The key inference from this study was that local adaptation to specific streams was important to the evolution and maintenance of spatial reproductive isolation between conspecific populations.

Taken together, studies of hybrids in salmonids across different scales of genetic divergence (from taxonomically distinct species to “species pairs” to conspecific populations) offer rich opportunities to study the importance and kinds of exogenous (and endogenous) selection in initiating divergence and speciation. The observation that many of these sympatric populations, hybrid zones, and genetically divergent populations have formed post-glacially (i.e., over the last 15,000 years) argues that such selection may operate rapidly (see also Hendry et al. 2000b; Hendry 2001).

Another issue relevant to speciation is the role of reinforcement, that is, when natural selection acts directly on mate choice to reduce the production of unfit hybrids (e.g., Noor 1995; Liou and Price 1994). Liou and Price (1994) and Jiggins and Mallet (2000) presented arguments that reinforcement should be most likely when there is a high degree of assortative mating that has already evolved, that is, in bimodal hybrid zones. Further, mosaic hybrid zones that seem to be common in salmonids should also promote reinforcement owing to
a high initial level of assortative mating via differences in habitat use (Jiggins and Mallet 2000). Consequently, a comparative analysis of the degree of reproductive isolation between sympatric and allopatric populations of naturally hybridizing salmonids would appear to provide excellent models to test for reproductive character displacement predicted under reinforcement. A possible example of such a process may be found in the Dolly Varden–bull trout system. The two species show greater differences in size at maturity when sympatric than when allopatric; sympatric Dolly Varden are much smaller than bull trout (e.g., Hagen and Taylor 2001), but they are similar in size at maturity when allopatric (Baxter 1997; Underwood et al. 1996; McPhail and Baxter 1996; E. Taylor unpublished). Selection against hybrids in sympatry may drive the evolution of different sizes at maturity, with correlated effects on size-dependent mate choice and habitat utilization, increasing reproductive isolation in sympathy. A complication for this hypothesis, and for the more general idea that reinforcement may be more likely in bimodal hybrid zones, however, is the “sneaking” mating behavior of small-sized male salmonids. As discussed above, sneaking of smaller male Dolly Varden on larger bull trout mating pairs may promote hybridization and compromise selection for size-based assortative mating.

6.2. Hybrid Speciation

Hybrid speciation encompasses the idea that hybridization events may produce recombinant lineages that are more fit than the parental genotypes, either in parental or novel environments, and become the basis for new evolutionary lineages. This idea is broadly accepted by botanists and many plant species may have arisen by hybridization (e.g., Arnold 1997; Riesberg 1998). In addition, genome duplications stemming from hybridization and subsequent divergent resolution of duplicate genes is thought to have been important in speciation within the Catostomidae (North American suckers) (Ferris et al. 1979). Furthermore, there is good evidence that the cyprinid fish *Gila seminuda* has arisen by homoploid (non-polyploid) speciation stemming from hybridization between *G. robusta* and *G. elegans* (DeMarais et al. 1992).

Perhaps the best hypothetical example of hybrid speciation in salmonids is the work of Svärdson (1979, 1998) who summarized an extensive dataset describing hybridization and introgression in Scandinavian *Coregonus*. Kosswig (1963) and Svärdson (1998) suggested that secondary contact between two to five formally allopatric species had resulted in hybridization and introgression with the production of novel recombinant genotypes that may have fostered adaptation to distinct environments resulting in up to five sympatric species (Svärdson 1998). Given the importance of trophic characters in resource partitioning in whitefishes and its possible role in reproductive isolation (e.g., Bernatchez 2003—*this volume*), as well as the possible novel trophic morphologies that may result from hybridization (McElroy and Kornfield 1993), hybrid speciation in these fish remains an interesting possibility. It will require rather careful phylogenetic analyses (e.g., DeMarais et al. 1992) to test this idea rigorously. Similarly, Mina (1992) suggested that a stable hybrid lineage of lenok...
(Brachymystax) had been established in eastern Russia via hybridization between formerly allopatric “blunt-nosed” and “sharp-nosed” forms of B. lenok. Another interesting case of hybridization that may have promoted diversification and speciation in salmonids are charrs of the Pacific basin, in particular, charrs found in waters of the Russian far east. These fish consist of a complex of forms that at one time had been described as consisting of up to 13 distinct species and a large number of undescribed sympatric forms (Behnke 1972, 1980; Savvaitova 1980). Reassessment of the systematic relationships of these Salvelinus suggests that these forms are part of a relatively recent (10,000–20,000 year) divergence of the S. alpinus–S. malma (Arctic charr and Dolly Varden) complex resulting from vicariant events surrounding the high Arctic and Pacific coast of North America (Savvaitova 1980; Brunner et al. 2001). Given evidence both for historical and contemporary hybridization and introgression among Pacific basin charr (McPhail 1961; Baxter et al. 1997; Redenbach and Taylor 2002a,b), some of the extant diversity in the genus, including sympatric forms, may stem from evolutionary novelties stemming from hybridization (Behnke 1980).

6.3. Hybrid Zones and Molecular Aspects of Speciation

Finally, both natural and experimental hybridization and introgression can be exploited to resolve the genetic bases of ecological divergence and speciation. There are three basic approaches that can be adopted to address this fundamental unknown in evolutionary biology. First, high-resolution genetic markers such as tandem-repeat DNA loci that are so useful for individual parentage determinations (e.g., Bentzen et al. 2001; Taggart et al. 2001) could be used to study the fitness of genotypes in nature. The documentation of genotype-based fitness across multiple generations within a population consisting of parental and hybrid matings and their degree of association with habitat offer the possibility of directly assessing competing hybrid zone models (e.g., tension vs. mosaic models, endogenous vs. exogenous selection against hybrids, Rieseberg 1998). A second approach is to exploit natural variation in extent of hybridization/introgression among localities across a natural zone of contact. Harrison (1990) suggested that genes contributing to reproductive isolation could be identified by their association with linked genetic markers that introgress at lower than expected rates. Genetic mapping of these linked markers would then make it possible to determine the general number and genomic locations of “speciation genes.” Rieseberg (1998) reviewed the application of this approach to a natural hybrid zone in Helianthus sunflowers. The integration of this approach with surveys in hybrid zones with different levels of hybridization and introgression and the possible environmental correlates of such variation (Lu and Bernatchez 1999; Lu et al. 2001; Redenbach and Taylor 2002b) could reveal which ecological factors influence introgression and the genomic regions where such variable introgression is concentrated. Furthermore, a chief advantage of using salmonid hybrid zones for resolving the genetic architecture of divergence and speciation is the rich literature on
ecological differentiation between species, including sympatric species pairs (e.g., Taylor 1999), that hybridize in nature. In addition, a comparably rich literature and understanding exists for traits that are of importance in sexual isolation (Craig and Foote 2001; Fleming and Reynolds 2003—this volume). This knowledge, coupled with the increasing base of mapped molecular markers (Danzmann and Gharbi 2001), means that it will be possible to map the genomic distribution of quantitative trait loci (body size, growth rate, trophic characters, secondary sexual characters) that contribute to reproductive isolation in natural hybrid zones. Although work on plant species (e.g., reviewed by Rieseberg 1998) has exploited this approach the best to date, recent work on whitefish Coregonus species pairs exemplifies the steps and possibilities applied to salmonids. Rogers et al. (2001) described the production of a preliminary linkage map in experimentally produced hybrids between “dwarf” and “normal” populations of lake whitefish. Segregation across 998 amplified fragment length polymorphism (AFLP) loci was used to infer the number of linkage groups and genomic locations of genes that may be responsible for ecological and reproductive divergence in sympatric ecotypes of whitefish.

The large number of genome mapping projects in salmonids (Danzmann and Gharbi 2001) means that this approach offers great promise, although a general constraint may be the relatively lengthy generation time of salmonid fishes. Hybrid zones in salmonids, however, may be particularly useful to explore these issues because specific systems are in various stages of evolutionary divergence and it may be possible, therefore, to examine the genetic basis of divergence at different stages in speciation. For example, it would be interesting to know if the same genomic regions are involved in divergence between recently derived sympatric “species pairs” (e.g., dwarf and normal lake whitefish) compared to older divergences. Furthermore, the independent evolution of species pairs that still hybridize in different lakes or streams offers the opportunity to test whether or not similar genomic regions have been involved in replicate divergences and, if so, would support the argument that natural selection is central to evolutionary diversification. Replicate species pairs of dwarf and normal whitefish are discussed by Bernatchez (2003—this volume) as an excellent model system to examine this question. Other replicate species pairs, such as anadromous and nonanadromous sockeye salmon (Taylor 1999) offer similar opportunities.

Hybrid zones between more divergent lineages (e.g., Dolly Varden and bull trout; rainbow and cutthroat trout) that have formed independently (i.e., through independent episodes of secondary contact) could also be examined in terms of levels of introgression of loci associated with quantitative ecological or genetic differences between species relative to that for neutral loci. As Rieseberg (1998) detailed, the idea that ecological differences contribute to reproductive isolation predicts that introgression of such traits should be constrained relative to independently segregating neutral traits. Such genetic mapping of traits under conditions of differential introgression within hybrid zones can help identify the traits important to isolation, but the comparative analysis of introgression of such traits across hybrid zones and their correlation with environmental differ-
ences could help identify the crucial ecological factors initiating speciation (cf. Lu et al., 2001; Redenbach and Taylor 2002b).

A further contribution that salmonid hybrid zones could make to speciation involves their use in identifying specific genes important to reproductive isolation. In particular, comparative analysis of hybridization between pairs of species within genera that show differing levels of post-zygotic isolation may help resolve genetic regions important in, for instance, hybrid sterility. Comparative sequencing projects have, for instance, identified a gene important in hybrid sterility and inviability among species of Drosophila (Ting et al. 1998). Although the genomes of Drosophila are comparatively simple compared to salmonids (i.e., they have only four chromosomes), in principle, comparative sequence analysis of charr species that produce fertile offspring (e.g., Dolly Varden and bull trout) and those that produce sterile hybrids (e.g., bull trout and brook charr) may resolve the gene (or genes) contributing to sterility.

Finally, molecular genomics offers the opportunity to study the role of variable levels of gene expression in phenotypic divergence and speciation. Molecular genomics involves, in part, the identification and characterization of particular sets of genes that are expressed differentially during particular stages of development (Schulte 2001). By exploiting natural and experimental hybrids in salmonids and developing gene expression technology it will be possible, for instance, to characterize genes that are differentially expressed in different phenotypes (e.g., closely related species), including the resultant phenotypic novelties in hybrids. For instance, genes that contribute to endogenous selection against hybrids may have dysfunctional gene expression during development. Further, the incorporation of measures of differential expression in different phenotypes and the fitness of such phenotypes in different environments could provide insights into the potential adaptive nature of differential gene expression and how this contributes to exogenous selection against hybrids.

7. Conclusions

Given their importance to humans in a variety of ways and their conspicuous presence in natural ecosystems, salmonids have been well studied ecologically and genetically relative to most other fish. Such a scientifically high profile has resulted in numerous reports of natural hybridization between species across the Holarctic range of salmonids, as well as the study of numerous experimental hybrid populations. Nevertheless, salmonid hybrid zones remain relatively poorly characterized in terms of their structure and ecological and genetic factors that influence that structure. In large part, the underexploitation of salmonid hybrid zones results from the difficulty in working in aquatic ecosystems that are often geographically remote. Consequently, most salmonid hybrid zone studies have been opportunistic in nature (e.g., Baxter et al. 1997; Wilson and Hebert 1998). More recent and focused studies of salmonid hybrid zones (e.g., Campton and Utter 1985; Glémet et al. 1998; Lu et al. 2001; Redenbach and Taylor 2002a,b), however, have helped to better characterize the genetic structure of
hybrid zones, which, in general, appear to form mosaics owing to the tight linkage between salmonids and their aquatic ecosystems, and the role of watershed interconnectedness in promoting secondary contact between species (e.g., Redenbach and Taylor 2002a). These studies, aided by the development of molecular approaches to hybrid studies, have identified: (1) both historical and contemporary components to hybridization (Bernatchez et al. 1995; Redenbach and Taylor 2002a,b), (2) the assessment of endogenous (Lu and Bernatchez 1998) and exogenous selection (Hagen and Taylor 2001; Lu and Bernatchez 1998) in structuring hybrid zones, and (3) the possible adaptive consequences of introgression (Glémet et al. 1998; Redenbach and Taylor 2002b). These contributions have helped to integrate salmonids into the broader context of hybrid zones and their significance to evolution. Encouragingly, salmonid hybrid zones will probably increase in their profile and utility for understanding the genetic and ecological factors important to reproductive isolation and speciation because: (1) they offer a broad range of divergences (involving hybridization between post-glacial species pairs to divergences between lineages that are on the order of millions of years old), (2) the ecological and reproductive factors that differentiate species are well characterized, and (3) there is an increasing supply of genetic markers to probe the genomes for the loci that control these differences.

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