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Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon

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Abstract

Populations exposed to different ecological environments should diverge for phenotypic traits that influence survival and reproduction. This adaptive divergence should reduce gene flow between populations because immigrants become less fit than residents and because hybrids perform poorly in either environment (i.e., ecologically-dependent reproductive isolation). Here I demonstrate adaptive divergence and the evolution of reproductive isolation in populations of sockeye salmon (*Oncorhynchus nerka*) introduced from a common ancestral source into a new lake system (Lake Washington, Washington). The introduced fish founded several new populations, two of which experience very different environments during breeding and early development (Cedar River v.s. Pleasure Point beach). Over 13 generations, the two populations diverged for adult traits (female body size, male body depth; measured in the wild) and embryo traits (survival to hatching, development rate, size at emergence; measured in a common environment). The rates of divergence for these characters were similar to those observed in other examples of 'rapid evolution', and can best be attributed to natural selection. Partial reproductive isolation has evolved in concert with adaptive divergence: the rate of exchange of adults between the populations (determined using natural tags) is higher than the rate of gene flow (determined using DNA microsatellites). The demonstration that adaptive divergence can initiate reproductive isolation in less than 13 generations suggests that the first signs of 'ecological speciation' may appear soon after new environments are first colonized.

Introduction

In this paper, I outline a research program that has sought to elucidate evolutionary rate, pattern, and process within an empirical system. Specifically, I have documented adaptive divergence and the evolution of partial reproductive isolation between contemporary populations of sockeye salmon (*Oncorhynchus nerka*) that were recently derived from a common ancestral source. I begin with a general conceptual framework and then describe the study system and my results.

Adaptive divergence begins when a new population is founded, often through the colonization of a formerly unused environment or resource (allopatric, sympatric, or parapatric). The initial colonists are presumably farther from an adaptive peak in their new environment than they were in their ancestral environment. This initial mismatch between phenotypes and the environment probably contributes to the frequent failure of efforts to establish new populations. Of course some introduced populations do become very successful, perhaps owing to release from natural predators or competitors. Within the native range of a species, however, the distribution of phenotypes in a new population should fall some distance from the theoretical optima. Under this assumption, several patterns might be expected during subsequent adaptive evolution.

First, the strength of directional selection should be greatest immediately after colonization (Figure 1). As generations pass, the strength of selection should decline because the population is approaching a new

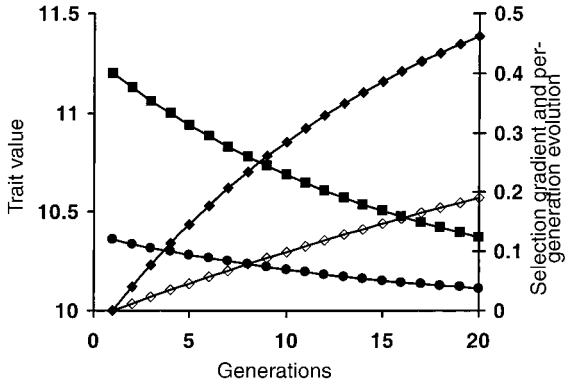

Figure 1. A graphical depiction of adaptive divergence, illustrating how a population changes after introduction into a new environment. Evolution is modeled as $\Delta z = G\beta$, where Δz is the change in mean trait value each generation, G is the additive genetic variance for the trait, and β is the regression of the trait on relative fitness (selection gradient). I consider stabilizing selection around a phenotypic optima in the new population: $\beta = [\theta - X]/\lfloor\omega^2 + P\rfloor$, where θ is the optimal trait value, X is the current mean trait value, ω is the strength of stabilizing selection around the optima, and P is the phenotypic variance. For the curves with filled symbols, $G = 0.3$, $P = 1$, $X = 10$, $\theta = 12$, and $\omega = 2$. Theory predicts an asymptotic approach of mean phenotype to the optimum (diamonds), initially strong selection gradients that asymptote to zero (squares), and initially large per-generation evolutionary responses that also asymptote to zero (circles). Adaptation under weaker stabilizing selection ($\omega = 4$) is shown with open diamonds.

optimum. I do not know of any studies in the wild that have quantitatively examined temporal variation in the strength of directional selection after a colonization event. This is not surprising because accurate measurements of selection can be extremely difficult even within a single generation (Endler, 1986; Schluter, 2000; Kingsolver et al., 2001).

Second, assuming some additive genetic variance, evolutionary responses should mirror selection intensities, starting high and decreasing with time (Figure 1). This pattern of asymptotically declining rates of evolution with time following a colonization event has been observed in the laboratory (e.g., Lenski & Travisano, 1994). Although more difficult to test in the wild, the same pattern has been inferred (based on samples at three times) in an experiment where guppies (*Poecilia reticulata*) were transferred from high-predation to low-predation environments (Reznick et al., 1997).

Third, average population fitness should increase in rough proportion to the rate of evolutionary change: most rapidly at first and then at a declining rate with time. This pattern of asymptotically increasing fitness in new populations has been demonstrated in the laboratory (e.g., Lenski & Travisano, 1994) but studies in the wild have not yet been undertaken. A complicating factor may be that density-dependence can decrease average fitness as populations approach an adaptive peak.

Fourth, as populations adapt to new conditions, they should diverge from their ancestral counterparts. Adaptive divergence has been confirmed in numerous studies of organisms introduced to new environments (Hendry & Kinnison, 1999; Gilchrist, Huey & Serra, 2001; Losos et al., 2001; Reznick & Ghalambor, 2001; Haugen & Vøllestad, 2001). Divergence in phenotypic traits should be mirrored by divergence in fitness, whereby populations adapting to new environments become less fit in their ancestral environments. Such fitness trade-offs have been demonstrated for laboratory populations (e.g., Cooper & Lenski, 2000), post-glacial fishes (Schluter, 2000), and insect host races (e.g., Filchak, Roethele & Feder, 2000; Via, Bouck & Skillman, 2000). The rate at which these trade-offs evolve remains unknown.

Fifth, ecologically-dependent reproductive isolation should evolve in concert with adaptive divergence. Reproductive isolation can evolve if mate choice is based on the traits under divergent selection (Lande & Kirkpatrick, 1988; Nagel & Schluter, 1998), if hybrids or backcrosses are inferior to pure-type individuals (Via, Bouck & Skillman, 2000; Rundle & Whitlock, 2001), or if 'reinforcement' leads to assortative mating (Liou & Price, 1994; Higgie, Chenoweth & Blows, 2000). These mechanisms are often combined under the umbrella of 'ecological speciation', in which reproductive isolation evolves as a consequence of adaptation to divergent ecological environments (Mayr, 1942; Dobzhansky, 1951; Rice & Hostert, 1993; Schluter, 1996a; Schluter, 2000). An important aspect of this process is that hybrid inferiority often has an ecological context: hybrids are inferior in each parental environment but may not have an intrinsic genetic disadvantage (Rice & Hostert, 1993; Rundle & Whitlock, 2001).

Reproductive isolation resulting from divergent natural selection is often seen in laboratory experiments (Rice & Hostert, 1993) but evidence from natural populations has, until recently, accumulated very slowly (Schluter, 2000). The recent work has shown that reproductive isolation can evolve after several thousand generations (e.g., post- glacial fishes: Schluter, 1996b; Lu & Bernatchez, 1999; Taylor, 1999; Rundle et al., 2000) or several hundred generations (e.g., insect host races: Feder et al., 1994; Via, 1999; Filchak, Roethele & Feder, 2000; Via, Bouck & Skillman, 2000). What remains unknown is how

rapidly reproductive isolation actually evolves when new environments are first colonized. If substantial adaptive divergence can take place over short time intervals (Hendry & Kinnison, 1999), ecologically-dependent reproductive isolation may evolve at similar rates.

Adaptive divergence and the evolution of reproductive isolation are dynamic processes and deserve to be examined at a variety of temporal scales. Unfortunately, no studies in the wild have followed the trajectory of adaptive divergence (other than to sample a couple of times), or tested for reproductive isolation after less than 100 generations. I argue that to understand how natural selection generates biological diversity, we must begin to follow the temporal trajectory of adaptive divergence and reproductive isolation, particularly during the earliest stages.

Experimental approaches

Long-standing natural populations or species that are well-adapted to their local environments are of limited use when attempting to infer processes acting during adaptive divergence. A solution is conduct studies of experimental evolution in the wild (e.g., Endler, 1980; Reznick, Bryga & Endler, 1990; Reznick et al., 1997). Such studies are rare, however, because of logistical difficulties. An easier approach, albeit less refined, is to study populations occupying different environments that were introduced from a common ancestral source at some known time in the recent past (Hendry & Kinnison, 1999; Reznick & Ghalambor, 2001).

My study focused on sockeye salmon introduced into Lake Washington, Washington, in the 1930s and 1940s. I contrasted two populations that currently breed in very different ecological environments (a river v.s. a lake beach) but shared common ancestors less than 13 generations previously. Other studies have examined adaptive divergence over similar time frames but none has also tested for the evolution of reproductive isolation. Another novel aspect of my study is that colonization of the divergent environments was natural (after introduction to the lake system). Moreover, natural mixing of the populations has been maintained so that they are not strictly allopatric. My study therefore represented a case of divergence-with-gene flow (*sensu* Rice & Hostert, 1993).

In the present paper, I (1) review the relevant life history and behavior of sockeye salmon, (2) provide a short history of sockeye salmon in Lake Washington, (3) show how genetic markers confirmed a common ancestry for the two focal populations, (4) provide evidence of adaptive phenotypic divergence in adult life history and morphology, (5) provide evidence of adaptive genetic divergence in the developmental biology of embryos, (6) show that partial reproductive isolation has evolved between the populations, (7) show that rates of divergence within Lake Washington are comparable to those observed in other studies, (8) demonstrate that natural selection rather than genetic drift is the most plausible mechanism for divergence, and (9) argue that alternative explanations are unlikely.

The study system

Sockeye salmon

Here I outline a generalized sockeye salmon life history. Detailed information, citations, and descriptions of exceptions can be found in Burgner (1991), Wood (1995), and the papers cited below. Life for a wild sockeye salmon begins in the late summer or fall (depending on latitude and altitude) as an egg buried in the gravel of a stream or lake beach. The eggs develop at a rate that depends on water temperature, hatching after about 51–198 days (12.0–2.0°C). The hatched embryos (called 'alevins') remain in the gravel for another 29–86 days (12.0–2.0°C), during which time they absorb their yolk sac but do not feed. At the end of the incubation period, embryos wriggle their way up through the gravel and emerge as free-swimming larvae (called 'fry'), which immediately migrate to a nearby lake. Dates of emergence seem to have evolved so that the entry of fry into a lake is matched to the best conditions for survival and growth (Brannon, 1987). For the next 1–2 years, juveniles feed within the lake and attain a size of 2–30 g (depending on the lake), after which they migrate as 'smolts' to the ocean (anadromy). After 1–3 years of feeding in the ocean, during which time they attain a size of 1–6 kg (depending on age and population), they begin to mature and swim back to their natal lakes.

Anadromous salmon returning to breed cease feeding when they enter freshwater and then complete maturation, which takes weeks to months, within their natal lake. Females develop large gonads (up to 25% of body mass) and males develop large secondary sexual characters (Figure 2). After maturation is complete, the fish congregate at their natal breeding areas. Females select specific nesting sites, dig in the gravel to construct nests within which they bury their

Figure 2. A female (left) and a male (right) sockeye salmon breeding along a beach in Iliamna Lake, Alaska. Note the male's deep body, a trait exaggerated through sexual selection. The female is above her nest, where eggs are buried in the gravel. Photograph by Andrew P. Hendry.

eggs, and then defend their nests from encroachment by other females. Males compete with each other for opportunities to breed with females. Females usually complete oviposition within a few days and then remain at their nest site for the duration of their life. Males compete for mating opportunities over their entire reproductive life span, often breeding with several females. All sockeye salmon then die within a few weeks of when they started breeding.

Sockeye salmon have evolved distinctive reproductive ecotypes, with populations of one type breeding in streams and populations of another type breeding along lake beaches (we use the term 'ecotype' in Turesson's (1922) original sense: populations are grouped into different ecotypes based on their residence in, and adaptations to, distinct environments). Within a given lake system, stream and beach populations are partially reproductively isolated because of natal homing (Varnavskaya et al., 1994; Quinn, Volk & Hendry, 1999; Burger et al., 2000) and show adaptive differences in life history, morphology, and behavior (Blair, Rogers & Quinn, 1993; Wetzel, 1993; Quinn, Hendry & Wetzel, 1995; Wood, 1995; Hamon et al., 2000). Each ecotype may be represented by several populations within a given lake system, and the different populations within an ecotype often adapt to population-specific selective pressures (e.g., egg size is positively correlated with gravel size – Quinn, Hendry & Wetzel, 1995).

In the present study, I contrast the beach ecotype with a specific representative of the stream ecotype: populations in large streams and rivers. Selective factors that differ between river and beach environments include (1) breeding adults in rivers but not at beaches must contend with fast-flowing water, (2) eggs and alevins in rivers but not at beaches are susceptible to gravel movement (scour) caused by floods (ice scour does not occur within Lake Washington because the lake does not freeze), and (3) newly-emerged fry in rivers but not at beaches must migrate to reach their lake. River and beach embryos may also experience very different incubation temperatures.

Lake Washington

Here I briefly review the last century's history of Lake Washington and its sockeye salmon. Details can be found in Woodey (1966), Hendry, Quinn and Utter

(1996), Gustafson et al. (1997), Hendry and Quinn (1997), and Hendry, Hensleigh and Reisenbichler (1998). The historical distribution and abundance of *O. nerka* within the Lake Washington watershed are only partly understood. At the turn of the century, indigenous kokanee (the non-anadromous form of *O. nerka*) were present in large numbers but indigenous sockeye salmon (the anadromous form of *O. nerka*) were absent or very rare (Gustafson et al., 1997). In 1912–1916, dramatic anthropogenic changes to the watershed (the Cedar River was diverted into the lake, a new lake outlet was constructed, lake level dropped 3 m) are generally assumed to have caused the extinction or severe depletion of any sockeye salmon that may have been present.

Large numbers of juvenile *O. nerka* have been introduced into Lake Washington. The first known introduction, apparently unsuccessful, was of sockeye salmon from an unknown source in 1917 (Woodey, 1966; Hendry, Quinn & Utter, 1996; Gustafson et al., 1997). The largest set of subsequent introductions was of sockeye salmon originally from Baker Lake (propagated at Birdsview Hatchery, Washington). Other introductions included sockeye salmon juveniles from Cultus Lake, British Columbia, (Figure 3) and kokanee from Lake Whatcom, Washington.

Sockeye salmon currently breed at several places in the Lake Washington watershed but are only abundant in a few locations. The populations most relevant to the present study include the Cedar River and the Pleasure Point beach. The Cedar River is the largest tributary to the watershed (11.6 m^3/s mean daily discharge, October–November 1992–1993) and has the largest sockeye salmon population (76,000–359,000 breeders per year, 1967–1991). The Pleasure Point beach is a small area along the lake shore (~700 m^2) situated 7 km north of the Cedar River (Figure 3). Breeding was first documented at Pleasure Point in 1957 and numbers of breeders have since been estimated at 100–1,000 (1963–1965, Woodey, 1966) and 520–8,180 (1976–1991, R. Egan, personal communication). In my experience, numbers of breeders in the early 1990s at Pleasure Point were < 1,000 per year.

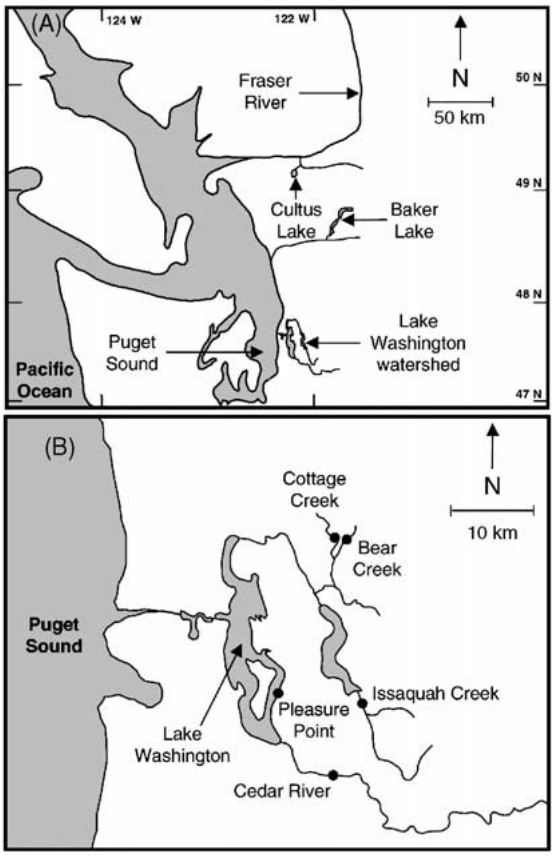

Figure 3. Locations of Cultus Lake, Baker Lake, and Lake Washington (A), and collection sites within the Lake Washington watershed (B). Filled circles indicate locations from which fish were collected. Numerous rivers and lakes are omitted from panel (A) and some streams are omitted from panel (B). Reprinted with permission from Hendry and Quinn (1997), with modification.

Empirical results

Ancestral origins

I began my research by investigating ancestral origins for the various Lake Washington sockeye salmon populations. One set of possible origins included sockeye salmon indigenous to the watershed or introduced from Baker Lake or Cultus Lake. Another set included introduced or indigenous kokanee, which might have adopted an anadromous tendency or interbred with anadromous sockeye salmon. I evaluated these possibilities using allelic variation at presumed-neutral genetic markers. In 1992 and 1993, I collected adult sockeye salmon breeding at each of five different sites within the Lake Washington watershed (Cedar River, Issaquah Creek, Bear Creek, Cottage Creek, Pleasure Point, Figure 3). I also obtained samples of adult sockeye salmon from Baker Lake (where breeding currently takes place in artificial ponds), juvenile sockeye salmon from Cultus Lake, and kokanee from Lake Washington (primarily Issaquah Creek). I then used protein electrophoresis to screen for allelic variation at 22 allozyme loci, of which seven were polymorphic (for details see Hendry, Quinn & Utter, 1996).

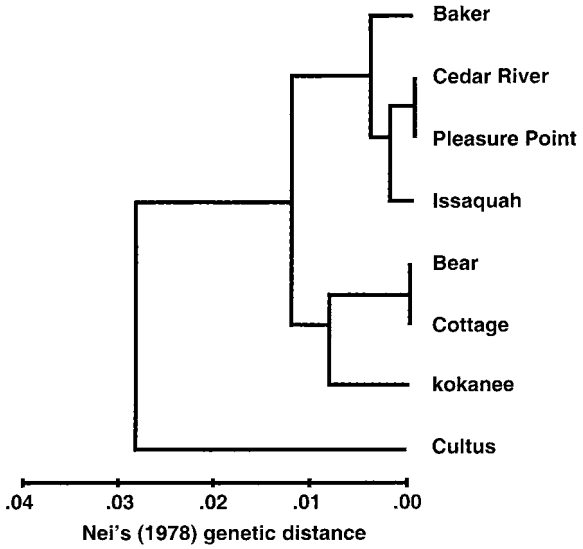

Figure 4. Genetic relationships among the sampled populations, shown as a UPGMA cluster diagram based on pair-wise comparisons of Nei's unbiased D (seven polymorphic loci and 16 monomorphic loci). All pair-wise differences are statistically significant, except for Pleasure Point versus the Cedar River, and Bear Creek versus Cottage Creek. Reprinted with permission from Hendry, Quinn and Utter (1996), with modification.

At a coarse level, three major genetic groups were evident (Figure 4). The first group consisted solely of Cultus Lake sockeye salmon, which were distinct from all other collections (average Nei's unbiased $D = 0.025$). I concluded that the Cultus Lake introductions did not contribute substantially to the present Lake Washington populations (Hendry, Quinn & Utter, 1996; see also Seeb & Wishard, 1977). The second group consisted of Lake Washington kokanee and Bear and Cottage Creek sockeye salmon, with the latter two populations indistinguishable from each other (Figure 4). I concluded that Bear/Cottage Creek sockeye salmon represent descendents of fish indigenous to the watershed (Hendry, Quinn & Utter, 1996; see also Seeb & Wishard, 1977). This conclusion is controversial (Gustafson et al., 1997) but is supported by (1) large genetic differences from the putative ancestral populations (Baker and Cultus lakes) and from all other Lake Washington populations (average $D = 0.014$, Hendry, Quinn & Utter, 1996; see also Anderson, 1997), and (2) moderate frequencies (0.226−0.277) in the Bear/Cottage Creek fish of an allele that is very rare in the other populations. A recent study showed that straying from the Cedar River into Bear Creek is minimal: 11.8 million fry were marked in the Cedar River from 1995–1997 (47.6% of the population) but not one was found in 1,219 breeding adults that were examined in Bear Creek from 1998 through 2000 (K. Fresh, unpublished data).

The third group consisted of sockeye salmon from Baker Lake, the Cedar River, Issaquah Creek, and Pleasure Point. The genetic affinity of these Lake Washington populations to Baker Lake fish has been confirmed in other analyses of variation in allozymes (Shaklee, Ames & LaVoy, 1996; Winans, Aebersold & Waples, 1996; Gustafson et al., 1997) and DNA microsatellites (P. Bentzen, unpublished data). I concluded that these three Lake Washington populations had their origin in the introductions from Baker Lake. Two of the populations (Cedar River and Pleasure Point) were particularly well suited for the study of adaptive divergence because they are closely related genetically and now breed in substantially different environments (a river v.s. a lake beach).

It seems most likely that the Cedar River population was established first (by the introductions from Baker Lake) and that the Pleasure Point population was established later (by 'straying' from the Cedar River). I make this inference because (1) sockeye salmon were introduced directly into the Cedar River but not at Pleasure Point, (2) sockeye salmon were observed breeding in the Cedar River by 1940 (Royal & Seymour, 1940) but not at Pleasure Point until 1957 (Woodey, 1966), and (3) the only introduction that could have directly produced breeders by 1957 was at a location far from Pleasure Point (Issaquah Creek). The earliest possible year for the start of evolutionary divergence was 1937 (first successful introduction) and the latest possible year was 1957 (first observed breeding at Pleasure Point). When my study began in 1992, the populations had therefore been diverging for a minimum of 35 years (1957–1992, about 8 generations) and a maximum of 55 years (1937–1992, about 13 generations). I adopt the latter time frame for estimating divergence, even though the actual time frame was probably less, because the 1937 date is unambiguous and conservative.

Phenotypic divergence: adult life history and morphology

Having identified two populations of a recent common origin that now breed in very different environments (Cedar River v.s. Pleasure Point), the next step was to test for adaptive divergence. Most studies of introduced populations begin with a test for phenotypic differences in the wild (e.g., Reznick, Bryga & Endler, 1990; Losos, Warheit & Schoener, 1997; Kinnison

et al., 1998a; Haugen, 2000a). I took approach for two adult traits (male body depth, female body length) that should diverge in response to selection in beaches versus rivers.

Male body depth in sockeye salmon (from the anterior insertion of the dorsal fin to the bottom of the abdomen, Figure 2) evolves as a compromise between the opposing forces of natural and sexual selection. In the polygynous salmon mating system, males compete for access to females but females do not show strong mate choice (Foote, 1990; Quinn, Adkison & Ward, 1996). Variance in male reproductive success is very high, leading to intense intra-sexual selection (Fleming & Gross, 1994; Quinn & Foote, 1994). The primary factor determining male dominance (and mating success) is body size (Foote, 1990) but male dominance is also positively correlated with body depth (absolute size, and after standardizing to a common body length, Quinn & Foote, 1994). Although sexual selection thus favors larger and deeper-bodied males, such males are at a disadvantage in certain breeding environments, such as small streams (e.g., Ruggerone, Hanson & Rogers, 2000; Quinn, Hendry & Buck, 2001). Deep-bodied males are also at a disadvantage in fast-flowing water because their shape will compromise hydrodynamic performance during breeding. In accord with these expectations, males in beach populations, where the water is deep and the current is weak, have deeper bodies (for a given body length) than do males in stream and river populations (Blair, Rogers & Quinn, 1993; Wetzel, 1993; Hamon et al., 2000). Because the Cedar River has strong currents but the Pleasure Point beach does not (Hendry & Quinn, 1997), *I predicted that adaptive divergence would result in deeper bodied males at Pleasure Point than in the Cedar River.*

Female body length in salmon evolves to balance a complex set of trade-offs. Natural selection favors large females because they produce more eggs and are more successful at obtaining and defending high-quality nest sites (Foote, 1990; Fleming & Gross, 1994). When nesting environments are prone to gravel scour (movement of the gravel during periods of high flow, Montgomery et al., 1996), larger body size is favored to an even greater extent because large females bury their eggs deeper in gravel, which protects them from disturbance (Steen & Quinn, 1999). Other factors, however, can select against large female body size. A universal cost of attaining large size in salmon is maturing at a later age or adopting riskier foraging strategies, which generate a higher probability of mortality prior to reproduction (Healey, 1987). Another cost to large size is incurred when breeding environments are very shallow because large fish are more likely to 'strand' themselves (Quinn, Hendry & Buck, 2001) and are more susceptible to predation by bears (Ruggerone, Hanson & Rogers, 2000). For the Lake Washington populations, the above selective factors would likely be similar, except that gravel scour is a source of very high embryo mortality in the Cedar River (Thorne & Ames, 1987) but is entirely absent from Pleasure Point (Hendry & Quinn, 1997). Based on this difference, *I predicted that adaptive divergence would result in larger females in the Cedar River than at Pleasure Point.*

I tested these two predictions by measuring breeding fish collected in 1992 and 1993 (for details see Hendry & Quinn, 1997). Both predictions were strongly supported (Figure 5). Relative to Cedar River males, Pleasure Point males were 13.0% deeper bodied in 1992 and 13.8% deeper bodied in 1993 (standardized to a common body length using allometric adjustments). Relative to Pleasure Point females, Cedar River females were 7.1% longer in 1992 and 5.5% longer in 1993 (at the most common age-at-maturity, 4 years). These differences were highly statistically significant (Figure 5) and I expect they are also biological significant. First, differences of this magnitude are comparable to differences between river and beach populations in lake systems that were colonized thousands of years ago (Blair, Rogers & Quinn, 1993; Wetzel, 1993). In Iliamna Lake, for example, males were 3.3–24.3% deeper bodied in three beach populations than they were in four river populations (2 years of data, standardized to a common body length), with an average difference for all possible river-beach contrasts within years of 12.8% (from Table 3 in Blair, Rogers & Quinn, 1993). For females, the difference in body length within Lake Washington corresponds to an estimated difference in egg burial depth of 2.3 cm (14.8% deeper) in 1992 and 1.8 cm (11.6% deeper) in 1993 (using the solid line in Figure 2 of Steen & Quinn, 1999). This difference could easily determine success or failure of a nest because average egg burial depths within a given stream are often very close to the average depth of gravel scour (Montgomery et al., 1996).

Demonstrating rapid phenotypic divergence does not reveal what fraction of that divergence was the result of genetic change versus phenotypic plasticity (Howard et al., 2001; Losos et al., 2001). The best way to directly address this ambiguity would have been to

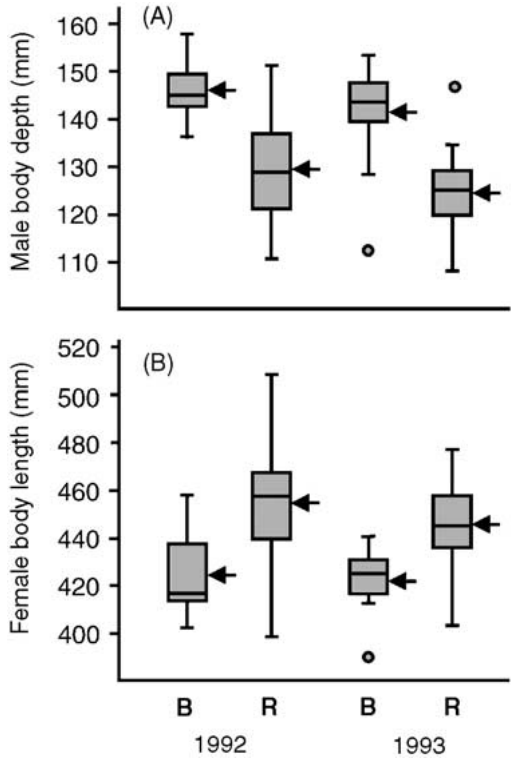

Figure 5. Differences between Pleasure Point and the Cedar River for (A) standardized body depth of males (standardized using allometric adjustments, slopes did not differ in ANCOVA), and (B) the body length of females maturing at 4 years of age. Boxes contain 50% of the data, whiskers contain the remainder, horizontal lines indicate medians, arrows indicate means, and the circle indicates an outlier. In two-way ANOVAs, non-significant interaction terms ($p = 0.901$ and $p = 0.461$, respectively) were removed to reveal that male body depth differed between sites ($p < 0.001$) and years ($p = 0.004$), and that female body length differed between sites ($p < 0.01$) and years ($p = 0.015$).

raise Cedar River and Pleasure Point fish to maturity in each other's environment (i.e., a reciprocal transplant experiment). This approach is prohibitively difficult for salmon because of their large size, inconvenient generation length (about 4 years), and extended periods of lake and ocean rearing. Instead, the genetic basis for phenotypic traits in salmon is usually determined within single populations (released into the wild or held in captivity) or occasionally for multiple populations reared in a common hatchery environment. This work has demonstrated that female body length is heritable in salmon (e.g., $h^2 = 0.3 \pm 0.2$ SE, Smoker et al., 1994), and that standardized adult male body depth varies among sockeye salmon populations (e.g., Moore, 1996). I attempted to rear the progeny of Cedar River and Pleasure Point fish in a hatchery but non of them survived to maturity. Thus, although I can say that female body length and male body depth have diverged in an apparently adaptive direction, and that these traits often have a genetic basis, I cannot state to what degree the observed divergence between the Cedar River and Pleasure Point populations was genetic. It is very unlikely, however, that plasticity caused all of the difference because the two populations share a common environment for the entire growth phase of their life (Hendry et al., 2001b).

Genetic divergence: embryo survival, development rate, and size-at-emergence

After documenting phenotypic differences among populations in the wild, a typical next step when studying introductions is to use common-garden experiments for estimating the amount of genetically-based adaptive divergence (e.g., Reznick, Bryga & Endler, 1990; Reznick et al., 1997; Carroll et al., 2001; Quinn, Kinnison & Unwin, 2001; Haugen & Vøllestad, 2000). As noted above, I was unable to do this for the two adult traits in Lake Washington. Instead, I tested predictions for eggs, alevins, and newly-emerged fry (together 'embryos') using traits that could be assayed in a common laboratory environment (survival, development rate, size-at-emergence).

Natural incubation temperatures differ dramatically between the populations. Wild Pleasure Point embryos incubate at an isothermal 9.9°C (SD = 0.1°C across temperature loggers and over the entire incubation period, Figure 6), owing to upwelling groundwater (see also Woodey, 1966). In contrast, wild Cedar River embryos incubate in variable and usually colder temperatures (Figure 6). An additional nuance is the protracted breeding season in the Cedar River (early October through early January), which causes embryos that begin incubating on different dates to experience different temperature regimes (Figure 6). I compared Pleasure Point embryos (from mature adults collected on November 18) and three temporally-distinct groups of Cedar River embryos (from mature adults collected on October 21 – Early Cedar, November 20 – Middle Cedar, and December 21 – Late Cedar). Embryos from each of six full-sib families for each of the four groups were split among each of three laboratory temperatures (5, 9, and 12.5°C). Hendry, Hensleigh and Reisenbichler (1998) provide details on this experiment.

The survival of salmonid embryos in a hatchery environment is strongly influenced by water temperature. When temperatures are moderate (4–12°C), survival

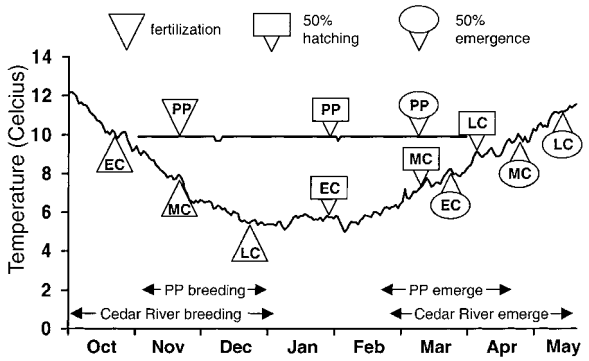

Figure 6. Temperature profiles for embryo incubation sites at Pleasure Point (upper line) and in the Cedar River (lower line). Fertilization dates are shown for the Early Cedar (EC), Middle Cedar (MC), Late Cedar (LC), and Pleasure Point (PP) groups. Hatching and emergence dates are shown for each group if they had incubated in the wild. Temperature records for Pleasure Point are the average of two different automatic temperature loggers buried in the gravel at locations where eggs were incubating. Temperature records for the Cedar River depict the 16-year average at a gauging station. Reprinted with permission from Hendry, Hensleigh and Reisenbichler (1998), with modification (see also Quinn, Volk & Hendry, 1999).

is usually high (>95%), but when temperatures are higher or lower, survival can decline precipitously (Beacham & Murray, 1989). Wild Pleasure Point eggs remain within the suitable temperature range for their entire incubation period (Figure 6) and Cedar River eggs do not experience lethally low temperatures. However, the eggs of early-breeding Cedar River adults can experience high temperatures (Figure 6), which should select for improved survival at such temperatures. *I predicted that adaptive divergence would result in increased survival to hatching at the highest laboratory temperature (12.5°C) for Early Cedar eggs but not for the other groups.*

The development rate of embryos is determined primarily by incubation temperature: warmer water = faster development (Beacham & Murray, 1989). When temperatures vary among incubation sites, breeding date appears to have evolved so that the emergence of fry from different populations is synchronized to a comparatively narrow time window, centered around the presumed optimal time (Brannon, 1987). When populations breed at times and in temperatures that would result in asynchronous emergence, they sometimes show compensatory differences in development rate: embryos that would otherwise emerge late will speed up their development per unit of temperature (e.g., Brannon, 1987; Tallman & Healey, 1991). Pleasure Point and Middle Cedar embryos would begin incubating at the same time but would experience different average temperatures (9.9°C v.s. 6.7°C), leading to peak emergence in the wild after 110 d (March 6) and 154 d (April 22), respectively (Figure 6). Embryos in the different Cedar River groups (Early, Middle, Late) would begin incubating at different times but would experience similar average temperatures (6.7, 6.7, and 7.3°C), leading to peak emergence in the wild after 155 d (March 22), 154 d (April 22), and 144 d (May 11, Figure 6), respectively. Assuming selection for synchronized emergence within Lake Washington, *I predicted that adaptive divergence would result in Pleasure Point embryos developing more slowly than Middle Cedar embryos (i.e., hatching and emerging later at a common temperature), and Early Cedar embryos developing more slowly than Late Cedar embryos.*

Body size at emergence from the gravel is a critical trait for juvenile salmonids. Mortality can be very high during the first few weeks of free-swimming life and larger fry tend to survive at higher rates (e.g., Einum & Fleming, 2000a). Females can produce large fry by producing large eggs but increasing egg size is constrained by adverse effects on maternal fitness through reduced fecundity (Quinn, Hendry & Wetzel, 1995; Einum & Fleming, 2000b). Selection for large fry should therefore favor efficient conversion from yolk tissue in the embryo to body tissue in the fry. This conversion efficiency is influenced by water temperature, and well-adapted populations should show their greatest efficiency at temperatures they typically experience in the wild (Beacham & Murray, 1989). Average incubation temperatures in the wild were 6.7 (Early Cedar), 6.7 (Middle Cedar), 7.3 (Late Cedar), and 9.9°C (Pleasure Point). *I therefore predicted that adaptive divergence would result in Pleasure Point fry being their largest at emergence in the intermediate laboratory temperature (9°C) but that this would not be the case for Cedar River fry, which would be their largest at the coldest temperature (5°C).*

Some of the above predictions regarding adaptive divergence were supported, others were not. Survival-to-hatching was excellent for all groups (except an occasional family) in the cold and intermediate temperatures. In the warm temperature, however, the survival of all groups except the Early Cedar was dramatically reduced (Figure 7). Early Cedar embryos have thus evolved the ability to tolerate the high pre-hatching temperatures they often experience in the wild. Development rates did not differ between the Pleasure Point and Middle Cedar groups. In contrast, Late Cedar embryos developed faster than Early Cedar embryos (Figure 8), as was predicted based on

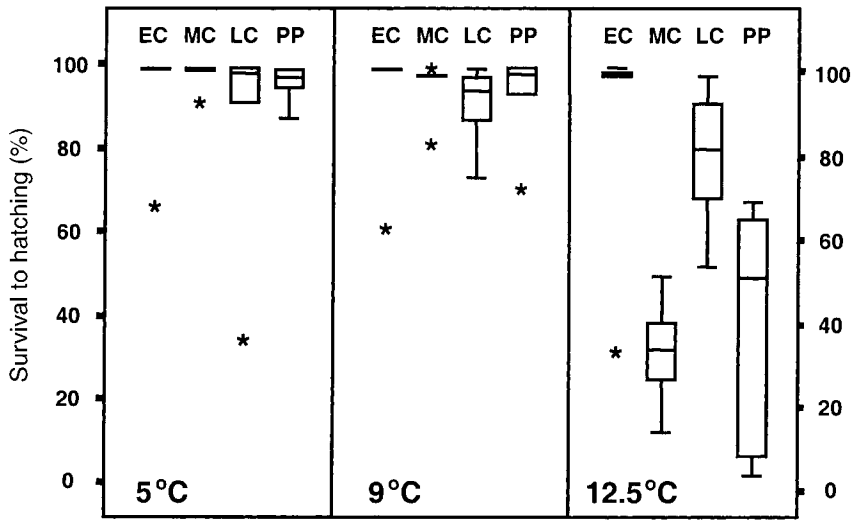

Figure 7. Survival from fertilization to hatching at the three incubation temperatures for eggs from the Early Cedar (EC), Middle Cedar (MC), Late Cedar (LC), and Pleasure Point (PP) groups. The data are shown as the median, quartiles, and extreme values (asterisks) for family means. The interaction term is statistically significant ($p < 0.001$). Reprinted with permission from Hendry, Hensleigh and Reisenbichler (1998), with modification.

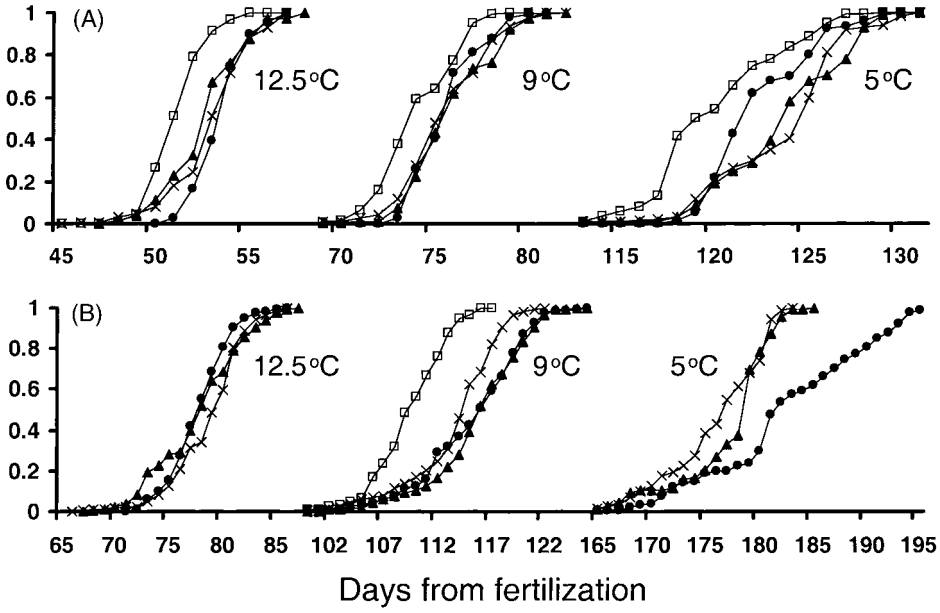

Figure 8. Cumulative hatching (A) and emergence (B) profiles for embryos and alevins from each collection at each incubation temperature. Points depict the proportion of fry that had hatched or emerged by a given day for the Early Cedar (filled circles), Middle Cedar (filled triangles), Late Cedar (open boxes), and Pleasure Point (crosses). The only significant difference for days to hatching was between Early Cedar and Late Cedar embryos ($p = 0.043$) at 12.5°C. The only significant difference for days to emergence was between Late Cedar embryos and all other groups ($p < 0.010$) at 9°C. *X*-axes are not continuous because some days were omitted between the temperatures. Reprinted with permission from Hendry, Hensleigh and Reisenbichler (1998), with modification (including corrections to labeling mistakes in the original).

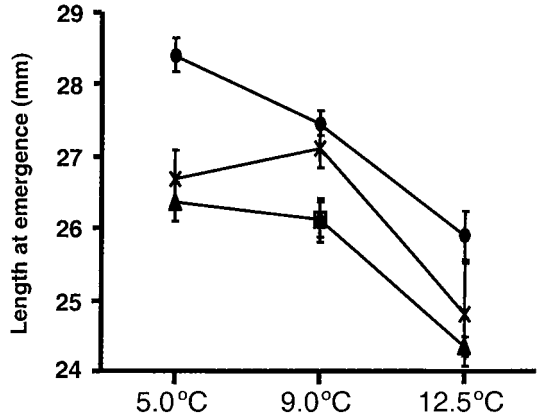

Figure 9. The length of fry at emergence for the Early Cedar (filled circles), Middle Cedar (filled triangles), Late Cedar (box), and Pleasure Point (crosses). Error bars represent one standard error around the mean. Data were not available for the Late Cedar at 5 or 12.5°C. The interaction term is statistically significant ($p = 0.007$). Reprinted with permission from Hendry, Hensleigh and Reisenbichler (1998), with modification.

selection for synchronized emergence. It thus seems that adaptive divergence in development rate is possible but has not occurred between the Pleasure Point and Cedar River populations when they are standardized for breeding time. As predicted, Pleasure Point fry were their largest at the intermediate temperature whereas Cedar River fry were not (Figure 9). This pattern suggests the adaptive evolution of reaction norms linking fry size to incubation temperature. Haugen and Vøllestad (2000) provide similar evidence for the evolution of adaptive reaction norms in embryo traits of another salmonid species.

My experiment considered adaptive divergence in space (Pleasure Point v.s. Middle Cedar) and time (Early Cedar v.s. Late Cedar). The divergence between Pleasure Point and the Cedar River likely occurred *de novo* within Lake Washington (see below). Some of the adaptive divergence between the Early Cedar and Late Cedar groups, however, could have predated the colonization event. This would be the case if considerable variation in breeding date existed in the fish used to initiate the Baker Lake hatchery stock, if those fish had developmental adaptations to breeding dates (as above), and if those adaptations remained associated with breeding times during many years of hatchery reproduction. Historical records are not sufficient to determine if these speculations might be true.

Reproductive isolation

If adaptive divergence has taken place, ecologically-dependent reproductive isolation should evolve as a byproduct (Schluter, 2000). Some isolation is expected simply because individuals moving between populations, as well as any hybrids, will be less fit than pure-type fish remaining in their home environments. Isolation could also evolve if the traits undergoing adaptive divergence influence mate choice. Tests for ecologically-dependent reproductive isolation typically involve controlled experiments quantifying the strength of pre-zygotic or post-zygotic isolation (Rice & Hostert, 1993; Via, 1999; Rundle et al., 2000; Via, Bouck & Skillman, 2000). Disadvantages of such tests are that they quantify isolation in a specific experimental context (usually not a natural one) and that they usually quantify only one aspect of isolation (e.g., mate choice). An alternative approach, having neither of these disadvantages, compares the rate of immigration of breeders into a population to the resulting rate of gene flow. The difference between these two rates reflects the absolute (or 'total') amount of intrinsic isolation. Disadvantages of this approach are that it is 'indirect' and that it does not discriminate pre-zygotic from post-zygotic isolation. I used the indirect approach to compare the rate of immigration of Cedar River adults into Pleasure Point to the resulting rate of gene flow. I assumed immigration was unidirectional into the Pleasure Point population because the Cedar River population is two orders of magnitude larger.

I estimated the rate of immigration of Cedar River adults into the Pleasure Point population by examining daily growth increments on otoliths (calcified elements of the inner ear). Characteristic dark and light banding patterns on otoliths are influenced by variation in diurnal water temperature. When temperatures fluctuate, increments are dark and highly contrasted against their background. When temperatures are constant, increments are less distinctive and weakly contrasted (for images and details see Quinn, Volk & Hendry, 1999). Because wild Pleasure Point embryos incubate in constant temperatures whereas Cedar River embryos incubated in variable temperatures (Figure 6), I could sample breeding adults at Pleasure Point and examine the region of their otoliths that was formed during incubation, thereby determining whether each adult had been 'born' (i.e., incubated and hatched) in the Cedar River or at Pleasure Point. This method revealed that over 2 years, about 39% of the breeders at Pleasure Point had actually immigrated from the Cedar River. Although this immigration rate is very high, it nevertheless reflects strong natal homing. The only reason that so many breeders at Pleasure Point were immigrants is that the Cedar River

population is two orders of magnitude larger. If the two populations actually intermixed freely, 98% of the adults breeding at Pleasure Point would have come from the Cedar River (Quinn, Volk & Hendry, 1999).

The otolith analysis also revealed that 12.3% of Cedar River breeders had incubated in constant temperatures. These fish were probably not immigrants from Pleasure Point because that population was so small that even in its entirety, it would contribute <2% of possible breeders in the Cedar River. Instead, Cedar River breeders with constant-temperature otolith patterns probably reflected some thermal heterogeneity within the river (i.e., some sites may have relatively constant temperatures, Quinn, Volk & Hendry, 1999). If this is true, our estimate of immigration into Pleasure Point may be a slight underestimate. All subsequent analyses omitted Cedar River adults with constant-temperature otolith patterns because their origin was not certain.

If the observed level of immigration into the Pleasure Point population (∼39%) translated directly into gene flow, genetic divergence of the two populations at selectively-neutral loci would not be possible. Thus, genetic divergence between 'residents' at the two sites would indicate that immigrants have reduced reproductive success, and that reproductive isolation has evolved. I assayed variation at six nuclear DNA microsatellite loci in Pleasure Point residents (born and breeding at Pleasure Point, $N = 22$), Cedar River residents (born and breeding in the Cedar River, $N = 35$), and Pleasure Point immigrants (born in the Cedar River but breeding at Pleasure Point, $N = 12$). This analysis revealed that Cedar River residents and Pleasure Point immigrants were genetically similar ($p = 0.365$, $F_{ST} = 0.008$, Nei's unbiased $D = 0.010$), confirming that immigrants to Pleasure Point were from the Cedar River. It also revealed that residents at the two sites were genetically different ($p = 0.002$, $F_{ST} = 0.025$, $D = 0.054$), and that this difference could not be attributed to linkage between a microsatellite locus and a locus under selection (Hendry et al., 2000). For more details see Howard et al. (2001) and Hendry et al. (2001b).

These microsatellite data have been criticized because only a single year of data was available (another year of samples was destroyed by a freezer failure) and the sample sizes were small (Gustafson et al., 2001). However, the smallest sample was for Pleasure Point immigrants, which were simply the noise that needed to be removed when testing for reproductive isolation between the resident populations (Hendry et al., 2001b). Moreover, the observed pattern of genetic relationships (Cedar residents = Pleasure Point immigrants ≠ Pleasure Point residents) was exactly as expected if reproductive isolation had evolved, despite the fact that five different patterns were possible if the results had arisen at random. Thus, the observed divergence is best explained by lower reproductive success of immigrants, relative to residents, at Pleasure Point (Hendry et al., 2000, 2001a,b).

The observed genetic divergence between Cedar River residents and Pleasure Point residents was slight (Howard et al., 2001); less than that typically observed among native populations of sockeye salmon within other lake systems (Varnavskaya et al., 1994). Large differences, however, were not expected because the two Lake Washington populations have been diverging for less than 13 generations (Hendry et al., 2001b). Even if *no* gene flow was taking place, the amount of genetic divergence expected between two such populations after 13 generations would be only $F_{ST} = 0.034$ (using equations in footnote 27 of Hendry et al., 2000, with beach $N_e = 50$ and river $N_e = 10{,}000$). Howard et al. (2001) argued that the significant microsatellite differences conflict with the finding of no differentiation at allozyme loci (Hendry, Quinn & Utter, 1996). The studies are actually not in conflict, however, because the allozyme work did not attempt to separate immigrants from residents, which must be done when estimating genetic differentiation (otherwise immigrants are considered part of the resident gene pool, Hendry et al., 2001b).

We considered potential ecological mechanisms contributing to reproductive isolation by comparing residents and immigrants for female length and male body depth, traits subject to divergent selection between the populations (see above). In accord with adaptive expectations, Cedar River resident females were 7.8% longer than Pleasure Point resident females, and Cedar River resident males were 9.4% deeper-bodied than Pleasure Point resident males (Figure 10). Pleasure Point immigrants were intermediate for both traits (Figure 10), suggesting that the divergence had both a genetic basis (otherwise Pleasure Point immigrants and residents would be the same) and an environmental basis (otherwise Cedar River residents and Pleasure Point immigrants would be the same). Intermediacy of the Pleasure Point immigrants might also be explained by morphology-influenced site selection (if smaller river females and deeper-bodied river males are more likely to stray to the beach) or breeding-site selection by hybrids (if hy-

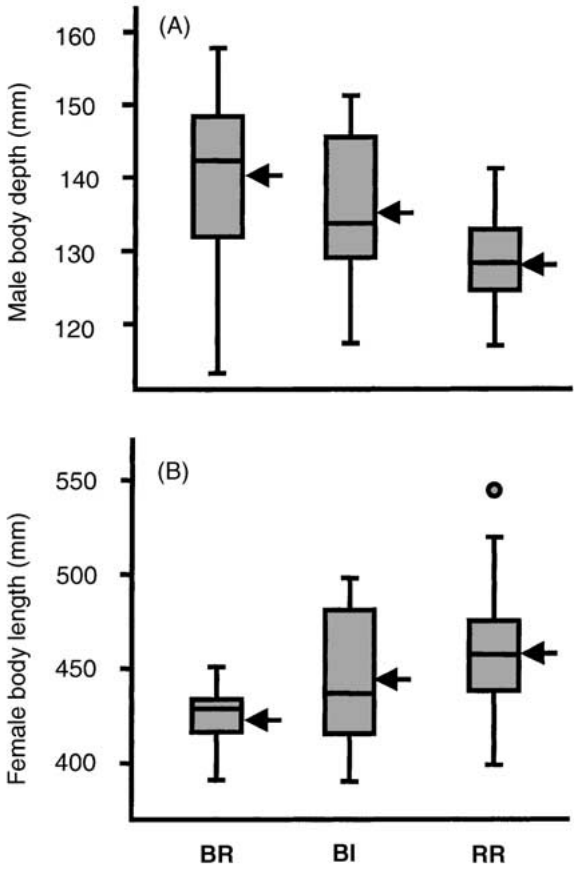

Figure 10. Differences in male standardized body depth (A) and female body length (B), between beach residents (BR), beach immigrants (BI), and river residents (RR). Presentation is the same as for Figure 5. Based on Tukey tests, river residents and beach immigrants had similar female body lengths ($p = 0.365$) and male body depths ($p = 0.076$), river residents and beach residents had different lengths ($p = 0.003$) and body depths ($p < 0.001$), and beach residents and beach immigrants had similar lengths ($p = 0.256$) and body depths ($p = 0.289$). Reprinted (and modified) with permission from Science 290: 516–518. Copyright 2000 American Association for the Advancement of Science.

brids are produced in the river and then breed at the beach). Regardless of the reason, immigrants and residents at Pleasure Point were not identical for traits under selection, suggesting an ecological basis for the observed reproductive isolation. Many other traits subject to divergent selection also differ between the populations (e.g., Hendry, Hensleigh & Reisenbichler, 1998) and immigrants are likely also compromised for those traits.

Our results suggest that ecologically-dependent partial reproductive isolation has evolved after less than 13 generations. This finding dramatically reduces the maximum rate at which such isolation has been observed to evolve in the wild. The best examples thus far are for postglacial fishes diverging for several thousand generations (Schluter, 1996b; Lu & Bernatchez, 1999; Taylor, 1999) and insect 'host races' adapting to new plants for hundreds of generations (Feder et al., 1994; Via, 1999; Via, Bouck & Skillman, 2000). My results imply that even in these previous examples, a substantial amount of the adaptive divergence and reproductive isolation may have arisen very early in the process of divergence. We might therefore expect to find partial reproductive isolation in other instances of rapid adaptive divergence.

Rates of divergence

How does the rate of divergence between sockeye salmon ecotypes within Lake Washington compare to other examples of 'rapid' evolution? Addressing this question requires quantifying rates of divergence using a metric that standardizes for generation length and allows comparisons across diverse traits and taxa. The best such rate measure is the 'haldane', which expresses the amount of change in standard deviations per generation (Haldane, 1949; Hendry & Kinnison, 1999). I calculated rates of divergence in haldanes for female body length and male body depth (Pleasure Point v.s. Cedar River), and for days to hatching and emergence (Pleasure Point v.s. Middle Cedar; Early Cedar v.s. Late Cedar). Parametric bootstrapping was used to estimate 95% confidence limits for each rate, and randomization tests were used to determine the probability that each rate was different from zero (for details see Hendry & Kinnison, 1999). I assumed divergence took place over 13 generations but the actual interval was probably shorter (see above).

Rates of divergence were highly significant for female length and male body depth. In the first set of data (adults collected from the two sites, corresponding to Hendry & Quinn, 1997; Figure 5), female body length diverged at 0.117 haldanes in 1992 ($CL = 0.071 - 0.175$, $p < 0.001$) and 0.110 haldanes in 1993 ($CL = 0.067 - 0.171$; $p = 0.001$), and male body depth diverged at 0.157 haldanes in 1992 ($CL = 0.119 - 0.213$, $p < 0.001$) and 0.153 haldanes in 1993 ($CL = 0.086 - 0.260$, $p < 0.001$). In the second set of fish (residents at the two sites identified using otolith microstructure, corresponding to Hendry et al., 2000; Figure 10), female body length diverged at 0.089 haldanes ($CL = 0.057 - 0.133$, $p = 0.003$) and male body depth diverged at 0.098 haldanes ($CL = 0.054 - 0.167$, $p < 0.001$). These two sets of

rates are not identical because they used different subsets of fish (e.g., otoliths were not available for all fish). The lower rates in the second set of data arose because 2 years were pooled, which increased variances.

Rates of divergence for days to hatching and emergence were generally significant for the Early Cedar versus Late Cedar comparison but not the Pleasure Point versus Middle Cedar comparison. Days to hatching diverged between Pleasure Point and Middle Cedar at 0.003 haldanes in 5°C water (CL = $-0.121 - 0.111$, $p = 0.493$), 0.008 haldanes in 9°C water (CL = $-0.166 - 0.114$, $p = 0.447$), and 0.016 haldanes in 12.5°C water (CL = $-0.171 - 0.152$, $p = 0.456$); and between Early Cedar and Late Cedar at 0.087 haldanes in 5°C water (CL = $-0.001 - 0.450$, $p = 0.057$), 0.105 haldanes in 9°C water (CL = $0.030 - 0.309$, $p = 0.035$), and 0.198 haldanes in 12.5°C water (CL = $0.129 - 0.494$, $p = 0.008$). Days to emergence diverged between Pleasure Point and Middle Cedar at 0.033 haldanes in 5°C water (CL = $-0.066 - 0.150$, $p = 0.233$), 0.074 haldanes in 9°C water (CL = $-0.002 - 0.188$, $p = 0.061$), and 0.000 haldanes in 12.5°C water (CL = $-0.274 - 0.272$, $p = 0.537$); and between Early Cedar and Late Cedar at 0.249 haldanes in 9°C water (CL = $0.182 - 0.678$, $p = 0.002$; data for other temperatures were not available, see Hendry, Hensleigh & Reisenbichler, 1998). These confidence intervals for development rates should be viewed with caution because they were based on mean values for only 3–6 surviving full-sib families per comparison.

I compared rates of divergence within Lake Washington to rates estimated for other studies (by Kinnison & Hendry, 2001). Here I include studies of divergence (i.e., synchronic comparisons) over 34 generations or less (an obvious break in the data). I compared rates for female body length and male body depth to studies that measured divergence in wild-captured individuals ('phenotypic' haldanes), and rates for days to hatching and emergence to studies that measured divergence in a common environment ('genetic' haldanes). This comparison showed that divergence within Lake Washington was within the range of rates in comparable studies. Lake Washington divergence was sometimes at the high end of the range, particularly for the Early Cedar versus Late Cedar contrasts (Figure 11). This divergence highlights the importance of temporal isolation ('isolation-by-time') to adaptive divergence ('adaptation-by-time') even within continuous breeding populations (see also Hendry, Hensleigh & Reisenbichler, 1998; Hendry, Berg & Quinn, 1999; Quinn, Unwin & Kinnison, 2000). Note, however, that if preexisting differences in development rate were present in fish breeding at different times (see above), the true rates of divergence for Early Cedar versus Late Cedar would be less than that reported here.

Natural selection or genetic drift?

I have thus far interpreted my results as adaptive divergence in response to natural selection. The comparison of Lake Washington rates of divergence to those observed in other studies suggests that this inference is at least plausible. However, it is also useful to provide a more formal appraisal of the theoretical efficacy of different evolutionary mechanisms. Here I first estimate the intensity of selection required to generate the observed divergence in the absence of ongoing gene flow. I then estimate the required intensity of selection with ongoing gene flow. Finally I test whether random genetic drift could have led to the observed divergence in the absence of selection. For convenience, I analyze only female body length (mature females at 4 years of age) and male body depth (standardized to a common body length).

Assume that (1) the Cedar River population is ancestral, (2) Pleasure Point was colonized from the Cedar River in a single event, (3) founder effects were minimal, (4) no subsequent gene flow took place, and (5) trait heritabilities and selection intensities remained constant through time. Under these conditions, evolution in the Pleasure Point population should proceed according to $R = h^2 S$, where R is the evolutionary response per generation, h^2 is the narrow-sense heritability, and S is the selection differential (I use single-trait equations because genetic correlations between the traits are not known). The average per-generation response to selection (R) in the Pleasure Point population was 1.3 mm for male body depth (16.9 mm over 13 generations, from Figure 5) and -2.1 mm for female body length (-26.8 mm over 13 generations, from Figure 5). Assuming a reasonable heritability ($h^2 = 0.3$), selection differentials (S) would be 4.0 mm for male body depth and -6.3 mm for female body length. Dividing these differentials by the phenotypic standard deviation (average of 1992 and 1993 in the Cedar River, 8.6 mm for male body depth, 18.4 mm for female body length) generates standardized selection differentials (or 'net selection intensities') of 0.47 and -0.34, respectively. These selection intensities are high but fairly common in nature

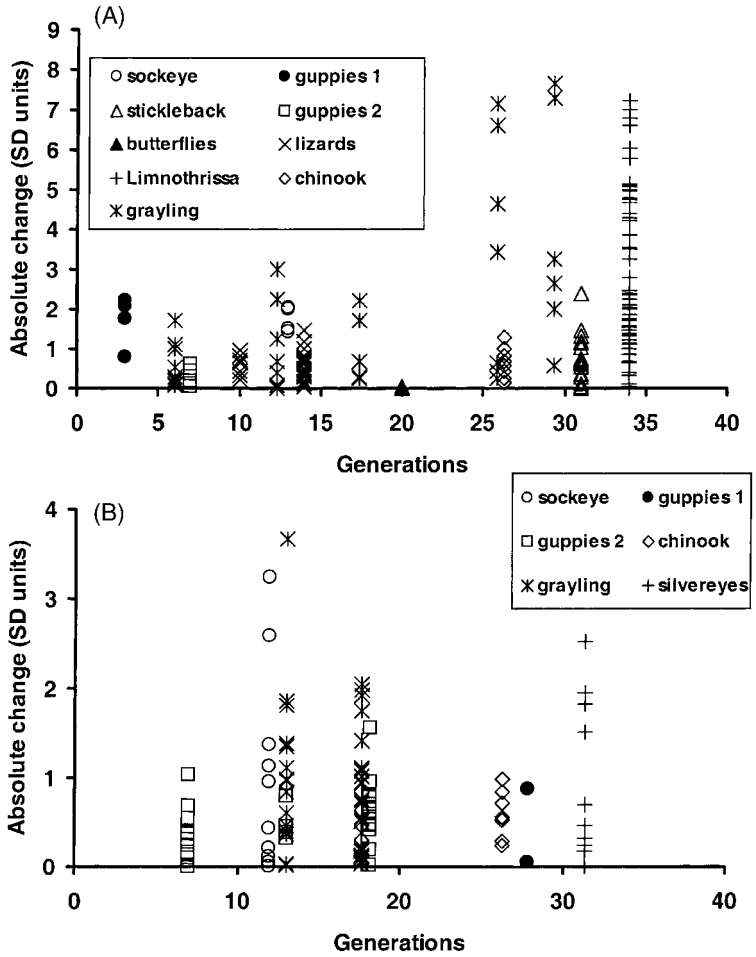

Figure 11. Rates of divergence for Lake Washington sockeye salmon in comparison to other studies of divergence. 'Phenotypic' rates (A) and 'genetic' rates (B) are shown as the absolute amount of divergence (numerator of haldane) versus the number of generations. Sources of data for Panel (A): sockeye (female body length and male body depth in the present study), guppies 1 (Endler, 1980), stickleback (Klepaker, 1993), guppies 2 (Reznick & Bryga, 1987), butterflies (Hill, Thomas & Blakeley, 1999), lizards (Losos, Warheit & Schoener, 1997), *Limnothrissa* (Hauser, Carvalho & Pitcher, 1995), chinook salmon (Kinnison et al., 1998a), and grayling (Haugen, 2000a,b,c). Sources of data for Panel (B): sockeye (days to hatching and days to emergence in the present study; generations set at 12, rather than 13, so that the points are visible), guppies 1 (Magurran et al., 1995), guppies 2 (Reznick, Bryga & Endler, 1990; Reznick et al., 1997), chinook salmon (Kinnison, Unwin & Quinn, 1998; Kinnison et al., 1998b), grayling (Haugen & Vøllestad, 2000), and silvereyes (S. Clegg, unpublished data).

(Endler, 1986; Kingsolver et al., 2001). For example, Endler (1986, p. 211) found that the geometric mean of the absolute value of statistically significant selection intensities was 0.59. Using all of the 749 standardized selection differentials from Kingsolver et al.'s (2001) review (absolute values, significant and nonsignificant), 0.34 falls into the 81st percentile and 0.47 falls into the 90th percentile. In the absence of gene flow, selection thus seems a reasonable mechanism for phenotypic divergence within Lake Washington.

When gene flow continues after the founding event, the equation for evolutionary change needs to be modified (Hendry, Day & Taylor, 2001). Adopt again the above assumptions (except the second), and further assume that the Cedar River population does not evolve, the rate of gene flow to Pleasure Point remains constant over time, and gene flow from Pleasure Point back to the Cedar River is minimal. The appropriate equation for evolution in the Pleasure Point population is then $R_B = m(Z_R - Z_B) + h^2 S$, where m is the proportion of the Pleasure Point population made up of immigrants from the Cedar River each generation, and Z is the mean phenotype of the subscript population (Cedar River, R; Pleasure Point, B). I recursed this equation for 13 generations using different values of S until simulated Pleasure Point

mean phenotypes matched those observed in nature. I started each simulation with $Z_B = Z_R = 126.7$ mm for male body depth and 450.0 mm for female body length (average Cedar River values), and used $m = 0.1$ (a high but reasonable value for effective gene flow, see Figure 1 in Hendry et al., 2000) and $h^2 = 0.3$. This analysis yielded estimated net selection intensities of 0.88 for male body depth and -0.65 for female body length. These intensities are higher than those without gene flow, because now adaptation has to counteract genes continually being reintroduced by immigrants, but they are still within the range of selection intensities observed in nature (Endler, 1986; Kingsolver et al., 2001). Using all of the standardized selection differentials from Kingsolver et al.'s (2001) review (absolute values), 0.65 falls into the 94th percentile and 0.88 falls into the 96th percentile. It is not surprising that the Lake Washington values are at the high end of the range because it clearly represents a case where mean phenotypes started some distance from their optima. If the starting phenotypes were intermediate between the present-day Cedar River and Pleasure Point populations, net selection intensities would be lower. This exercise shows that even with ongoing gene flow, the observed amount of phenotypic divergence between the two populations could be achieved by natural selection. However, I do not have precise estimates of m or h^2, and certain combinations of these parameters could generate unrealistically high estimates of selection intensity.

Random genetic drift has the potential to change quantitative traits in the absence of natural selection. It is therefore useful to compare observed divergence to that expected under a model of change by drift (mutation need not be considered because of the short time interval). Lande (1976, 1977) developed expectations for the amount of among-population variance in quantitative traits owing to drift. These equations are inappropriate in the present context because they do not allow for ongoing gene flow. I therefore used a Monte Carlo simulation model (QDUN, developed by M. Kinnison), which is based on a random sampling of individuals from the distribution of breeding values in each population, with a specified number of individuals exchanged among populations (gene flow). If gene flow is set at zero, the results from QDUN are the same as those from Lande's deterministic equations (M. Kinnison, unpublished data). I used QDUN to determine the expected distribution, based on random genetic drift, of differences in female body length and male body depth between the Cedar River and Pleasure Point populations after 13 generations. I assumed $N_e = 100$ in each population (harmonic mean of $N_e = 50$ and $N_e = 10{,}000$), $h^2 = 0.30$, and $m = 0.1$ into Pleasure Point from the Cedar River ($N_e m = 10$). Phenotypic variances were set at 18.4 mm for female body length and 8.6 mm for male body depth.

These simulations revealed that random genetic drift could not have caused the observed divergence. Expected median differences under drift between the populations were 1.62 mm (upper 95% percentile = 4.64 mm) for female body length and 0.75 mm (upper 95% percentile = 2.18 mm) for male body depth. These values are well below the observed differences for female body length (1992: mean = 30.2 mm, 95% CI = 16.9 − 43.4; 1993: mean = 23.4, 95% CI = 11.8 − 35.0) and male body depth (1992: mean = 16.8 mm, 95% CI = 11.6 − 21.2; 1993: mean = 17.0, 95% CI = 11.4 − 22.6). Natural selection thus seems the most plausible mechanism driving divergence within Lake Washington. As noted above, however, we cannot be certain as to how much of this divergence had a genetic basis.

Alternatives

I have argued that the Cedar River and Pleasure Point populations evolved their adaptive differences and reproductive isolation *de novo* and *in situ*. An alternative is that 'river' and 'beach' genotypes were present in the original introduced population, and that Pleasure Point was founded by 'beach' genotypes whereas the Cedar River was founded by 'river' genotypes. This possibility is worth considering (Gustafson et al., 2001; Hendry et al., 2001a). As a precedent, sockeye salmon were introduced into Frazer Lake, Alaska, from an inlet river (Red Lake, Alaska), a beach (Karluk Lake, Alaska), and an outlet river (Ruth Lake, Alaska). Introduced beach and inlet fish contributed to new populations at both beaches and inlets in Karluk Lake but introduced inlet fish contributed disproportionately to the new inlet populations and introduced beach fish contributed disproportionately to the new beach populations (Burger et al., 2000). If something similar happened within Lake Washington, the observed divergence and reproductive isolation could reflect habitat-specific segregation of fish having pre-existing differences. Because Baker Lake is the ancestral source for both Cedar River and Pleasure Point fish (see above), the question becomes whether or not both stream and beach fish were introduced from Baker Lake into Lake Washington.

Several generalizations can be made regarding the ancestral Baker Lake population (for citations see Hendry & Quinn, 1997). First, substantial stream and beach populations were present in Baker Lake at the turn of the century but the relative abundance of each type is not known. Second, hatchery production was initiated in 1896 using a mixture of stream fish (captured in the upper Baker River) and beach fish (captured in the lake using gill nets). The relative contributions of these sub-populations to hatchery production cannot be estimated because the relevant records were destroyed in a series of fires. Third, between 1899 and 1933 virtually all sockeye salmon returning to Baker Lake were captured in a weir at the lake's outlet and spawned artificially. Fourth, the fish ultimately introduced into Lake Washington continued to be maintained in a hatchery from 1933 through the introductions. Hatchery propagation as a single mixed population for generations thus accounted for all (or nearly all) of the fish introduced into Lake Washington. I conclude that although both beach and stream fish probably contributed to the initial hatchery stock, subsequent artificial propagation achieved genetic admixture prior to the introductions. A final point arguing against pre-existing differences is that the Pleasure Point population appears to have been colonized by immigrants from the Cedar River (see above). The most likely scenario was thus that stream and beach divergence occurred *de novo* within Lake Washington. The substantial genetic variation for beach and stream traits originally present in the introduced fish may simply have facilitated rapid divergence in response to selection.

Closing comments

I have outlined a study that documented adaptive divergence and the evolution of partial reproductive isolation in the wild after less than 13 generations. These results are not novel from the standpoint of 'rapid' evolution (e.g., Endler, 1980; Reznick et al., 1997; Hendry & Kinnison, 1999; Gilchrist, Huey & Serra, 2001; Losos et al., 2001; Quinn, Kinnison & Unwin, 2001; Reznick & Ghalambor, 2001; Haugen & Vøllestad, 2001). Nor are they novel in demonstrating that adaptive divergence leads to reproductive isolation (e.g., Feder et al., 1994; Via, 1999; Filchak, Roethele & Feder, 2000; Rundle et al., 2000; Via, Bouck & Skillman, 2000, for others see Schluter, 2000). Instead, the greatest novelty lies in demonstrating that adaptive divergence can quickly lead to partial reproductive isolation. This conclusion may remain controversial, at least for Lake Washington sockeye salmon, but it is certainly plausible given theoretical expectations and numerous empirical demonstrations in the laboratory (Rice & Hostert, 1993). The real question is: why haven't other studies reported reproductive isolation after similar time intervals?

It isn't that other researchers have looked for reproductive isolation and failed to find it (barring a file-drawer problem). Instead, no one seems to have looked for isolation after this short a time interval in nature. Perhaps this reluctance stems from a psychological hurdle dating back to Darwin, a hurdle which modern evolutionary biology has only recently passed. Darwin consistently emphasized the slow pace of changes wrought by natural selection. This emphasis and its adoption by later evolutionary biologists probably discouraged research into evolution on shorter time scales. Although a few studies, such as Industrial Melanism, stand out as exceptions, the current emphasis on evolutionary changes in contemporary populations really began in the last few decades. Now that ample evidence has accumulated that evolution does work quickly (reviewed by Hendry & Kinnison, 1999; Reznick & Ghalambor, 2001), the next logical step is to test whether this evolution can just as quickly lead to reproductive isolation. I expect that Lake Washington sockeye salmon will ultimately be just one of many examples of partial reproductive isolation evolving after 10–20 generations. The future lies in further development of model systems for empirically examining the dynamics of adaptive divergence and reproductive isolation.

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