



Rapid Evolution of Reproductive Isolation in the Wild: Evidence from Introduced Salmon

Andrew P. Hendry, *et al.*
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ing depends not only on initial porosity but also on the relative time scales for soil deformation and pore pressure diffusion (18). If fluid pressure can diffuse into or away from contracting or dilating soil as quickly as the soil deforms, pressure equilibration keeps pace with deformation and the effects of porosity change diminish. However, the time scale for pore pressure diffusion is h^2/D , where h is the typical thickness of the deforming soil mass and D is its typical hydraulic diffusivity. Even sandy soils with high diffusivity commonly have $D < 100 \text{ cm}^2/\text{s}$ (Table 1). Thus, the time scale for diffusive pore pressure equilibration in deforming soil masses with $h \sim 1 \text{ m}$ typically surpasses 10 s. In comparison, the time scale for landslide acceleration in response to basal pore-pressure change is $\sqrt{h/g}$ (21), which yields values $< 1 \text{ s}$ for $h \sim 1 \text{ m}$. We conclude that pore pressure diffusion can seldom keep pace with soil deformation and that relatively small variations in porosity can influence landslide behavior profoundly.

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2. Soil porosity (pore volume/total soil volume) ranges naturally from about 0.3 to 0.7 as a result of geological and biological modification of parent sediment or bedrock. An alternative measure of pore space is void ratio (pore volume/soil solids volume). Critical-state porosity depends not only on the physical properties of soil but also on the ambient state of stress and stress history.
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9. On the scale of individual grains and pores, the coupling of fluid pressure and displacement of adjacent solids is described by viscous lubrication theory. On a continuum scale involving millions of grains and pores, the same coupling can be described by porosity change and attendant development and diffusion of pore fluid pressure.
10. Soil strength typically obeys the Coulomb-Terzaghi equation $\tau = (\sigma - p)\tan\phi + c$, where τ is mobilized shear strength, σ is total normal stress, and p is pore fluid pressure, all on the same failure plane; ϕ is the soil angle of internal friction; and c is soil cohesion. Increased pore fluid pressure therefore reduces soil strength if all other factors are constant.
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14. All compaction loads were applied normal to the slope. The longest compaction periods produced the lowest porosities, and vibratory compaction produced more uniform porosities than did foot traffic.
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16. Tiltmeters were Crossbow model CXTA01-CAN, rigidly mounted in smooth cylindrical tubes 2.5 cm in diameter, fitted with rough exterior vanes 1 cm high to provide good frictional contact with soil. Exten-

someters were Celesco model PT101-250AS, attached to anchors embedded in the soil surface. Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. government.

17. Dynamic piezometers were fabricated to have a configuration that promoted rapid dynamic response and minimal signal attenuation (17). The sensing elements in these piezometers were Honeywell Micro-switch differential pressure transducers (model 26PCCFA3D, with nominal range 0 to 15 psi). Identical pressure transducers mounted to the pressure ports of Soil Moisture Equipment Jet-Fill tensiometers (model 2725, equipped with porous ceramic tips with nominal 1-bar air entry pressures) were used to measure pore water pressures less than atmospheric.
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20. As illustrated in Fig. 2, dense soils generally display a prominent peak in strength (due to dilation), which impedes landslide initiation. After peaking, the strength of dense soils gradually decays. With sufficiently large deformations, dense soils and loose soils hypothetically approach a state of constant porosity (the critical state) and constant (residual) strength. Breakage of soil aggregates complicated this behavior in our ring-shear experiments (Fig. 2).
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25. Supported in part by grant EAR9803991 from NSF. We thank K. Swinford for assistance with experiments and S. Ellen, J. Roering, and B. Muhunthan for critiquing the manuscript.

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Rapid Evolution of Reproductive Isolation in the Wild: Evidence from Introduced Salmon

Andrew P. Hendry,^{1*} John K. Wenburg,² Paul Bentzen,^{2,3} Eric C. Volk,⁴ Thomas P. Quinn³

Colonization of new environments should promote rapid speciation as a by-product of adaptation to divergent selective regimes. Although this process of ecological speciation is known to have occurred over millennia or centuries, nothing is known about how quickly reproductive isolation actually evolves when new environments are first colonized. Using DNA microsatellites, population-specific natural tags, and phenotypic variation, we tested for reproductive isolation between two adjacent salmon populations of a common ancestry that colonized divergent reproductive environments (a river and a lake beach). We found evidence for the evolution of reproductive isolation after fewer than 13 generations.

Ecological speciation occurs when organisms exposed to divergent selective regimes evolve reproductive isolation as a by-product of adaptation (1–3). Mechanisms contributing to ecological speciation include mate choice based on traits under divergent selection (4, 5), hybrid or backcross inferiority (2), and reinforcement of assortative mating when hybrids are inferior (6, 7). Ecological speciation appears to be relevant in allopatry and sympatry and has been supported by theoretical models, laboratory experiments, and studies of natural systems (1–9). Here we

focus on an unknown aspect of ecological speciation: How quickly can reproductive isolation evolve?

Rapid evolution of adaptive traits often occurs in populations exposed to divergent ecological environments (10, 11). Although this implies that reproductive isolation may also evolve rapidly, the best examples of ecological speciation are seen in groups that began diverging thousands of years ago (12, 13). Unfortunately, inferring evolutionary rates on the basis of long-standing groups is questionable, because averaging disparate rates across time will obscure biologically important short-term evolution (11). Thus, reproductive isolation might evolve in only a few generations, or it may require a long and gradual accumulation of isolating mechanisms. Some insects that colonized new host plants 100 to 200 years ago have evolved ecologically mediated reproductive isolation (14, 15). We ask whether reproductive isolation can evolve even faster by testing for evidence of intrinsic barriers to gene flow between two populations of sockeye salm-

¹Organismic and Evolutionary Biology Program, University of Massachusetts, Amherst, MA 01003–5810, USA. ²Marine Molecular Biotechnology Laboratory, University of Washington, 3707 Brooklyn Avenue Northeast, Seattle, WA 98105–6715, USA. ³School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, WA 98195, USA. ⁴Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501, USA.

*To whom correspondence should be addressed. E-mail: ahendry@bio.umass.edu

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on (*Oncorhynchus nerka*) derived from a common source less than 13 generations previously.

Sockeye salmon form distinct reproductive “ecotypes,” with adults breeding in streams or along lake beaches (16, 17). After continental glaciers receded about 10,000 years ago, sockeye salmon colonized hundreds of new lake systems, many of which now contain reproductively isolated populations that are adapted to beaches and streams (16). Postglacial reproductive isolation was presumably achieved through natal homing, divergent sexual ornamentation, and ecological selection against hybrids. Parallel evolution of these ecotypes within many different lake systems provides a robust interpretive framework.

Introductions of salmon to new locations have provided excellent opportunities to study rates and patterns of evolution (18–21). Sockeye salmon were introduced into Lake Washington, Washington, from Baker Lake, Washington, between 1937 and 1945. These introductions gave rise to a large (currently 100,000 to 350,000 breeders) self-sustaining population in the major tributary to Lake Washington (Cedar River). In 1957, a new population was first documented breeding along a Lake Washington beach (Pleasure Point) about 7 km north of Cedar River. The beach site was apparently colonized by fish from the river or, if not, both populations are at least of the same introduced lineage [inferred from historical records and allozyme variation (18–20)]. The two populations have been diverging for a maximum of 56 years (1937 to 1992), which is equivalent to approximately 13 generations (20), and the beach population is less abundant by about two orders of magnitude (22). As a starting point for divergence, we adopted 13 generations (from 1937) rather than 8 generations (from 1957) because the former is unambiguous and conservative.

We examined reproductive isolation between the beach (Pleasure Point) and river (Cedar River) populations on the basis of collections of breeding adults (23). We used population-specific natural marks to identify

river fish that were immigrants to the beach, DNA microsatellite loci to estimate genetic differentiation between river and beach residents, population-genetic models to infer the relative reproductive success of immigrants, and adult life history and morphology to consider the ecological basis for isolation.

We quantified immigration of river-born adults to the beach using natural marks that are produced on otoliths (calcareous ear stones) while embryos incubate in the gravel. Because the incubation environment is isothermal at the beach but variable in the river (22), we could examine the otoliths of adults to determine whether each had been born (incubated) at the beach or river (24). This analysis, conducted blind with respect to collection location, microsatellite variation, and phenotypic traits, revealed that most breeding adults collected from the river had indeed incubated under a variable thermal regime (34 of 35 in 1992 and 30 of 38 in 1993) but that many adults collected from the beach had also incubated under a variable thermal regime (14 of 32 in 1992 and 12 of 34 in 1993). Thus, approximately 39% of adults breeding at the beach (48% of females and 34% of males) were immigrants from the river (22).

This estimated immigration rate of river fish into the beach population is so high that unless reproductive isolating mechanisms had evolved, the two populations could not have diverged at neutral genetic loci. We used allelic variation at six microsatellite loci (25) to quantify genetic differences between three groups categorized by otolith patterns and breeding location: beach residents (born and breeding at the beach), river residents (born and breeding in the river), and beach immigrants (born in the river but breeding at the beach). River residents and beach immigrants showed no evidence of genetic divergence (Table 1), which is consistent with the expectation that immigrants to the beach were from the river. In contrast, beach residents were genetically distinct from river residents and from beach immigrants (Table 1). This pattern of genetic differentiation could only have arisen if beach immigrants have

reduced reproductive success relative to beach residents.

We considered the extent of reproductive isolation by comparing the proportion of immigrant breeding adults in the beach population (adult migration, determined from otoliths) to the proportion of immigrant genes (gene flow, determined from microsatellites). If gene flow were less than adult migration, we would have evidence for the evolution of reproductive isolation. The standard approach to estimating gene flow from genetic data requires assumptions that most natural populations violate (26). We therefore estimated gene flow using recursion equations that avoided these assumptions, allowing for two populations of different sizes, asymmetric gene flow, and nonequilibrium conditions (27). Gene flow from the river to the beach was less than adult migration (39%), as long as the beach effective population size was not exceptionally low ($N_e > 8$; Fig. 1). Breeding population sizes range from 100 to 8180 at the beach (20), suggesting that $N_e \gg 8$. Thus, the reproductive success of river fish breeding at the beach must be lower than that of beach residents, despite their recent common ancestry.

We considered potential isolating mechanisms by examining two adult traits that are subject to divergent selection between rivers and beaches. Male body depth is sexually selected (28) and reaches extremes in beach populations where it is unopposed by predation, water flow, or water depth (29). In rivers, males have shallower bodies (29), presumably owing to selection for increased swimming efficiency. Female body size differs between beaches and rivers because large

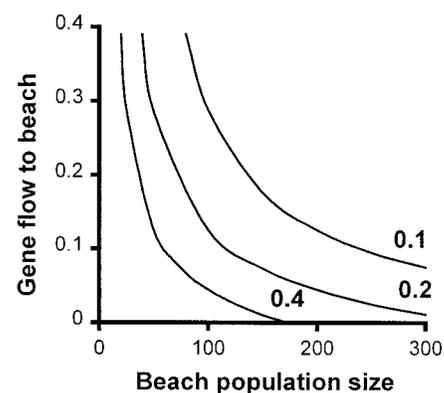


Fig. 1. Estimated rates of gene flow from the river to the beach. Curves were determined with recursion equations (27) to estimate gene flow (m_1) that would lead to the observed F_{ST} (0.025) after 13 generations. We assumed that beach colonizers were representative of the river population (that is, founder effects were minimal), that all gene flow was from the river to the beach ($m_2 = 0$), and that $N_e = 10,000$ in the river. Recursions were started with an IBD of 1/10,000. Curves represent a range of possible N_e/N ratios (0.1, 0.2, and 0.4).

Table 1. Genetic differentiation at six microsatellite loci (25) between beach residents ($N = 22$), river residents ($N = 35$), and beach immigrants ($N = 12$). Nei’s unbiased genetic distance (D) and F_{ST} (bootstrapped 95% confidence intervals are shown in parentheses) were calculated with TFGA (35). The significance of genotypic differentiation (GD) was determined with GENEPOP (36). Observed differentiation cannot be attributed to linkage of any one locus to a locus under divergent selection, because even after deletion of the locus that best differentiated river residents from beach residents (Ssa85, $F_{ST} = 0.054$), divergence was still substantial.

Comparison	F_{ST}	F_{ST} (no Ssa85)	D	GD (P value)
River residents versus beach immigrants	0.008 (0.002–0.013)	0.008	0.010	0.365
River residents versus beach residents	0.025 (0.008–0.042)	0.017	0.054	0.002
Beach residents versus beach immigrants	0.015 (0.001–0.033)	0.012	0.026	0.030

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females dig deeper nests, thereby protecting their eggs from disturbance during flooding (30). Flooding is absent from beaches, and females are correspondingly smaller (17, 29). In our study, beach males had deeper bodies (standardized to average body length) and beach females were shorter, with beach immigrants somewhat intermediate for both traits (Fig. 2). These results suggest that beach immigrants are not as well suited for the beach environment as are beach residents, perhaps contributing to reduced mating success or offspring survival. Many other traits are subject to divergent selection between beaches and rivers (20), and beach immigrants are probably also compromised for those traits.

Significant reproductive isolation (albeit partial) after fewer than 13 generations implies that much of the isolation observed in ecological speciation can arise soon after initial divergence.

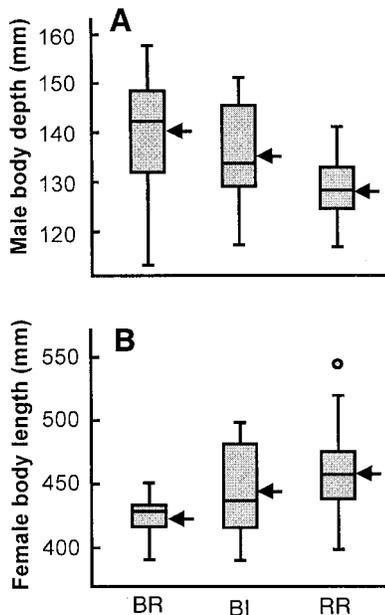


Fig. 2. Differences in (A) standardized male body depth and (B) female body length (37) between beach residents (BR), beach immigrants (BI), and river residents (RR). Boxes contain 50% of the data and bars contain the remainder; horizontal lines indicate medians, arrows indicate means, and the circle indicates an outlier. On the basis of Tukey tests, river residents and beach immigrants had similar female 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 ($P = 0.289$). The morphological intermediacy of beach immigrants could arise because of phenotypic plasticity (if swimming in rivers reduces body depth), morphology-influenced site selection (if smaller river females and deeper bodied river males are more likely to breed at the beach), or site selection by hybrids (if hybrids were produced in the river and then bred at the beach).

Our results may seem exceptional but are clearly biologically possible, as evidenced by laboratory studies in which reproductive isolation often evolves over similar time frames (8). Our study was based on indirect methods (patterns of genetic variation), which measure total isolation (postzygotic and prezygotic). Direct tests of reproductive isolation, such as mate preference, would be complementary because they quantify the prezygotic contribution to isolation (9). Experimental demonstrations of speciation in the wild have been considered intractable because isolation is assumed to require a long period. Our findings suggest that when organisms colonize different environments, experimental studies of speciation may prove feasible.

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- In 1992 and 1993, breeding adult fish were collected at a weir 10.4 km upstream from the mouth of the Cedar River; at Pleasure Point, fish were collected with fishing gear (18, 19). Fish were killed by a blow to the head and measured for length [from the middle of the eye to the hypural plate (19)]. Males were also measured for body depth [from the anterior insertion of the dorsal fin to the bottom of the abdomen (19)]. Otoliths were removed (22), and tissue samples were frozen (18).
- Characteristic dark and light otolith banding patterns are influenced by variations 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 (37). We processed otoliths to reveal banding patterns in the core region that correspond to the incubation period (22). Blind trials were then used to classify each otolith as having been produced by a variable (river) or constant (beach) thermal regime (22).
- DNA was extracted from frozen tissue, and six dinucleotide microsatellite loci (One μ 1, One μ 2, One μ 8,

One μ 11, One μ 14, and Ssa85) were amplified as described elsewhere (32). Microsatellite alleles were separated with an Applied Biosystems 373A automated DNA sequencer and were analyzed with GeneScan 672 and Genotyper software (33, 34). Only 1992 samples were analyzed, because 1993 samples were destroyed by a freezer failure. Loci were in Hardy-Weinberg equilibrium, except for One μ 2 locus in river residents ($P = 0.023$), and locus pairs were in linkage equilibrium, except for One μ 2 versus One μ 14 ($P = 0.046$) and One μ 8 versus One μ 11 ($P = 0.041$) in beach residents. These deviations were not significant after sequential Bonferroni corrections.

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$$f'_1 = \frac{1}{2N_{e1}} + \left[1 - \frac{1}{2N_{e1}} \right] [(1 - m_1)^2 f_1 + 2m_1 (1 - m_1) f_{12} + m_1^2 f_2]$$

$$f'_2 = \frac{1}{2N_{e2}} + \left[1 - \frac{1}{2N_{e2}} \right] [(1 - m_2)^2 f_2 + 2m_2 (1 - m_2) f_{12} + m_2^2 f_1]$$

$$f_{12} = (1 - m_1)(1 - m_2) f_{12} + m_1(1 - m_2) f_2 + m_2(1 - m_1) f_1 + m_1 m_2 f_{12}$$

$$F_{ST} = \frac{\frac{f_1 + f_2}{2} - \frac{f_1 + f_2 + 2f_{12}}{4}}{1 - \frac{f_1 + f_2 + 2f_{12}}{4}}$$

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- Female body length differed ($F = 6.02$, $P = 0.004$) among beach residents ($N = 13$), river residents ($N = 42$), and beach immigrants ($N = 12$). Analysis of covariance (ANCOVA) revealed that male body depth/body length (\log_{10}) slopes were homogeneous ($F = 1.08$, $P = 0.347$) among beach residents ($N = 27$), river residents ($N = 22$), and beach immigrants ($N = 14$). After removal of the interaction term, body length ($F = 107.26$, $P < 0.001$) and group ($F = 9.05$, $P < 0.001$) had significant effects on body depth. Body depth was standardized to average body length (444.7 mm) using $D_{std} = D_0(444.7/L_0)^{1.346}$, where D is body depth, L is body length, 1.346 is the ANCOVA slope, and subscripts std and 0 refer to standardized and observed measurements.
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Daniel J. Howard, *et al.*

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Examining Evidence of Reproductive Isolation in Sockeye Salmon

The study of speciation has recently undergone a revival, with much controversy centering on whether new species can originate quickly and within the geographic range of their ancestor. Hendry *et al.* (1) described a case of reproductive isolation arising between two sockeye salmon populations in only 13 generations. If true, this finding would deserve considerable attention. However, Hendry *et al.* have failed to make the case that the two populations of salmon are indeed reproductively isolated.

First, the evidence cited by Hendry *et al.* for significant genetic divergence of the beach and river populations is not convincing. Their conclusion that reproductive isolation has evolved rests on a small amount of genetic differentiation between a Cedar River population of sockeye salmon in Lake Washington and a nearby beach population, both apparently founded some time after 1937. That genetic differentiation, they claimed, provides evidence for the rapid evolution of reproductive isolation in the wild (i.e., a reduced reproductive success of fish who migrate from river to beach), because the differentiation occurred despite supposedly large amounts of migration between the populations.

The observed level of differentiation between the two populations at six microsatellite loci ($F_{ST} = 0.025$), however, was substantially lower than the level of differentiation found among populations within most anadromous fish species [median $F_{ST} = 0.081$ (2)]. Also, although the F_{ST} of 0.025 was significantly greater than zero, it was not significantly greater than the F_{ST} between Cedar River residents and Pleasant Point Beach immigrants, groups of individuals presumed to come from the same population [see overlap in 95% confidence intervals in table 1 of (1)]. Moreover, Nei's unbiased genetic distance (D), another measure of genetic differentiation, was 0.000 between river residents and beach residents at 20 allozyme loci (3)—an indication of no perceptible difference in the combined frequency of 20 non-microsatellite genes.

A second problem is that the evidence for substantial migration from river to beach is weak at best. Hendry *et al.* estimated that 39% of adults breeding at the beach were immigrants from the river. This estimate was based on natural marks found in otoliths of adults collected from Pleasant Point Beach. Otoliths of sockeye salmon born in variable thermal regimes differ from otoliths of salmon born in

isothermal regimes (4). Because Pleasant Point Beach seemed to have an isothermal regime (4), Hendry *et al.* assumed that adult salmon collected from this population that have “variable-regime” otoliths actually originated from Cedar River, which was characterized as having a fluctuating thermal regime (4).

Otoliths of fry born at Pleasant Point Beach, however, were not examined by Hendry *et al.* Thus, without further study, one cannot assume that fry born at this site have otoliths characteristic of an isothermal regime. In the absence of this crucial control, we can conclude nothing about the rate of migration between beach and river populations. Emphasizing the need for caution in using otoliths to estimate migration is the observation by Hendry *et al.* that in 1993, 21% of the fry from the Cedar River population actually had otoliths typical of salmon that had developed in isothermal conditions. The close morphological similarity between supposed beach immigrants and beach residents [figure 2 of (1)] also advises caution. Until better estimates of migration between the beach and river populations are available, it is premature to regard the low level of genetic differentiation between these populations as indicating even a slight amount of reproductive isolation.

Third, the evidence that river and beach salmon evolved different sizes and shapes—differences that the authors believe may cause reproductive isolation—is nonexistent. Hendry *et al.* have provided no evidence that observed phenotypic differences have a genetic basis. They did not rear fish from both populations in a constant environment, yet such “common garden” experiments are essential for demonstrating whether size and shape differences represent evolved adaptations, the plasticity of genetically similar organisms developing in different environments (5, 6), or a combination of these genetic and nongenetic factors.

Fourth, Hendry *et al.* did not adequately consider reasonable alternative explanations for genetic differentiation in the face of gene flow. For example, habitat-specific selection may be operating on either the assayed microsatellite loci or genes closely linked to them. This possibility seems plausible in view of the lack of differentiation at allozyme loci, and is strengthened if differentiation between the two populations is attributable to only one or two microsatellite loci. Hendry *et al.* explored this possi-

bility by examining the effects of removing the most divergent microsatellite locus on overall F_{ST} values. They claimed that, after deleting this locus, interpopulation divergence was still substantial, but they provided no P values or 95% confidence intervals for the revised F_{ST} values [table 1 of (1)]. The absence of these significance tests may mean that, when one excludes the most divergent locus, the F_{ST} of 0.017 between river residents and beach residents is not significantly greater than zero. Such a result would implicate selection, not reproductive isolation, as the factor responsible for genetic differentiation of these populations. One should also consider (although Hendry *et al.* did not mention it) that native sockeye salmon existed in Lake Washington before the introduction of sockeye salmon from Baker Lake, Washington, in 1937 (3). Differential introgression of alleles from these genetically distinct native populations, which still exist in the lake, could explain the slight genetic divergence between the river and beach populations.

We have no quarrel with the idea that reproductive isolation may arise quickly in the wild; indeed, we encourage research in this area. Nevertheless, much more work must be done before the sockeye salmon in Lake Washington can be seen as a compelling example of rapidly evolving reproductive isolation. These salmon may represent only populations that have evolved some genetic differences by adapting to different habitats, a common occurrence in animal species (7). But, as with *Homo sapiens*, most differentiated populations do not go on to become new species or even evolve any reproductive isolation. Population differentiation is not a sufficient condition for incipient speciation.

Daniel J. Howard
Jeremy L. Marshall
W. Evan Braswell

Department of Biology
New Mexico State University
Las Cruces, NM 88003, USA
E-mail: dahoward@nmsu.edu

Jerry A. Coyne
Department of Ecology and Evolution
The University of Chicago
1101 East 57th Street
Chicago, IL 60637, USA

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TECHNICAL COMMENT

Response: Hendry *et al.* (1) reported that two new salmon populations have diverged in response to natural selection (spawning at a beach versus in a river) and now show partial reproductive isolation. This result implied that the initial stages of “ecological speciation” can occur much faster than had previously been supposed.

Howard *et al.* raise four concerns. First, they argue that the genetic differences are too small to be noteworthy. The populations were derived from a common ancestor fewer than 13 generations previously, however, so large genetic differences were neither expected nor crucial to our conclusions. Even if no gene flow was taking place, the expected F_{ST} would be only about 0.034 [equations in reference 27 of (1), with beach $N_e = 50$ and river $N_e = 10,000$]. An earlier allozyme study did indeed yield a genetic differentiation estimate of approximately zero (2), but that study did not attempt to separate immigrants from residents. Immigrants may have lower reproductive success than residents, so they must be separated when estimating differentiation; otherwise immigrants are considered part of the resident gene pool. The immigrant sample size was small ($N = 12$), a fact criticized by Gustafson *et al.* (3); the small sample size does not pose a problem, however, because immigrants were simply the “noise” that needed to be removed when estimating differentiation. Our microsatellite result ($F_{ST} = 0.025$ between beach residents and river residents) is thus consistent with partial reproductive isolation.

Second, Howard *et al.* maintain that our estimate of adult migration from the river to the beach (39%), which was based on examination of otoliths from mature salmon, is too high. Although we have not examined otoliths of juveniles from the Pleasure Point beach, we have examined otoliths from many juveniles that incubated under variable or constant temperatures, and the observed patterns of otolith microstructure are consistent with those expected (4). This inference is bolstered by controlled experiments validating the correspondence between temperature regimes and otolith microstructure (5). In short, the otolith method reliably distinguishes fish that incubate in variable temperatures from those that incubate in constant temperatures. Howard *et al.* point out that about 21% of adults collected from the river in 1993 (3% in 1992) appeared to have incubated in constant temperatures. As we have discussed elsewhere (4), this probably reflects the presence of some isothermal incubation sites in the river, rather than immigration from the beach into the river. If some river fish incubated in constant temperatures, our estimate of immigration to

the beach was conservative, a fact that strengthens our conclusion that gene flow is reduced relative to adult movement.

The third concern of Howard *et al.* is that the morphological differences (river females larger, beach males deeper bodied) were not confirmed in a “common garden” experiment. Logistical constraints precluded such experiments, but the observed differences were at least partially genetic. Wild salmon from the two populations experience common environments from emergence until breeding. Any environmental effects would have to arise before emergence, which is unlikely for adult size and shape, or during breeding. The different breeding environments were unlikely to cause the differences in female length—females in rivers actually shrink slightly during breeding (6)—but might have influenced male body depth. However, beach immigrants had shallower bodies than beach residents (nonsignificant, owing to low power), despite their common breeding environment. This suggests that plasticity (if indeed present) did not entirely obscure genetic differences. Furthermore, common garden experiments with juveniles from these populations have demonstrated adaptive genetically based differences for other traits (7).

Finally, Howard *et al.* suggest that we failed to adequately consider alternative explanations. One alternative, that a microsatellite locus was linked to a gene under selection, is unlikely given the small number of randomly chosen microsatellites in comparison to the large salmon genome. Nevertheless, we did recalculate genetic divergence after removing the locus (Ssa85) that best differentiated beach residents from river residents. The level of divergence inevitably decreased, but the pattern of differentiation remained the same, and the difference between beach and river residents remained significant [F_{ST} (95% CI) = 0.003–0.038; genotypic differentiation, $P = 0.015$]. Although another lineage of fish (perhaps native) persists elsewhere in the watershed, they have not introgressed significantly (if at all) with the introduced lineage. The introduced and native lineages are very distinct genetically, the native fish are few and geographically isolated, and the beach and river populations did not appear until after the introductions (2, 7).

Howard *et al.* conclude that our results “may represent only populations that have evolved some genetic differences by adapting to different habitats.” We would agree entirely—after adding that the differentiation has contributed to their partial reproductive isolation. We did not claim that the two populations are separate species or that they will eventually become so. Any such claim would obscure the consideration of speciation as a process, rather than a precisely defined point in time. We merely claimed to have demon-

strated that adaptive divergence can lead to the rapid onset of reproductive isolation.

Howard *et al.* close by pointing out that “[p]opulation differentiation is not a sufficient condition for incipient speciation.” Of course not—but each new “species” initially went through a stage in which it was a newly derived population, with only minor genetic differences from its colonizing source (allopatric, sympatric, or otherwise). For this reason, the study of adaptive population divergence, and any corresponding reproductive isolation, remains crucial for understanding ecological speciation. Lake Washington sockeye salmon provide one example of how quickly this process may take place, and other examples will likely follow. We certainly do agree with Howard *et al.* and others (3, 8) that much work remains to be done.

Andrew P. Hendry

*Organismic and Evolutionary
Biology Program
University of Massachusetts
Amherst, MA 01003–5810, USA
E-mail: ahendry@bio.umass.edu*

John K. Wenburg

*Division of Biological Sciences
University of Montana
Missoula, MT 59812, USA*

Paul Bentzen

*Marine Molecular Biotechnology
Laboratory
University of Washington
3707 Brooklyn Avenue N.E.
Seattle, WA 98105–6715, USA
and School of Aquatic
and Fishery Sciences
University of Washington
Box 355020
Seattle, WA 98195, USA*

Eric Volk

*Washington Department of Fish
and Wildlife
600 Capitol Way North
Olympia, WA 98501, USA*

Thomas P. Quinn

*School of Aquatic and Fishery Sciences
University of Washington*

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