

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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