

COUNTERGRADIENT VARIATION AND SECONDARY SEXUAL COLOR: PHENOTYPIC CONVERGENCE PROMOTES GENETIC DIVERGENCE IN CAROTENOID USE BETWEEN SYMPATRIC ANADROMOUS AND NONANADROMOUS MORPHS OF SOCKEYE SALMON (*ONCORHYNCHUS NERKA*)

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Abstract.—Genetically distinct anadromous (sockeye) and nonanadromous (kokanee) morphs of the Pacific salmon, *Oncorhynchus nerka*, develop identical, brilliant red color at maturity during sympatric breeding in freshwater streams. The marine and lacustrine environments they occupy prior to maturity, however, appear to differ in the availability of dietary carotenoid pigments necessary to produce red coloration. We tested the hypothesis that kokanee, which occupy carotenoid-poor lakes, are more efficient at using the dietary pigments than are sockeye, which occupy the more productive North Pacific Ocean. In a 2-year controlled breeding study, flesh and skin color of mature and immature crosses fed a low-carotenoid diet were quantified with both a chromameter and by chemical extraction of carotenoid pigments. Results revealed striking countergradient variation in carotenoid use, with kokanee approximately three times more efficient at sequestering the pigments to the flesh musculature than similar age sockeye. This difference translated into virtually nonoverlapping differences between pure crosses in secondary sexual color at maturity, when the pigments are mobilized and transported to the skin. Kokanee crosses turned pinkish red over most of their body, whereas sockeye turned olive green. The olive green was similar to the breeding color of residuals in the wild, the progeny of anadromous sockeye that remain in fresh water and are believed to have given rise to kokanee on numerous independent occasions. Reciprocal hybrids were similar to each other and intermediate to the pure crosses, indicating additive genetic inheritance. Mate choice trials with sockeye males in the wild showed the ancestral morph strongly preferred red over green models. These results suggest a preference for red mates maintained in nonanadromous breeding populations drove the reevolution of the red phenotype in kokanee via more efficient use of dietary carotenoid pigments. This is a novel, yet hidden, mechanism by which sexual selection promotes the genetic differentiation of these sympatric populations.

Key words.—Color vision, countergradient variation, kokanee, mate choice, *Oncorhynchus nerka*, sensory drive, sexual selection, sockeye, sympatric populations.

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Sexual selection is often considered a directional force leading to rapid phenotypic divergence in secondary sexual traits, and ultimately speciation, in allopatric (Lande 1981; Lande and Kirkpatrick 1988; Schluter and Price 1993), parapatric (Lande 1982), and sympatric (Turner and Burrows 1995; Payne and Krakauer 1997) populations. Interpopulation phenotypic variation in sexual traits may be indicative of underlying adaptations to the different environmental conditions under which sexual signals are produced, transmitted, and received (Reimchen 1989; Hill 1993; Endler and Houde 1995; Ryan et al. 1996). If biases exist in animal sensory systems, however, mate choice criteria can constrain phenotypic variation in sexual traits via sensory drive (Endler 1992; Ryan and Keddy-Hector 1992; Endler and Basalo 1998). In this case, sexual selection may act as a stabilizing force, limiting the range of phenotypes that can be used as signals or possibly selecting for a particular sexual phenotype.

Countergradient variation occurs when genetic effects on a trait oppose or compensate for environmental effects such that phenotypic differences among populations are minimized

(Levins 1968, 1969; reviewed in Conover and Schultz 1995). For traits subject to countergradient selection, substantial genetic differences may underlie similar phenotypes (Berven et al. 1979; Conover and Present 1990; Parsons 1997; Arendt and Wilson 1999). Many sexual traits, such as body size and coloration, are condition dependent such that their expression depends on the ability to acquire and use environmental resources (Kodric-Brown and Brown 1984; Andersson 1994; Johnstone 1995). Thus, if populations occupy environments that differ in the availability of resources, mating preferences arising from sensory biases may promote phenotypic convergence in the expression of sexual traits via genetic divergence in aspects of resource use. Given this possibility, do similar sexual phenotypes among populations necessarily imply a lack of genetic divergence with respect to these traits? Although no examples exist for sexually selected traits or sympatric populations, countergradient selection has been identified as a largely overlooked means by which natural selection promotes the genetic differentiation of allopatric populations (Conover and Schultz 1995).

Sockeye salmon and kokanee are incipient species that occur as two habitat morphs: the large, anadromous sockeye (freshwater and marine phase) and the small, nonanadromous kokanee (wholly freshwater; Ricker 1940; Wood and Foote 1996). The young are spawned in freshwater streams and subsequently migrate to a nursery lake (Burgner 1991). Sock-

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eye then migrate to the ocean, where they spend 1–3 years, whereas kokanee remain in the lake until maturity and return to natal freshwater streams to spawn and die. Molecular genetic evidence indicates the morphs are genetically distinct, even where they spawn in sympatry, and polyphyletic, such that sockeye and kokanee inhabiting the same lake are usually more closely related than either is to the same morph from other lakes (Foote et al. 1989; Taylor et al. 1996; Winans et al. 1996; Wood and Foote 1996). This presumably neutral molecular genetic differentiation is maintained by strong selective differences between the marine and lacustrine environments in a suite of characters including life-history traits, swimming performance, seawater adaptability, and feeding morphology (Wood and Foote 1996). Genetic differentiation is facilitated by the large, mostly environmentally induced, size difference between sockeye and kokanee at maturity (Wood and Foote 1990, 1996), which results in assortative mating by morph (Foote and Larkin 1988). In some systems, some progeny of sockeye remain in the nursery lake until maturity (residuals; Ricker 1938; Krogius 1981). Being the nonanadromous progeny of anadromous parents, residuals are the presumed intermediary by which sockeye gave rise to kokanee on numerous independent occasions over the last 15,000 years (Ricker 1940, 1959). The pattern of genetic differentiation among and within lakes supports this interpretation (references cited above). Also, across the range of *O. nerka*, the distribution of kokanee is contained within that of sockeye, and kokanee occur on many marine islands that could only have been colonized by the anadromous form (Nelson 1968). Finally, sockeye introduced to new systems have given rise to nonanadromous populations on independent occasions (Ricker 1959; Scott 1984). Like kokanee, residuals are much smaller than sockeye due to the lower productivity of freshwater environments (Wood and Foote 1990, 1996) and are distinguishable from both morphs by their olive green skin color at maturity (Ricker 1938; Krogius 1981).

Sockeye and kokanee turn from silver to bright red during maturation, while the head is olive green and the fins are blackish red. The red color of mature *O. nerka*, as well as some other salmonids, results from the transport of carotenoid pigments (mainly astaxanthin) to the skin, which are deposited in the flesh during the immature phase (Steven 1947; Crozier 1970; Kitahara 1984; Hatlen et al. 1996). Animals cannot synthesize carotenoids *de novo*, but must acquire them from their diet (Goodwin 1984). If dietary carotenoids are limited, both flesh color of immature fish (Steven 1947; Torrissen et al. 1995; Olsen and Mortensen 1997) and subsequent skin color at maturity (Steven 1947; Scheidt et al. 1988) are depressed (see also Kodric-Brown 1989).

For a number of reasons, we suspected that phenotypic similarity in breeding color of sockeye and kokanee masks associated genetic divergence in the use of dietary carotenoids. First, carotenoids are plant-synthesized pigments and their abundance is related to primary productivity (Sanger 1988; Leavitt 1993), which is greater in the northern temperate marine environment inhabited by sockeye than in the generally oligotrophic lakes occupied by kokanee (Gross et al. 1988). Second, given their much smaller size at maturity, kokanee have a greater surface area-to-volume ratio than sockeye so that carotenoids stored in the flesh must be de-

posited over a relatively greater skin area. Third, residuals turn olive green at maturity, in contrast to the red of their anadromous counterparts (Ricker 1938; Krogius 1981). However, kokanee introduced to the same lakes containing residuals (Ricker 1940, 1959) as well as other lakes (Averett and Espinosa 1968) turn red. Further, sockeye that become landlocked upon introduction to other systems also turn olive green to dusky reddish green at maturity (Ricker 1959; Scott 1984; Quinn et al. 1998). Given these observations, the fact that kokanee turn red at maturity, similar to sockeye, suggests they are able to use available carotenoids more efficiently.

To test the hypothesis that selection for the red breeding phenotype results in genetic divergence in carotenoid use, we measured color of breeding adults in the wild and the progeny of crosses reared under controlled conditions. We predicted that progeny of kokanee crosses would use dietary carotenoids more efficiently than sockeye and that this difference would translate into marked differences in skin color between the morphs at maturity. We also addressed the question of whether the ancestral sockeye morph possesses a preference for red over green mates that could act as a selection pressure for the reemergence of red breeding color in kokanee from green residuals. This study provides the first test of the hypothesis that countergradient selection has resulted in phenotypic convergence in a sexual trait or in sympatrically spawning populations.

METHODS

Broodstock Collection and Genetic Design

Mature sockeye and kokanee were collected from Narrows Creek, Takla Lake, British Columbia (55°23'N, 125°50'W) on 15–16 August 1991, and their forklength (FL, tip of snout to fork of tail) and color (see below) measured. Thirty full-sib families of *O. nerka* were created: nine families of pure kokanee (PK, kokanee female × kokanee male) and seven families each of hybrid kokanee (HK, kokanee female × sockeye male), hybrid sockeye (HS, sockeye female × kokanee male), and pure sockeye (PS, sockeye female × sockeye male); for specific details of rearing methodology, see Craig et al. 1996). Crosses were reared at Rosewall Creek Experimental Hatchery on Vancouver Island, British Columbia, under identical conditions for approximately 2 years (16 August 1991 to 6 October 1993).

Rearing and Sampling Procedures

Fertilized eggs were incubated in $9.0 \pm 0.8^\circ\text{C}$ well water. Juveniles were fin-clipped to identify cross type, tagged with a coded wire to identify family, and reared in indoor 480-L tanks at a density of 109 fish per tank (two families per tank) on a simulated natural photoperiod. On 10 November 1992 all fish were interspersed between two outdoor concrete channels (17,000 L each), where they were reared until September 1993 under a natural photoperiod for 49°N latitude (see Craig et al. 1996).

Fish were fed identical diets supplemented with astaxanthin, the major carotenoid found in salmonids (Torrissen et al. 1989). Feeding began in December 1991 on a salmon starter diet. In April 1992, fish were switched to diet con-

taining 16 mg astaxanthin/kg derived from krill. In December 1992, fish were switched to diet containing 10 mg astaxanthin/kg from krill. In June 1993, fish were switched to diet containing 25 mg synthetic astaxanthin/kg. Feed was dispensed by automatic feeders every half hour during daylight hours at a rate of 3.8–1.0% body weight per fish each day, decreasing over the course of the experiment.

Fish were randomly sampled from 14 September to 6 October 1993 after a proportion had matured. Mature females had ovulated and males were producing milt. Fish were killed with tricaine methanesulfonate (MS-222) and their FL (± 1 mm) and color (see below) measured. A 1-cm thick sample of the dorsal musculature (2 cm \times 3 cm) was excised from each fish beneath the origin of the dorsal fin and just above the lateral line on the left side of the body, stored immediately at -20°C , and transferred to -80°C within 1–4 weeks for carotenoid analysis.

Color Measurement

Flesh and skin color was measured on parental fish; mature, wild fish; and their progeny with an electronic chromameter (Minolta CR-100 model; Minolta Corp Ramsey, NJ). The chromameter was set for type C illumination (6744 K) and chromaticity using the $L^*a^*b^*$ international standard (CIE 1986). Increasing a^* values appear as more intense red color (Hunter 1987). Flesh color of mature and immature fish was measured on the dorsal muscle sample described above. Skin color of all mature fish and a subsample of immature fish was measured at three points on the left side of the body: (1) midway between the origin of the dorsal fin and the lateral line; (2) directly below the adipose fin and centered on the lateral line; and (3) on the head between the posterior margin of the eyes.

Carotenoid Analysis

Total carotenoid concentration of flesh samples from a subsample of immature fish, as well as flesh and skin samples from nearly all mature fish was determined by spectrophotometric analysis according to the method of Saito and Regier (1970). Samples were wet-weighed, dehydrated, dry-weighed, and the skin separated from the underlying tissue prior to grinding and repeated extraction with acetone. The optical density of the extracted solution was measured at the peak of the absorption spectrum of astaxanthin dissolved in acetone (477 nm wavelength; Ando and Hatano 1987). Total carotenoid concentration (C , mg/kg) was calculated according to Saito and Reiger (1970) as: $C = AV/WE$, where A is the absorbance at 477 nm wavelength; V is the final volume of the solution (ml), W is the dry weight of the sample (g), and E is the extinction coefficient of a 1% solution at a pathlength of one centimeter ($E = 0.22$; Ando et al. 1994).

Statistical Analysis

Statistical analyses are based on the mean of dorsal and adipose color scores; results were similar when analyzed separately (Craig 1995). For immature crosses, there was no difference in flesh color or carotenoid concentration between sexes (two-way ANOVA; color, $F_{1,471} = 2.6$, $P = 0.11$; ca-

rotenoid, $F_{1,283} = 1.5$, $P = 0.23$); therefore, sexes were pooled. Two PK families contained only one immature individual and were removed from the statistical analysis, resulting in a balanced design consisting of seven independent full-sib families for each cross type. Variation in flesh color and carotenoid concentration of immature fish was analyzed by model III nested ANOVA with family (random effect) nested within cross type (fixed effect). By incorporating family effects, this analysis reflects genetic variation between sockeye and kokanee populations in the wild.

Sample sizes were limited for mature fish due to the overall maturation rate (21%), variation in maturation rate by cross type (number of mature individuals: 68 PK, 19 HK, 26 HS, 16 PS), and by sex (78 males; 45 of 51 mature females were PK; no PS females matured). Statistical comparisons were limited to males because they displayed significantly redder skin than females (two-way ANOVA, $F_{1,107} = 6.4$, $P = 0.013$), which precluded pooling. Due to limited family sample sizes, mature males were pooled by cross type and analyzed by model I ANOVA. A model III nested ANOVA was conducted on a^* scores from a subset of mature males for which sufficient samples were available (number of families: 5 PK, 3 HK, 4 HS).

Particular cross types were compared using three a priori statistical contrasts: (1) PK were compared to PS to test for differences between sockeye and kokanee morphs; (2) the reciprocal hybrids (HK and HS) were compared to test for differences due to maternal effects and/or sex linkage; and (3) the mean of the reciprocal hybrids (HS and HK) was compared to that of the pure crosses (PS and PK) to test for additive genetic inheritance. If the trait is additive, HS and HK should be intermediate between PS and PK and not significantly different from the mean of the two pure cross types.

Mate-Choice Experiments

To test the hypothesis that a behavioral preference for red over green mates is present in sockeye, 30 independent mate-choice trials were conducted on native sockeye spawning beaches at Lake Iliamna, Alaska. For each trial, a model was placed in each of two white PVC grids (1.8 cm diameter, 1 m²) positioned 2 m apart in less than 3 m water depth. Female models consisted of a rectangular (30 \times 23 cm) piece of 4-mm thick plexiglass with a perpendicular rib (30 \times 11.5 cm) that ran lengthwise down the middle. Models were painted with either acrylic banner red (CWA1261) with peak reflectance at 700 nm or azure green (CWA1258) with peak reflectance at 525 nm (Krylon[®], Division of The Sherwin-Williams Co., Bedford Heights, OH). The red model reflected virtually no light from 400 to 560 nm, above which the amount of reflected light increased dramatically, peaking at about 700 nm. The green model reflected little light from 400 nm to 460 nm and above 600 nm; its peak reflectance at about 525 nm was only about 23% as bright as that of red at 650 nm. Five-minute observation periods began immediately after a green and red model were placed in the center of each quadrat simultaneously. Mate color preference was tested by comparison of the maximum number of males with their heads in the quadrat at any one time, number of physical contacts with the models, number of sexual displays per-

formed (quivers; Foote and Larkin 1988), and spawning events (sperm release) observed. Divers switched models between trials to control for possible observer effects and each trial was conducted on a new area of the spawning grounds to ensure new males were tested each time. Data were analyzed with Wilcoxon signed-rank tests (Sokal and Rohlf 1995).

Statistical analyses were conducted with Systat version 8.0. All experiments were conducted under the guidelines of the University of Washington Animal Care Committee.

RESULTS

Variation between Sockeye and Kokanee Morphs in the Wild

Mature sockeye and kokanee breeding in Narrows Creek were both bright red over most of their body, with green heads, white to grayish black bellies, and dusky black fins (Fig. 1). There were no significant differences in skin color between the morphs (Fig. 1A; two-way ANOVA, $F_{1,101} = 2.5$, $P = 0.12$). Sockeye were approximately threefold longer than kokanee in the wild (mean \pm SD FL [cm]: sockeye males, 58.2 ± 2.9 , $n = 7$; kokanee males, 19.4 ± 0.6 , $n = 22$; sockeye females, 54.1 ± 1.4 , $n = 9$; kokanee females, 19.1 ± 1.0 , $n = 9$), but skin color was not correlated with length within sexes of either morph (all $P > 0.5$). Males were redder at maturity than females ($F_{1,101} = 7.3$, $P = 0.01$), with this difference similar between the morphs (morph \times sex, $F_{1,101} = 0.3$, $P = 0.61$). Flesh color was opaque white and similar between the morphs (two-way ANOVA, $F_{1,43} = 1.8$, $P = 0.18$) and sexes ($F_{1,43} = 3.0$, $P = 0.09$), indicating nearly all carotenoids had been mobilized from the flesh (mean \pm SD flesh a^* : sockeye males, 4.67 ± 1.2 , $n = 6$; kokanee males, 5.92 ± 5.9 , $n = 15$; sockeye females, 6.36 ± 2.1 , $n = 11$; kokanee females, 8.26 ± 5.3 , $n = 15$). The morphs also exhibited similar green head color (Fig. 1B, two-way ANOVA, $F_{1,50} = 0.5$, $P = 0.49$). Head color varied little (range = 7.3) compared to body color (range = 27.2) and was similar between the sexes ($F_{1,50} = 1.3$, $P = 0.27$).

Variation within and among Immature Progeny of Sockeye and Kokanee Crosses

After a 2-year rearing period, immature progeny of sockeye and kokanee crosses were uniformly silver over the flanks of the body, with bluish backs, white bellies, bluish gray heads, and slightly darkened fins. There were no significant differences in skin or head color among the four immature cross types (mean \pm SD skin a^* : PK, 4.1 ± 1.2 , $n = 10$; HK, 2.6 ± 1.2 , $n = 12$; HS, 2.8 ± 1.4 , $n = 8$; PS, 2.9 ± 1.6 , $n = 6$; $F_{3,32} = 2.9$, $P = 0.053$; mean \pm SD head a^* : PK, -0.2 ± 0.6 , $n = 10$; HK, -0.7 ± 0.4 , $n = 12$; HS, -0.6 ± 0.6 , $n = 8$; PS, -0.6 ± 1.2 , $n = 6$; $F_{3,32} = 2.3$, $P = 0.1$). In contrast to fish in the wild, size varied little among immature crosses (mean \pm SD FL [cm]: PK, 24.0 ± 1.6 , $n = 59$; HK, 26.9 ± 2.3 , $n = 113$; HS, 25.2 ± 2.2 , $n = 167$; PS, 27.5 ± 1.5 , $n = 142$). Although differences in FL among the cross types were significant (one-way ANOVA, $F_{3,477} = 63.2$, $P < 0.0001$), they were small (3.5-cm range of means among cross types) relative to the morphs in the wild (39.1-cm difference

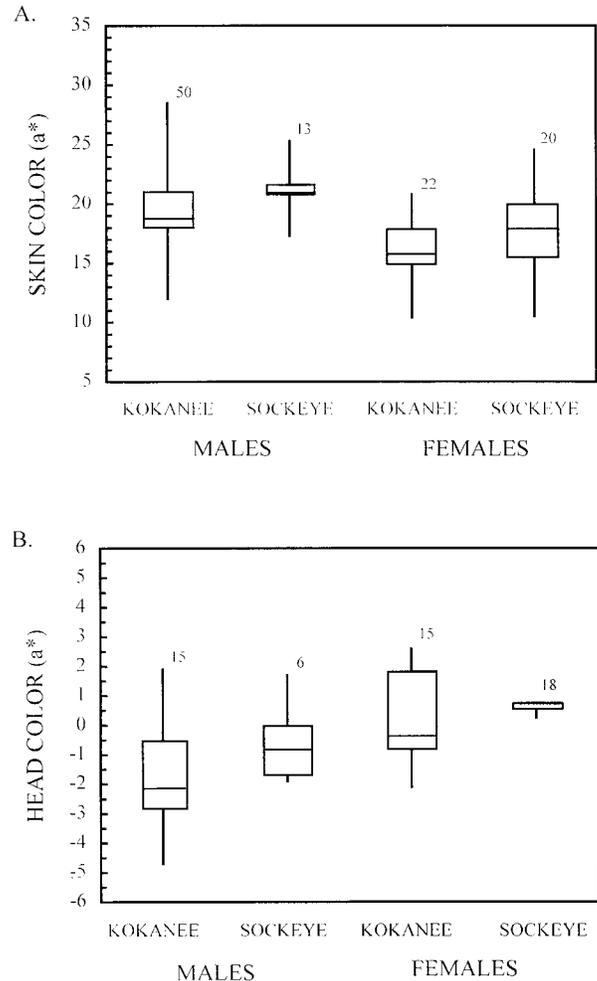


FIG. 1. (A) Red skin color and (B) green head color of mature, sympatric sockeye and kokanee morphs on the spawning grounds in Narrows Creek, Takla Lake, British Columbia during 1991. Horizontal bar shows the median, box delineates the 25th and 75th percentiles, and vertical lines span the maximum and minimum values. Sample sizes given above each bar. Note the y-axis is scaled differently for the two panels.

in mean size between morphs), with PS the largest followed by HK, HS, and then PK. Correlations between color and FL were observed for some cross types, but were generally low and not consistent for different measures of color (a^* : HK, $r^2 = 0.10$, $n = 113$; HS, $r^2 = 0.08$, $n = 167$; carotenoids: HK, $r^2 = 0.12$, $n = 65$; PS, $r^2 = 0.10$, $n = 78$; all other $P > 0.5$). Size adjustment had little effect on the results here and in all subsequent cases; as such, only analyses based on unadjusted data are reported (for further details, see Craig 1995).

Although similar in external appearance, immature sockeye and kokanee crosses differed dramatically in flesh color and associated carotenoid concentration (Fig. 2). There was highly significant variation in both a^* scores and carotenoid concentration among immature crosses (a^* : $F_{3,24} = 62.3$, $P < 0.001$; carotenoids: $F_{3,24} = 49.1$, $P < 0.001$) as well as families within cross type (a^* : $F_{24,442} = 6.9$, $P < 0.001$; carotenoids: $F_{24,256} = 3.4$, $P < 0.001$). Cross type explained

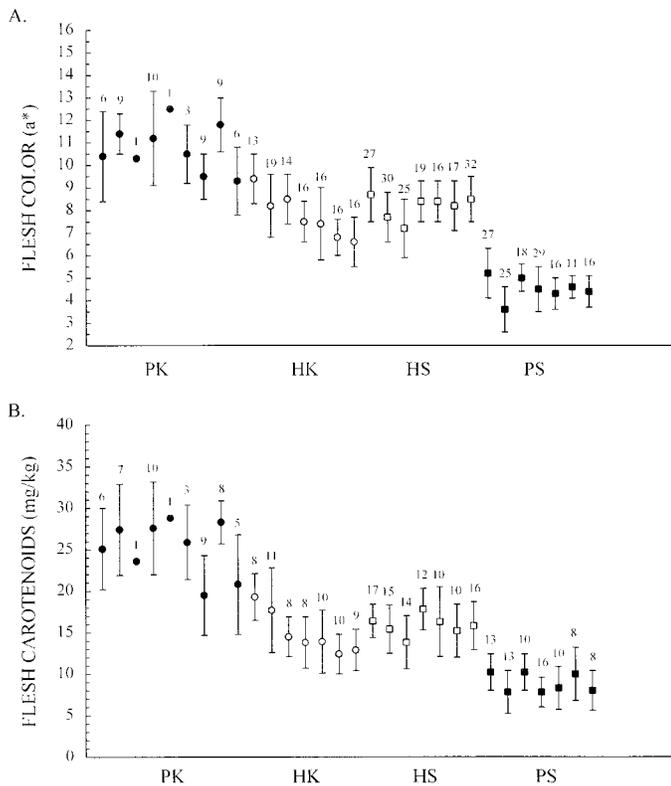


FIG. 2. Flesh (A) color and (B) carotenoid concentration of immature sockeye and kokanee crosses. Low values of a^* appear light pink, whereas high values appear orangey red. PK, kokanee female \times kokanee male, solid circle; HK, kokanee female \times sockeye male, open circle; HS, sockeye female \times kokanee male; open square; PS, sockeye female \times sockeye male, closed square. Values are mean \pm standard deviations for each family. Families with one individual are included for comparison, but were excluded from statistical analyses. Number of individuals per family given above each bar. Cross-type mean \pm SD a^* : PK, 10.7 ± 1.7 ; HK, 7.7 ± 1.4 ; HS, 8.1 ± 1.2 ; PS, 4.5 ± 1.0 . Cross-type mean \pm SD carotenoid concentration (mg/kg): PK, 25.9 ± 5.7 ; HK, 14.9 ± 4.0 ; HS, 15.8 ± 3.1 ; PS, 8.8 ± 2.6 .

much more of the variation in flesh color than did family (a^* : 73.9% vs. 7.0%; carotenoids: 69.0% vs. 7.6%; see Sokal and Rohlf 1995, p. 294). Relative to immature PS, PK exhibited much redder flesh (Fig. 2A, $F_{1,24} = 141.5$, $P < 0.001$) and threefold greater flesh carotenoid concentrations (Fig. 2B, $F_{1,24} = 145.9$, $P < 0.001$). There was virtually no overlap between PK and PS crosses in flesh a^* scores (PK: 6.8–15.0; PS: 1.0–8.1; 6.5% overlap, 13 of 201 individuals) or carotenoid concentrations (PK: 14.01–37.58 mg/kg; PS: 4.70–14.98 mg/kg; 5.1% overlap, six of 133 individuals). The reciprocal hybrids, HK and HS, were similar to each other, indicating that maternal effects or sex linkage were not significant (a^* : $F_{1,24} = 1.1$, $P = 0.31$; carotenoids: $F_{1,24} = 0.6$, $P = 0.43$). In addition, the mean of HK and HS did not differ from the mean of the pure types, indicating additive genetic inheritance (a^* : $F_{1,24} = 1.7$, $P = 0.2$; carotenoids: $F_{1,24} = 3.2$, $P = 0.09$). Broad-sense heritability of flesh color (a^* scores) based on full-sib correlations were significantly different from zero within each of the four cross types (one-way ANOVA, all $P < 0.0001$), but differed considerably

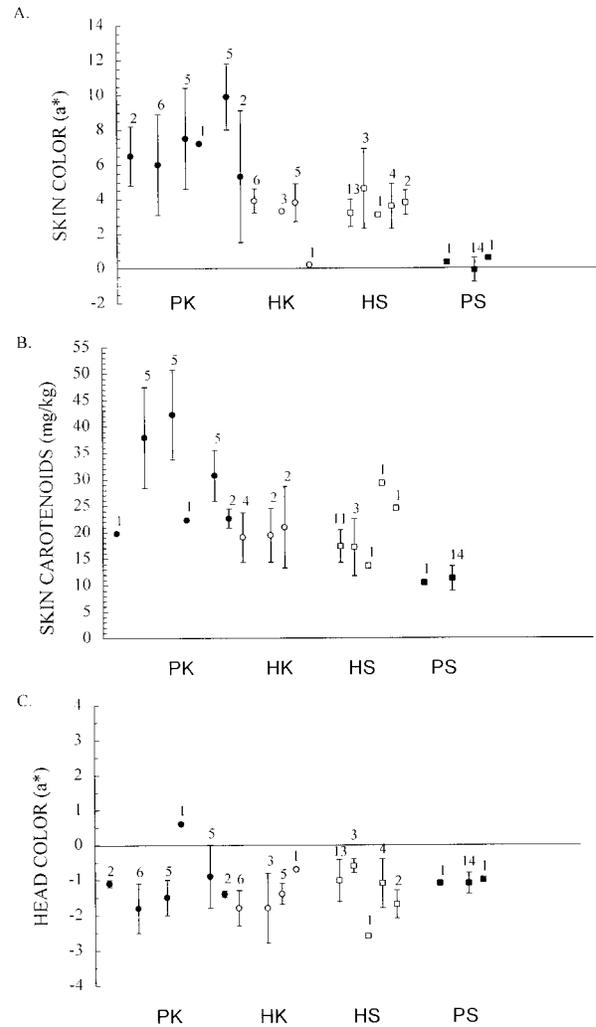


FIG. 3. (A) Red skin color, (B) skin carotenoid concentration, and (C) green head color of mature sockeye and kokanee crosses. Low values of a^* appear olive green, whereas high values appear pinkish red. Refer to Figure 2 for cross-type designations. Values are mean \pm standard deviations for each family. Number of individuals per family given above each bar. Cross-type mean \pm SD skin a^* : PK, 7.2 ± 2.7 ; HK, 3.5 ± 1.1 ; HS, 3.5 ± 1.2 ; PS, -0.01 ± 0.7 . Cross-type mean \pm SD carotenoid concentration (mg/kg): PK, 33.4 ± 9.9 ; HK, 19.6 ± 1.7 ; HS, 18.2 ± 4.7 ; PS, 11.2 ± 2.2 . Cross type mean \pm SD head a^* : PK, -1.2 ± 0.8 ; HK, -1.6 ± 0.6 ; HS, -1.1 ± 0.7 ; PS, -1.1 ± 0.3 .

among cross types ($h^2 \pm$ SE: PK, 0.68 ± 0.001 ; HK, 0.79 ± 0.002 ; HS, 0.26 ± 0.001 ; PS, 0.28 ± 0.001).

Variation within and among Mature progeny of Sockeye and Kokanee Crosses

In contrast to immature progeny, which were similar in external appearance but differed in flesh color, mature fish differed dramatically in external color but had similar, opaque white flesh color (Fig. 3). Mature PK were pinkish red over the body with green heads. In contrast, mature PS were olive green over the entire head and body, sometimes with a faint streak of red along the lateral line. The reciprocal hybrids appeared intermediate, with red color concentrated along the

midline of the body, extending dorsally and toward the caudal region. The mean a^* score for male PK crosses was 37.5% of that for wild male kokanee in Narrows Creek, indicating carotenoid pigments were indeed limiting to crosses reared in the hatchery (compare Figs. 1A, 3A). As for immature fish, differences in size among mature male crosses were small but significant and in the same direction as for immature crosses (mean \pm SD FL [cm]: PK, 28.4 ± 2.1 , $n = 23$; HK, 30.6 ± 1.7 , $n = 16$; HS, 28.9 ± 1.4 , $n = 23$; PS, 31.0 ± 1.1 , $n = 16$; one-way ANOVA, $F_{3,74} = 11.3$, $P < 0.001$). Only color scores of PK were significantly correlated with size ($r^2 = 0.45$, $n = 23$; all other $P > 0.5$; size-adjustment did not alter the results; see Craig 1995).

The threefold difference in flesh color and carotenoid concentration between immature PK and PS crosses translated into striking differences in skin color at maturity (Fig. 3A, B). Variation in skin color among mature male crosses was highly significant (one-way ANOVA; color: $F_{3,74} = 55.7$, $P < 0.0001$; carotenoids: $F_{3,56} = 35.8$, $P < 0.001$), with the mean of PK approximately threefold that of PS (Fig. 3A, B; a^* , $F_{1,74} = 163.3$, $P < 0.001$; carotenoids, $F_{1,56} = 163.5$, $P < 0.001$). There was virtually no overlap between PK and PS crosses in a^* scores (Fig. 3A; range: PK, 1.15–13.05; PS, –1.05–1.55; 5.1% overlap, two of 39 individuals) and no overlap in skin carotenoid concentration (Fig. 3B; range: PK, 19.80–49.02 mg/kg; PS, 6.5–15.19 mg/kg). As for immature crosses, the reciprocal hybrids were similar to each other (a^* : $F_{1,74} = 0.01$, $P = 0.93$; carotenoids: $F_{1,56} = 0.3$, $P = 0.62$) and did not differ from the mean of the pure types (color: $F_{1,74} = 0.06$, $P = 0.81$; carotenoids: $F_{1,56} = 3.6$, $P = 0.06$), supporting an additive genetic basis for the trait. Results of nested ANOVA on the reduced dataset of mature male fish were consistent with those for the pooled analysis and indicate variation in mature skin color reflects variation between sockeye and kokanee morphs in the wild. Family effects on skin a^* values for mature fish were significant but small ($F_{9,57} = 2.9$, $P = 0.006$; 12.5% variance explained) and did not obscure differences among cross types ($F_{3,9} = 18.6$, $P < 0.001$; 49.9% variance explained). The pattern in skin color among mature female crosses was similar to that of males (mean \pm SD a^* : PK, 5.11 ± 2.1 , $n = 45$; HK, 2.45 ± 1.3 , $n = 3$; HS, 2.03 ± 1.3 , $n = 3$).

All crosses exhibited similar green head color (Fig. 3C; one-way ANOVA, $F_{3,74} = 1.9$, $P = 0.14$). In addition, there was no difference in head color between mature male PK and PS crosses reared in the hatchery and wild male sockeye and kokanee on the spawning grounds (compare Figs. 1B, 3C; one-way ANOVA, $F_{3,56} = 2.2$, $P > 0.1$).

The flesh of all mature crosses was opaque white (mean \pm SD flesh a^* : PK, 3.0 ± 1.0 , $n = 23$; HK, 3.5 ± 1.7 , $n = 16$; HS, 2.2 ± 1.1 , $n = 23$; PS, 2.6 ± 0.9 , $n = 16$) like that of their parents and essentially devoid of carotenoids (mean \pm SD flesh carotenoids: PK, 1.2 ± 0.2 , $n = 8$; HK, 1.3 ± 0.3 , $n = 8$; HS, 1.0 ± 0.2 , $n = 4$; PS, 0.9 ± 0.2 , $n = 4$). Slight differences in flesh color among mature cross types were inconsistent for the two measures of color and driven by a few individuals (one-way ANOVA followed by Bonferroni post hoc pairwise comparisons; HK > HS based on a^* scores, $P = 0.01$; HK > PS based on carotenoid concentration, $P = 0.04$; all other $P > 0.05$). These slight differ-

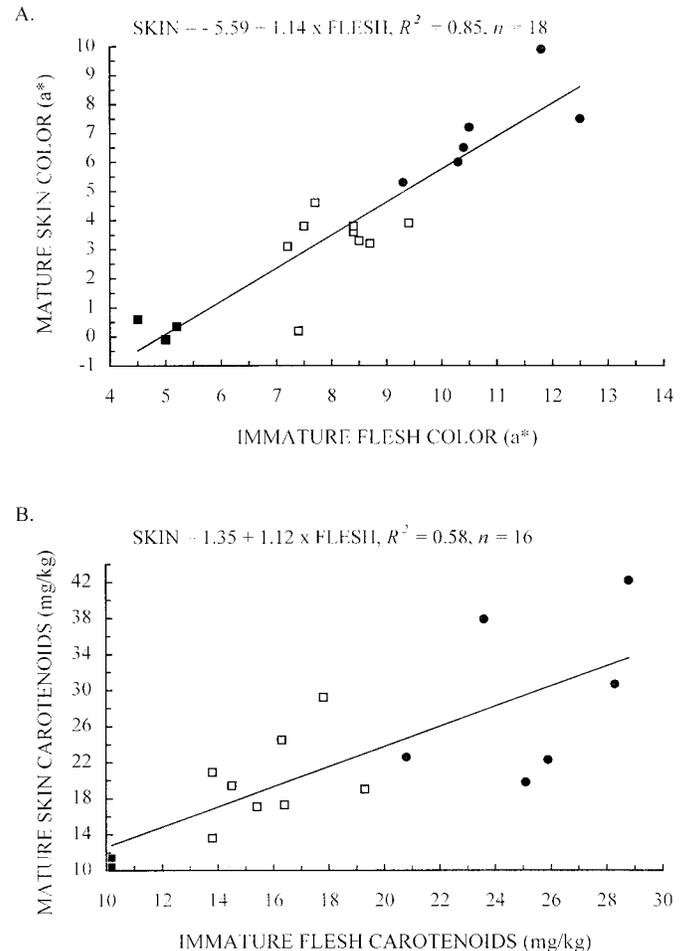


FIG. 4. Relationship between immature flesh and mature skin color based on family means for (A) chromameter a^* scores and (B) carotenoid concentration for crosses reared in the hatchery. Refer to Figure 2 for cross-type designations. PK, solid circle; HK and HS (pooled), open square; PS, closed square. Each symbol represents the mean of mature and immature individuals within the same family. Regression equations given (both $P < 0.05$).

ences, however, cannot account for the large differences in skin color among mature crosses.

Relationship between Immature Flesh Color and Mature Skin Color

To examine the relationship between immature flesh color and mature skin color, we compared immature (males and females) and mature siblings (males) within families across cross types. Based on family means, there was a strong relationship between the mean flesh color of immature fish and the corresponding mean skin color of mature males measured over the cross types (Fig. 4; a^* , $P < 0.0001$; carotenoids, $P = 0.001$). Within cross type, relationships between immature and corresponding mature family means were generally positive but not significant (a^* : PK, $r = 0.72$, $n = 6$, $P = 0.08$; HK/HS pooled, $r = 0.35$, $n = 9$, $P = 0.36$; PS, $r = -0.57$, $n = 3$, $P = 0.62$; carotenoids: PK, $r = 0.45$, $n = 6$, $P = 0.37$; HK/HS pooled, $r = 0.29$, $n = 8$, $P = 0.43$), most likely due to the limited number of families, individuals per family,

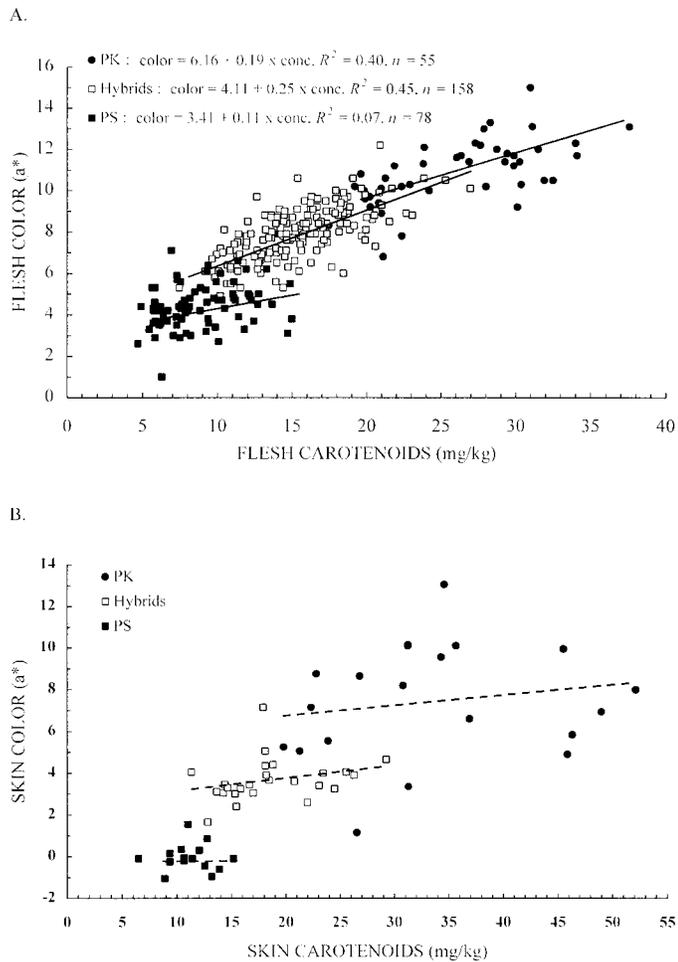


FIG. 5. Relationship between color and carotenoid concentration for (A) the flesh of immature crosses and (B) the skin of mature crosses of sockeye and kokanee reared in the hatchery. Refer to Figure 2 for cross-types designations. PK, solid circle; HK and HS (pooled), open square; PS, closed square. Solid lines represent significant relationships ($P < 0.05$, regression equations given) and dashed lines nonsignificant relationships ($P > 0.05$).

and the relatively small range in flesh and skin color among families within cross type (Figs. 2, 3).

Relationship between a^* Scores and Carotenoid Concentration

Higher carotenoid concentrations in the flesh and skin were reflected by higher chromameter a^* scores (Fig. 5). For immature fish the relationship was significant within each of

the cross types (Fig. 5A). Prior studies have reported an asymptotic relationship between a^* scores and carotenoid concentration for the flesh of salmonids (Torrissen et al. 1989; Smith et al. 1992; Hatlen et al. 1998). However, when pooled over cross type asymptotic (color = $26.2 \times \text{conc}/(36.3 + \text{conc})$, $n = 292$, $R^2 = 0.77$) and linear (color = $2.35 + 0.34 \times \text{conc}$, $n = 292$, $R^2 = 0.77$) models explained similar amounts of the variation. For skin color of mature crosses, the relationship was more variable (Fig. 5B), but again there was little difference between asymptotic (color = $106.5 \times \text{conc}/(522.6 + \text{conc})$, $n = 63$, $R^2 = 0.52$) and linear (color = $-0.87 + 0.24 \times \text{conc}$, $n = 63$, $R^2 = 0.54$) models.

Mate-Choice Experiments with Sockeye in the Wild

Sockeye males reacted in an unmistakable sexual manner to red female models and virtually ignored green models (Table 1). The maximum number of males in the quadrat at any one time was over twice as high for quadrats containing the red model. Further, the total number of contacts with the models was approximately 7.5 times greater for the red versus green model. The reaction to the red model was so strong that males, either singly or in groups of up to seven, participated in 63 discrete spawning events with the red model in 150 min of observation. In contrast, no spawnings occurred with the green model, positioned 2.5 m away.

DISCUSSION

As sockeye reinvaded freshwater habitats following the retreat of the Wisconsin ice sheets, they repeatedly gave rise to green-colored, rather than red-colored, nonanadromous breeding populations. This immediate and dramatic phenotypic shift in breeding color is demonstrated by repeated observations from independent systems of green, nonanadromous progeny of anadromous sockeye parents (i.e., residuals; Ricker 1940, 1959; Krogius 1981; Scott 1984; Quinn et al. 1998). The green breeding color of residuals is most likely due to a scarcity of available carotenoid pigments in lacustrine relative to marine environments. Diet differences within the lacustrine environment are an unlikely explanation because sockeye and kokanee morphs have near identical diets when both are resident in the nursery lake as juveniles (Wood et al. 1999). Although comparable estimates of carotenoids available to sockeye and kokanee do not exist, primary productivity, to which carotenoid abundance is correlated (Sanger 1988; Leavitt 1993), is greater in the marine than lacustrine environments (Gross et al. 1988). The large, environmentally induced size difference between anadromous and nonanadromous fish is a result of this difference

TABLE 1. Result of mate-choice trials of male sockeye salmon from Iliamna Lake, Alaska with red and green female models. Each value represents the mean \pm standard deviation for 30 independent, 5-min trials. Refer to text for definition of behaviors. Results of Wilcoxon signed rank test are given.

| Maximum no. males | | Total no. contacts | | Total no. quivers | | Total no. spawnings | |
|-------------------|------------------------------|--------------------|------------------------------|-------------------|------------------------------|---------------------|------------------------------|
| Red | Green | Red | Green | Red | Green | Red | Green |
| 6.2 \pm 2.9 | 3.0 \pm 1.5 | 52.4 \pm 26.2 | 7.0 \pm 6.6 | 3.0 \pm 3.0 | 0.04 \pm 0.2 | 2.1 \pm 2.5 | 0 |
| | $Z = -4.31$, $P < 0.001$ | | $Z = -4.70$, $P < 0.001$ | | $Z = -4.40$, $P < 0.001$ | | $Z = -3.90$, $P < 0.001$ |

in productivity (Ricker 1940; Wood and Foote 1990, 1996; this study). We argue that maintenance of a behavioral preference for red despite probable environmental variation in carotenoid availability drove the reevolution of red breeding color in kokanee via more efficient use of this resource. Below we discuss the proximate basis of differences in carotenoid use, sensory drive as the mechanism underlying phenotypic convergence in breeding color, and the role of breeding color and associated carotenoid use in the sympatric genetic differentiation of the morphs.

Proximate Basis of Adaptive Genetic Divergence in Carotenoid Use

Immature pure kokanee (PK) deposited approximately three times more carotenoids in their flesh than did pure sockeye (PS) when fed the same carotenoid-limited diet under controlled conditions. This difference in efficiency as juveniles translated into nonoverlapping differences in secondary sexual color at maturity. Mature PK crosses developed pinkish red skin, similar to their parents in the wild, whereas PS turned olive green, sometimes with a faint streak of red, similar to residuals (Ricker 1938, 1959; Krogius 1981) but in marked contrast to their bright red parents.

Males were redder at maturity than females in the wild (Fig. 1) and within each cross type reared in the hatchery (see Methods). In contrast to differences among the cross types, the redder breeding color of males does not reflect differences in carotenoid use from the diet but, rather, in the partitioning of stored carotenoids at maturity. There was no effect of sex on the color or amount of carotenoids stored in the flesh of juveniles (see Methods), indicating the differences between the sexes in mature skin color arose after the initial deposition of carotenoids in the flesh. Sockeye and kokanee produce large, carotenoid-rich eggs (Crozier 1970; Wood and Foote 1996), so it appears that females allocate pigments to the developing eggs at the apparent cost of allocation to their skin. Blount et al. (2000) noted that the biochemical importance of carotenoids in egg development could result in a trade-off with its use in the production of secondary sexual color in animals.

The more efficient use of carotenoids by kokanee probably results from an increased rate of absorption from the diet. Salmonids are inefficient at absorbing carotenoids (Torrissen et al. 1989, 1990; Hardy et al. 1990; March et al. 1990), indicating that significant variation in this trait likely exists. However, variation in rates of deposition into the flesh, as well as pigment metabolism and excretion may also exist (Torrissen et al. 1989). Whatever the mechanism underlying variation in flesh pigment concentration, use of stored carotenoids at maturity appears similar between the morphs. Each of the cross types mobilized virtually all of the pigment in the flesh during maturation, and differences in the amount of pigment deposited in the skin varied in relation to differences in carotenoids stored in the flesh (Fig. 4).

It is unlikely that other factors could account for the striking differences in color and carotenoid concentration observed. Because crosses were reared in fresh water, an alternative hypothesis is that sockeye require a saline environment for the absorption of carotenoids. However, there is no

known relationship between salinity and carotenoid use (Goodwin 1984), and studies with a closely related species (*O. mykiss*) revealed no difference in carotenoid use when reared in fresh or salt water (Storebakken and Choubert 1991; No and Storebakken 1992). Diet, density, and other potential differences in rearing environment were strictly controlled. Indeed, crosses shared the same rearing channels during the second year of the experiment when the majority of weight gain occurred. The small size differences among crosses arising from additive genetic variation in growth rate (Wood and Foote 1990, 1996) cannot account for observed differences in color (Craig 1995). Similarly, one could postulate that the switching on of a regulatory gene associated with smoltification (a series of physiological changes in preparation for migration to the sea) is required for sockeye to utilize carotenoids. However, this is not the case. All fish used in this study had undergone the smoltification process, yet differences persisted (Foote et al. 1994). Finally, the primary source of carotenoid pigments in the diet was krill (of marine origin), yet kokanee turned red and sockeye did not.

Color and associated carotenoid use in *O. nerka* appear under additive genetic control as has been reported in several other salmonid species (Torrissen and Naevdal 1984, 1988; Iwamoto et al. 1990; Elvingson and Nilsson 1994; Withler and Beacham 1994). The similar and intermediate color of both immature (flesh) and mature (skin) reciprocal hybrids indicates maternal or sex-linked effects were not important. Variation among families within cross type accounted for a small but significant amount of the variation in color (7–12%). Although our estimates of heritability (range = 0.26–0.79) include effects of dominance and common environment and are thus maximum estimates (Falconer 1989), they are reasonable given reported estimates for other salmonids (range = 0.2–0.5, references cited above). The significant additive genetic variation within morphs indicates the potential for carotenoid use to evolve rapidly in response to directional selection, giving rise to genetic differences between morphs.

Countergradient Sexual Selection in Breeding Color Promotes Sympatric Genetic Divergence

Our results indicate that as kokanee evolved from sockeye via residuals they converged on the same original red phenotype through genetic divergence in the use of carotenoids. Whereas this process is typically thought to occur in allopatric populations (Conover and Schultz 1995), several lines of evidence indicate that countergradient selection on breeding color promotes sympatric differentiation of sockeye and kokanee morphs. First, sockeye give rise to green nonanadromous progeny (residuals), not red ones (kokanee). Second, sockeye and kokanee occupy identical freshwater reproductive environments. Third, salmon, including sockeye and kokanee, are able to see red (Hawryshyn 1998), which is a common sexual signal in freshwater fishes (Rowland 1989; Milinski and Bakker 1990; Evans and Norris 1996; Houde 1997). Finally, red coloration is an important component of mate choice in sockeye, whereas green alone is not (Table 1). We argue that these conditions have facilitated the reevolution of the red phenotype in kokanee from green

residuals via sensory drive and that this process is an important mechanism contributing to the sympatric genetic differentiation of the morphs.

In general, sensory drive refers to interactions between the physical environment and animal sensory systems that bias the direction of trait evolution through effects on the generation, transmission, and detection of signals (Endler 1992; Endler and Basalo 1998). The visual system of salmonids (*Oncorhynchus* spp.) contains four cone types ranging in sensitivity from ultraviolet to red wavelengths (Hawryshyn 1998). During maturation and return to freshwater breeding habitats, there is a shift in the relative proportion of visual pigments vitamin A₁ and A₂ (rhodopsin and porphyropsin; Beatty 1966). In sockeye and kokanee, this shift affords a greater sensitivity to long (red) wavelengths, with peak absorption at about 625 nm and sensitivity up to about 710 nm (Novalles-Flamarique 2000). Similar shifts have been found in other salmonids (Beatty 1966) and other diadromous species (Lythgoe 1979; Hawryshyn 1998). These are generally considered adaptations to the long-wavelength-biased transmission properties of shallow, freshwater environments (Lythgoe 1979; Novalles-Flamarique et al. 1992; Novalles-Flamarique and Hawryshyn 1993; Hawryshyn 1998). The increased sensitivity likely depends on red-green color opponent neurons that are sensitive to red signals on the typical green background of freshwater environments (Lythgoe 1979; Endler 1990; McDonald and Hawryshyn 1995). Return by sockeye and kokanee to the same breeding habitat with spectral properties conducive to the use of red as a visual signal could lead to the evolution of carotenoid-based red color as a sexual signal in both morphs, as is relatively common in other freshwater fishes (references cited above).

In support of this hypothesis, sockeye males strongly preferred red female models and virtually ignored green models based on every mate choice criteria considered. Although not conclusive proof that sockeye exhibit true color (hue) preference, because brightness and brightness contrast also differed between the models, such color choice is likely. The specific spectral properties used by sockeye in selecting mates are the subject of ongoing studies. Whereas the green model elicited virtually no response from males when presented alone (Table 1), the green head of *O. nerka* may function as a chromatic amplifier of carotenoid-based red color as has been suggested in other species (Endler 1991; Houde 1997). All hatchery-reared crosses of *O. nerka* showed the same green head color as sockeye and kokanee in the wild (Figs. 1B, 3C).

The preference for red over green in sockeye suggests that residuals would possess the same preference, even though they themselves are green at maturity. Color preferences are heritable in many fishes (Houde 1992, 1997) and likely also in sockeye because they are semelparous and have little opportunity to learn color preference. Supporting this, we know that kokanee, like sockeye, prefer red over green models (Drake 1999). Given a preference for red over green in the ancestral morph (sockeye) and the green breeding color of the freshwater progenitors of kokanee (residuals), our results are consistent with the hypothesis that sensory drive for red resulted in the reevolution of red breeding color in kokanee via more efficient use of the limited dietary carotenoids avail-

able in fresh water. Thus, sensory drive may promote genetic divergence not only by selecting for different sexual phenotypes (Lande 1981; Endler 1992; Schluter and Price 1993), but through selection for the same phenotype. This is a novel, yet hidden, means by which sexual selection promotes divergence of these sympatric populations.

Although variation in color can lead to assortative mating and even speciation in fishes (Seehausen et al. 1997; Galis and Metz 1998; Seehausen and Alphen 1998), it is unclear if phenotypic convergence in color of sockeye and kokanee has resulted in a decrease or increase in interbreeding. Males and females of both morphs select mates based on relative size (Foote 1988, 1989; Foote and Larkin 1988). Whereas the morphs successfully interbreed in the wild (Foote et al. 1997) and hybrids are viable and fertile under hatchery conditions (Wood and Foote 1996), the direction of gene flow is almost totally limited to kokanee males sneaking on spawning sockeye pairs. Given that female sockeye select strongly against small males, apparently independent of color (Foote and Larkin 1988), and have little control over the spawning success of sneaker males, the color of the much smaller residual or kokanee males may have little bearing on their reproductive success relative to interbreeding with sockeye. However, if kokanee females discriminate mates based on color, selection may act against residuals because they are green rather than red. We do not know mate color preferences of female kokanee or of residuals. However, in Takla Lake, British Columbia both sockeye and kokanee males prefer red over green models (Drake 1999).

It is more likely that variation in breeding color and associated carotenoid use promote sympatric genetic differentiation as one of a suite of traits at which hybrids are at a disadvantage. In Narrows Creek, we have found evidence of quantitative genetic divergence in color (this study), early development (Craig et al. 1996), growth (this study; Wood and Foote 1996), maturity (this study; Wood and Foote 1996), and gill raker morphology (Foote et al. 1999). We have also found differences in smoltification (Foote et al. 1992), swimming performance, and body morphology (Taylor and Foote 1991) in other systems. These differences appear associated with selective differences between the marine and lacustrine environments and collectively are thought to result in the near complete mortality of hybrids (Wood and Foote 1996). In contrast to previously examined traits, however, genetic differences in color were virtually nonoverlapping between sockeye and kokanee, suggesting countergradient variation in carotenoid use is a particularly important, but subtle process contributing to postzygotic isolation.

In conclusion, our results demonstrate that phenotypic convergence in red breeding color masks striking genetic divergence in the use of dietary carotenoid pigments between sympatric morphs of the same species. Further, a bias in the sensory system toward red wavelengths of light appears to be the mechanism underlying countergradient variation in carotenoid use. Such countergradient variation for condition-dependent sexual traits may be important in other systems. Whether environmental variation in resources promotes phenotypic convergence in sexual traits will depend in part on the existence and amount of genetic variation in mating preferences and the trait itself. In contrast to sockeye and ko-

kanees, where available evidence suggests mating preferences for red breeding color are maintained, guppy (*Poecilia reticulata*) populations vary extensively in their preference for the area of carotenoid-based orange color (Houde and Endler 1990; Endler and Houde 1995). Furthermore, this variation in preference generally parallels phenotypic variation in the expression of the trait. However, similar preferences for carotenoid-based red color have been identified between populations that differ in expression of the trait (guppies: Houde and Hankes 1997; sticklebacks: Reimchen 1989; McKinnon 1995; house finches: Hill 1993, 1994; reviewed in Houde 1997), in some cases attributed to limited availability of carotenoids (Reimchen 1989; Hill 1993). Whereas alternative sexual traits not dependent on the acquisition of carotenoid pigments may evolve under these circumstances, selection for more efficient use of carotenoids is possible as well. Whatever the case, given the widespread use of carotenoid-based red color as a signaling trait and the dependence of animals on the environment for acquisition of these pigments, genetic divergence in carotenoid use not evident in phenotypic comparisons may be present in other systems as well.

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