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Consequences of farmed–wild hybridization across divergent wild populations and multiple traits in salmon

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Abstract. Theory predicts that hybrid fitness should decrease as population divergence increases. This suggests that the effects of human-induced hybridization might be adequately predicted from the known divergence among parental populations. We tested this prediction by quantifying trait differentiation between multigenerational crosses of farmed Atlantic salmon (*Salmo salar*) and divergent wild populations from the Northwest Atlantic; the former escape repeatedly into the wild, while the latter are severely depleted. Under common environmental conditions and at the spatiotemporal scale considered (340 km, 12 000 years of divergence), substantial cross differentiation had a largely additive genetic basis at behavioral, life history, and morphological traits. Wild backcrossing did not completely restore hybrid trait distributions to presumably more optimal wild states. Consistent with theory, the degree to which hybrids deviated in absolute terms from their parental populations increased with increasing parental divergence (i.e., the collective environmental and life history differentiation, genetic divergence, and geographic distance between parents). Nevertheless, while these differences were predictable, their implications for risk assessment were not: wild populations that were equally divergent from farmed salmon in the total amount of divergence differed in the specific traits at which this divergence occurred. Combined with ecological data on the rate of farmed escapes and wild population trends, we thus suggest that the greatest utility of hybridization data for risk assessment may be through their incorporation into demographic modeling of the short- and long-term consequences to wild population persistence. In this regard, our work demonstrates that detailed hybridization data are essential to account for life-stage-specific changes in phenotype or fitness within divergent but interrelated groups of wild populations. The approach employed here will be relevant to risk assessments in a range of wild species where hybridization with domesticated relatives is a concern, especially where the conservation status of the wild species may preclude direct fitness comparisons in the wild.

Key words: conservation; domestication; F_1; F_2; intraspecific hybridization; life history; outbreeding depression; population differentiation; population divergence; salmon.

Introduction

Predicting the consequences of multigenerational hybridization between divergent populations has long been a challenge in ecology. Indeed, in many instances, recombination between generations will dramatically change gene combinations in hybrids with unpredictable and either beneficial or negative fitness outcomes (Dobzhansky 1948, Templeton 1986, Coyne and Orr 1989, Barton 2001, Edmands 2002, 2007, Hufford and Mazer 2003). As the rate of human-induced, intraspecific hybridization increases, there is a growing need to understand its consequences for several conservation issues. In some cases, information is required on the possible benefits to wild species from intentional hybridization, such as when small, fragmented populations are outbred to offset known effects of inbreeding (Edmands 2007). In other cases, information is needed on the potentially negative impacts of accidental hybridization between artificially selected and wild organisms (Ellstrand 2003).

Although evolutionary theory predicts greater reductions in hybrid fitness with increasing divergence between parental populations (Barton 2001, Edmands 2002), this has rarely if ever been empirically tested in cases dealing with human-induced intraspecific hybridization, despite its potential value for conservation. Consider the frequent concern regarding hybridization between artificially selected and wild organisms (Ellstrand 2003, McGinnity et al. 2003, Bowman et al. 2007, Randi 2008). If the reduction in mean fitness in different wild populations resulting from hybridization could be adequately predicted a priori from the

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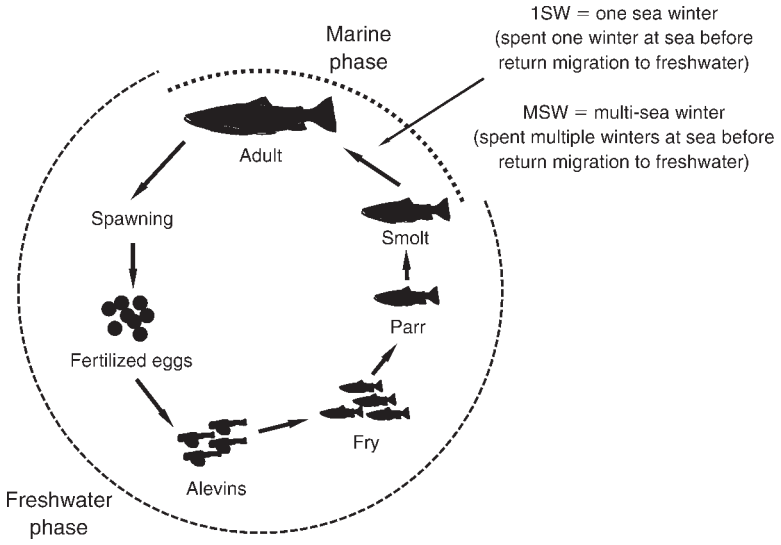

FIG. 1. The Atlantic salmon life cycle.

collective environmental and life history differentiation, genetic divergence, and the geographic distance between parental populations (a proxy for the degree of adaptive and/or evolutionary divergence between populations; Edmands 2002), this would be beneficial in two ways. First, it would provide insight into which populations might be most negatively impacted from hybridization. Second, we might be able to learn enough to predict reasonably well the effects of hybridization.

Multigenerational hybridization studies that test theoretical predictions may be especially necessary for fish species important to both fisheries and aquaculture. For at least 25 years, concerns have been raised about the potential loss of local adaptation and outbreeding depression that could occur in declining wild populations when escaped farmed fish enter the wild and interbreed with wild fish (International Council for the Exploration of the Sea 1984, Hansen 1989, Verspoor 1989, Hindar et al. 1991, 2006, Hutchings 1991*a*, Waples 1991, McGinnity et al. 1997, 2003, Fleming et al. 2000, Naylor et al. 2005, Bekkevold et al. 2006, McClelland and Naish 2007, Thorstad et al. 2008). However, the usually long generation times (several years) and large adult body sizes (several kilograms) of exploited/farmed fishes render them as expensive and time consuming models for studying multigenerational hybridization (Hutchings and Fraser 2008). Hence, few studies have carried out multigenerational crosses between farmed and wild fish, and these have been restricted to single interpopulation comparisons (McGinnity et al. 2003, McClelland et al. 2005, Tymchuk et al. 2006, 2007).

A remaining challenge hindering risk assessments is in predicting the potentially different effects of farmed–wild hybridization among different wild populations. Within a given region, different effects are likely even if only one farmed strain is escaping into the wild. This is because within freshwater or marine fish species, the extent of population differentiation is a result of the interplay between selective pressures at different life stages, gene flow, genetic drift and/or mutation arising through periods of historical isolation or vicariance (Hutchings and Fraser 2008).

Herein we test the prediction of greater reductions in farmed–wild hybrid fitness as parental divergence increases in Atlantic salmon (*Salmo salar*), a species with a complex, migratory life cycle (Fig. 1). We specifically examine the consequences of interbreeding between farmed Atlantic salmon and population representatives from two groups of ecologically and genetically distinct wild populations. Wild population representatives from these groups, Tusket (TUSK) and Stewiacke (STEW) Rivers (Fig. 2), are both severely depleted and within 250 km of most salmon farming activity in eastern North America (Department of Fisheries and Oceans Canada 2003, Committee on the Status of Endangered Wildlife in Canada 2006). Regional farmed salmon (FARM) are derived from the Saint John River, a population within a third, recognized group of wild salmon populations (Fig. 2). Thus, while FARM salmon are "locally" derived, their ancestor differs genetically from many surrounding wild populations, including TUSK and STEW (Tables 1 and 2; Appendix A). In addition, when our research was initiated in 2001, FARM salmon had already undergone four generations of artificial selection, primarily for faster growth and delayed maturity (Glebe 1998). For the first two generations of artificial selection, it is known that mass gains of $\approx$10% per generation (SD = 0.7–0.9) in FARM salmon were generated (Friars et al. 1995, O'Flynn et al. 1999). Details of later generation selection intensities are lacking (B. Glebe, *personal communication*). Escaped FARM salmon have been detected in most rivers within 300 km of farming, including TUSK and STEW (Morris et al. 2008), and

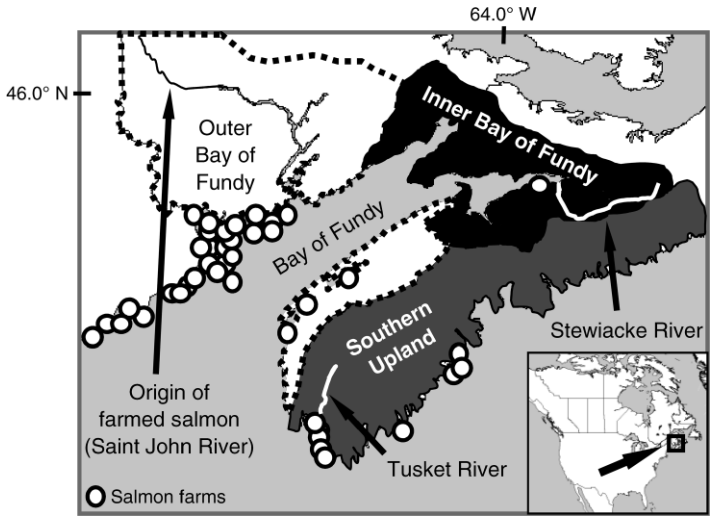

FIG. 2. Map of the location of Atlantic salmon study populations, the regional groups of wild populations from which they are derived (Outer Bay of Fundy, Inner Bay of Fundy, Southern Upland), and the general location of regional salmon farms (adapted from Committee on the Status of Endangered Wildlife in Canada [2006] and Morris et al. [2008]). Some farm sites may include other salmonids.

likely interbreed with regional wild salmon (O'Reilly et al. 2006).

A review of existing population divergence data between TUSK, STEW, and the FARM ancestor reveals that, based on the geographic distance separating populations and neutral genetic divergence, TUSK and STEW are more divergent from one another than either is from the FARM ancestor (Tables 1 and 2). For environmental and life history differentiation, TUSK is more differentiated at early-life and juvenile stages from the others, with the differences between STEW and the FARM ancestor being the smallest. Conversely, however, STEW is more differentiated from the others at subadult/adult stages (Table 1). Collectively, one might expect (1) a greater reduction in hybrid fitness resulting from TUSK–STEW hybridization than for hybridization between TUSK or STEW with FARM and (2) intermediate reductions in hybrid fitness for each farmed–wild comparison, but in varying ways with respect to adaptive divergence owing to differences between FARM and TUSK (mainly earlier stages) vs. FARM and STEW (mainly later stages).

Such predictions must remain tentative because they are based on a three interpopulation comparison (i.e.,

TABLE 1. The collective divergence known a priori between parental populations used in this study, based on environmental and life history differentiation.

Age and parameter	Population			References
	TUSK	STEW	FARM†	
Environmental/life-history differentiation				
Juvenile				
Water temperature (winter)	2–4°C‡	1–2.5°C	1–2.5°C§	1, 2
Air temperature (winter)	−2.1°C	−4.8°C	−5.2°C	2
River pH	4.6–5.2	>6.0	6.0–6.47	1, 3, 4, 5
Smolt age at migration	2.1 years	2.6 years¶	2.54 years	6, 7, 8
Smolt migration timing	early–mid May	Jun–Jul¶	Jun	6, 9, 10
Surface geology of rivers	metamorphic rock	limestone	limestone	3, 4, 5, 11
Subadult or adult				
Adult arrival time in rivers	late May–Jul#	Aug–Oct	late May–Aug	6, 7, 9, 12
Adult age composition	1SW (65%), 2–3SW	1SW (94%)	1SW (60%), 2SW	8, 12, 13, 14
Percentage of 1SW females	40–60%	72%	14%	8, 12, 13, 14
Marine feeding areas	Greenland	Gulf of Maine	Greenland	6, 9, 14, 15
Marine migration distance	2500–3000 km	500–1500 km	2500–3000 km	6, 9, 14, 15

Notes: Geographic distance represents the shortest geographic distance between river mouths via sea water. References: 1, Lacroix (1985); 2, Environment Canada (2005); 3, Watt (1986); 4, Watt (1987); 5, Lacroix and Knox (2005); 6, Jessop (1986); 7, Amiro et al. (2000); 8, Hutchings and Jones (1998); 9, Ritter (1989); 10, Amiro (2003); 11, Roland (1982); 12, Marshall (1986); 13, Amiro (2000); 14, Committee on the Status of Endangered Wildlife in Canada (2006); 15, Jessop (1976). 1SW = one sea winter (salmon that return to spawn after one winter at sea); 2SW = two sea winters; 3SW = three sea winters (see Fig. 1).

† Ancestor of the farmed strain used in regional aquaculture (Saint John River, New Brunswick, Canada).

‡ Data from four tributaries within a geographically proximate river (Medway River; Lacroix 1985).

§ Data from a geographically proximate river (Digdeguash River; Lacroix 1985).

¶ Data from another population representative within the inner Bay of Fundy (Big Salmon River; Jessop 1986).

Data from the LaHave River population (another Southern Upland representative population; Amiro et al. 2000).

TABLE 2. The collective divergence known a priori between parental populations used in this study, based on genetic divergence and geographic distance separating populations.

Population	STEW		FARM	
	Geographic distance (km)	Genetic distance	Geographic distance (km)	Genetic distance
TUSK	340	0.431†, 0.405‡, 0.058§	220	0.198†, 0.167‡, 0.033§
STEW			200	0.240†, 0.225‡, 0.035§

Note: See Appendix A for more on genetic distances.
† Nei's (1972) standard genetic distance (D_S; all values were significantly different from one another).
‡ Nei's (1978) unbiased genetic distance (D; all values were significantly different from one another).
§ Genetic differentiation (θ_{ST}; all values were significantly different from one another).

three data points; see Johnson 2000). They also assume that aquaculture elicited no or minimal evolutionary changes in FARM salmon, an issue we treat in more detail in *Discussion*. Bearing this in mind, we compared differentiation at a suite of traits between TUSK, STEW, and FARM salmon, and their multigenerational hybrids (F_1 = farmed or wild × wild, $F_2 = F_1 \times F_1$, backcrosses = F_1 × wild). We used common garden experimentation because the small size and critical conservation status of regional wild salmon prevented us from comparing their performance to farmed salmon in nature.

MATERIALS AND METHODS

Parental populations and crosses in 2001

In 2001, unfertilized gametes were collected from sexually mature adults originating from FARM (fourth-generation, artificially selected), TUSK, and STEW. TUSK adults were obtained from the wild; STEW adults had been collected as one or two year old juveniles in the wild and subsequently raised to sexual maturity in captivity. Gametes were transferred to Dalhousie University (Halifax, Canada) and used to generate 10 full-sibling families of each of the three parental and three F_1 hybrid crosses (Lawlor et al. 2009; Fig. 3). These crosses were then raised until maturity under common environmental conditions (i.e., temperature ± 0.1–0.15°C, food regimes, densities, dissolved oxygen, pH = 7.0; tank dimensions for fertilized egg to parr stages, 67.3 cm diameter, 45.7 cm height, or 100 L water volume; smolt to adult stages, 201.9 cm diameter, 76.2 cm height, or 1800 L water volume). We cannot discern whether these rearing conditions might be more similar to one parental environment than the other. For instance, water originated from a natural watershed near Dalhousie University (Pockwock Lake, at a latitude between TUSK and STEW rivers). Water temperatures were also allowed to fluctuate naturally over the incubation period and from juvenile to adult stages, ranging from 3° to 6°C and 7° to 21°C, respectively. Thus, incubation and rearing temperatures approximated those to which regional wild salmon are naturally exposed (2–4°C, 5–22°C, respectively; Lacroix 1985, MacMillan et al. 2005). However, for practical reasons, rearing densities were higher than found in the wild, being more similar to those to which FARM salmon are normally exposed (Thorstad et al. 2008). Similarly, experimental pH (7.0) more typified the conditions that FARM and STEW salmon were normally exposed (pH = 6.0–6.5; TUSK pH = 4.6–5.2; Fraser et al. 2008).

Crosses in 2005

Crosses generated in 2001 reached maturity in 2005; these were then used to re-generate the same three

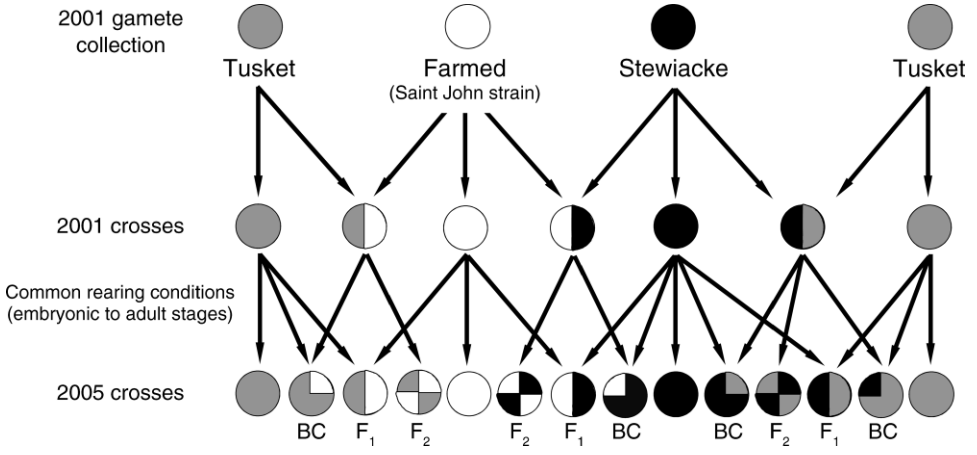

FIG. 3. A general flow diagram of the study's cross design.

parental populations and three F_1 hybrid crosses, as well as to create three F_2 hybrids and four backcrosses (i.e., for a total of 13 crosses; Fig. 3). Crosses were performed on 22, 25, and 29 November and 6 December 2005. Prior to generating these crosses, all adults (i.e., from crosses generated in 2001) were individually tagged and fin clipped for DNA analysis (five polymorphic microsatellite loci). To avoid inbred matings (i.e. full-sib, half-sib, or cousin), parentage assignments were then performed to assign adults back to their respective families (Duchesne et al. 2002). The pool of potential parents against which offspring were compared was always relatively small ($n = 20$). Nevertheless, we elected to use a likelihood-based rather than an exclusion method here because the latter makes use of allele frequency information (Jones and Ardren 2003), permitting assignment of offspring when two or more sets of parents are compatible with a given offspring across all loci examined. This was important in our study because a few of the female–male pairings within certain crosses in 2001 did not exhibit diagnostic suites of alleles, even with the high polymorphism (mean = 17 alleles per locus; range 11–26) and heterozygosity (mean = 0.86; range 0.78–0.93) of the five microsatellite loci employed. Despite this, the overall parentage assignment error rate based on simulated data was low, averaging 2.6% and ranging from 0.9 to 5.8% within crosses.

From the context of *within* a cross, a cross consisted of 9–23 chiefly full-sibling families, except for TUSK and all F_2 hybrids in which each female was crossed to two or three different males (details in Appendix B). Families comprised ≈500 eggs each and were randomly allocated to one of three compartments nested within one of 60 circular 100-L (water volume) tanks that received the same flow-through water source. Compartments were of equal size, separated by equal distances, and had their bottoms and sides drilled out and filled with a thin-mesh screen to ensure sufficient oxygenation for eggs and hatching alevins. Under common environmental conditions as described above, eggs were incubated in the dark at temperatures of predominantly 3.4–4°C until hatching commenced in March 2006.

Trait differentiation

Detailed trait descriptions, measurements, and sample sizes are found in Appendix C. We first quantified and compared differentiation in maternal body size and egg size of the mature parental and F_1 hybrid mothers used to generate 2005 crosses. We then compared cross differentiation at five early life history traits: length at hatch, yolk sac volume at hatch, length at yolk absorption, yolk sac conversion efficiency, and embryonic survival. These traits (or changes to them) may be linked to fitness as early growth and size influence the probability of surviving to maturity in salmonids (Metcalfe and Thorpe 1992, Koskinen et al. 2002). Subsequently, six juvenile/subadult traits were compared. First, three body size traits, and as a proxy for growth, changes to these traits over time, were measured up to 1108 days post-fertilization (length, mass, condition factor). Second, we compared two body morphology "traits" between crosses on days 341–342 (shape differences along two multivariate axes), as well as the percentage of two year old "smolts," the life stage when juveniles undergo physiological transformations before migrating to sea water in the wild (Fig. 1). Salmonid body size and growth can influence the age/size at which individuals migrate or reach maturity, which in turn may affect individual fitness (Hutchings 1991*b*, Beckman and Dickhoff 1998, Garcia de Leaniz et al. 2007). Additionally, salmonid body morphology may influence swimming efficiency in relation to flow regime, migration distance, or predator avoidance, with purported links to fitness among populations (Taylor and Foote 1991, Hawkins and Quinn 1996).

Trait statistical analyses

For each interpopulation comparison, we were primarily interested in testing whether (1) parental populations differed in mean trait values from one another and (2) hybrids differed from their wild parental populations. Depending on trait sampling characteristics, we used either generalized linear models (GLMs) or generalized linear mixed models (GLMMs) to test our hypotheses (Appendix D). These were fitted with appropriate error distributions to account for overdispersion, nonnormality and/or heterogeneous variance in the data; data transformation was also carried out if this improved model fit (Appendix D). Where applicable, GLMMs included cross and/or day as fixed effects and either mother (egg size), family (length at hatch, yolk sac volume, length at first feeding), or tank (body size attributes or morphology) as random effects (Appendix D). To complement body morphology GLMMs, discriminant function analysis (DFA) was used to assess how confidently individuals from parental populations could be reassigned correctly using a jackknifed classification procedure. Finally, for each trait, we compared variability in trait values between crosses (coefficient of variation = CV = SD/mean) using GLMs (or GLMMs if permissible by data structure) as outlined above. For the five early life history traits, CVs calculated across families were combined in one analysis. Body size and morphology CVs were estimated for each tank and compared separately.

The early life history traits we measured are well-known to be influenced by maternal effects in salmonids (Beacham and Murray 1990, Perry et al. 2005). Thus, when crosses differed from one another at one of these five traits, we carried out additional GLMs or GLMMs to account for potential maternal effects, using the subsets of families that originated from the same mothers within an interpopulation comparison (Appendix B). These models included mother and cross

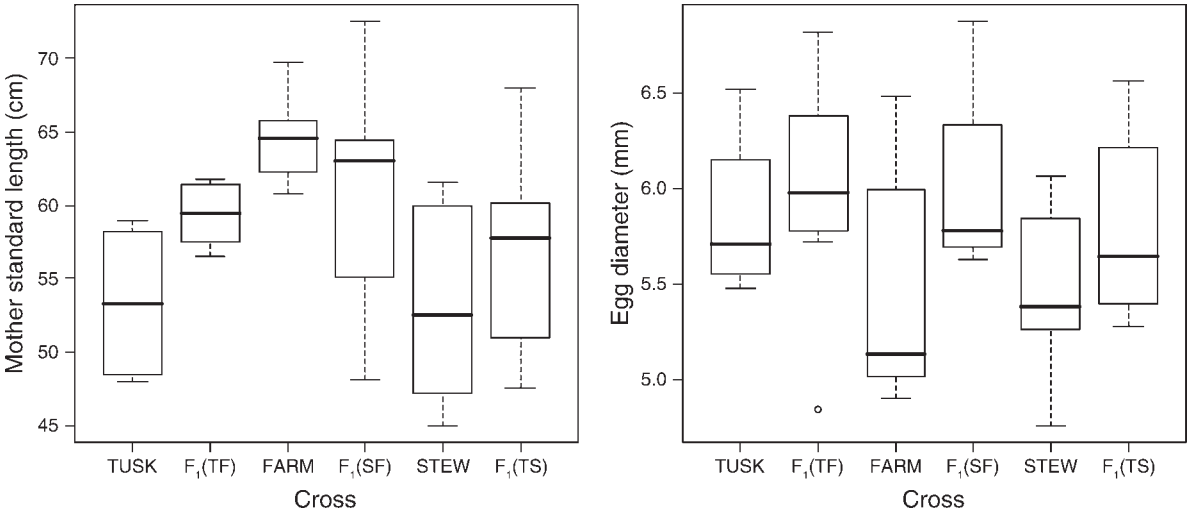

FIG. 4. Boxplots of maternal standard length and egg diameter for different crosses. The order of the three interpopulation comparisons from left to right is TUSK–FARM (TF), FARM–STEW (SF), STEW–TUSK (TS). The lower and upper ends of each box represent the 25th and 75th quartiles, respectively. Medians are represented by the bold bar in each box. Skewness is reflected by the position of the median relative to the ends of each box. Whiskers extend from the top and bottom of each box to data no more than 1.5 times the inter-quartile range; values beyond this range (outliers) are represented by open circles.

as fixed effects; GLMMs included random mother effects.

Evaluating the overall effects of hybridization

We considered several approaches to evaluate outbreeding effects, as no general consensus on how to do so exists across diverse taxa (Lynch 1991, Edmands 2007). To increase the breadth of our assessment, we included three additional juvenile traits from previous studies involving the same crosses: two anti-predator responses (Houde et al. 2010) and embryonic developmental rates (D. J. Fraser et al., *unpublished manuscript*). All methods assumed that each trait was independent. Note that anti-predator responses were not assessed in TUSK–STEW hybrids.

TABLE 3. Summary of trait differences for various interpopulation crosses relative to wild parental Tusket (TUSK) or Stewiacke (STEW) values (as intercepts), based on GLM or GLMM. Directions of arrows indicate whether mean trait values were significantly higher (↑) or lower (↓) than wild parental values. For length and weight, only interactions between cross and day are listed, as these signify whether or not changes in body size over time differed between crosses (see Figs. 4–8 for a visual representation). Note that only traits with significant differences in CV among crosses are reported.

Trait	FARM	F_1 (TF)	F_2 (TF)	BC (T × TF)	FARM	F_1 (SF)	F_2 (SF)	BC (S × SF)
Maternal body size (cm)	↑***	↑**	NA	NA			NA	NA
Maternal egg diameter (mm)			NA	NA		↑*	NA	NA
Length at hatch (mm)					↑*	↑*	↑**	↑**
Yolk sac volume (mm^3)	↓*							
Length at first feeding (mm)			↑*		↑*	↑*		↑*
Yolk sac conversion efficiency (mm/mm^3)						↓*	↓*	↓**
Embryonic survival			↓*					
Length (cross × day)‡	↓*	↓*	↓*	↓*	↑**	↑**	↑*	↑*
Mass (cross × day)‡					↑***	↑***	↑***	↑*
Condition factor ($g/cm^3 \times 10\,000$)	↓*		↑*					
Condition factor (cross × day)			↓*	↓*				
Percentage age-2 smolts		NA	NA	NA	↑***	↑***	↑***	↑*
Body morphology (RW1)	↓***	↓***			↑***	↑†		
Body morphology (RW2)	↑***	↑***	↑***		↑***			
CV body morphology (RW1)	↑*							
CV body morphology (RW2)			↑*					

Note: Abbreviations are: T, TUSK; F, FARM; S, STEW.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.10$; NA, not applicable or not available.

‡ By day 930, there were no statistical differences in body size attributes between TUSK, FARM, and their hybrids. By day 1108, FARM and F_1 TUSK–FARM hybrids weighed significantly more than TUSK, and were marginally longer (see Appendix C: Table C1).

Line cross analysis.—To explore the genetic basis underlying population differentiation, we employed the joint-scaling procedure based on weighted least-squares regression (outlined in Lynch and Walsh 1998). The procedure firstly evaluated the fit of the data for each trait (means and variances) in each interpopulation comparison to a simple additive model of genetic differentiation. If this initial model was not adequate to fit the data, model building proceeded to a more complex model incorporating dominance. With only five crosses in two of the three interpopulation comparisons, genetic models accommodating epistatic interactions were only subsequently tested in the TUSK–STEW comparison (six crosses) if the model incorporating dominance was not adequate to fit the data. Significance was assessed using a χ^2 goodness-of-fit test statistic; a likelihood-ratio test was used to determine whether the additive-dominance model yielded a significantly better fit over the simpler additive one (or in the TUSK–STEW comparison, whether an epistatic model yielded a better fit than an additive-dominant one; Lynch and Walsh 1998). We considered the results of this procedure cautiously for traits with a potential maternal influence. Note that the test also assumed trait normality which was not met with some of our data. The test was also based on a diploid model of inheritance but a suitable model accounting for residual tetraploidy in Atlantic salmon currently does not exist (McClelland and Naish 2007, Fraser et al. 2008).

Hybrid deviations from parental population midpoint values.—We assessed whether hybrid means across all traits differed from expected values assuming additive gene action. The degree to which hybrid trait means deviated from parental population midpoint values was calculated using $[(X_{\text{hybrid}}/X_{\text{midparent}}) - 1]$ (Edmands 2007). Midpoint means for comparisons involving F_1 and F_2 hybrids were calculated as $1/2(P_i + P_j)$, and as $3/4P_i + 1/4P_j$ for BC hybrids, where P_i and P_j were the means for parental population crosses i and j, respectively. Although this analysis assumed that traits were normally distributed and it did not account for potential cross differences in variance, it permitted a standardized comparison between hybrid and parental population means across all traits.

Magnitude of trait differentiation between hybrids and parental populations.—Across all traits, we estimated the absolute proportional change in the mean of each hybrid class (F_1, F_2, BC) relative to its parental populations. For farmed–wild comparisons, this was calculated relative to the wild parental population mean and relative to STEW in the TUSK–STEW comparison. Similarly, we estimated by how many parental standard deviations (SD) the hybrid mean departed from the parental population mean. These calculations also assumed normality in each trait. We also tested for differences in skewness and kurtosis between hybrids and their parental populations, based on the proportion of traits showing significantly positive/negative skew or kurtosis values, or differences in the means of these values across traits. For example, F_2 generation recombination may generate novel variation and result in flattened distributions (Rieseberg et al. 1999).

TABLE 3. Extended.

TUSK	F_1 (TS)	F_2 (TS)	BC (T × TS)	BC (S × TS)
		NA	NA	NA
		NA	NA	NA
↑*	↑*	↑**	↑*	↑*
↑*	↑*		↑*	
↑*	↑*		↑*	
↓†	↓*	↓*	↓*	
		↓†	↓†	
↑***	↑***	↑**	↑***	
↑***			↑**	
↓*	↓*	↓*	↓*	
↑*	↓*	↑*	↑**	
↑***	NA	NA	NA	NA
↑***	↑***	↑***	↑***	
↓***	↓***	↓†	↓***	
	↑*			
↓*	↓*		↓*	↓*

RESULTS

Trait differentiation

Maternal body size and egg size.—Maternal standard lengths differed between populations in comparisons involving FARM, with FARM mothers being longer than TUSK or STEW; F_1 hybrids were generally intermediate in length relative to both parents (Fig. 4; Table 3). Egg diameter was only significantly different (larger) in F_1 STEW–FARM hybrids relative to both parents (Table 3), but all three F_1 hybrids exhibited a tendency to exceed parental trait values (Fig. 4).

Early life-history traits.—Length at hatch differed only in comparisons involving STEW, with STEW being shorter than all other parents and interpopulation hybrids; STEW also had smaller yolk sacs relative to TUSK and some TUSK–STEW hybrids (Fig. 5; Table 3). Length at hatch was not correlated with yolk sac volume at hatch across crosses (Pearson's $r = 0.36$, $P = 0.23$), and generally, within each interpopulation comparison, crosses with larger yolk sacs had poorer yolk sac conversion efficiencies (Fig. 5). Thus, by the time of first feeding, length relationships between hybrids and parental populations exhibited similar trends to length at hatch, the notable exception being F_2 TUSK–FARM hybrids which were longer than both parental populations (Fig. 5; Table 3). For all four traits, analyses

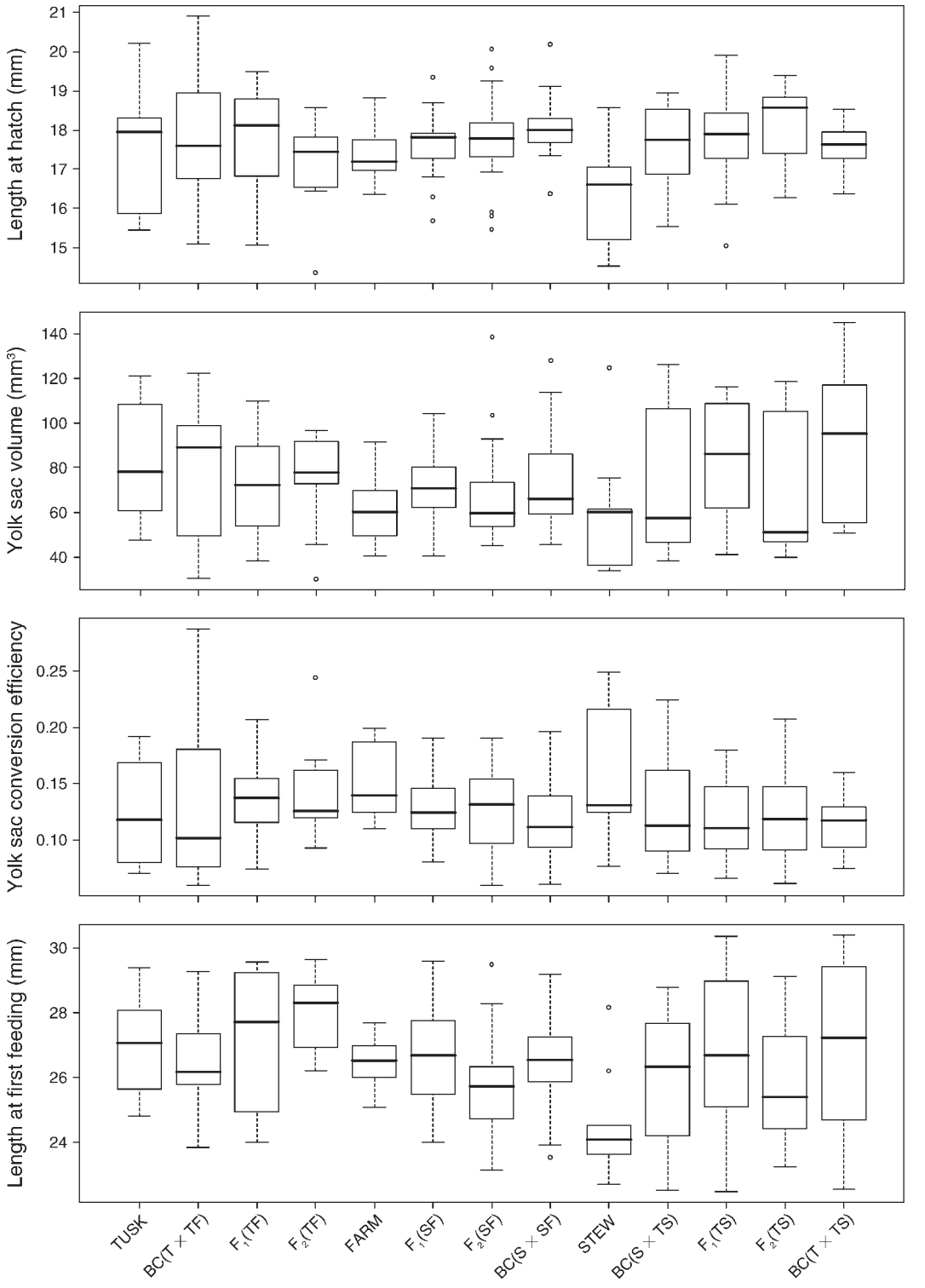

FIG. 5. Boxplots of length at hatch, yolk sac volume at hatch, yolk sac conversion efficiency, and length at first-feeding. The order of the three interpopulation comparisons from left to right is TUSK–FARM (TF), FARM–STEW (SF), STEW–TUSK (TS). Note the differences in units along the *y*-axes.

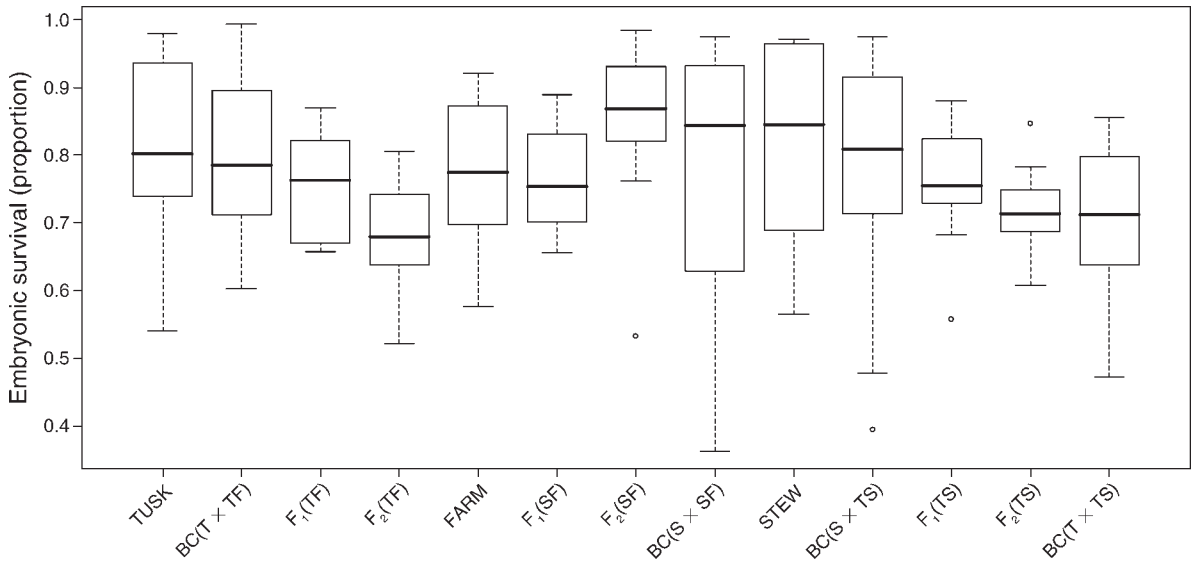

FIG. 6. Boxplots of embryonic survival for different crosses. The order of the three interpopulation comparisons from left to right is TUSK–FARM (TF), FARM–STEW (SF), STEW–TUSK (TS).

accounting for maternal effects indicated that both cross (GLMs, all $P < 0.001$) and mother (GLMs, all $P < 0.052$) explained a significant amount of the variation in the data. Thus, each trait was influenced by individual maternal effects, but trait differences existed between crosses even after accounting for these effects.

The only differences in embryonic survival involved F_2 TUSK–FARM hybrids, which had significantly lower survival than their parental populations, and a trend for F_2 TUSK–STEW and BC TUSK–STEW hybrids (backcrossed to TUSK) to have reduced survival relative to parental populations (Fig. 6; Table 3). However, based on the subsets of families that originated from the same mothers within an interpopulation comparison, survival differences were attributable to significant differences between mothers (GLMs: all $P < 0.034$), and not crosses (GLMs; all $P > 0.706$). Across all early life history traits, no differences in CV were detected between crosses in each interpopulation comparison (GLM; all $P > 0.769$).

Juvenile to subadult body size.—All three interpopulation comparisons exhibited differences in body size-at-age and growth (changes to body size over time) (Table 3; Appendix C: Table C1). STEW salmon grew slower than TUSK or FARM (Fig. 7). Body size changes varied less between TUSK and FARM, with TUSK growing faster than FARM to day 482, but FARM growing faster than TUSK by day 1108 (Fig. 7; Table 3; Appendix C: Table C1). Over time, TUSK salmon generally had a higher condition factor than STEW salmon (Table 3).

TUSK–FARM hybrids fluctuated in their body size over time relative to each parental population but were generally intermediate to TUSK and FARM; the exception was F_2 TUSK–FARM hybrids which were larger than either parental population following yolk absorption (day 211), though these differences diminished by day 344 (Fig. 7; Table 3; Appendix C: Table C1). All STEW–FARM hybrids were slightly more similar in body size to FARM than to STEW; all TUSK–STEW hybrids were intermediate in body size relative to both parental populations to day 482, and slightly more similar in body size relative to TUSK than STEW by days 930 and 1108 (Fig. 7; Table 2; Appendix C: Table C1). TUSK and STEW also had higher and lower condition factors, respectively, than most or all TUSK–STEW hybrids (Table 2). Condition factor differences between crosses changed over time (i.e., a significant cross × day interaction in our models) but with no consistent trends (Table 3).We also detected no differences in CV of body size between crosses in any interpopulation comparison (GLMs; all $P > 0.20$).

Percentage age-2 smolts.—STEW had a lower percentage of smolts (68.2%) than TUSK (93.1%) or FARM (96.4%) (Appendix C: Fig. C1; Table 3). STEW–FARM hybrids also had significantly different smolt proportions than either parental population ($F_1 = 86.2\%$; $F_2 = 90.3\%$; BC = 73.7%; Appendix C: Fig. C1). Multigenerational hybrids exhibited intermediate smolt proportions in all three interpopulation comparisons (Appendix C: Fig. C1).

Juvenile body morphology.—The first relative warp (RW1) of geomorphometric analyses (details in Appendix C: Fig. C2) summarized shape variation in body depth with caudal region and peduncle length. STEW had slightly deeper heads, deeper bodies, shorter caudal regions, and shorter caudal peduncles than FARM or TUSK (FARM being intermediate) (Fig. 8; Table 3). RW2 summarized variation in head length, dorsal fin placement, and within-caudal region features.

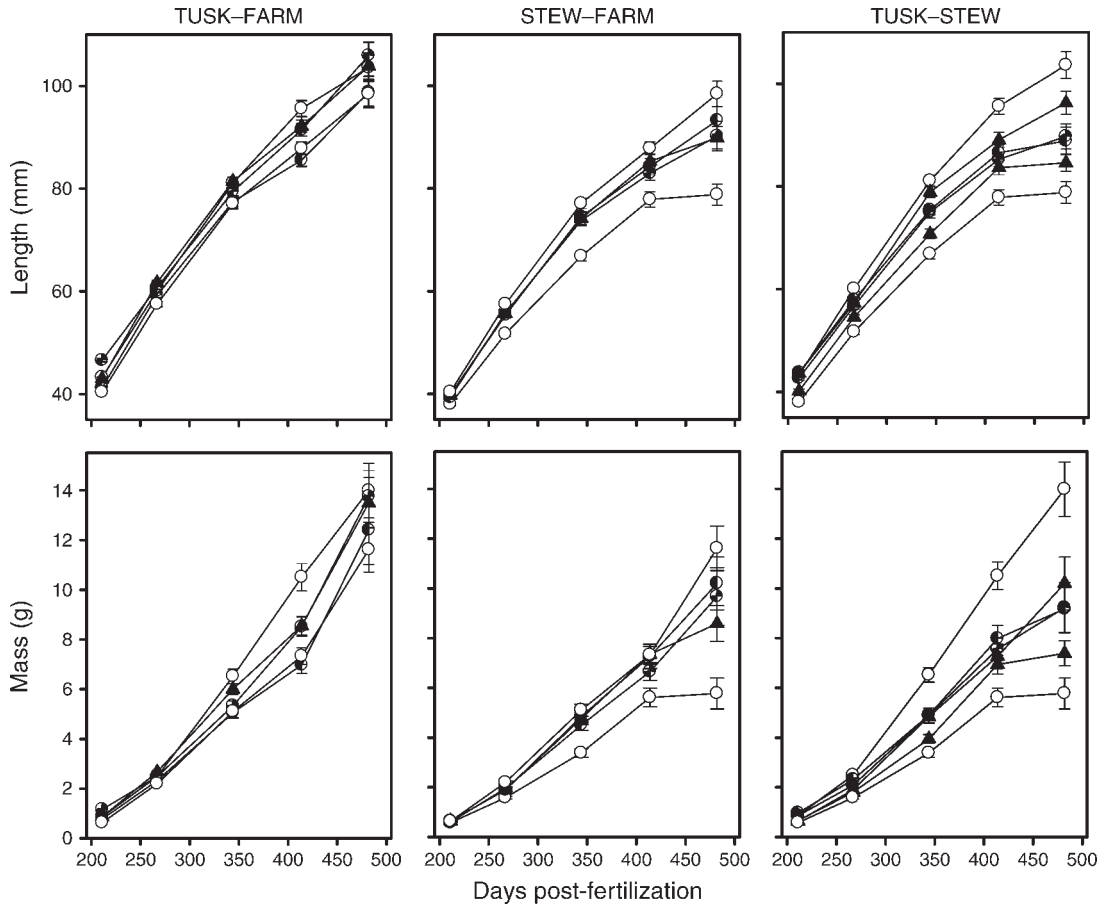

FIG. 7. Changes in juvenile body size (length and mass, ±SE) over time between crosses. Only data up to day 482 are shown to more easily visualize the main differences within and between interpopulation populations (see also Appendix C: Table C1). Key: white circles, parental populations; black triangles, backcrosses; half black-half white circles, F_1 hybrids; checkered circles, F_2 hybrids.

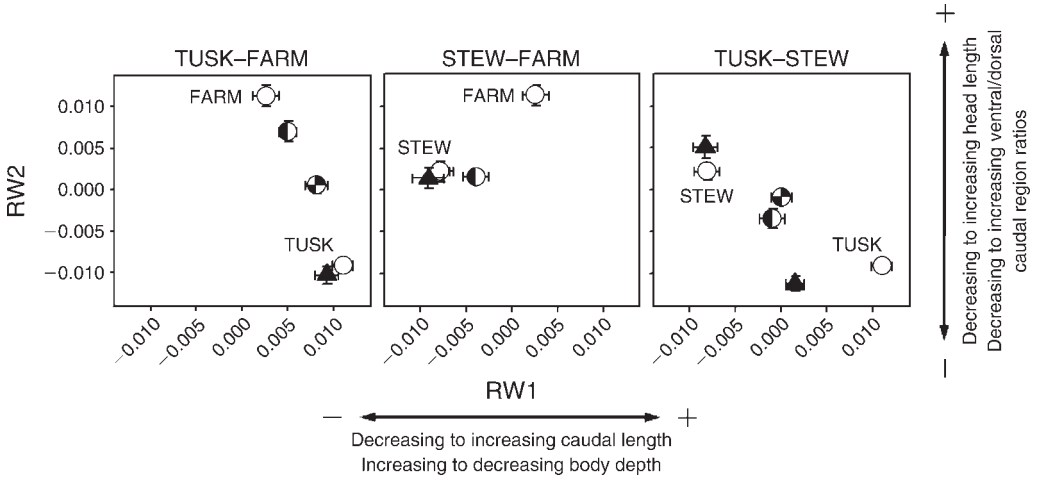

FIG. 8. Juvenile (parr) body morphology between crosses, along the first two relative warps (RW1 and RW2; mean scores ±SE). Key: white circles, parental populations; black triangles, backcrosses; half black-half white circles, F_1 hybrids; checkered circles, F_2 hybrids.

TABLE 4. Results of line-cross analyses across 13 different traits.

Trait	TUSK–FARM χ^2 (df = 3)	STEW–FARM χ^2 (df = 3)	TUSK–STEW χ^2 (df = 4)
Length at hatch	0.17	1.63	0.87
Yolk sac volume	0.02	0.41	0.21
Yolk sac conversion efficiency	0.07	0.68	0.39
Length at first feeding	1.93	0.69	0.46
Embryonic survival	0.45	3.16	0.85
Embryo developmental rate§	1.23	0.56	0.34
Length¶	0.10	0.04	0.38
Mass¶	0.22	0.11	0.13
Condition factor¶	0.10	0.08	0.08
Body morphology (RW1)	6.26‡, 4.23†	1.93	0.16
Body morphology (RW2)	20.73***, 16.91*	4.12	0.65
Anti-predator response 1#	0.26	0.46	NA
Anti-predator response 2#	0.14	0.15	NA

Notes: The χ^2 values are based on an additive model of genetic differentiation. Deviations from an additive model are noted with symbols that reflect the degree of statistical significance. Where such deviations occurred, χ^2 values from an additive-dominant model are included. Where applicable, standard errors and variances of different traits were estimated from family means. The analysis did not include percentage age-2 smolts. Degrees of freedom for the additive-dominant model were 2 (farmed–wild comparisons) or 3 (TUSK–STEW). "NA" indicates not available.

* $P \leq 0.05$; *** $P < 0.001$; † $P < 0.1$; ‡ $P \leq 0.15$.

§ Based on data from D. J. Fraser et al., *unpublished manuscript*.

¶ Only day-930 results presented.

Based on data from Houde et al. (2010).

TUSK had slightly shorter heads, shorter ventral relative to dorsal caudal regions, and shorter, more posterior-placed dorsal fins than FARM or STEW (STEW being intermediate) (Fig. 8; Table 3). On average, 83.8% of individuals were re-assigned to their respective parental populations (TUSK 93.8%; FARM 77.5%; STEW 80.0%); most mis-assigned fish were between FARM and STEW (26 of 39; 66.7%). Hybrids did not always exhibit statistical differences in body shape from both parental populations and/or clear intermediate body shapes relative to parents; notably, F_1 TUSK–FARM hybrids were more similar to FARM than TUSK, perhaps due to a maternal influence (Fig. 8; Appendix B). The only clear trend for differences in CV of RW scores between crosses was for STEW to be more variable at RW2 than TUSK and most of their hybrids (Table 3).

Overall hybridization effects

Line-cross analyses.—With one exception (F_1 TUSK–FARM body morphology), a simple additive genetic model adequately explained differentiation at the 13 traits × 3 interpopulation comparisons measured between parental and hybrid crosses (Table 4).

Hybrid deviations from parental midpoint values.—Across all traits, 95% confidence intervals around hybrid mean values all overlapped with 0, suggesting no general deviation from an additive genetic basis for trait differentiation (Table 5). Details of hybrid deviations from parental midpoint values at individual traits are found in Appendix E.

Magnitude of trait differentiation.—Within each interpopulation comparison, the absolute degree of phenotypic change across all traits from the wild parental population mean was always greatest in F_1 and F_2 hybrids and least in BC hybrids, though it did not always differ statistically between hybrid classes (Table 5). Across interpopulation comparisons, the magnitude of trait differentiation was greatest in TUSK–STEW hybrids, and similar and more intermediate in TUSK–FARM and STEW–FARM hybrids (Table 5). Details of the magnitude of trait differentiation at individual traits are found in Appendix E.

Over all crosses and traits, 8 of 161 skewness comparisons and 25 of 161 kurtosis comparisons had values that were significantly different from zero (student t tests, all $P < 0.05$). Skewness was always right-sided; kurtosis mainly involved narrow peaks (20 out of 25 comparisons). However, within each interpopulation comparison, there were no differences between any hybrid and their parental populations in the proportion of traits with either significant skewness or kurtosis (all $\chi^2 < 1.65$, df = 1, all $P > 0.20$) or in mean skewness or kurtosis values (GLMs; data not shown).

DISCUSSION

Our study has produced five key results that pertain directly to the risks faced by wild species resulting from hybridization with their domesticated counterparts. First, we detected a variety of genetically based trait differences between a "locally" derived farmed Atlantic salmon strain and divergent wild populations in the Northwest Atlantic. Second, trait differences between wild populations were broadly associated with their contrasting life histories in nature. Third, at the spatiotemporal scale examined, many traits appeared to respond to outbreeding in a similar (additive) way. Fourth, wild backcrossing did not completely restore hybrid trait distributions to presumably more optimal, wild states. Finally, the degree to which hybrids deviated from their parents in absolute terms increased predictably with increasing parental divergence.

TABLE 5. Average effects of hybridization across 13 traits relative to midparent trait means ($[X_{hybrid}/X_{midparent}] - 1$), as well as the absolute degree of phenotypic change (proportion, SD) in hybrids relative to the wild parent (see *Materials and methods* for details).

Effect	TUSK × FARM		
	F_1(TF)	F_2(TF)	BC(T × TF)
Difference from midparent mean	0.10 (0.15)	0.00 (0.07)	0.08 (0.10)
Change from parental mean (proportion)	0.26[a]† (0.11)	0.20 (0.08)	0.06[b]† (0.01)
SD from parental mean	0.67[a] (0.18)	0.53[a] (0.10)	0.21[b] (0.05)

Notes: The degree of phenotypic change in the TUSK–STEW comparison is presented relative to STEW. The analysis did not include percentage of age-2 smolts. Number in parentheses are standard errors. Within an interpopulation comparison, crosses with different superscript letters differed statistically ($P < 0.05$) using GLM.
† $P < 0.10$.

Regional farmed and wild trait differentiation.—Based on the present and previous research, FARM salmon differ significantly from wild salmon at 11 of 17 (64.7%; TUSK) and 11 of 16 studied traits (68.8%; STEW). Differentiated traits in the present study related to early life history, juvenile and subadult body size, smolt age, and body morphology; previously detected differences included aggression, anti-predator behavior, embryo developmental rate, pathogen resistance, and acid tolerance (Fraser et al. 2008, Lawlor et al. 2009, Houde et al. 2010; D. J. Fraser et al., *unpublished manuscript*), as well as gene expression underlying primarily metabolism and growth (Normandeau et al. 2009). Traits observed to differ in each farmed–wild comparison were sometimes different. Trait differences were also not always at the same life history stages or of the same magnitude in each farmed–wild comparison. Moreover, the direction of a trait difference between farmed and wild salmon changed over successive life history stages in at least one case: TUSK salmon grew faster than FARM at early juvenile stages but the opposite trend was observed at later juvenile and subadult stages.

Our study was not designed to discern the degree to which farmed–wild trait differences may be attributable to the ancestry of FARM vs. the farming process per se, but they were likely influenced by both processes. For example, FARM salmon have been selected for faster growth and delayed maturation (e.g., O'Flynn et al. 1999), whereas their FARM ancestry is the most parsimonious explanation for their reduced acid tolerance (Fraser et al. 2008). However, regardless of the origin of trait differences between regional FARM and wild salmon, FARM salmon used in this study are representative of the FARM salmon being mass-produced in regional aquaculture and escaping repeatedly into eastern North American rivers (Morris et al. 2008). From a risk assessment perspective, therefore, our work parallels that of others showing that domesticated and wild organisms can be considerably different at a variety of traits that are likely of import to fitness in the wild (Malmkvist and Hansen 2002, Mercer et al. 2006, Hutchings and Fraser 2008, Randi 2008, Thorstad et al. 2008).

Potential for wild local adaptation.—Wild Atlantic salmon conservation biology, like that of many subdivided species, incorporates the assumption that individual populations are locally adapted (see Garcia de Leaniz et al. 2007), and hence that farmed–wild interbreeding will reduce adaptation in the wild. The genetically based trait differences between our wild populations do not demonstrate that local adaptation exists, but their linkages with the known habits of these populations (see Tables 1 and 2) merit discussion.

The chief known difference between STEW and TUSK salmon is migratory behavior. STEW salmon are reported to have a localized migration between river and marine feeding areas in the Bay of Fundy (Committee on the Status of Endangered Wildlife in Canada 2006, Hubley et al. 2008). Conversely, TUSK salmon are long-distance migrants, travelling to marine feeding areas off of Greenland (Ritter 1989). We found that body shape and growth corresponded with the contrasting migrations of each population. Relative to short-distance STEW migrants, TUSK migrants grew faster and were more streamlined. In other salmonids, larger and more streamlined body forms improve swimming and presumably energetic efficiency for longer migrations (Taylor and Foote 1991, Hawkins and Quinn 1996). Faster growth may be favored in long-distance TUSK migrants because (1) subadults are in transit longer to and from nonbreeding areas and thus face greater time constraints for growth (Fraser et al. 2007*b*) and (2) juveniles have less time to reach critical threshold smolt size before migrating to sea, given that they out-migrate earlier each spring than STEW (Tables 1 and 2). Such threshold smolt sizes are linked to marine survival in other populations (Garcia de Leaniz et al. 2007). Growth and behavior were also linked; faster-growers (TUSK) were more aggressive and had slightly reduced anti-predator responses relative to slower-growers (STEW; Houde et al. 2010). Such a pattern is consistently observed across diverse taxa, and relates to individual fitness trade-offs between being larger but more aggressive, or less-aggressive but smaller (Biro and Stamps 2008). Faster embryo development rates to hatching were also characteristic of TUSK (D. J. Fraser et al., *unpublished manuscript*), the population found at the lowest latitude. Faster embryo developmental rates are often found in salmon populations from lower latitudes (Beacham and Murray 1990, Hodgson and

TABLE 5. Extended.

STEW × FARM			TUSK × STEW			
F_1(SF)	F_2(SF)	BC(S × SF)	F_1(TS)	F_2(TS)	BC(S × TS)	BC(T × TS)
0.04 (0.08)	0.07 (0.16)	0.14 (0.19)	−0.14 (0.16)	−0.17 (0.11)	0.21 (0.21)	−0.07 (0.24)
0.20 (0.05)	0.15 (0.05)	0.12 (0.03)	0.47 (0.23)	0.35 (0.14)	0.21 (0.11)	0.14 (0.08)
0.55 (0.09)	0.52 (0.10)	0.45 (0.09)	0.86[a] (0.11)	0.75[a]† (0.13)	0.48[b]† (0.07)	0.37[b] (0.08)

Quinn 2002). This is perhaps because incubation periods are shortened between later spawning in the fall and an earlier onset of spring, when conditions favorable to growth and survival are optimal (Beacham and Murray 1990, Hodgson and Quinn 2002).

Repeat breeding and the frequency of maturation after one winter at sea are also higher in salmon from STEW than TUSK (Committee on the Status of Endangered Wildlife in Canada 2006; Tables 1 and 2). Interestingly, despite a low proportion of mature females on day 1108 in each cross (mean: 6.2%, range 0–21.4%), their proportion was higher in STEW relative to TUSK ($\chi^2 = 5.05$, df = 1, $P = 0.024$) or FARM ($\chi^2 = 3.40$, df = 1, $P = 0.065$), and mature female hybrids were found only in crosses involving STEW. Across different salmonids, long-distance migrants often experience reduced post-breeding survival (Brett and Glass 1973, Schaffer and Elson 1975). The evolution of short- vs. long-distance migration, smaller vs. larger body size, and increased vs. reduced repeat-breeding (i.e., STEW vs. TUSK) are believed to be inseparably linked in salmonid diversification (Crespi and Teo 2002).

Collectively, there is substantial circumstantial evidence from this and recent works that *some* local adaptation *may* exist in wild salmon at the geographic scale between our study populations (TUSK and STEW). We are less certain as to what the early life history differences might reflect. Maternal effects influenced these traits, and no information currently exists on microhabitats and spawning areas in each river, or relationships between female reproductive investment (quality, size, and number of eggs) and juvenile/adult survival (Crespi and Teo 2002).

Genetic basis of population differentiation.—We found little evidence at the spatial scale examined here (200–340 km) that quantitative trait differentiation deviated from an additive model. Additive-dominant models in line-cross analyses rarely improved model-fitting of our data over a simple additive model. Similarly, overall hybrid trait means did not differ from expected mid-parent values in all classes of hybrids (F_1, F_2, BC). Thus, the spatial and/or temporal scale at which detrimental nonadditive genetic mechanisms of outbreeding are manifested may be greater than the scale we studied or time since our study populations likely diverged (10 000–12 000 years ago; Pielou 1991, King et al. 2001). Ongoing gene flow between regional groups of wild populations in our study region, although restricted (Fraser et al. 2007*a*), could also have retarded the formation of coadapted gene complexes, especially if selective pressures are not strong (Templeton 1986, Kawecki and Ebert 2004). Other common-garden studies on salmonids at comparable spatial scales have also found that trait differentiation was largely additive (McClelland et al. 2005, Tymchuk et al. 2007). Studies in the wild have found evidence for reductions in lifetime survival of F_1 or F_2 hybrids among populations (farmed or wild) separated $\geq$1000 km (McGinnity et al. 2003, Gilk et al. 2004) or isolated for 10 000–12 000 years (Gharrett et al. 1999), or no evidence among wild populations separated by 300 km but perhaps diverged for 13 000 years (Smoker et al. 2004). However, it is difficult to gauge from these works whether outbreeding depression was due to additive vs. nonadditive mechanisms. Formal tests to distinguish the relative roles of these mechanisms were not possible or not carried out (Gharrett et al. 1999, Gilk et al. 2004), or alternative explanations to detrimental nonadditive mechanisms in the F_2 generation (e.g., paternal sperm quality effects) could account for reduced survival in hybrids (cf. McGinnity et al. 2003). In reality, a range of genetic mechanisms probably underlie outbreeding depression in many fishes (McClelland and Naish 2007), as they do in other subdivided species (Edmands 1999, Etterson et al. 2007). Thus, similar assessments to ours in Atlantic salmon and other fish species but at greater spatiotemporal scales are merited.

Our conclusions regarding the lack of detrimental nonadditive genetic mechanisms must be tempered with the following uncertainties. Trait variability observed within crosses, the modest numbers of families used, and the polygenic basis of trait expression may have reduced statistical power for detecting deviations from additivity at some traits (e.g., maternal egg size, body size). We also did not compare the performance of multigenerational hybrids at all traits likely to be important to survival in migratory salmonids at later, nonbreeding stages (e.g., habitat selection; Fraser and Bernatchez 2005) and/or where coadapted gene complexes may underlie trait expression (e.g., disease resistance; Goldberg et al. 2005). We are currently examining whether epistatic breakdown of coadapted gene complexes might not arise in Atlantic salmon until the F_3 generation because of their residual tetraploid ancestry (McClelland and Naish 2007, Fraser et al. 2008). Finally, our research was necessarily carried out under

laboratory conditions, but detrimental nonadditive expression of certain traits might only be manifested upon exposure to natural environmental stressors (Montalvo and Ellstrand 2001, Edmands 2007).

Magnitude of trait differentiation between hybrids and parental populations.—We have shown that outbreeding depression via the loss of local adaptation may be the chief potential consequence of regional farmed–wild interbreeding because trait differentiation between study populations appears to have a largely additive basis. Put another way, the magnitude of the differentiation between hybrids and parental populations in absolute terms may largely govern hybrid fitness in nature. On average, the trait values of F_1 and F_2 farmed–wild hybrids deviated 15–26% (or about 0.5–0.7 SD) from wild parental population means. A complete reversion back to the wild phenotypic state did not occur after one generation of backcrossing. Farmed–wild backcrosses still deviated 6–12% from wild parental population means (or 0.21–0.46 SD) and were statistically differentiated from wild fish at 25–35% of traits studied in this and related studies.

Provided farmed escapes are not frequent and numerous, these results suggest that, at the scale examined, it will take at least three generations of backcrossing for natural selection in the wild to completely restore the presumably more optimal genetic constitution of different regional wild salmon populations. Additionally, other work (Edmands and Timmerman 2003) has suggested that outbreeding depression via the loss of local adaptation may be stronger but more transient than outbreeding depression arising from nonadditive mechanisms. If this is a general phenomenon, our research findings raise a key question for risk assessment: can depleted wild populations persist in the face of the reduced population growth that will be incurred most severely in the early generations of farmed–wild hybridization? The demographic consequences of farmed–wild hybridization may also be exacerbated in the Northwest Atlantic because regional farmed escapes are often numerous and often are found in proximate rivers where wild salmon breed (Morris et al. 2008). Furthermore, the extent to which farmed–wild hybrids differ from wild fish might also be greater as of 2009 because FARM salmon are in their seventh generation of artificial selection (as opposed to four generations when our study was initiated in 2001). Fortunately, a more predictable, additive-only basis of outbreeding depression should mean that farmed–wild hybrid impacts on different wild populations can be more easily modeled (Hutchings 1991*a*, Hindar et al. 2006).

Many of the changes to behavioral, life history, morphological, and developmental trait expression in farmed–wild hybrids may, on average, reduce hybrid fitness in the wild, especially given the above discussion on putative local adaptation at the spatial scale between TUSK and STEW rivers. To determine how much the mean fitness of wild populations might be reduced from farmed–wild hybridization, a more complete analysis would need to evaluate all studied traits simultaneously (as well as other correlated traits), to account for phenotypic and genetic covariation between traits (e.g., Lande 1976, Hard 2004).

Eco-evolutionary considerations of farmed–wild interbreeding: population divergence and hybrid fitness.—Assuming that each study population exhibits a phenotype that is closer to the optimum in its respective environment, our results are consistent with the theoretical prediction of a greater reduction in hybrid fitness with increasing population divergence between parental populations (Barton 2001, Edmands 2002). Of all three interpopulation comparisons, TUSK–STEW hybrids deviated the most phenotypically from parental populations across all traits, whereas deviations in TUSK–FARM and STEW–FARM hybrids were similar and more intermediate (i.e., relative to no phenotypic differences). These results suggest that the magnitude of fitness consequences to different wild populations from farmed–wild interbreeding may be predicted by the collective baseline information on the farmed strain and wild populations, especially if life history and environmental information is available.

Although the wild populations studied may be equally distant from farmed Saint John salmon in divergence terms (Tables 1 and 2), they nevertheless differ considerably from farmed salmon depending on the life stage and individual trait. Equal rates of farmed–wild interbreeding may therefore have very different consequences for population growth rates in each wild population. In fact, we suggest that, of our two study populations, STEW could be more at risk from the potential effects of farmed–wild interbreeding than TUSK. Available information suggests that dispersal of farmed escapees is higher into the STEW than TUSK population (Morris et al. 2008). Pre-mating isolation between FARM and STEW salmon may be lower because environmental and life history divergence (i.e., run timing) between the FARM ancestor and STEW is not as great (Tables 1 and 2). The conservation status of STEW (endangered) is more severe than TUSK (Department of Fisheries and Oceans Canada 2003, Committee on the Status of Endangered Wildlife in Canada 2006). Increases in marine mortality, implicated in regional wild salmon declines, are also most severe in inner Bay of Fundy populations such as STEW (Cairns 2001, Committee on the Status of Endangered Wildlife in Canada 2006, Hubley et al. 2008). This suggests that farmed–wild interbreeding could be especially influential on STEW population growth rate because FARM and STEW salmon differ most at this survival-limiting life stage. Collectively, it is imperative that future work link phenotypic/fitness changes brought on by farmed–wild interbreeding with demographic changes of import to the persistence of different wild populations.

Could the observed relationship between hybrid fitness and population divergence be extended to other salmon populations within regional groups? Life-history and phenotypic traits of populations are generally more similar within than between regional groups (Department of Fisheries and Oceans Canada 2003, Committee on the Status of Endangered Wildlife in Canada 2006, Fraser et al. 2007*b*, 2008). Thus, we expect that those populations most similar to STEW or TUSK within their respective regional groups would generate farmed–wild hybrids deviating from the wild state to a similar degree, and perhaps experience a similar reduction of fitness in nature. Again, though, any assessment of risk faced by other populations from farmed–wild interbreeding would have to consider additional factors, such as wild population size, the dispersal rate of farmed escapees, and the distance from farming activity. These factors vary dramatically within regional groups in eastern North America (Department of Fisheries and Oceans Canada 2003, Morris et al. 2008). Moreover, the relationship between hybrid fitness and parental divergence might be a non-linear one at greater spatiotemporal scales (Moll et al. 1965, Edmands 1999, Willi and Van Buskirk 2005).

Other caveats: population divergence and hybrid fitness.—Certain traits may be more directly related to fitness than others, but all traits were assumed to have equal influence on fitness in our overall assessments. Furthermore, while we were careful to consider a holistic approach towards characterizing parental population divergence, we did not consider all available proxies of divergence. For instance, our cross design did not permit quantification of genetic divergence between populations based on quantitative trait divergence (Q_{ST}). However, Q_{ST} estimates could most certainly produce more meaningful predictions of outbreeding effects than traditional measures of neutral genetic differentiation (F_{ST}) in many instances (McClelland and Naish 2007). Nevertheless, as our study illustrates, there is merit in considering even the general (and often known) environmental and life history differentiation between populations before such intensive studies are conducted.

Predictions regarding the relationship between population divergence and hybrid fitness were also based on characteristics of the FARM ancestor, not the artificially selected FARM strain. Many traits we examined may not be under strong directional selection during farming, but how much did artificial selection change FARM salmon (four generations as of the study's initiation in 2001)? And had farmed–wild introgression already occurred prior to 2001, dating back as far as the inception of the eastern North American salmon aquaculture industry in the early 1980s? Mean body mass at later life stages, known to be under strong directional selection in farming, provides a basis to explore these possibilities, with the caveat that our fish were raised in fresh water rather than salt water (see Fleming et al. 2002). Available data indicate that artificial selection yielded mass gains of $\approx$10% per generation (0.7–0.9 SD) for the first two generations of the FARM breeding program (Friars et al. 1995, O'Flynn et al. 1999). A 30% to 40% increase in mass (1.5–2.0 SD) would be expected after four generations based on several other salmonid aquaculture breeding programs (Gjerde 1986, Hershberger et al. 1990, Gjedrem 2000). Relative to FARM then, wild populations exhibiting smaller body mass differences may have already experienced farmed–wild introgression. On day 1108 of our study, FARM mass was 74.3% (2.07 SD) and 17.0% (0.56 SD) greater than STEW and TUSK salmon, respectively. This suggests that farmed introgression may have occurred in one of two study populations. However, extensive F_1 farmed–wild interbreeding without introgression could have occurred in either population. Moreover, an alternative explanation to TUSK–FARM introgression could be that wild Saint John salmon were not the fastest growing population for initiation of regional aquaculture: based on their life-history characteristics, TUSK salmon have likely always been favored for faster growth (Tables 1 and 2; see *Discussion: Potential for wild local adaptation*). In short, our data are insufficient to determine to what extent farmed–wild interbreeding may have already occurred in the Northwest Atlantic.

Other considerations: possible future changes to hybridization effects.—The lack of a consistent trend for F_1 hybrid vigor across different traits in this study is notable because our wild populations (TUSK, STEW) have been severely depleted over the past few salmon generations (10–15 years) (Department of Fisheries and Oceans Canada 2003, Committee on the Status of Endangered Wildlife in Canada 2006). Additionally, neither wild population (nor the FARM strain) had reduced neutral genetic diversity or heterozygosity relative to either historical samples from time periods prior to population declines, or to other, more abundant, regional wild populations (Fraser et al. 2007*a*; P. O'Reilly, *unpublished data*). These observations suggest that, currently, our wild populations may not be experiencing appreciable levels of inbreeding. Hence, farmed–wild interbreeding may very well generate outbreeding depression. However, should regional wild populations such as STEW and TUSK remain depleted in future generations, inbreeding will increase. At some point, this could shift the balance between inbreeding and outbreeding depression wherein farmed–wild interbreeding might conceivably generate hybrids that have improved rather than diminished fitness. Although potentially very problematic for risk assessments, we suggest that such a continuum of hybridization effects in relation to wild population health merits further consideration in future research.

Conclusion.—Unprecedented increases in world aquaculture production, coupled with the severe decline of many wild fish populations, have made it crucial to rigorously assess the potential risks associated with

interactions between wild and farmed individuals. Our study has contributed to this process by examining the differential effects of multigenerational farmed–wild hybridization across divergent wild populations, a key to assessing risk at larger spatiotemporal scales in fishes (Hutchings and Fraser 2008). We found that the extent to which farmed–wild hybridization may alter the phenotypic and genetic composition of divergent wild populations is considerable, across generations and among many traits that may be of importance to fitness in the wild. Combined with several recent works, our study therefore raises concerns that continued farmed–wild hybridization may have contributed to both the declines and the lack of recovery of many wild Atlantic salmon populations in the Northwest Atlantic, especially those with a higher degree of farmed immigration (Cairns 2001, Ford and Myers 2008, Fraser et al. 2008, Morris et al. 2008, Normandeau et al. 2009, Houde et al. 2010). Future work will incorporate the life stage-specific changes in phenotype/fitness brought about by farmed–wild hybridization into demographic modeling on the short- and long-term consequences to wild population persistence.

More generally, assessments of the risk posed to wild species from hybridizing with their domesticated relatives are increasingly needed in many taxonomic groups (Ellstrand 2003, Bekkevold et al. 2006, Mercer et al. 2006, Bowman et al. 2007, Hutchings and Fraser 2008, Randi 2008, Kidd et al. 2009). The approach we have employed should provide a scientifically-sound rationale by which to initiate such risk assessments, particularly in the recurrent situation where the wild species is subdivided into ecologically and genetically distinct populations, and where the conservation status of the wild species may prevent direct fitness comparisons in the wild.

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APPENDIX A

Neutral genetic divergence between parental populations (*Ecological Archives* A020-032-A1).

APPENDIX B

Experimental crosses generated and the number of families generated per cross (*Ecological Archives* A020-032-A2).

APPENDIX C

Detailed descriptions of traits measured and methodology (*Ecological Archives* A020-032-A3).

APPENDIX D

Final model structure of GLMs and GLMMs for analyzing different traits (*Ecological Archives* A020-032-A4).

APPENDIX E

Effects of hybridization at 13 individual traits (*Ecological Archives* A020-032-A5).