Interspecific Hybridization, a Matter of Pioneering? Insights from Atlantic Salmon and Brown Trout

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Interspecific hybridization may occur in situations of recent contact between a colonizer and a resident species, being more intense in the colonization front. Atlantic salmon Salmo salar and brown trout S. trutta have been sympatric species since their origin and they share spatial and temporal spawning niches, exhibiting low levels of bi-directional interspecific hybridization and introgression throughout their distribution range. Different causes have been identified for increased hybridization, from escapes or deliberate releases of domesticated fish to sneaking male behavior. We have examined hybridization rates and direction in different situations of advance of one of these species into a territory formerly inhabited by the other (247 samples were analyzed in northern Spain and 487 in Kerguelen Islands). In all cases, hybrids found in the colonization front were offspring of colonizer females and resident males. We hypothesize that these findings are the result of adaptive relaxed mate choice of colonizing females, regardless of the relative abundance of each species.

Key words: colonization, hybridization, relaxed mate choice, Salmo salar; Salmo trutta

Introgressive hybridization can be interpreted as an invasion of the genome by foreign genes (Mallet 2005). Increased interspecific hybridization is one of the consequences of species translocations (e.g., Seehausen et al. 2001) and invasions (e.g., Grosholz 2002) because secondary contacts between formerly isolated species contribute to break reproductive barriers and lead to introgressive hybridization (e.g., Kinziger and Raesly 2001).

When a fraction of a nonnative species enters in a new area and encounters a native species, a contact wave front is originated where the new species (the colonizer) is generally much scarcer than the native one. In such wave fronts, hybridization and introgression are more likely to occur as they are the prime area of secondary contact. Newly arrived females (colonizers) may relax mate choice (e.g., Kaneshiro 2006; Tinghitella and Zuk 2009) for avoiding gamete losses in absence of high-quality or just suitable conspecific breeders; they would mate with the most abundant males, conspecific or not, whereas females of the native species would reject mating with (scarce) alien males. The situation could be also considered as a matter of relative abundance, the scarcer species providing generally the female in interspecific crosses (Wirtz 1999). As a consequence of such relaxed mate choice, asymmetrical interspecific hybridization would be expected with the female corresponding to the colonizer species.

Aquatic organisms provide examples of interactions between native and colonizer species due to escapes or deliberate releases of farmed fish and shellfish (Bartley and Subasinghe 1996; Cambray 2003). There are many examples of foreign colonizers in freshwater (Baigun and Ferriz 2003; Townsend 2003) and marine environments (Semmens et al. 2004), and their interaction with wild native species may lead to interspecific hybridization (Naylor et al. 2005; Vellend et al. 2007). Recent escapes or releases of colonizer species in an exotic area may therefore provide good occasions for studying the initial stages of secondary interspecific contact and following the advance of colonizers since the beginning.

Atlantic salmon (Salmo salar) and brown trout (Salmo trutta) are sympatric species that exhibit introgressive hybridization in the wild in all their distribution areas. Interspecific hybridization seems increased by different factors, such as escapes or releases of domestic individuals and alternative mating behavior like male sneaking (Table 1). Both species are intensively cultured and exploited worldwide, and it is possible to find reciprocal situations where one species acts as a recent colonizer of an ecosystem already inhabited by the other species. The aim of this work, employing recently restored Atlantic salmon populations in north Spain and expansive brown trout introduced in the Kerguelen Islands...
Materials and Methods

Case Studies

Atlantic Salmon as a Colonizer

In North Spain (South Europe; lat 43°N), streams support brown trout and Atlantic salmon sympatric populations. Rivers are short and subject to variable conditions of water level and flow (e.g., Moran et al. 2005) depending on snowfall, rainfall, and temperature. Therefore, access of migratory fish to many upstream river areas and small tributaries varies each year depending on such climatic conditions. All river areas are occupied by resident brown trout, whereas river zones accessible from the sea are inhabited by the 2 species (Atlantic salmon is anadromous in this region and migrates to sea for growing before maturity).

Some upstream areas generally inaccessible to fish migrating from the sea were selected for sampling. Relatively small physical obstacles (small hydroelectric facilities or dams of mills) created during the 1950s interrupted anadromous fish run in the Narcea, Esva, and Porcia rivers; heavy pollution in downstream river sections isolated upstream areas of the River Las Mareas, a tributary of the River Sella, when a big factory was constructed by the river in the 1960s. In very exceptional years, high water flow (i.e., floods) allows fish passage over the physical obstacles and dilutes pollution. When an upstream area is made accessible, adult salmon may colonize it from downstream searching for new spawning areas and enter in secondary contact with resident brown trout. We have sampled upstream areas of the 4 above cited rivers the year after the floods, that is, the summer/autumn after anadromous fish accessed there due to exceptional environmental circumstances of high water levels: Porcia and Esva rivers, in 1995 and Narcea and Las Mareas rivers, in 1999.

In addition, the upstream section of the River Sella was followed in more detail. Also isolated since the 1950s by a hydroelectric dam, it was made accessible to migratory fish by a fish ladder (Escala Tejera) in 1995. This area was sampled in 2003 and again 5 years later (2 generations, generation length for salmon being approximately 2.6 years at this latitude; Consuegra et al. 2005), in order to follow its evolution after the first secondary contact.

Brown Trout as a Colonizer

Atlantic salmon from Denmark and Scotland were introduced in the lake–river systems of Korrigans and Armor, respectively, in the remote Kerguelen Islands (Southern Ocean; lat 49°S) where they acclimatized and reproduced naturally, establishing populations of very small effective population size (Ayllon, Davaine, et al. 2004). After salmon acclimatization, brown trout arrived in the Korrigans system.

### Table 1  Examples of natural hybridization between *Salmo salar* and *S. trutta*

<table>
<thead>
<tr>
<th>Region</th>
<th>Maternal species</th>
<th>% Hybrids</th>
<th>Situation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>North America</td>
<td>Brown trout</td>
<td>4.67%</td>
<td>Nonnative brown trout</td>
<td>McGowan and Davidson (1992)</td>
</tr>
<tr>
<td>North America</td>
<td>Brown trout</td>
<td>0.81%</td>
<td>Salmon parr males in hybrid crosses</td>
<td>Gephard et al. (2000)</td>
</tr>
<tr>
<td>Kerguelen Islands</td>
<td>Brown trout</td>
<td>5.6–6.6%</td>
<td>Both species are nonnative</td>
<td>Ayllon, Martinez, et al. (2004)</td>
</tr>
<tr>
<td>South Europe (France, Spain)</td>
<td>Atlantic salmon</td>
<td>0.74–3.46%</td>
<td>Parents of domestic origin in hybrid crosses</td>
<td>Castillo et al. (2008)</td>
</tr>
<tr>
<td>England</td>
<td>Atlantic salmon, brown trout</td>
<td>18%</td>
<td>Old contact area</td>
<td>Hartley (1996)</td>
</tr>
<tr>
<td>Sweden</td>
<td>Brown trout</td>
<td>41.5%</td>
<td>Both species reintroduced in a river section</td>
<td>Jansson and Öst (1997)</td>
</tr>
<tr>
<td>Scotland</td>
<td>Atlantic salmon</td>
<td>0.97%</td>
<td>Females of domestic origin in hybrid crosses</td>
<td>Youngson et al. (1993)</td>
</tr>
</tbody>
</table>
by migration from neighbor rivers. In only 3 generations, brown trout displaced completely Atlantic salmon from Korrigans spawning areas and the latter species disappeared from the 2 areas A and B (upstream and downstream, respectively) of the system, leaving hybrids as a signal of interspecific mating (Ayllon, Martinez, et al. 2004). In the Armor system, the salmon population was resident. Contact between the 2 species in spawning areas started in 1993, according to fish inventories conducted routinely. We sampled the Armor system for genetic analyses in 2002 (more or less 2 generations at that latitude; Davaine and Beall 1997) and again in 2009.

Sampling
When a new species colonizes a river, hybridization occurs in the lower portions of the river systems, and the occurrence and degree of hybridization decrease with upstream distance from the source of the colonizing (nonnative) species (Boyer et al. 2008; Muhlfeld et al. 2009). The field sampling methodology has taken into account the spatial dynamics of hybridization by sampling individuals all along the tension zone. Indeed, hybridization rates could be higher or lower depending on where the samples were collected. Surveys were conducted by electrofishing along the longitudinal profile of the river systems upstream the obstacle recently overcome or demolished (Spanish rivers) or upstream the initial point of secondary contact between the species (Kerguelen rivers). Those areas were systematically electrofished: the first 200 m just upstream the obstacle and sections 100–200 m long every 500 m for several kilometers upstream (whenever possible). Sampling was carried out in summer/autumn (August–October in Spain and December–January in Kerguelen). Juvenile salmonids were caught by 2 or 3 electrofishing passes in the selected stream sections to obtain adipose fin biopsies, which were preserved in ethanol until DNA analysis.

Genetic Analyses
DNA was extracted from tissue samples employing a Chelex protocol (Estoup et al. 1996). For determining the species of each sample, the 5S ribosomal RNA nuclear marker was polymerase chain reaction amplified with the primers of Pendas et al. (1995), which yield amplification fragments of 255 and 276 bp for Atlantic salmon and brown trout, respectively. For determining the maternal species of hybrids, the mitochondrial cytochrome b gene was amplified and subjected to restriction digestion with the restriction enzyme PstI (Ayllon, Martinez, et al. 2004) that yields 2 fragments of 166 and 183 bp for Atlantic salmon and 5 bands of 53, 57, 73, 79, and 87 bp for brown trout.

Results
The percentage of interspecific hybridization between these species has been very different according to the cases studied until now (Table 1). These percentages ranged between 0.74% in the case of parents of domestic origin in hybrid crosses (Castillo et al. 2008) and 41.5% when both species were reintroduced in a river section (Jansson and Öst 1997).

Inventory of individuals of each species (Atlantic salmon and brown trout) and interspecific hybrids are shown in Table 2, grouped by the colonizer species. When Atlantic salmon colonized rivers where brown trout was the only resident (Las Mareas, Porcia, Esva, and Narcea rivers), the percentage of interspecific hybrids ranged in our samples between 3.03% and 9.84% (mean 5.84%), and the maternal species was Atlantic salmon irrespective of the relative abundance of each species (Table 2a). In the 2 cases where brown trout was the colonizer (Korrigan’s systems A and B; Table 2b), the percentage of interspecific hybrids was 5.56% and 6.58% (mean 5.88%) and the maternal species was brown trout, again independently of the relative abundance of each species. Therefore, independently of the colonizer’ species and the relative abundance of each species, in all cases the colonizing females spawned with resident males. The hybrids were found preferentially near the first contact areas in the 2 cases (Atlantic salmon or brown trout colonizers).

The evolution of hybridization processes after colonization of a river area by Atlantic salmon or brown trout can be seen in Table 2 (advanced colonization). In the upstream River Sella, the colonizer species was Atlantic salmon (Table 2a). Eight years after the opening of the area to migratory salmon, brown trout was more abundant than Atlantic salmon (88.8% and 8.5%, respectively), and the hybrids (2.7%) were offspring of Atlantic salmon females. Five years later, Atlantic salmon was more abundant than brown trout. The percentage of hybrids was similar (2.75%) and generated from the 2 reciprocal crosses: 7 hybrids were progeny of Atlantic salmon females and one of a brown trout female. A spatial pattern of hybridization was found in this case study: The hybrids were concentrated near the first contact area (in the 200 m sampled upstream the ladder). As only one nuclear marker was analyzed, it is not possible to know if hybrids were of first, second, or third generation.

In the Armor system (Kerguelen Islands), the colonizer species was brown trout (Table 2b, advanced colonization). At the time of the contact (1993), Atlantic salmon individuals were more abundant than brown trout in all parts of the system. In 2002, one interspecific hybrid (0.5%), offspring of a brown trout female, was found in the first contact area. Seven years later, brown trout was much more abundant than Atlantic salmon (84.3% and 15.7%, respectively). Interspecific hybrids were not found among the 312 individuals sampled in the system.

Discussion
Our data, obtained from very different situations of recent secondary contact between Atlantic salmon and brown trout, strongly suggest that interspecific hybridization, which is relatively common in salmonid species (Rosenfield et al. 2000; Redenbach and Taylor 2003; Ostberg et al. 2004), is a consequence of colonization, as also suggested by other authors (Kinzig and Raesly 2001; Seehausen et al. 2001;
Grosholz (2002). Wirtz (1999) suggested that in hybrid crosses, females are generally provided by the scarcer species. In our data, however, the maternal species of the hybrids, which appeared in first contact areas, was always the colonizer species, irrespective of the relative abundance of each species in the newly colonized area.

As reported by Kaneshiro (2006) for Hawaiian Drosophila, colonizer salmonid females exhibited relaxed mate choice and hybridized since the beginning of the secondary contact, likely because flexible behavior is advantageous in such circumstances. When the colonizers have expanded after few generations of colonization, hybridization patterns seem to change, decreasing or disappearing (the case of the Armor system), or giving chance to crosses in the opposite direction (the case of the River Sella): when Atlantic salmon became more abundant than brown trout, relaxed female mate choice would be no longer necessary. The rates of hybridization found in this work (0.5–9.8%) were consistent across study areas (Kerguelen, North Spain) and also with those found in other regions and latitudes (Table 1). The highest hybridization rate, 41%, was found in a case of simultaneous reintroduction of Atlantic salmon and brown trout in a small river section (Jansson and Öst 1997), suggesting extremely relaxed female mate choice of brown trout in presence of another co-colonizer. Very small hybridization rates when domestic individuals are involved in crosses (Youngson et al. 1993; Castillo et al. 2008) could be due to the fact that hatchery fish are generally poorer spawners than wild individuals (Fleming et al. 1996; Hansen et al. 2000; Weir et al. 2004; Jonsson N and Jonsson B 2005).

Breakage of 2 premating barriers between Atlantic salmon and brown trout could be at work in interspecific hybridization processes: male dominance (especially brown trout males; Garcia-Vazquez et al. 2002) and female behavior (Beall et al. 1997). High aggressiveness of brown trout males would impede small resident Atlantic salmon males from approaching and courting brown trout females.

Table 2  Number of individuals of each species and interspecific hybrids (BT, AS, and H as brown trout, Atlantic salmon and interspecific hybrids, respectively), maternal species (identified by mitochondrial DNA, mtDNA) and percent of hybrids, in areas recently (sporadically in the case of the Brook Las Mareas) colonized by one species and the evolution of hybridization processes in the contact area of Atlantic salmon and brown trout upstream River Sella and in the Armor System

<table>
<thead>
<tr>
<th>Maternal species</th>
<th>BT</th>
<th>AS</th>
<th>H</th>
<th>mtDNA</th>
<th>% Hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Atlantic salmon colonizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent colonization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brook Las Mareas</td>
<td>55</td>
<td>0</td>
<td>6</td>
<td>AS</td>
<td>9.84%</td>
</tr>
<tr>
<td>Upstream River Porcia</td>
<td>9</td>
<td>23</td>
<td>1</td>
<td>AS</td>
<td>3.03%</td>
</tr>
<tr>
<td>Upstream River Esva</td>
<td>29</td>
<td>1</td>
<td>1</td>
<td>AS</td>
<td>3.23%</td>
</tr>
<tr>
<td>Upstream River Narcea</td>
<td>28</td>
<td>0</td>
<td>1</td>
<td>AS</td>
<td>3.45%</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>24</td>
<td>9</td>
<td></td>
<td>5.84%</td>
</tr>
<tr>
<td>Advanced colonization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upstream River Sella</td>
<td>32</td>
<td>3</td>
<td>1</td>
<td>AS</td>
<td>2.70%</td>
</tr>
<tr>
<td>Upstream River Sella</td>
<td>94</td>
<td>196</td>
<td>8</td>
<td></td>
<td>2.75%</td>
</tr>
<tr>
<td>b) Brown trout colonizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent colonization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korrigan’s system area A</td>
<td>142</td>
<td>11</td>
<td>9</td>
<td>BT</td>
<td>5.56%</td>
</tr>
<tr>
<td>Korrigan’s system area B</td>
<td>6</td>
<td>65</td>
<td>5</td>
<td>BT</td>
<td>6.58%</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>76</td>
<td>14</td>
<td></td>
<td>5.88%</td>
</tr>
<tr>
<td>Advanced colonization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Armor system</td>
<td>9</td>
<td>76</td>
<td>123</td>
<td>1 BT</td>
<td>0.50%</td>
</tr>
<tr>
<td>Armor system</td>
<td>9</td>
<td>263</td>
<td>49</td>
<td>—</td>
<td>0%</td>
</tr>
</tbody>
</table>

Samples are grouped by the colonizer species (Atlantic salmon and brown trout, A and B, respectively).

under normal circumstances, but when they are absent or very scarce, sneaker salmon can fertilize trout ova as they do with conspecific females. This has been demonstrated experimentally (Garcia-Vazquez et al. 2002) and observed in the wild (Gephard et al. 2000). On the other hand, as explained above, relaxed female mate choice would be advantageous when conspecific males are scarce. Atlantic salmon male behavior can also contribute to such relaxed mate choice in salmon females, which may accept courtship by heterospecific males and even so the greatest part of their ova will be fertilized by conspecific sneakers (Castillo et al. 2010), representing an advantage when conspecific anadromous males are scarce or absent.

As a final remark, although introgressive hybridization can lead to a species’ extinction (Rhymer and Simberloff 1996; Epifanio and Nielsen 2000), it does not seem to be the case for the 2 model species investigated here. Instead, extinction of an Atlantic salmon population (in the case for the 2 model species investigated here, but in the wild (Gephard et al. 2000). On the other hand, as explained above, relaxed female mate choice would be advantageous when conspecific males are scarce. Atlantic salmon male behavior can also contribute to such relaxed mate choice in salmon females, which may accept courtship by heterospecific males and even so the greatest part of their ova will be fertilized by conspecific sneakers (Castillo et al. 2010), representing an advantage when conspecific anadromous males are scarce or absent.

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