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Genetic diversity and population structure of domestic brown trout (*Salmo trutta*) in France

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ABSTRACT

Animals in captivity are subject to similar evolutionary forces that act on natural populations, which can facilitate the generation of population genetic structure. Understanding the extent of genetic differentiation among captive populations provides insights into industry practices and the domestication process. We investigated the genetic structure of domestic brown trout (*Salmo trutta*) in France by surveying fish collected from 20 fish farms. Using microsatellite markers, we calculated basic measures of genetic diversity and differentiation among these various farms. We also evaluated population structure using tree-based approaches, model-based clustering methods, and ordination techniques. Differences in genetic diversity reflected founding histories and source stocks among the fish farms. Fish farms that raise trout originating from Mediterranean watersheds had lower levels of genetic diversity and much higher divergence than populations of Atlantic origin. Stocks believed to originate from the common Atlantic-based trout strain demonstrated low-levels of population structure. We observed fish of mixed ancestry in some fish farms and the presence of multiple genetic stocks within the same facility. Our findings reveal patterns of genetic structure that reflect differences in founding practices and movement of individuals and strains between fish farms. Such findings have consequences for fisheries managers stocking natural ecosystems with captive-reared fish, biologists attempting to understand the interactions between wild and domestic brown trout, and fish farmers involved in stocking or restoration activities.

Statement of relevance

Genetics resolve the history and practices of aquaculture.

1. Introduction

As in natural populations a variety of biological and anthropogenic forces act on captive populations to shape complex patterns of genetic structure, particularly those propagated for commercial interests (Bruford et al., 2003; Mignon-Grasteau et al., 2005; Zenger et al., 2007). Captivity artificially creates subpopulations from a global genetic resource and the degree of structure oscillates depending on forces that either homogenize gene pools or facilitate differentiation. When a particular strain is identified as having high potential value, the global genetic structure of captive populations can become homogenized as propagators adopt the same genetic resource (Notter, 1999; Signorello and Pappalardo, 2003; Tisdell, 2003). Movement of organisms between propagators and the acquisition of seed stocks from similar populations also facilitate homogenization (Zenger et al., 2007).

Opposing forces can create a heterogeneous structure among captive populations and lead to genetic divergence. Typical small population processes, such as genetic drift, inbreeding, and founder effects, can generate such patterns (Knibb et al., 2014; Skaala et al., 2004; Weigel, 2001). Propagators may select broodstocks from genetically differentiated wild populations and develop unique strains from those stocks (Giuffra et al., 2000; Holtsmark et al., 2008; Mickett et al., 2003; Rengmark et al., 2006). Artificial selection plays an important role in promoting divergence, whether individual propagators select for specific traits for economic objectives or organisms adapt to captive surroundings (Zenger et al., 2007; Frankham, 2010; Christie et al., 2012).

All of these forces interact to shape the global population structure of captive-held species. Understanding the extent of genetic divergence between captive populations has value for cultivators and the biological community at-large. Application of molecular markers provides insight into the extent that domestic production has shaped global population structure (Liu and Cordes, 2004). Information such as the loss of genetic diversity has value to propagators attempting to maximize fitness of their stocks (Appleyard and Ward, 2006; Bentsen and Olesen, 2002; Gallardo et al., 2004). For propagators and regulators, the scope of

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intermixing and movement of individuals between captive populations is important for understanding industry practices. Molecular markers can also be used to identify the source population for a harvested individual and differentiate specific stocks, which is useful for tracing source populations for regulatory purposes (Hayes et al., 2005).

The domestication of brown trout (*Salmo trutta*) provides an interesting case study to examine how various forces can shape the genetic structure of a species composed of wild and captive sub-units with implications for economic production and conservation. Native to much of the European continent along with portions of northern Africa and western Asia, the species historically occupied a large number of watersheds across this range, which generated intricate patterns of genetic structure due to colonization events and isolating processes (Bernatchez, 2001; García-Marín et al., 1999; Krieg and Guyomard, 1985). In their native range domestically-raised brown trout are actively stocked in natural water bodies to augment wild populations for angling purposes and raised for food production.

Production of brown trout in France is particularly notable, for domestication of this species has a long and complex history with important commercial and environmental implications. The demonstration of artificial reproduction in brown trout in 1840 (Haxo, 1853) stimulated the government to invest in the first "piscifacture" in Huningue in 1853 to support stocking in French rivers (Coste, 1861). Since then, stocking was supported by the creation of the non-profit units in parallel to private fish farms with the advice of successive governmental technical organizations. In the 1960s brown trout stocking became influenced by the demand for domesticated and highly heritable (Blanc et al., 1994) red spotted strains farmed in the north of France and Denmark. The so-called "Atlantic strains" became one of the major strains stocked worldwide (Vivier, 1956).

Later, experimental within-group selection to improve growth (Chevassus et al., 1992) provided significant improvement in body weight in captive-reared brown trout (Chevassus et al., 2004). An unknown number of farms reared stocks derived from this experimentally selected population. This strain was also monosexed to produce all-female diploid or sterile triploids (Chevassus et al., 1992). Production of all-female sterile triploids was promoted to limit genetic impact of escapees or the deliberate stocking of domesticated individuals (see Piferrer et al., 2009). The impact of monosexing technology on genetic characteristics of the broodstocks and the prevalence of stocking all-female triploid individuals are also not known. However, it is known that some fish farms capture wild fish (mainly mature males) and cross them with their domestic stocks to provide inputs of wild characteristics and local adaptation.

Also, in response to the growing awareness of the natural differentiation between wild populations, some fish farms have initiated the production of local wild stocks for stocking. Research has revealed that the major strains of domestic trout interbreed with wild fish, potentially homogenizing brown trout populations and causing the extinction of locally adapted populations (Barbat-Leterrier et al., 1989; Berrebi et al., 2000; García-Marín et al., 1999; Hansen, 2002; Madeira et al., 2005; Poteaux et al., 1998). The French government stopped granting funds for stocking in 2002, taking into account the impacts of stocking on native populations. However, governance of fishing practices in France is assigned to local angling federations and numerous federations continue to stock domestic brown trout, while some others limit or ban this practice. Some managers in Europe have adjusted stocking practices to preserve the genetic composition of wild brown trout populations and raise stocks derived from specific watersheds for stocking.

These activities suggest that the domestic brown trout population in France forms a heterogeneous assemblage of populations with potential inputs from wild fish. Initial studies using allozymes showed that domesticated stocks exhibited high genetic variation and belonged mainly to the Atlantic populations (Chevassus et al., 1992). However, polymorphic nuclear markers have the potential to reveal patterns reflective of the complex history of trout production in France. From a commercial perspective, it is important for propagators and those involved in the industry to understand common practices and the genetic composition of these populations. For ecologists and fisheries managers, identifying these genetic differences is vital to understanding stocking patterns and discriminating between the fitness of various strains. Using microsatellite data from fish farms in France, our goal was to examine the genetic diversity and population structure of the captive component of the larger global brown trout gene pool.

2. Methods

2.1. Sampling distribution

The data utilized for this study are part of a larger effort to characterize the genetic structure of brown trout across France (local and national projects such as Genesalm and Genetrutta). Tissue samples used here were obtained between 2004 and 2014 from 20 domestic populations sampled in 19 fish farms distributed across 18 administrative departments in France. Sampling was standardized so that approximately 30 fish were sampled per fish farm. One fish farm (Roquebillière) was sampled separately in both 2001 and 2008: for our analyses these two samples were treated as separate populations. On average about 29 fish were sampled per population, with a range from 20 to 41 individuals. Some fish farms are private companies: in this paper we will refer to these farms by the administrative department in which they are located.

Fish farms in France take advantage of different source populations when developing their stocks. Along with the main strains developed in France, several farms raise fish derived from local rivers. This includes rivers in the southwest of France that join the Atlantic Ocean and rivers that are part of the Mediterranean Sea basin. For comparison purposes, we classified fish farms into four categories based on supposed origin of the stock (Table 1). These categories were based on a priori designations given by managers of the fish farms. The first category was 'ComATL', which refers to the common, internationally-distributed Atlantic strains of fish. 'LocATL' refers to fish farms that raise stocks derived from local rivers within the Atlantic watershed. Similarly, 'LocMed' is applied to stocks are derived from local rivers within the Mediterranean watershed. 'MIX' are stocks believed to produce unique strains that are a purposeful mix of the 'ComATL' and Mediterranean-based strains. Based on information provided by fish farm managers, seven fish farms were considered as 'ComATL', six as 'LocATL', four as 'LocMed', and two as 'MIX'.

2.2. Microsatellite analysis

We used an improved Chelex extraction procedure based on the method of Estoup et al. (1996) for DNA extraction (Berrebi et al., 2013). A set of 12 nuclear microsatellite loci were selected for analysis that were polymorphic and gave PCR products of satisfactory quality. PCR reactions were optimized by adjusting $MgCl_2$ concentrations as well as annealing temperatures. The PCR program consisted of 5 min of pre-denaturation (95 °C) followed by 35 cycles of 30 s annealing, 30 s of extension (72 °C) and 30 s of denaturation (94 °C). The annealing temperatures are given in supplementary information (Table S1). One of each primer pair was labeled with fluoresceine, CY5 or CY3. PCR products were separated on 6% acrylamide gels and analyzed on a Hitachi FMBIO II scanner according to the manufacturer's recommendations. Gels were scored using the FMBIO ANALYSIS 8.0 program.

2.3. Population genetic analysis

We performed probability tests of deviations from Hardy-Weinberg Equilibrium (HWE) for each population using GenePop (Rousett, 2008). For the Markov Chain parameters we utilized a dememorization step of 1000 with 100 batches of 1000 iterations each. A number of loci deviated from HWE for certain populations (see Section 3), so we checked for the presence of null alleles using Micro-Checker (Van Oosterhout et al.,