

NEWS AND VIEWS

PERSPECTIVE

Expression of interest: transcriptomics and the designation of conservation units

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An important task within conservation genetics consists in defining intraspecific conservation units. Most conceptual frameworks involve two steps: (i) identifying demographically independent units, and (ii) evaluating their degree of adaptive divergence. Whereas a plethora of methods are available for delineating genetic population structure, assessment of functional genetic divergence remains a challenge. In this issue, Tymchuk *et al.* (2010) study Atlantic salmon (*Salmo salar*) populations using both microsatellite markers and analysis of global gene expression. They show that important gene expression differences exist that can be interpreted in the context of different ecological conditions experienced by the populations, along with the populations' histories. This demonstrates an important potential role of transcriptomics for designating conservation units.

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The correspondence between neutral genetic differentiation as measured by molecular markers, and adaptive divergence, such as that identified by analysis of quantitative traits is still not well established (Reed & Frankham 2001; Leinonen *et al.* 2008). Hence, defining conservation units based solely on neutral genetic variation reflects the degree of reproductive isolation and demographic history of populations, but does not necessarily capture components of differentiation resulting from adaptive divergence (Waples 1991; Crandall *et al.* 2000; Fraser & Bernatchez 2001). On the other side, analysing adaptive divergence is a challenging task. Testing local adaptation at the quantitative genetic level using, e.g. a Q_{ST} – F_{ST} approach (Leinonen *et al.* 2008) requires common garden experimental set-ups that may become unfeasible depending on the life history of the species and the number of populations to be tested. Resorting

to identification of adaptive variation at the genome level is a very promising avenue (Luikart *et al.* 2003), but current low-resolution genome scans typically identify only a handful of genes under possible selection (Vasemägi *et al.* 2005; Namroud *et al.* 2008). As a result, many studies attempting to define conservation units do a good job in testing demographic independence, but provide more circumstantial evidence regarding adaptive variation (see Hansen *et al.* (2008) for an example and discussion).

Analysing divergence among populations at the transcriptome level provides phenotypic data, but also represents a first step on the pathway from genotype towards functional phenotype (Fay & Wittkopp 2008). Hence, gene expression variation among individuals and populations can be interpreted as physiological acclimation and/or adaptation of individuals and populations. This dual nature of the data is reflected in different studies which on the one side use transcriptome level analyses for studying phenotypic plasticity (e.g. Giger *et al.* 2006; Aubin-Horth & Renn 2009) and on the other side, use transcriptome variation as indicators of adaptive divergence (e.g. Derome *et al.* 2006; Larsen *et al.* 2007; Roelofs *et al.* 2009). Could transcriptome data assist in the designation of conservation units? This is ultimately the question Tymchuk *et al.* (2010) set out to address.

Tymchuk *et al.* focused on 12 Atlantic salmon populations in the Bay of Fundy region and the Southern Uplands region of Nova Scotia, Canada. Important environmental variation exists throughout these regions which may have involved adaptive divergence among populations. In particular, it is known that populations from the Outer Bay of Fundy undertake marine feeding migrations to Greenland waters, whereas populations from the Inner Bay of Fundy undertake shorter migrations. Important heritable compensatory growth response differences have been observed between these population groups (Fraser *et al.* 2007b). Moreover, the populations are declining and of considerable conservation concern (Fraser *et al.* 2007a), which highlights the need for defining conservation units.

Neutral genetic population structure was analysed using seven microsatellite loci. Global gene expression was studied using a 16K cDNA salmonid microarray and based on RNA extracted from whole fry. The fry represented hatchery-reared offspring of wild parents that had spent at least part of their life cycle in the wild, and in four populations analyses were replicated over 2 years. Significant gene expression differences among populations were observed at 389 genes (22%) in the year where most populations were sampled and after controlling for rearing of fry in two different hatcheries. Hierarchical clustering based on these genes revealed a distinct grouping of populations from the southern Uplands, the inner and the outer Bay of

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Fundy. Important expression differences were also evident within regions. These patterns corresponded well with the relationships inferred from microsatellite markers. The combined microsatellite and transcriptome data would suggest that groups of populations at the regional level constituted evolutionarily significant units, although in the inner Bay of Fundy, the presence of two or more evolutionarily significant units might be argued.

Tymchuk *et al.* further noted that hatchery supplementation or introgression by escaped farmed salmon could have affected patterns of gene expression. This issue has been treated in more detail in another recent study, incidentally focusing on two of the same populations (Normandeau *et al.* 2009). This study confirms on the one side that spawning intrusion by farmed salmon may lead to homogenization of transcriptome variation among populations. On the other side, changes of gene expression profiles in admixed individuals may differ considerably among populations, suggesting that differences among wild populations in the genetic architecture underlying gene expression may cause unpredictable effects of introgression by farmed salmon.

The interesting study by Tymchuk *et al.* clearly suggests a potential for using transcriptome variation to assist in defining conservation units, but also highlights some important challenges in this respect. First, since the data is essentially phenotypic, evidence for the genetic basis of interpopulation variation should be provided by rearing or at the very least acclimating the individuals to be studied in a common garden set-up. The populations studied were reared in captivity but in two different hatcheries, thus potentially confounding different hatchery environments and real genetically based differences in gene expression. This problem was accommodated by removing genes showing different expression between the two hatcheries. In general, however, this is an example of a problem that is likely to arise in other studies encompassing a large number of populations where rearing in a single common garden is not feasible.

Second, even if the data have a genetic basis, a case has to be made that transcriptome variation among populations reflect selection as opposed to drift. This could not be specifically tested in Tymchuk *et al.*'s study, although there was a tendency that differences in gene expression and in the functional categories of genes showing expression differences coincided with environmental conditions and migratory life histories. A number of approaches have been applied for identifying the evolutionary forces underlying gene expression differences (Fay & Wittkopp 2008), but relatively few methods are suitable for fine-scale population studies. Rigorous testing could be conducted by comparing F_{ST} and gene expression Q_{ST} , or by identifying the quantitative trait loci underlying gene expression (eQTL) and analyse eQTLs within a genome scan framework; both approaches have been applied in non-model organisms (Roberge *et al.* 2007; Whiteley *et al.* 2008), but would be resource-demanding for analysing many populations. An alternative approach consists in testing correlation between gene expression and environmental parameters (e.g. tem-

perature) and control for population history based on neutral molecular markers (Whitehead & Crawford 2006). This sort of 'landscape transcriptomics' analysis appears to be a realistic option for testing adaptive divergence at the gene expression level and integrating the results into the designation of conservation units.

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