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NEWS AND COMMENTARY

Adaptive genomic changes in Antarctic fish

Copious copies keep out the cold

DH Lunt and SCP Renn

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Life in the sub-zero temperatures of the Southern Ocean requires special adaptation to extreme cold, and the notothenioid fish radiation—which dominates the biomass and species diversity of Antarctic fish—has provided textbook examples of molecular evolution and adaptation to thermal challenge. Previous studies have typically investigated single genes or phenotypes, such as antifreeze proteins, but in an exciting new survey, Chen *et al*. (2008) take a novel approach to characterizing the genome-wide changes in gene duplication and transcription, relating these to adaptation to the cold Antarctic waters. The broad implications and excitement generated by this research are because of its tripartite approach.

First, the authors investigated the transcriptome of the cold-adapted fish *Dissostichus mawsoni* by sequencing cDNA libraries made from multiple tissues. Chen *et al*. (2008) used unnormalized libraries in order to quantify EST (expressed sequence tag) abundance, and this revealed a highly biased transcription pattern, beyond ordinary expectations for simple tissue specificity. They identified a small number of dominant transcripts in each tissue, and a transcriptional shift towards a functional theme that suggests an elevated stress response.

Second, the frequency of recovery of gene transcripts was compared with published high-volume EST data sets for similar tissues in five model fish species (zebrafish, salmon, stickleback, mummichog and medaka). Over the 11 possible comparisons, 177 genes were found to be upregulated in *D. mawsoni*, of which 85 (48%) were already known to be upregulated with cold response in carp (Gracey *et al*., 2004). This result suggests that, not surprisingly, evolutionary adaptation to the Southern Ocean has co-opted some of the mechanisms that underlie typical physiological response to cold.

Third, and importantly, the authors investigated the contribution of gene duplication to transcriptional upregulation, using a *D. mawsoni* cDNA microarray based on the EST data. They employed array comparative genomic hybridizations (aCGH) to compare gene copy number at 10 700 loci in three Antarctic and two non-Antarctic notothenioid fishes. Using a hybridization threshold ratio set for a single, known, gene duplication event between two of these species, Chen *et al*. (2008) identified 101 protein-coding genes for which the average hybridization ratio across the cold-adapted species was above this threshold—indicating likely gene duplication. These candidate loci include 15 with potential activity in cold response, and many that were also among those upregulated in *D. mawsoni* relative to temperate species according to the EST analysis (Figure 1). It is perhaps unusual to apply a threshold analysis technique to this experiment, as Chen *et al*. (2008) do, rather than a statistical analysis that is more appropriate to the hybridization design. A more rigorous statistical analysis of aCGH results would help to quantify hybridization variation within and between species and ecotypes (for example, Emerson *et al*., 2008). Even so, successful identification of copy number variation (CNV) here supports the microarray technique as a rapid, sensitive and cost-effective method to assay genome-wide CNV in less traditional model organisms. Together these experiments provide a tantalizing glimpse of the genome-wide changes accompanying adaptation and radiation in an extreme environment.

In retrospect, we may not be surprised to find that genes required for short-term cold adaptation are well represented among duplicated loci in Antarctic fish inhabiting frigid environments. The adaptive potential of such duplications seems obvious, with increased copy number facilitating increased expression, and therefore being favoured in genes whose products promote survival in the cold. In other systems, multiple gene copies are known to influence fitness by increasing the quantity of mRNA transcripts (for example, Daborn *et al*., 2002). Gene duplication has long been thought to underlie adaptation and evolutionary novelty through processes such as subfunctionalization (for example, novel temporal or spatial expression) and neofunctionalization (novel gene function) (Taylor and Raes, 2004), and it is now clear that CNV is a very frequent type of genomic variation contributing significantly to phenotypic variation both within and between species (for example, Korbel *et al*., 2008; Dumas *et al*., 2007). This study has set the stage for further sequence analysis and expression profiling that will allow researchers to assess the relative contributions of the duplicate's divergence in expression pattern, sequence and function.

When designing interspecies hybridizations, however, careful controls against the influence of sequence divergence on hybridization efficiency should be considered from the start. In this study, genomic DNA from other Antarctic species was found to bind to the *D. mawsoni* microarray with greater affinity than DNA from the non-Antarctic species that diverged ~37 million years ago (Near, 2004). This reduced hybridization due to sequence divergence could give the appearance of lower copy number in the non-Antarctic species, even when no CNV exists, and it is unfortunate that a confounding effect exists in the same direction as the result. Although it is, of course, difficult to find a perfect experimental design for evolutionary adaptation when presented only with species available in nature, careful independent controls are still needed. Although the authors have attempted to validate the results by Southern blot hybridization, this suffers from exactly the same problems of reduced hybridization efficiency with sequence divergence, as shown in their Figure 3. Most studies do show that CNV can be reliably detected even in the presence of extensive sequence divergence (Dumas *et al*., 2007), but a reciprocal blot, also isolating the probes from temperate species, would have provided a more convincing validation. Quantitative PCR (with conserved primers) is a better choice, and can provide a validation that is not influenced by sequence divergence.

Identifying the way in which natural selection on the genome has brought about adaptation to novel environments is a fundamental aim of biology. Although the decreasing cost of DNA sequencing may provide a solution for some projects, the broad survey of adaptive radiations still stands to benefit from cost-effective microarray approaches. The study of Chen *et al*.

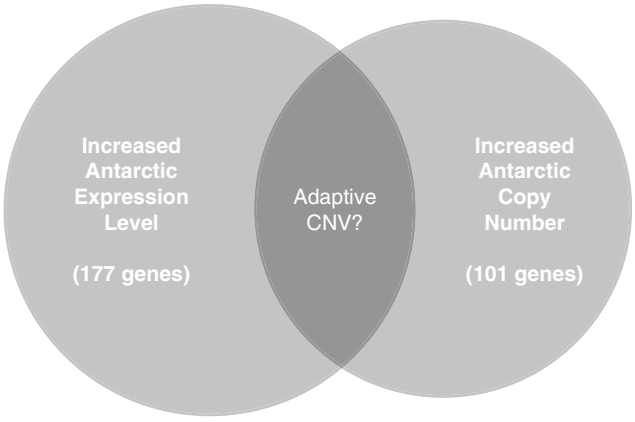

Figure 1 Chen *et al*. (2008) report candidate loci for adaptive copy number variation in Antarctic notothenioid fish. A subset of genes (centre) are both overrepresented in the Antarctic expressed sequence tag set relative to temperate species data sets (left) for four tissues and also appear to be overrepresented in genomic content according to comparative genomic hybridization to a cDNA microarray (right).

(2008) makes great progress in identifying candidate genomic changes in a classic system of adaptive radiation in an extreme environment. As adaptive genomic variation is unavoidably mixed with other changes that accrue with time, improved validation techniques, greater statistical rigor and future functional studies will ultimately be essential for deriving conclusions of general importance. Yet, the study by Chen *et al*. (2008) is significant and clearly shows this system could be one of the first textbook examples of the molecular basis of evolutionary adaptation at a genomic scale.

Dr DH Lunt is at the Department of Biological Sciences, The University of Hull, Hull HU6 7RX, UK and Dr SCP Renn is at the Department of Biology, Reed College, Portland, OR, USA.

e-mails: d.h.lunt@hull.ac.uk, renns@reed.edu

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