1. FINAL PUBLISHABLE SUMMARY REPORT

The PARAPOPGENE project addressed the application of a novel comparative molecular approach at different evolutionary, ecological and spatial scales to assess the processes acting on microevolutionary scales to generate variability among populations of parasites, to turn later into the processes acting on macroevolutionaly scales to generate species diversity in parasites.

The main objectives were:

1. To explore how population genetic structure and infection levels of parasites with different host's dispersal abilities were influenced by habitat structure, in particular, the unidirectional river flow.
2. To contrast the population genetics and phylogeographic structure of four freshwater trematode species with different life-history strategies and host's dispersal abilities.
3. To validate a DNA-based diagnostic test of identification of larval stages of *Diplostomum* spp. and develop a rapid method to distinguish between the most frequent *Diplostomum* species.
4. To evaluate the effect of geography and microhabitat preference on the diversity of *Diplostomum* species.

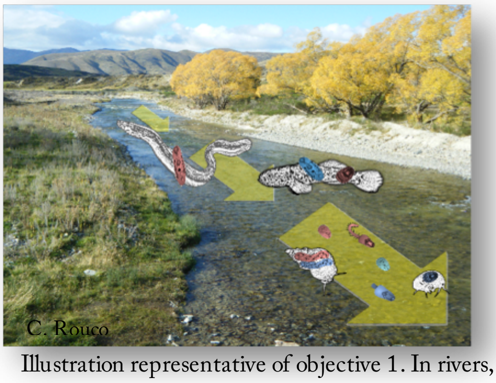

Illustration representative of objective 1. In rivers, while downstream dispersal of infective trematode stages (free-living larvae and metacercariae infecting amphipods) might be passively driven by river current alone, upstream re-colonisation of parasites requires active fish dispersal.

We used two different host-parasite model systems to address the above objectives during the outgoing (objectives 1and 2) and return (objectives 3 and 4) phase of the project.

In rivers, a common process originating from the unidirectional water flow, stream drift, favours the displacement and downstream dispersal of invertebrates. This process could also generate a gradient in infection levels and parasite species genetic diversity, leading to decreasing numbers of parasites per host and decreasing species genetic diversity as one moves upstream from the river mouth. First, to test the effect of unidirectional river current on parasite genetic diversity, we sampled native upland bully fish host (*Gobiomorphus breviceps*) and collected their trematode species at 10 sites along a 70 km stretch of the Manuherikia River (New Zealand). We analysed the genetic structure of two freshwater trematode species with different life histories (*Stegodexamene anguillae* and *Coitocaecum parvum*) using mitochondrial cytochrome c oxidase I gene (*cox*1) sequences. Second, we tested if the unidirectional water flow, stream drift, could generate a gradient in infection levels, leading to decreasing numbers of parasites per host as one moves upstream from the river mouth. We used four trematode species infecting upland bully fish in the same Manuherikia River settings.

The results from the **first objective** have been **published in *Molecular Ecology*** and ***Parasitology***. In brief, genetic structure, genetic diversity loss and drift were stronger in parasites whose most mobile hosts have low dispersal abilities and small home ranges. A prediction can be made for parasites under unidirectional drift: those parasites that stay longer in their benthic intermediate hosts or have more than one benthic intermediate hosts would have relatively high local recruitment and hence increased retention of upstream genetic diversity. We found a longitudinal gradient in trematode abundance along the river with a decreasing downstream-to-upstream continuum. Thus, the unidirectional river flow and a major process like drift in lotic systems, which influences the dynamics and distribution of invertebrate hosts, can also affect the distribution of trematodes. Host properties such as habitat preference, and parasite traits, particularly those related to transmission mode can influence the strength of the observed gradient, as may do other environmental and biotic factors. These results are highly relevant to the aquaculture industry and the policy makers for fish conservation and disease monitoring in freshwater habitats.

The **second objective** was to contrast the population genetics and phylogeographic structure of four phylogenetically distant freshwater trematode species with different life-history strategies and host's dispersal abilities. We sampled the trematodes' fish hosts (upland bully and common bully) at 44 sites throughout their entire geographic range in New Zealand. We used mitochondrial *cox*1 sequences and Amplification Fragment Length Polymorphism (AFLP) to address our second objective (results are summarized in a **manuscript in preparation**).

Following up from the above objectives, we performed the first test of the universality of the type of life-cycle as a driver of parasite dispersal. For some parasites, hosts used in their life-cycle may all be aquatic, i.e. invertebrates, amphibians or fish; in this case, the life-cycle is completed 'locally' (autogenic life-cycle). Alternatively, the final host may be a bird or mammal, which therefore allows passage of parasites from one aquatic locality to another (allogenic life-cycle). We carried out a meta-analysis of 16 published studies of population genetic structure in 16 trematode species using the fixation index, Fst, as a measure of population genetic differentiation. We applied generalised linear mixed models to answer our questions. Results showed a clear general pattern likely applicable to other taxa (a **manuscript** with these results **is under review**).

Hosts provide another habitat dimension for parasites. Parasite species with broad host ranges have often been shown to represent complexes of cryptic species (i.e. morphologically similar but genetically distinct species), being separated by different host preferences. The **third objective** was to validate a DNA-based diagnostic test of *Diplostomum* spp. identification *via* matching precisely identified life-cycle stages with mitochondrial and ribosomal DNA sequences, which enabled us to accurately distinguish between the most frequent *Diplostomum* species combinations in naturally infected fish. Metacercariae of *Diplostomum* spp. infecting three fish second intermediate host species (*Gasterosteus aculeatus*, *Salmo trutta fario* and *Salvelinus alpinus*) and cercariae from the first intermediate snail host, *Radix peregra*, in Iceland, were investigated, using a barcoding approach complemented with sequencing of the internal transcribed spacers 1 and 2 of the ribosomal RNA gene (ITS), and morphological characterisation the larval stages. We successfully matched three of the six *Diplostomum* spp. found and provided character-based molecular barcodes for the *cox*1 and ITS sequences to distinguish Icelandic *Diplostomum* spp. Multivariate analysis allowed as to find the morphometric features that best distinguish among metacercariae of distinct *Diplostomum* spp.

The **fourth objective** addressed the effect of geography and microhabitat preference on the diversity of *Diplostomum* spp. sequenced so far. We reconstructed the evolutionary history of the group using maximum likelihood, Bayesian and coalescence phylogenetic methods. The association of their evolutionary history with the geography and microhabitat specialisation (lens, vitreous humour, retina and brain) was assessed by Bayesian binary analysis and statistical dispersal-vicariance analysis. A **manuscript** on the foreground from objectives 3 and 4 is **in advance preparation**.

Our results are of heuristic value and provide important and broad insights into both the ecological and evolutionary aspects of host-parasite interactions. They contribute to EU excellence in parasite population genetics and increase competitiveness in parasite molecular ecology (e.g. by reinforcing molecular epidemiology studies whose importance is increasingly being recognised). The knowledge gain from this project is highly relevant to policy makers, to EU environmental and biodiversity policies, to the Global Change and Ecosystems priority of FP7 (e.g. contributing to obtaining baseline population genetics data for important fish pathogens which are likely to experience changes in their natural ranges in Europe as a consequence of climatic changes; as well as to protect native freshwater species from alien species and disease spread) and perhaps for the aquaculture industry.

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