Molecular markers, analysis and the population genetics of parasites

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

In this study different molecular techniques are contrasted (RAPD's, allozyme, sequencing mtDNA, sequencing ribosomal spacers) and appropriate analytical methods (allelic and infinite-sites approaches; inbreeding and coalescent models) used for estimating population genetic parameters in parasites. A range of population genetic questions at different scales were chosen to emphasise the importance of tailoring techniques and analytical methods to the particular question being investigated.

The realisation that each question formulated has a particular scale means the appropriate technique and markers must be useful at that scale to attempt to answer the question. The useful scale of a technique depends several factors including the region of DNA examined, the density of sampling of the technique, and the mode of evolution of the markers. Each technique will produce a useful range of variability. Below the lower limit there is no variation, above the upper limit the variation is too high to produce useful comparisons.

Parasites are of interest for many reasons, primarily because they can cause disease and thus impact on their host's population dynamics. They are often closely associated with their hosts and may undergo co-evolution, as well as causing an ongoing immunological "arms race" with their hosts. The parasitic mode of live is found throughout nearly all taxonomic groupings and thus classical models of population genetics based on sexual, diploid vertebrates do not fit well with the entire diversity of parasite groups.

Genetic diversity within and among populations of *Echinococcus granulosus* was examined contrasting a RAPD dataset with an allozyme dataset. Two models of variation in *Echinococcus* have been proposed, those of Smyth and Rausch, and the expected genetic structure from each was compared to the observed genetic structure. The premise of Smyth's model, predominant self-fertilisation, was supported, but the resultant pattern of genetic variation followed Rausch's model.

RAPD data, being dominant, present challenges to analysis. An approach to overcome this dominance problem and allow standard allelic frequency analysis is described using

the selfing rate estimated from allozyme data. The RAPD data were also analysed using both band-sharing and nucleotide diversity approaches.

A population genetic study of *Ostertagia ostertagi* in the USA was extended to two different scales: within an Australian state and between the USA and Australian continents. Three alternative explanations for the observed discrepancy between genetic structure and differentiation in an important biological trait, hypobiosis, were explored. A number of programs and analyses were compared including coalescent geneflow estimates.

Variation among multiple copies of two spacer regions of rDNA was examined within individuals of *Ostertagia ostertagi*. Both the intergenic spacer and internal transcribed spacer 1 regions were found to include repeat regions, with different numbers of repeats creating length differences in clones from the same worm. Multi-copy genes present extra challenges in analysis to ensure that only homologous copies are being compared. Many studies fail to look for variation within populations or within individuals.

The two major conclusions from these examples are that:

1). The study of variation necessarily involves an implicit scale, and markers must be chosen that are appropriate to the question being explored.

2). Using several methods of analysis of genetic data allows contrasts to be made, and if different methods produce similar results gives much more confidence in the conclusions drawn. Incongruence in results leads to new questions and reexamination of the assumptions of each analysis.

Publications

Book chapter:

Thompson, R.C.A., Lymbery, A.J., Meloni, B.P., Morgan, U M, Binz, N, Constantine, C.C. and Hopkins, R.M. (1994). Molecular epidemiology of parasite infections. **In**: Biology of Parasitism (Edited by Ehrlich, R and Nieto, A). Edicio Trilce, Montevideo, Uruguay, pp 167-185.

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List of abbreviations

Fst, Fis, Fit See Table 3.5 and sections 1.5.3.3, 3.2.4.
Fst is a measure of genetic differentiation over subpopulations. The correlation between the probability that two randomly chosen gene copies picked from a subpopulation share an ancestor in the last generation relative to picked from the total population. Uses the partitioning of total genetic variation into variability within and between populations. Fst = (Fit-Fis)/(1-Fis) where t is total, i is individual and s is subdivision.

Gst See Table 3.6 and section 1.5.3.3.
A measure of genetic variation between populations relative to that within populations, a generalised version of Fst. Originally devised by Nei, with the following formula by Slatkin: Gst = 1/(4Nm[k/(k-1)]2 + 1)

Ho, Hs, Ht See Tables 3.2 and 3.5.
Ho = Average observed heterozygosity within groups. Ht = Total heterozygosity in the entire data set. Hs = Gene diversity within groups averaged over the entire data set. See Fst for general concept of subpopulation genetic differentiation.

ITS See Chapter 5 especially Figure 5.1.
Internal Transcribed Spacers are non-coding regions that are transcribed and later excised from the final gene products. There are two ribosomal ITS regions, one between 18S and 5.8S genes, and one between the 5.8S gene and 28S gene. These regions are more variable than the surrounding coding regions.

IGS See Chapter 5 especially Figure 5.1.
Inter genic spacers are non-coding regions beween genes that are not transcribed (although they often include functional motifs such as transcription initiation). Ribosomal IGS is often a large (>1kb) region which is highly variable.

Nm or NeM See Table 3.5 and section 1.5.3.4
A measure of gene flow. The product of effective population size (Ne) and effective migration rate (m). Often estimated using $N_e m = 0.25(1\text{-Fst})/\text{Fst}$ (Nei, 1987)

Nst See section 1.5.3.3.
A specific estimator equivalent to Fst (Lynch & Crease 1990).

RAPD See section 1.4.4.5 and Chapter 3 especially 3.1.4.
Random Amplified Polymorphic DNA is a molecular technique which uses short (10-16bp) single primers which bind wherever complementary sequence is found and when by chance two bind facing each other, close enough for PCR to amplify a fragment is produced which appears as a band when run on a gel. This technique can be applied to any DNA without any knowledge of its sequence. It is highly sensitive to changes in PCR conditions.

UPGMA See section 1.5.3.5.
Unweighted pair group method with arithmetic average to estimate genetic distance between groups. Produces a matrix of distance estimates that is then used by a clustering algorithm to produce a tree.