Population Ecology and Genetics of the Marsh Fritillary Butterfly

*Euphydryas aurinia.*

Submitted by Melanie Rose Smee to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences in June 2011.

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: .................................................................
ABSTRACT

The past two decades have witnessed an unprecedented decline in Lepidopteran species, with more than a third of the UK’s butterflies now either considered threatened, or already lost from the country. The vulnerable marsh fritillary, *Euphydryas aurinia*, after a long term loss in the UK of 73% in abundance, has become an almost iconic species as the target of many well-funded conservation projects across the UK. Despite extensive ecological studies, populations of *E. aurinia* are shown in Chapter 2 to still be declining in south-west UK even after recommended management strategies have been implemented. This necessitates the need for prompt research beyond that of management requirements and butterfly habitat preferences.

In Chapter 3, microsatellite markers (EST-SSRs) were developed for *E. aurinia* and using these markers in Chapter 4, it is shown that *E. aurinia* populations in southern UK and Catalonia, Spain, are severely genetically differentiated at all geographical scales, and genetically depauperate, causing huge concerns for the conservation of this enigmatic and ecologically important species.

Dispersal is fundamental to metapopulation existence and survival. Phosphoglucose isomerase (PGI – an enzyme in the glycolysis pathway) is a well-endorsed candidate gene for dispersal, extensively studied in the Glanville fritillary (*Melitaea cinxia*) and Orange Sulphur (*Colias eurytheme*). In Chapter 5, an analysis across 27 sites in the UK discovered six non-synonymous SNPs (single nucleotide polymorphisms) within PGI. A single charge-changing SNP of interest showed no evidence of balancing selection, contrary to findings in *M. cinxia*, instead appearing to be neutral when analysed alongside microsatellite markers developed in Chapter 3. No link was found between genotype and flight, morphology or population trend. These findings challenge the emerging perspective that PGI could be used as an adaptive molecular marker for arthropods.

*Wolbachia* are endosymbiotic bacteria capable of dramatically altering the reproductive system of their host. In Chapter 6, a PCR-based diagnostic in conjunction with MLST (multi-locus sequence typing) identified 100% prevalence of a single strain of *Wolbachia* across all sampled *E. aurinia* populations in the UK. Total prevalence suggests that *Wolbachia* probably has little phenotypic impact on its host, but the
potential impacts of this endosymbiont on uninfected populations should be considered during any management plans for the conservation of *E. aurinia*.

Current management plans will need to incorporate all areas of research, from basic ecological requirements to molecular adaptation and unseen manipulators of host biology, to be able to fully and effectively conserve declining fragmented species.
ACKNOWLEDGEMENTS

There are a small number of people I couldn’t have done this without, and a large number of people I wouldn’t have wanted to do it without.

I will be forever grateful to my supervisors, Dave Hodgson (a.k.a. ‘Hodgy’) and Richard ffrench-Constant, for giving me the opportunity to do this PhD in the first place, fresh out of my Undergrad. The most humungous thanks go to Hodgy, for making me feel special, I quote “there’s only one Mel Smee”, but capable; for the door that was always open (and I had to creep past to avoid detection); and for letting me “fly away”, knowing I would come back. Thanks also to Richard, for his constant humour and updates on my grandmother (!). And to Caroline Bulman, my supervisor at Butterfly Conservation, for always being encouraging and having a few spare minutes for me even when pregnant.

To Yannick Pauchet I owe so much – there isn’t a big enough thank you for it. For always being there and supporting and encouraging me – picking me up when I was low, and bringing me back down to earth when my head was in the clouds. For nurturing my metamorphosis from an ecologist into a molecular ecologist, and so much more.

Two of the chapters of this thesis wouldn’t have been possible without the amazing generosity and willingness of Mike Singer and Sasha Mikheyev – I am hugely indebted to both for the opportunity given to me to visit Okinawa, and the chance to meet some wonderful people; Tanya, Yutaka, Hitomi, Pascal, Roxy, Laurent, Émile, Yoko & more.

There is no way I would have kept my sanity through these few years if it wasn’t for Julia Reger. Despite being a few hundred miles away, I am so thankful to Ju, just for always being there for me, and the constant emails preventing me from going completely crazy. It could never compare with sitting down with a cup of tea and biscuits and putting the world to rights, but it was damn close.

However, Kate Plummer and I did put the world to rights with endless cups of tea, giggles and chatting, and even tears. I am incredibly grateful for Kate’s friendship over the past few years, even from the other side of the globe. I couldn’t have asked to share a house for 3 years with better people than Kate and Xav Harrison, who provided much banter and makes the best chocolate cake in the world! A special thanks also goes to
Laura Bailey, for being so understanding over these years, and for just being you and always putting a smile on my face; and to Ceri Simmons, who also gave me many reasons to smile over these past few years, including her friendship and the chance to escape and be around horses whenever a spare minute allowed it. And to Pip and the Dorset crowd, my pseudo-family – I just love you loads!

There are endless lists of people who have made the past 3 or so years such brilliant fun. I would like to thank Andy – for my grey hairs. I attribute half to you and half to the PhD itself. Fieldwork would have been a much more tiresome task if it had not been for many helpers in the field: R. Hobson; M. Davey; M. Easter; M. Tunmore; the folk on the Lizard NNR Natural England team (for tea and giggles); Phil Harris for endless enthusiasm; MSc student Sian Rowland; and Nat Cumber, (Brown) Patch and Helen - for making life totally full to the brim of laughs (and Chelsea buns). Thanks also to NE, BC, CWT, DWT and all landowners allowing access to land, as well as all the voluntary collectors in Wales (CCW) and Scotland (SNH), especially Adrian Fowles and Tom Prescott, for all their help. To Blount and Hosken, for ‘corridor banter’ in the form of abusive shouts of ‘Smelly’ and ‘Smeegle’ down the hall way; to the ‘one month post-doc’ Sam – amazing how quickly you can form a firm friendship with someone; to Debbie Mason, the conservationist extraordinaire (marsh frits would be extinct now if you weren’t around to save them); to Casper Breuker, Chris Wheat, Paul Wilkinson, Michelle Hares, Nina Wedell, Zen Lewis, Anna Leonard and many more I’m sure – for help and advice freely given; and of course to all the past and present occupiers of the PhD office – it’s the people that make the experience, and mine has been a fantastic one at Tremough – Devi, Iain, Caro, Ruth, Cheryl, Damo, Lucy, Nic Chamberlain, Erika, Ross, Josie, Jan, Joe and Corrina, Sahran, Fran, Will, Stuart Hinchliffe, Andrea, Si Pickett, Dom & Dom, Ritika, Iva and many more.... A big thank you also goes to all the friends at home in Portsmouth, in Australia, and those made throughout my Undergrad who have stayed in touch and in some way or another helped me through the PhD.

And finally, huge thanks to my Mum, for knowing that ‘no phone call is a good sign’ and always understanding and supporting me; and to Dad and Alice – for constant encouragement and perspective. And finally to my brother Joe – for not leaving the planet entirely and coming back when I needed you most. I love you all very much.
## Table of Contents

Abstract 2  
Acknowledgements 4  
Table of Contents 6  
List of Figures and Tables 10  
List of Plates 12  
Author's Declaration 13  
Definitions and Abbreviations 15  

Chapter 1: Modern approaches to the conservation of lepidopteran species in fragmented landscapes. .................................................................................................................. 18  
1.1 THE CURRENT CONSERVATION CRISIS ......................................................... 19  
1.2 LEPIDOPTERA IN FRAGMENTED LANDSCAPES .............................................. 19  
1.3 CONSERVATION OF DECLINING SPECIES .................................................. 21  
1.3.1 ‘Visible’ factors to consider ........................................................................... 21  
1.3.2 ‘Invisible’ factors of importance .................................................................... 22  
1.3.3 Captive breeding and reintroductions ................................................................. 24  
1.4 CRITICAL FUTURE DIRECTIONS FOR CONSERVATION ECOLOGISTS .......... 25  
1.5 INTRODUCTION TO STUDY SPECIES ............................................................. 26  
1.6 SCOPE OF THESIS ........................................................................................... 29  

Chapter 2: Butterflies on the brink: habitat requirements for declining populations of the marsh fritillary (Euphydryas aurinia) in SW England .............................................................. 31  
2.1 ABSTRACT .......................................................................................................... 32  
2.2 INTRODUCTION .................................................................................................... 33  
2.3 MATERIALS AND METHODS ............................................................................... 35  
2.3.1 Study species .................................................................................................... 35  
2.3.2 Study area ......................................................................................................... 35  
2.3.3 Field Techniques ............................................................................................... 36  
2.3.4 Data Analysis .................................................................................................... 37  
2.4 RESULTS ............................................................................................................... 39  
2.4.1 Can habitat variables explain the presence of E. aurinia? ...................... 39  
2.4.2 Has management achieved optimum levels of habitat variables favoured by E. aurinia? ................................................................. 42  
2.5 DISCUSSION ........................................................................................................ 48
2.5.1 Using management tools to create suitable habitat............................... 48
2.5.2 Response of the butterfly – are we doing enough?............................... 49
2.5.3 Implications for the conservation of E. aurinia across the mid-Cornwall Moors, and beyond. .............................................................................. 51

Chapter 3: Development of microsatellites for the Vulnerable marsh fritillary butterfly by de novo transcriptome sequencing................................................................. 52

3.1 ABSTRACT .......................................................... 53
3.2 INTRODUCTION ..................................................... 54
3.3 MATERIALS AND METHODS ..................................... 55
3.4 RESULTS ................................................................ 59
3.5 DISCUSSION ............................................................ 60

Chapter 4: To Protect or Connect? Population genetic analyses inform conservation strategies for the marsh fritillary butterfly, Euphydryas aurinia........................................ 61

4.1 ABSTRACT .......................................................... 62
4.2 INTRODUCTION ..................................................... 63
4.3 MATERIALS AND METHODS ..................................... 66
  4.3.1 Sample collection and preparation.............................................. 66
  4.3.2 Genotyping........................................................................ 68
  4.3.3 Analysis of genetic diversity and variation................................... 69
  4.3.4 Population genetics – is sampling information needed?.............. 69
4.4 RESULTS ................................................................ 71
  4.4.1 International broad-scale analysis.............................................. 73
  4.4.2 Regional fine-scale analysis...................................................... 76
4.5 DISCUSSION ............................................................ 82
  4.5.1 Genetic variation............................................................ 83
  4.5.2 Isolation by distance .......................................................... 83
  4.5.3 Population structure........................................................... 84
  4.5.4 Comments on methods and future work .................................. 85
  4.5.5 Conclusions............................................................... 87

Chapter 5: Pgi’s Might Fly: A purported 'gene for' dispersal shows no signal of selection in the marsh fritillary butterfly. ........................................................................ 89

5.1 ABSTRACT .......................................................... 90
5.2 INTRODUCTION ..................................................... 90
5.3 MATERIALS AND METHODS ..................................... 94
  5.3.1 SNP genotyping ......................................................... 94
  5.3.2 Flight assays in the field ..................................................... 97
5.3.3 Statistical analysis of SNP genotype against ecological data .......................... 98
5.4 RESULTS ........................................................................................................ 99
  5.4.1 SNP variation ............................................................................................. 99
  5.4.2 Is PGI under selection? ............................................................................. 102
  5.4.3 Genotypic effects on ecological variables .............................................. 103
5.5 DISCUSSION .................................................................................................. 105
  5.5.1 Variation in *Pgi* genotype frequencies .................................................. 105
  5.5.2 Relating *Pgi* genotypes to flight and population trend ...................... 107
  5.5.3 Further studies needed for *E. aurinia* .................................................. 107
  5.5.4 Conclusions .............................................................................................. 108
5.6 SUPPLEMENTARY MATERIAL ..................................................................... 110
  5.6.1 RNA isolation and cDNA synthesis .......................................................... 110
  5.6.2 Sequencing of *Pgi* cDNA ....................................................................... 110
  5.6.3 Single nucleotide polymorphisms (SNPs) in *Pgi* across 27 sites ......... 111

Chapter 6: Friend or foe? *Wolbachia* infection in the threatened British butterfly

*Euphydryas aurinia* ............................................................................................... 114

  6.1 ABSTRACT .................................................................................................... 115
  6.2 INTRODUCTION ............................................................................................ 116
  6.3 MATERIALS AND METHODS ..................................................................... 119
    6.3.1 Sample collection and preparation ......................................................... 119
    6.3.2 Detecting *Wolbachia* ........................................................................... 120
    6.3.3 Multilocus sequence typing (MLST) ...................................................... 121
    6.3.4 Phylogenetic analysis ............................................................................ 122
  6.4 RESULTS ....................................................................................................... 123
    6.4.1 Screening for *Wolbachia* .................................................................... 123
    6.4.2 Multilocus sequence typing (MLST) ...................................................... 124
    6.4.3 Phylogenetic analysis ............................................................................ 125
  6.5 DISCUSSION ................................................................................................. 129

Chapter 7: General Discussion ............................................................................. 134

  7.1 GENERAL DISCUSSION .............................................................................. 135
    7.1.1 Baseline research of species ecology is still needed ......................... 135
    7.1.2 Neutral molecular markers: useful, but questionable? ...................... 136
    7.1.3 Adaptive molecular markers in lepidopteran conservation ............. 140
    7.1.4 Out of sight, out of mind – unseen foes must not be forgotten ......... 141
    7.1.5 Management recommendations ........................................................... 142
7.1.6 Conclusions ................................................................................................................. 144

Appendices ......................................................................................................................... 146

Appendix 1: Genomic DNA extraction protocol .................................................................. 147

Appendix 2: Sequencing the glycolytic enzyme phosphoglucose isomerase (Pgi) from

Euphydryas aurinia ................................................................................................................. 148

WHAT IS PGI? ....................................................................................................................... 148

WHY IS PGI OF INTEREST? ................................................................................................. 149

ATTEMPTS TO OBTAIN THE WHOLE GENOMIC LENGTH OF PGI................................. 150

References ............................................................................................................................ 153
List of Figures and Tables

CHAPTER 1

FIGURE 1.1 – The annual number of publications including the word metapopulation* in the abstract or title when ISI Web of Science was queried. .................................................. 20

FIGURE 1.2 – The change in the British distribution of E. aurinia from the period of 1690 – 1994 to more recent times of 2000 – 2004. ................................................................. 27

CHAPTER 2

FIGURE 2.1 - South-west England, showing the project area across the Mid-Cornwall Moors ........................................................................................................................................ 36

TABLE 2.1 - Results of a linear mixed effects model using multiple regression to relate habitat variables with larval web densities and adult butterfly densities. ............ 40

TABLE 2.2 - Results of linear mixed effects models using single regressions to determine the gross influence of each habitat variable on density of larval webs and adult butterflies. .......................................................................................................................... 41

FIGURE 2.2 - The significant quadratic effect of stock grazing on density of larval webs ........................................................................................................................................ 42

FIGURE 2.3 - The year when grazing was started has a significant effect on the occurrence of S. pratensis .................................................................................................................. 43

FIGURE 2.4 - Temporal comparison of S. pratensis density for each site over the five years of the project .................................................................................................................. 44

FIGURE 2.5 - Autumn sward height is significantly affected by the year in which grazing started and the intensity of grazing ........................................................................ 46

FIGURE 2.6 - Temporal comparison of log transformed larval web densities and adult densities across the five years of monitoring at all sites and Individual temporal trends of web densities at only those sites where E. aurinia has an extant colony 47

CHAPTER 3

TABLE 3.1 – Characteristics of 14 microsatellite loci in Euphydryas aurinia (Lepidoptera: Nymphalidae) .................................................................................................................. 58

FIGURE 3.1 – Hardy-Weinberg equilibrium (HWE) ‘exact test’ statistics for each of the 28 populations included in the study ........................................................................... 59
CHAPTER 4

FIGURE 4.1 – Map of samples sites across the UK and Catalonia area of Europe.

TABLE 4.1 – Microsatellite summary statistics for nine loci used to genotype *E. aurinia* across the UK and Catalonia area of Europe.

FIGURE 4.2 – LOSITAN results for all loci across all samples.

TABLE 4.2 - Sites across the UK and Catalonia area of Europe from which *E. aurinia* was sampled, showing genetic diversity statistics per ‘geographical’ site.

TABLE 4.3 - Euclidean distances (km) against genetic distance for the five regions studied.

FIGURE 4.3 – Isolation-by-distance across all samples.

FIGURE 4.4 - Output of STRUCTURE analysis for genetic clusters across the entire region studied.

TABLE 4.4 – Analysis of molecular variance (AMOVA) results at an international scale, comparing the use of geographical and genetic ‘populations’.

TABLE 4.5 - Pairwise *F*<sub>ST</sub> distances against Euclidean distances (km) for populations in southern England.

TABLE 4.6 - Pairwise *F*<sub>ST</sub> distances against Euclidean distances (km) for populations in Catalonia.

FIGURE 4.5 – IBD analyses for southern England and Catalonia.

FIGURE 4.6 – Output from a Bayesian clustering analysis in STRUCTURE v.2.3.3 for populations in southern England, and populations in the Catalonia area of Europe.

FIGURE 4.7 – Overall view of clustering analyses in STRUCTURE v.2.3.3.

TABLE 4.7 – Analysis of molecular variance (AMOVA) results at a regional scale, comparing the use of geographical and genetic ‘populations’.

TABLE 4.8 – A summary of studies investigating population genetics of *E. aurinia*.

CHAPTER 5

FIGURE 5.1 – Map of samples sites across the UK and Catalonia.

TABLE 5.1 - The observed frequencies of each genotype at SNP 373 across 30 sampling sites.

FIGURE 5.2 - Genotype frequencies of *Pgi* SNP 373 across the study area, including southern England, as well as Wales and Scotland and Catalonia.

FIGURE 5.3 - LOSITAN results for all samples in the UK for which there was data for both microsatellites and *Pgi*.
Figure 5.4 - Logged means ± standard errors of distance flown and duration of flight for butterflies of differing genotypes.................................................................103
Figure 5.5 – Population trend against the frequency of each genotype at a site ....... 104
Figure 5.5.1 – Twenty seven sites from which an individual larvae was sampled and sequenced for the coding region of phosphoglucone isomerase (Pgi)................112
Figure 5.5.2 – Synonymous and non-synonymous single nucleotide polymorphisms (SNPs) across the twelve exons of Pgi. ...............................................................113

Chapter 6
Figure 6.1 – Sample sites in the south-west UK, Wales and Scotland ................... 119
Figure 6.2 – An example agarose gel for scoring presence/absence of Wolbachia.... 121
Table 6.1 – List of primers used during screening and MLST ..............................122
Table 6.2 – Population information from each site sampled and numbers of individuals from each site screened with wsp primers .............................................123
Table 6.3 – Putative sex ratios at a sub-sample of populations ..............................124
Figure 6.3 – Comparison of wsp sequences from strains of differing phenotype, via sequence alignment and phylogenetic analysis ..............................................126

Chapter 7
Figure 7.1 – Interplay of population spatial structure, IBD and FST.........................139

Appendices
Figure A1 – The metabolic pathway of glycolysis.................................................149
Table A1 – Primer sequences tested for genomic length of Pgi ............................150
Figure A2 – Progress made to gain the genomic length of Pgi ............................152

List of Plates

Chapter 1
Plate 1.1 – Dorsal and ventral views of an adult E. aurinia at Lydlinch, 2009..........28
Plate 1.2 – A web of 4th instar E. aurinia larvae at Stithians, 2008........................29
AUTHOR’S DECLARATION

This thesis was made possible by funding from the Biotechnology and Biological Sciences Research Council (BBSRC) in conjunction with a CASE partnership from Butterfly Conservation (BC). All of the chapters presented in this thesis were written by M. R. Smee, with additional editing by D. J. Hodgson. Individual contributions to each chapter are listed below.

An initial 5 year partnership project was undertaken by Natural England; Cornwall Wildlife Trust; Butterfly Conservation; The Environment Agency and The Highways Agency, across central Cornwall as part of the Natura 2000 series of sites - sites of European importance for nature conservation. W. Smyth (Natural England) and M. Tunmore (Cornwall Environmental Consultants) were involved in this initial project. M. R. Smee continued the project for another year, analysed the 6-year dataset and wrote the manuscript. D. J. Hodgson assisted with statistical issues and reviewed the manuscript. W. Smyth and M. Tunmore reviewed the manuscript before it was sent for publication.

CHAPTER 3
M. C. Singer (University of Texas) was instrumental in making this study possible, and also provided samples of *E. aurinia* from European populations, as did B. Wee (National Ecological Observatory Network, USA). R. ffrench-Constant provided funding for the transcriptome of *E. aurinia* to be gained from 454-pyrosequencing and Y. Pauchet extracted RNA with M. R. Smee and prepared the normalized library. P. Wilkinson assembled the 454 data and ran the query script for perfect microsatellite repeats. A. S. Mikheyev (Okinawa Institute for Science and Technology) hosted M. R. Smee whilst the lab work was carried out and provided the funding for all lab consumables, travel and accommodation for the two month duration, as well as advice on microsatellite marker choice and analysis. Initial draft prepared by M. R. Smee and improved by Y. Pauchet and D. J. Hodgson.

CHAPTER 4
Contributions as for Chapter 3. X. Harrison provided initial training in the use of STRUCTURE, and R. ffrench-Constant provided useful comments on the initial draft.
CHAPTER 5
Initial idea to study PGI in *Euphydryas aurinia* suggested by R. ffrench-Constant, who also provided funding for lab consumables and sequencing throughout the project. Y. Pauchet provided M. R. Smee with training in molecular techniques, both in the laboratory and on computer programmes, from PCR and primer design to sequence alignment. P. Wilkinson did the sequencing and D. Smith carried out some final PCRs to complete the dataset for analysis. M. R. Smee carried out statistical analyses and wrote the initial draft, both of which were improved upon by D. J. Hodgson.

CHAPTER 6
**DEFINITIONS & ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 pyrosequencing</td>
<td>One method of next-generation sequencing (NGS), originating from a company called 454 Life Sciences but now owned by Roche Diagnostics, which uses a &quot;sequencing by synthesis&quot; approach - relying on the detection of pyrophosphate release on nucleotide incorporation rather than chain termination as in Sanger sequencing. See NGS definition for more general information.</td>
</tr>
<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphism PCR uses primers for known regions of the genome to amplify genomic DNA, which is then digested by restriction enzymes and run on polyacrylamide gels to visualise differences in fragment lengths.</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs of DNA.</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA synthesized from an mRNA template.</td>
</tr>
<tr>
<td>Co-dominant markers</td>
<td>Molecular markers in which both the alleles present at a locus contribute to the expressed phenotype and heterozygotes can be distinguished from homozygotes.</td>
</tr>
<tr>
<td>Contig</td>
<td>Often used to determine the original DNA sequence of the source material, contigs are sets of overlapping segments of DNA from a single genetic source.</td>
</tr>
<tr>
<td>Endosymbiont</td>
<td>An organism living in close contact – symbiosis – with another organism for the majority of its life.</td>
</tr>
<tr>
<td>EST</td>
<td>Expressed sequence tag: a short sub-sequence of a cDNA sequence.</td>
</tr>
<tr>
<td>HWE</td>
<td>Hardy-Weinberg equilibrium: allele and genotype frequencies in a population are in equilibrium, or remain constant, from generation to generation.</td>
</tr>
<tr>
<td>LIFE and Natura2000</td>
<td>LIFE (the Financial Instrument for the Environment), is one of the spearheads of the European Union's environmental policy and was introduced in 1992. It co-finances projects in three areas: LIFE-Nature, LIFE-Environment and LIFE-Third Countries. The LIFE-Nature fund supports the positive management and enhancement of sites of European importance for nature conservation, collectively known as the Natura 2000 series of sites - SPAs and SCIs (Site of Community Importance).</td>
</tr>
</tbody>
</table>
Metapopulation

Existing at the landscape level and consisting of several (>2) smaller geographically separated subpopulations. The metapopulation as a whole can persist if there is a balance between stochastic extinctions and recolonisations across the fragmented landscape.

Microsatellite, (EST-SSR)

Also known as simple sequence repeats (SSRs) or short tandem repeats (STRs). These are tandem repeating sequences of (perfect or compound) di-, tri- or tetra- (and so on) nucleotide motifs, for example, a tri-nucleotide repeat; CAACAACAACAACA. Microsatellites are co-dominant and typically neutral, hence their use as molecular markers in population genetics. If derived from a transcriptome, as is the case here, they are also referred to as EST-SSRs, which are SSRs derived from ESTs (see above).

MLST

Multi locus sequence typing: isolates of bacterial species are characterised using DNA sequences of internal segments of several housekeeping genes (usually five or six). Each loci may have several alleles, and so the combination of alleles across these genes defines the unique allelic profile (or sequence type - ST) of the bacterial strain.

Neutral variation

Variation that has a small selection coefficient relative to the population size, such that |s| < 1/2Ne, where s is either the selective disadvantage of a detrimental genetic variant or the selective advantage of an adaptive genetic variant and Ne is the effective population size.

NGS

Next-generation sequencing is massively parallel DNA sequencing, where millions of reads (sequences) can be generated over the course of a few hours. Generally, the reads generated are smaller (300 - 500 bp) than when using Sanger sequencing technologies (800 - 1000 bp). Roche 454 FLX Titanium system (454 pyrosequencing) is one of the better known platforms for NGS, along with Illumina's (Solexa) Genome Analyser and ABI's SOLiD.

Null Alleles

allele(s) that fail to amplify within an individual because of mutations at primer annealing sites, and hence cause an individual to appear homozygote for the second allele present at that locus. Null alleles can be a significant problem in studies of population genetics as they skew results towards an excess of homozygotes, where there actually might not be an excess.

PCR

Polymerase chain reaction: a technique to amplify thousands to millions of copies of a particular DNA sequence from a single or just a few original copies.
| **PGI** | **Phosphoglucone isomerase:** an enzyme in the metabolic pathway of glycolysis. PGI catalyses the second step of the pathway in which glucose-6-phosphate is converted to fructose-6-phosphate. High energy molecules of ATP are released during glycolysis, which are used for flight and other essential cellular functions. |
| **SNP** | **Single nucleotide polymorphism:** a difference of a single nucleotide (A, T, C or G) in the DNA sequence of different individuals. |
| **Subpopulation** | One unit of a metapopulation, containing a breeding population of the species. |
| **Thelytoky** | Parthenogenesis in which only female offspring are produced. |
| **Univoltine** | One generation a year. |
| **UTR** | **Untranslated region:** the sections either side of the coding region of a gene which are not translated into amino acids. |
| **‘vulnerable’** | IUCN Red Data List category: species which are classified as 'vulnerable' are likely to become 'endangered' unless trends are reversed. A decline over 10 years of 30 - 49% deems a species as 'vulnerable'. |