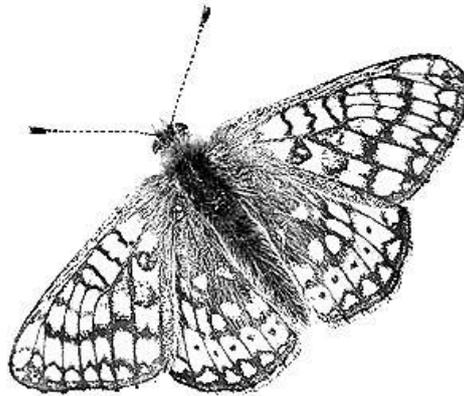


Population Ecology and Genetics of the Marsh Fritillary Butterfly *Euphydryas aurinia*.



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.

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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.

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AUTHOR'S DECLARATION

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DEFINITIONS & ABBREVIATIONS

454 pyrosequencing	One method of next-generation sequencing (NGS), originating from a company called 454 Life Sciences but now owned by Roche Diagnostics, which uses a "sequencing by synthesis" 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.
AFLP	<u>A</u> mplified <u>f</u> ragment <u>l</u> ength <u>p</u> olymorphism 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.
bp	<u>b</u> ase <u>p</u> airs of DNA.
cDNA	Complementary DNA synthesized from an mRNA template.
Co-dominant markers	Molecular markers in which both the alleles present at a locus contribute to the expressed phenotype and heterozygotes can be distinguished from homozygotes.
Contig	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.
Endosymbiont	An organism living in close contact – symbiosis – with another organism for the majority of its life.
EST	<u>E</u> xpressed <u>s</u> equences <u>t</u> ag: a short sub-sequence of a cDNA sequence.
HWE	<u>H</u> ardy- <u>W</u> einberg <u>e</u> quilibrium: allele and genotype frequencies in a population are in equilibrium, or remain constant, from generation to generation.
LIFE and Natura2000	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).

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	<u>M</u> ulti <u>l</u> ocus <u>s</u> equencing <u>t</u> yping: 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 < 1/2N_e$, where s is either the selective disadvantage of a detrimental genetic variant or the selective advantage of an adaptive genetic variant and N_e is the effective population size.
NGS	<u>N</u> ext- <u>g</u> eneration <u>s</u> equencing 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	<u>P</u> olymerase <u>c</u> hain <u>r</u> eaction: a technique to amplify thousands to millions of copies of a particular DNA sequence from a single or just a few original copies.

PGI	<u>P</u>hospho<u>g</u>lucose <u>i</u>somerase : 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	<u>S</u>ingle <u>n</u>ucleotide <u>p</u>olymorphism : 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	<u>U</u>n<u>t</u>ranslated <u>r</u>egion : 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'.