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Fifteen new polymorphic microsatellite loci for the meadow brown butterfly, *Maniola jurtina*

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ABSTRACT

We characterized fifteen microsatellite markers for the butterfly *Maniola jurtina*. For the six studied populations (96 samples) the total number of alleles per locus ranged from 3 to 55 and mean overall expected heterozygosity across all loci was 0.74. In spite of a high frequency of null alleles detected in part of the loci, a recurrent phenomenon in Lepidopteron, the estimation of pairwise F_{ST} seems rather insensitive to the presence of these null alleles as shown by the high correlation between F_{ST} calculated after correction for the presence of null alleles and non-corrected F_{ST}, indicating that the loci may be usable in population genetics, more specifically for the study of populations genetics structure.

1. Introduction

Maniola jurtina is a widespread univoltine species in north-western Europe. Although it is one of the most abundant butterflies in France, *M. jurtina* has declined over the last two decades (EEA, 2013) and suffered from habitats loss in intensively cultivated landscapes where patchiness of remnant suitable habitats makes dispersal ability crucial (Delattre et al., 2013). Quantifying gene flow within and between these remnant sites is thus key to understand population dynamics and to estimate the risks of high habitat fragmentation due to human activities, and ultimately to design conservation plans. Here we report the isolation and characterization of 15 unlinked microsatellite loci, validated, for their use in population genetics structure studies, on 96 individuals from six populations representing five regions all around France.

2. Methods

2.1. Microsatellite analysis and genotyping

Loci were developed by ECOGENICS GmbH (http://www.ecogenics.ch); Zürich, Switzerland using DNA from the head of 12 individuals (6 males and 6 females from Burgundy). Size-selected fragments from genomic DNA were enriched for SSR

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content by using magnetic streptavidin beads and biotin-labeled GATA and GTAT repeat oligonucleotides. The SSR-enriched library was analyzed on a Roche 454 platform using the GS-FLX Titanium reagents. The total 9509 reads had an average length of 427 base pairs. Of these, 646 contained a microsatellite insert with a tetranucleotide of at least 6 repeat units. Suitable primer design was possible in 374 reads. After testing for the quality of amplification and polymorphism, 15 loci were finally selected and tested on a larger sample of individuals from France (Table 1).

To test for their utility in population genetics, we amplified the 15 selected loci on a set of 96 individuals originating from 6 populations (16 individuals per population) from five regions all around France (from North-East to South-West: Lorraine, Franche-Comté, Burgundy, Midi-Pyrénées, Aquitaine). We used non-letal DNA sampling by collecting one leg per individual.

We extracted total DNA from individual butterflies leg using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). Before enzymatic digestion, each butterfly leg was cut in 4–8 pieces to facilitate DNA extraction. The 15 loci were amplified in three Multiplexes, in 10 μl reaction volumes using Qiagen Type-it Microsatellite kit with 5 μl of PCR MasterMix (HotStarTaq Plus DNA polymerase, PCR buffer, dNTP mix), 1 μl of template DNA (1–10 ng), 1 μL of primer mix (final concentrations: see Table 1)

Table 1
Characteristics of 15 microsatellite loci in *Maniola jurtina*, tested on 96 individuals from 6 populations, from several regions in France (from North-East to South-West: Lorraine, Franche-Comte, Burgundy, Midi-Pyrenees, Aquitaine).

Locus (GenBank AN)	Primer sequence (5′-3′)	MP	[Pp] (μM)	Repeat motif	Size-range (bp, n = 96)	NA (n = 12)	NA (n = 96)	Mean He (SE)	Mean Ho (SE)	Frequency of null alleles: mean (range)
Mj0008	F: PET-CGTGTCGCCTAAACCACATC	1	0.2	(ACAT)7	93–155	3	3	0.038	0.042	0.001 (0.000–0.001)
(KT264265)	R: TGGCAACCCTAAACCCTACG							(0.028)	(0.031)	
Mj3956	F: PET-CAACATCGGGAGTCGAAACG	2	0.12	(GATA)7	110–249	5	17	0.783	**0.356**	0.242 (0.183–0.290)
(KT264271)	R: CTCAGCCAGGATACCCACTC							(0.035)	**(0.057)**	
Mj5331	F: PET-TTAGACCGTGATCCCACTGC	3	0.12	(TATC) 10	100–131	11	19	0.876	0.833	0.031 (0.000–0.087)
(KT264274)	R: ATTTCGATAGGCAACGAGGC							(0.011)	(0.042)	
Mj5287	F: 6FAM-GCTAGCTCGTGGGTACTCTG	1	0.3	(GATA) 11	128–189	7	10	0.413	**0.094**	0.250 (0.189–0.346)
(KT264273)	R: CTCCAAGCAATAAGACCGCC							(0.072)	**(0.048)**	
Mj7232	F: 6FAM-AAGTTACAAGAGCGTTGGCG	2	0.24	(CTGT)7	146–221	9	12	0.803	0.677	0.082 (0.015–0.175)
(KT264279)	R: GCGGGAACTCTTGGGTTTTC							(0.011)	(0.057)	
Mj4870	F: 6FAM-ATGATCCATAGCTGCGTTGC	3	0.2	(ATGT)7	161–178	4	10	0.707	0.396	0.177 (0.000–0.330)
(KT264272)	R: CTCCTTAGCGCTTACACGTC							(0.015)	(0.100)	
Mj7132	F: NED-ATCTGCGGATTTGCAGTTGG	1	0.16	(TATG) 13	159–211	11	11	0.808	0.779	0.023 (0.000–0.058)
(KT264278)	R: CACTATTGAGCACGTGTGTCC							(0.016)	(0.037)	
Mj5522	F: NED-TGATCTTTGCCAGCAGGAAC	2	0.12	(GATA)8	156–207	9	17	0.846	**0.521**	0.180 (0.129–0.226)
(KT264275)	R: AGTGTAAGCTGGCCCTAAAC							(0.007)	**(0.026)**	
Mj3637	F: NED-CTTCCGCAAAATAACGTCTGC	3	0.12	(TCTA)7	171–207	5	8	0.734	0.490	0.143 (0.112–0.238)
(KT264270)	R: AGATACTCCATTGACCCGGC							(0.013)	(0.037)	
Mj5647	F: PET-GCGTTCTGATTACCACCCTG	1	0.3	(TATG) 13	172–246	9	28	0.860	**0.367**	0.261 (0.001–0.381)
(KT264277)	R: GCGACAGTCCCCTAAGATCG							(0.021)	**(0.082)**	
Mj0247	F: PET-ATTCCACAAACGAGCCAACG	2	0.24	(GATG)8	185–312	13	37	0.917	0.750	0.087 (0.021–0.160)
(KT264266)	R: ACTCCGATGGTAAGAGGTGC							(0.005)	(0.043)	
Mj2410*	F: PET-TAATTAGAGTTTGCGCGGGG	3	0.24	(TGTA)7	189–262	9	21	0.869	**0.573**	0.152 (0.001–0.290)
(KT264269)	R: CGCACACCGCAGTATAAGTG							(0.009)	**(0.078)**	
Mj5563	F: VIC-CGGTTTTGCCGATAGCGTAG	1	0.3	(ATCT)7	188–393	16	55	0.932	0.713	0.114 (0.033–0.219)
(KT264276)	R: CGCAAGGCAATAGACCACTC							(0.005)	(0.064)	
Mj0272# (KT264267)	F: VIC-GTTGCATTGGCACACTCCTC R: CAGCTGCACACTACGACAAG	2	0.3	(AGAT)7	209–336	8	15	–	–	– –
Mj0283	F: VIC-CCCTTAGAATAAGAACTCGGCTC	3	0.16	(AGAT)9	190–250	7	15	0.790	0.407	0.208 (0.001–0.318)
(KT264268)	R: TGTTCGCACATGCTTAGTCC							(0.012)	(0.082)	

Genbank AN: Genbank Accession Number, MP: PCR multiplex, [Pp]: primer pair concentration, NA: number of alleles (for the 12 individuals used for the development and the 96 from 6 populations used for the characterization), mean and SE (standard deviation): over the populations, He: expected heterozygosity, Ho: observed Heterozygosity (in bold: significant departure from Hardy–Weinberg equilibrium over the populations), *: sex-linked locus, #: null alleles in to high frequency, no estimations.