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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.

and 3 μl of high purity water. Polymerase chain reaction (PCR) conditions were set on Senso Quest thermal cycler as follows: initial denaturation 10 min at 94 °C; 35 cycles of 1.5 min (for the 10 first) or 30 s (for the 25 following) at 56 °C, 30 s at 72 °C; final elongation of 5 min at 72 °C. Total reaction volume was 10 μL, of which 1 μL was DNA. PCR products were run on an ABI 3730 DNA Analyzer (Applied Biosystems) with the GeneScan-500 LIZ size standard and we performed genotyping and manually confirmed all peaks with GeneMapper 4.0 (Applied Biosystems).

2.2. Genetic analyses

We tested for linkage disequilibrium among pairs of loci per population using Genepop (Rousset, 2008), we used GenAlEx (Peakall and Smouse, 2012) to obtain the number of alleles per marker, expected and observed Heterozygosity and departure from Hardy–Weinberg equilibrium. We used FreeNA (Chapuis and Estoup, 2007) and Micro-Checker (Van Oosterhout et al., 2004) to detect the presence of null alleles and quantify their frequency. FreeNA was also used for the estimation of the pairwise Fst.

3. Results

Table 1 summarizes the results. The total number of alleles per locus in the 96-samples set ranged from 3 to 55 and there was no linkage disequilibrium between pairs of loci across all populations (Appendix). Mean overall expected heterozygosity across all loci was 0.74. Five loci repeatedly departed from Hardy–Weinberg Equilibrium (bold values in Table 1). One locus had a very high frequency of null alleles (Mj0272) and was discarded from estimations of heterozygosity; seven others exhibited moderate frequency of null alleles (<15%). The high frequency of null alleles estimated for the locus Mj2410 was due to a sex-linkage: all females were homozygote (butterflies have a ZW sex-determination system, with heterogametic ZW females and homogametic ZZ males). Pairwise F_{ST} among the 6 populations are shown in Table 2, for 14 loci with or without the ENA correction for the presence of null alleles (Chapuis and Estoup, 2007) and for the 9 loci which do not exhibit a departure from Hardy–Weinberg equilibrium over all the populations. The correlation between these two F_{ST} estimates are shown in Fig. 1.

4. Discussion

The 454 GS-FLX Titanium technology allowed the isolation of 15 polymorphic microsatellite loci in M. jurtina. The presence of many null alleles is recurrent when developing microsatellite in Lepidopteran (Nève and Meglécz, 2000), even when a combination of biotin-enrichment protocol and 454 GS-FLX Titanium technology is used (Sinama et al., 2011). Here we had to discard only one locus that had too many null alleles to compute heterozygosity estimates. Seven other loci had a moderate frequency of null alleles. But the estimation of pairwise F_{ST} among the 6 populations seems rather insensitive to the presence of these null alleles: F_{ST} calculated after correction for the presence of null alleles were significantly correlated with

Table 2
Pairwise F_{ST} (14 loci) estimated with FreeNA, with or without the ENA correction described in Chapuis and Estoup (2007) and for 9 loci (with no significant departure from Hardy–Weinberg equilibrium over the populations) for the six populations (from North-East to South-West: Lorraine, Franche-Comte, Burgundy, Midi-Pyrenees, Aquitaine). The ENA correction method was found to efficiently correct for the positive bias induced by the presence of null alleles on F_{ST} estimation and provide accurate estimation of F_{ST} in presence of null alleles.

Fst:14 loci not using ENA						
Pop		Lorraine	Aquitaine	Burgundy 1	Burgundy 2	Franche-Comté
	Aquitaine	0.000704				
	Burgundy 1	0.003995	0.000601			
	Burgundy 2	−0.005661	−0.002679	−0.001216		
	Franche-Comté	0.000942	−0.003896	0.002051	−0.007594	
	Midi-Pyrénées	0.002657	−0.002965	0.017868	0.003523	0.008818
Fst: 14 loci using ENA						
Pop		Lorraine	Aquitaine	Burgundy 1	Burgundy 2	Franche-Comté
	Aquitaine	0.003057				
	Burgundy 1	0.006097	−0.001331			
	Burgundy 2	−0.00341	−0.000961	0.001809		
	Franche-Comté	0.006993	0.003866	0.006293	−0.001263	
	Midi-Pyrénées	0.005858	0.001406	0.019374	0.007722	0.013653
Fst:9 loci						
Pop		Lorraine	Aquitaine	Burgundy 1	Burgundy 2	Franche-Comté
	Aquitaine	0.001347				
	Burgundy 1	0.000941	−0.001282			
	Burgundy 2	−0.007556	−0.004825	−0.000196		
	Franche-Comté	0.003943	−0.008219	−0.002836	−0.005615	
	Midi-Pyrénées	0.014265	−0.001643	0.015859	0.015726	0.015726

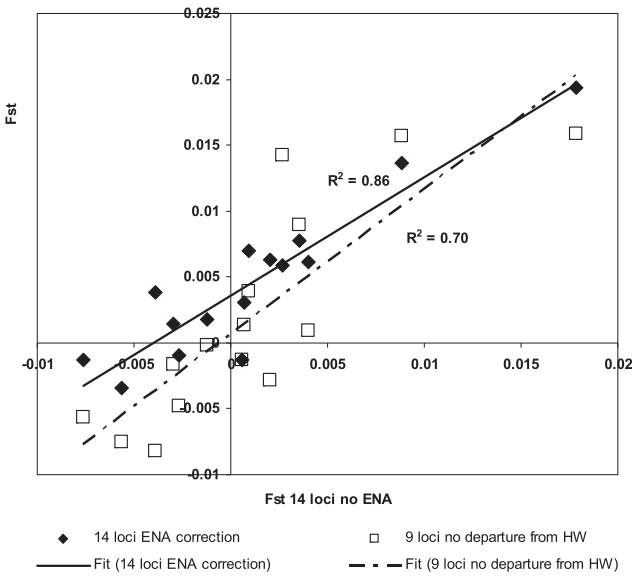

Fig. 1. Correlation (Pearson coefficient) between pairwise Fst with 14 loci without ENA correction and Fst with 14 loci with ENA correction or Fst with the 9 loci with no significant departure from Hardy–Weinberg equilibrium over the 6 populations.

non-corrected F_{ST} (Fig. 1, similar result for Fst calculated with the 9 loci which did not exhibit a significant departure from Hardy–Weinberg equilibrium), indicating that the 14 loci may be usable for studies of population genetics structure.

Pairwise F_{ST} values were rather low (all below 0.02) meaning that isolation by distance is negligible at the scale of our study. This is in agreement with two other studies using allozymes that reported mean F_{ST} values of 0.015 (Goulson, 1993) and 0.05 (Wood and Pullin, 2002) for populations sampled over 130 km and 30 km respectively.

The mean dispersal distance of Maniola jurtina is considered as rather low, in the magnitude of several hundred meters (Schneider, 2003). The very low F_{ST} values reported here might be due to either long distance dispersal underestimated until now, or to gene flow between regions by stepping stones. These two hypotheses are supported by contradictory evidences. The first one is supported by Schneider (2003), who showed that there was a positive linear relationship between dispersal distances and the scale of study sites. A mark-release-recapture experiment reported maximal dispersal distances up to 2 km (Schneider et al., 2003). Long-distance dispersal are thus possible. The second one is supported by Delattre (2010), who showed that dispersal events drastically decreased when suitable habitats were separated by more than 200 m. The willingness of leaving a suitable habitat is thus depending on its connectivity with neighbor patches. We fully agree that the temporal scales of these two contradictory evidences are quite different (days for mark-release-recapture experiment, 30 min for behavioral observations). However, each of them might support either long distance dispersal or dispersal between stepping stones. Using these new microsatellite markers within a landscape genetics framework will certainly shed light on this controversial issue. Indeed, it will now be possible to understand if and how landscape composition and configuration influence dispersal behaviors in this butterfly.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2015.10.006.

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