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Short Communication

Optimization and validation of a multiplex assay for microsatellite loci analysis in the field cricket, *Gryllus campestris* (Orthoptera: Gryllidae)

H. Panagiotopoulou [a,*], M. Baca [b], K. Baca [c], A. Stanković [a,c,d], M. Żmihorski [e,f]

[a] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland
[b] Centre for Precolumbian Studies, University of Warsaw, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland
[c] Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Pawińskiego 5a, 02-106 Warsaw, Poland
[d] The Antiquity of Southeastern Europe Research Center, University of Warsaw, Krakowskie Przedmieście 32, 00-927 Warsaw, Poland
[e] Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland
[f] Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, SE 750 07, Uppsala, Sweden

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ABSTRACT

We present an optimized multiplex of 14 microsatellite loci that can be amplified in four reactions and loaded as two separate panels on the ABI genetic analyzer. The assay was validated on 342 individuals of field cricket (*Gryllus campestris*, L., 1758) from natural populations. The chosen loci are polymorphic and allow for efficient population genetic analysis, although we observed some aberrations, which are discussed. The optimized multiplex sets show reproducible amplification pattern with low genotyping error rate and should be therefore very useful in analyses of both experimental as well as natural populations of crickets. The optimized multiplex outperforms previously developed sets as it allows for amplification of 14 loci in four instead of seven reactions. These panels may serve as an applicable tool in conservation programs in countries in which this species is endangered.

Introduction

The field cricket (*Gryllus campestris* L., 1758) is rare or endangered due to human-induced impacts comprising habitat alternations and increased chemical pollution in western parts of Europe. Recently, it became a subject to reintroduction and translocation programs in Germany and United Kingdom (Witzenberger and Hochkirch, 2008). These projects should be accompanied by assessments of current genetic diversity and correct source population choice, followed later by careful genetic monitoring. Considering the biology and population dynamics of this species, which include relatively large population sizes, flightlessness, very short generation time of 1 year only and strong habitat preferences, the field cricket may serve as an indicator for ecosystem changes or as a model for investigations of evolutionary processes (Bretman et al., 2008, 2011; Witzenberger and Hochkirch, 2008; Tyler et al., 2013).

The marker of choice for population genetic studies are microsatellite loci as they are commonly implemented in a very broad field of research, ranging from landscape ecology, through conservation genetics to past population dynamics (Manel et al., 2003; Schlötterer, 2004; Chistiakov et al., 2006). In such analysis, a number of loci are required, therefore a simultaneous amplification of several loci in one reaction reduces time and costs considerably. Multiplexing additionally reduces possible human errors that may occur during sample manipulations and rise with the number of analysis steps (Neff et al., 2000; Bonin et al., 2004). Newly designed multiplexes need to be optimized and validated on a sufficiently large group of individuals with the chosen loci exhibiting optimally high level of polymorphism and error-free amplification, in order to obtain confident results (Masi et al., 2003; Pompanon et al., 2005; DeWoody et al., 2006).

As the field cricket is commonly used as a model species in evolutionary studies and there are on-going genetic studies of the wild populations of this species (Bretman et al., 2008, 2011; Witzenberger and Hochkirch, 2008), an optimized multiplex set of microsatellite loci would be desirable.

Materials and methods

The *G. campestris* individuals (n = 342) were sampled in 21 different localities of the lower Oder River (52.89° N, 14.32° E; n = 319) and three localities of the lower Vistula River (53.05° N, 18.40° E; n = 23) valleys in Poland. DNA extraction from the hind leg, preserved in 75% ethanol, was performed using the Wizard SV96 Genomic DNA Purification System Kit (Promega). Seventeen loci designed for *Gryllus bimaculatus*, and cross-species amplified in *G. campestris*

* Corresponding author at: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland. Tel.: +48 22 5923233; fax: +48 226584176.
E-mail address: hpana@wp.pl (H. Panagiotopoulou).

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Table 1
Description and gene diversity indices of the 14 loci (Dawson et al., 2003; Bretman et al., 2008) used in the two multiplexes, including the attribution of the loci to two multiplex panels amplified in four multiplex PCR reactions A and B–D, respectively (Mixes 1 and 2), dye labeling and optimized primer concentrations (μM). GenBank–GenBank accession numbers. Size range refers to the observed size range in bp of the amplified alleles; N_A — number of alleles; H_O — observed heterozygosity; H_E — expected heterozygosity; PIC — polymorphic information content calculated for the whole sample set (n = 342). A_n — null allele frequency estimated with Micro-Checker (I) and GENEPOP (II) and averaged over the 16 populations of crickets (n = 263). In parentheses: for N_A, H_E, H_O and PIC — the range of the polymorphism indices values calculated for individual populations; for A_N I and A_N II — the number of populations for which the $A_N > 0.19$. HWE — departures from Hardy–Weinberg equilibrium, given as the number of populations for which the results were significant with $P < 0.05$ and $P < 0.001$, respectively. Loci: *Gbim34*, *Gbim48* and *Gbim52* were excluded from the final set and not listed in the table. The overall estimates were calculated for the set of 12 autosomal loci.

Locus/Genbank	Repeat motif	Primer sequences	Multiplex (Mix)	Fluorescent dye	μM	Size range	N_A	H_E	H_O	PIC	HWE	A_N I	A_N II
Gbim15	$(CA)_{16}$	(F) GACTGCGGGTACCCTTGTCG	A (1)	6-FAM	0.1	151–177	9	0.78	0.68	0.75	1/0	0.00	0.05
AJ315368		(R) ATCCGGAGCTTCAGCAAGGC					(2–7)	(0.32–0.85)	(0.13–0.95)	(0.26–0.79)		(0)	(1)
Gbim40	$(CA)_{18}$	(F) GATCTGTCCTATCATCACCTCTTGC	A (1)	HEX	0.1	132–162	14	0.65	0.58	0.63	2/0	0.00	0.03
AM398104		(R) ACGGCAGGCGGAGTTTCCTCC					(3–8)	(0.36–0.79)	(0.40–0.83)	(0.33–0.74)		(0)	(0)
Gbim57	$(CA)_{12}$	(F) TGCGAATGCCGGAGTAATACC	A (1)	TAMRA	0.25	155–169	7	0.34	0.31	0.32	0/0	−0.01	0.02
AM398121		(R) CGGGAGGACAAGCTCTCACC					(1–5)	(0.13–0.63)	(0.13–0.54)	(0.00–0.56)		(0)	(0)
Gbim49	$(GT)_{21}$	(F) TTGCCACATCTCCCGAGAAAG	A (1)	ROX	0.1	185–195	4	0.67	0.34	0.60	7/4	0.12	0.16
AM398113		(R) TTGGTCCGTGCGTGGTAATTC					(2–4)	(0.31–0.72)	(0.13–0.82)	(0.29–0.64)		(5)	(5)
Gbim59[*]	(CA)17	(F) CCTCTCCCCTCATGCTCACG	B (2)	HEX	0.2	82–90	3	0.33	0.36	0.28	–	−0.01	0.00
AM398123		(R) TTTAAGCTCCAGACTGGTGTAGTG											
Gbim72	(GT)17	(F) ACCAGGTGAATGTCGGAGCAG	B (2)	HEX	0.1	179–211	7	0.69	0.64	0.64	0/0	−0.02	0.01
AM398136		(R) CAGTGTGGCACCACAGCAATC					(2–6)	(0.39–0.76)	(0.45–0.91)	(0.31–0.69)		(0)	(0)
Gbim29	(CA)3A(CA)16	(F) GATCCATTTCCGCCACTTCG	B (2)	HEX	0.2	250–284	12	0.87	0.73	0.85	2/0	−0.01	0.04
AM398093		(R) AATGCAACGGCATCGTAGGG					(2–7)	(0.53–0.85)	(0.60–0.96)	(0.38–0.80)		(0)	(0)
Gbim58	(CA)5CG(CA)4CG(CA)4CG(CA)4	(F) TCCTCATACATGAGACGTACTCCCTTC	C (2)	6-FAM	0.3	83–113	15	0.76	0.21	0.73	16/11	0.25	0.26
AM398122	CG(CA)4CG(CA)6CG(CA)15	(R) TCTCGATTGGTCTCTAACAGGTAATGC					(2–7)	(0.34–0.84)	(0.00–0.33)	(0.27–0.77)		(12)	(12)
Gbim35	(CT)22	(F) ACTCGACAACACTTAACGGACTAATGC	C (2)	6-FAM	0.2	202–220	8	0.41	0.06	0.35	13/7	0.20	0.21
AM398099		(R) TGTGAACGGAAAGGCTTGACC					(1–4)	(0.14–0.67)	(0.00–0.27)	(0.00–0.57)		(10)	(5)
Gbim33	(GATA)14 (GATT)3	(F) GCTTCAGAAGGCGAAGACACG	C (2)	TAMRA	0.1	194–262	18	0.82	0.71	0.80	0/0	−0.01	0.01
AM398097		(R) TTGGTGGATTGTGACGATTATTGC					(3–13)	(0.50–0.91)	(0.60–0.92)	(0.42–0.86)		(0)	(0)
Gbim71[*]	(CA)16	(F) CACTGCCACGCAATATTTGGAC	D (2)	6-FAM	0.2	132–140	4	0.14	0.10	0.14	–	0.04	0.00
AM398135		(R) GAGTGCCGAAAGCCGTTAGC											
Gbim21	(GA)2TA(GA)3	(F) GACCGCCACTAACCCACCAC	D (2)	6-FA	0.2	256–324	27	0.91	0.57	0.90	8/1	0.10	0.11
AM398085	GG(GA)2GG(GA)5	(R) GGAACGGGCAGCAGTTTGTC		M			(4–13)	(0.59–0.91)	(0.22–0.86)	(0.54–0.85)		(2)	(4)
Gbim04	(GT)27	(F) CGACGTATGTAGGCCTGCGG	D (2)	ROX	0.1	201–215	6	0.76	0.64	0.72	1/0	0.01	0.15
AJ315356		(R) ATCCTACCAACACGGCACGG					(2–6)	(0.39–0.78)	(0.30–0.83)	(0.31–0.73)		(0)	(5)
Gbim66	(CA)20	(F) AAGCTCATTTACCCTGCTGTTTGC	D (2)	ROX	0.2	283–323	10	0.68	0.59	0.64	4/0	0.00	0.03
AM398130		(R) AACTCCAGGCAAGGGACACG					(2–7)	(0.27–0.74)	(0.29–0.86)	(0.25–0.68)		(0)	(0)
Overall							11.4	0.69	0.51	0.66	15/9	0.05	0.09
							(2.4–6.3)	(0.49–0.70)	(0.45–0.59)	(0.36–0.62)			

[*] Loci which were proven to be sex-linked to the X chromosome (Bretman et al., 2011). For these loci calculations of genetic polymorphism were conducted exclusively for females (n = 42).