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MICROSATELLITE LETTERS

A suite of multiplexed microsatellite loci for the ground beetle *Abax parallelepipedus* (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae)

Tamar Marcus · Thorsten Assmann ·
Walter Durka · Claudia Drees

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Abstract We report two sets of polymorphic, multiplexed microsatellite markers for the ground beetle *Abax parallelepipedus*. As the species is flightless, restricted to forests and affected by habitat fragmentation it can serve as a model species for landscape and conservation genetics. A complete set of 20 loci can be amplified in five PCR reactions and sequenced in two rounds, and a subset of 14 loci can be analyzed together in one PCR run and one sequencing round. In a scan of 3,432 individuals from across Germany using the 14 loci subset, we found between three and 14 alleles per locus. After accounting for two loci that are apparently sex-linked, no significant deviations from Hardy–Weinberg equilibrium were found. None of the loci showed evidence for the presence of null alleles. No overall linkage disequilibrium was detected. Some of the loci can also be used to study other *Abax* species.

Keywords *Abax parallelepipedus* · Carabidae · Landscape genetics · Primers

Introduction

Today's conservation practices mostly account for species diversity, although it is crucial to incorporate measures to conserve genetic diversity as well. In order to do this, we must understand the effects of current and historical landscape structure and land use on genetic parameters. *Abax parallelepipedus* has previously been used in such studies, as its biology and population dynamics are well known, it is strongly restricted to forests, and it has a low dispersal capability as it is flightless. Even recent fragmentation has been shown to have significant effects on the genetic composition of this species (Keller et al. 2004), and current distribution is influenced by habitat continuity (Assmann 1999). *A. parallelepipedus* has also been studied in the context of biological pest control (Kromp 1999). We report a set of 20 multiplexed microsatellite loci as well as a subset of 14 loci which can be amplified and sequenced in a single run. Some of these loci can also be used to study other *Abax* species.

Methods and results

We extracted DNA using the CTAB DNA extraction protocol from *A. parallelepipedus* individuals collected across Germany, and obtained 19,783 DNA sequences from a shot-gun sequencing run on a Roche 454 Genome Sequencer FLX Titanium done by GenoScreen (Lille, France). Primers were designed for 49 microsatellite loci using MSATCOMMANDER (Faircloth 2008). Primers were designed with a GTTT tag to prevent plus-A stutter bands and with either a M13R or a CAG tail. We additionally designed new primers for the five previously published microsatellite loci in *A. parallelepipedus* (Keller

T. Marcus (✉) · T. Assmann
Institute of Ecology, Leuphana University, Scharnhorststraße 1,
21335 Lüneburg, Germany
e-mail: tamar.marcus@leuphana.de

W. Durka
Department of Community Ecology (BZF), Helmholtz Centre
for Environmental Research UFZ, Theodor-Lieser-Strasse 4,
06120 Halle, Germany

C. Drees
Zoological Institute and Museum, Biozentrum Grindel,
University of Hamburg, Martin-Luther-King-Platz 3,
20146 Hamburg, Germany

Table 1 Summary of primer sets. Details for 20 polymorphic microsatellite loci and two multiplex sets developed for *A. parallelepipedus*

Locus	Forward primer ~~3′–5′~~ 5′-3′	Reverse primer ~~3′–5′~~ 5′-3′	Repeat type	M13/CAG tag set (n = 24)				
				Side tagged/color-tag	PCR group	Sequencing group	Size range	Number of alleles
apar_2	GCCGCACGATATTAGCGAC	TTGGGAGTAAGTCTGTCCGG	AC	F/VIC-M13R	c	1	185–187	2
apar_4	CCACTGCACGTTCACTACAC	CAGTGAGTCGGGAGTGTC	AG	R/VIC-CAG	d	2	116–118	2
apar_5	CAACAACATTACCGGCGGAG	GCCGAGTCACTTGTTACGTG	AG	F/PET-M13R	e	2	171–175	2
apar_6	AAACATTCTGCGGTGACACC	CTGCTGCCCTCTTGTAAACG	AG	F/PET-M13R	b	1	305–307	2
apar_11	TTCGCCCTCAATCTCACCC	TCGTAGTGATGGCTGTGAGG	AC	F/PET-M13R	b	1	255–257	2
apar_12	GACCGTCGAGTGTAATGACG	CAATCTGCTCCTCAAGTTCAAG	AG	R/VIC-CAG	d	2	150–152	2
apar_14	GACATCTCGACTGCACCTAC	CCCTGTCTTTCCAACATCGC	AG	F/NED-M13R	d	2	138–142	3
apar_16	CGGTACTGTTCACTCTCTTGC	TAGGGTGGTCGGGAAATCAC	AC	R/NED-CAG	a	1	119–123	2
apar_20	ACACTCCACTCAAAGTTGCG	AAACGGTCAACTTTCCACCC	AC	F/NED-M13R	d	2	206–208	2
apar_23	GTGCCTATCGTTCTTTGTCAC	GTTTGCGATATTGTCTCTTGGCGG	AC	F/NED-M13R	d	2	183–185	2
apar_24	GTTTAGACGGTTCATTGCTGCATG	ACAGTTTGGCCTATCGTTACC	ACAT	R/FAM-M13R	a	1	187–191	2
apar_25	GTTTCGTAGCGAAACAGTGCCTTG	ATACTCCGGCGCTACTTTGG	AC	R/VIC-M13R	c	1	221–225	3
apar_27	CCTCCTTACCAAGTAACGGG	GTTTGGAAGCGACAGTCAACGTG	AC	F/PET-M13R	e	2	266–270	2
apar_32	TTTACCAACACACGCAGGC	GTTTGGACCACAACACGTTAGCAC	AG	F/FAM-M13R	a	1	115–119	3
apar_34	GTTTGCCATACTAGGTGCTCTGG	ATCTCCCGTGAAATCAACGC	AC	R/PET-M13R	b	1	117–125	3
apar_41	CTGATAACAACTGTGAGTGCTG	GTTTCAAACCACCCACATCGATGG	AAG	F/PET-M13R	b	1	173–182	2
apar_44	GTTTCTTAATGTTCCATGCCGCG	TCTTCTTCGGCAAGCGTTAC	AG	R/PET-M13R	e	2	199–201	2
apar_46	CAGTTCAGTTCATCACGGGC	GTTTGGAACCCAACGCAGAAAGTC	AAC	F/FAM-CAG	e	2	242–243	2
apar_50	GCTGGACTATTACAGAAGTCTTTTGC	ATGTGGAGGAAGCACGTGTT	CATA	R/NED-CAG	a	1	278–286	2
apar_52	CGGAGGACGTCTCTGCAAA	TCTGGCGTCGTTTGAATGGA	CA	R/FAM-CAG	e	2	182–192	3

Table 1 continued

Locus	Directly labeled primer set (n = 3,432)							GenBank number
	Fluorescent label	Concentration in primer mix (μM)	Size range	Number of alleles	Mean H_O	Mean H_E	F_{IS}	
apar_2	PET	0.75	165–175	6	0.258	0.263	−0.35–1	KF048982
apar_4	–	–	–	–	–	–	–	KF048983
apar_5	FAM	0.5	149–159	6	0.407	0.415	−0.38–0.60	KF048984
apar_6	PET	0.8	286–290	3	0.17	0.186	−0.29–1	KF048985
apar_11	–	–	–	–	–	–	–	KF048986
apar_12	VIC	0.5	122–140	10	0.095	0.102	−0.15–0.66	KF048987
apar_14	NED	0.75	112–124	6	0.365	0.356	−0.59–0.56	KF048988
apar_16	–	–	–	–	–	–	–	KF048989
apar_20	FAM	0.75	180–190	5	0.346	0.344	−0.57–1	KF048990
apar_23	NED	0.5	161–167	4	0.025	0.026	−0.05–1	KF048991
apar_24	–	–	–	–	–	–	–	KF048992
apar_25	NED	0.75	203–209	4	0.427	0.439	−0.32–1	KF048993
apar_27	NED	0.5	244–250	4	0.437	0.441	−0.52–0.53	KF048994
apar_32	FAM	0.5	98–102	3	0.338	0.369	−0.42–0.66	KF048995
apar_34	PET	0.5	104–114	5	0.169	0.168	−0.21–0.66	KF048996
apar_41	–	–	–	–	–	–	–	KF048997
apar_44	VIC	0.5	180–190	6	0.125	0.227	−0.77–1	KF048998
apar_46	FAM	0.5	217–227	5	0.164*	0.344*	−0.11–1	KF048999
apar_50	FAM	0.5	250–294	14	0.153*	0.154*	−0.18–0.64	AJ510195
apar_52	–	–	–	–	–	–	–	AJ510196

In the CAG/M13R tag set 20 loci are amplified in five PCR runs and sequenced in two rounds. In the directly labeled primers set, a subset of 14 loci is amplified and sequenced in one multiplex run. Number of alleles is given for each of the sets as they were tested on different numbers of individuals. Values of observed (H_O) and expected (H_E) heterozygosity are given as the average for all 143 populations while F_{IS} values are given as the range for all of the populations. Values marked with asterisks indicate tests which were preformed only on the females due to apar_44 and apar_46 most probably being sex-linked

Table 2 Trans-species amplification of the directly labeled primer set (Table 1) in other *Abax* species

Species	Population	apar_2	apar_5	apar_6	apar_12	apar_14	apar_20	apar_23
A. carinatus	Boc (n = 23)							
	% working	100	96	100	96	100	100	100
	A	2	3	2	2	4	5	1
	HWE	1	0.0962	–	–	0.0005 (+)	0.0444 (+)	–
	H_O	0.13	0.727	0.043	0.045	0.957	0.652	0
	H_E	0.125	0.627	0.043	0.045	0.602	0.571	0
A. ovalis	Alb_15 (n = 24)							
	% working	96	100	100	96	100	100	100
	A	4	2	2	3	3	3	1
	HWE	0.4323	1	1	0.4194	0 (+)	0.0721	–
	Alb_49 (n = 24)							
	% working	100	100	100	100	100	100	100
	A	1	2	2	2	2	2	1
	HWE	–	0.5494	1	–	0 (+)	0.0144 (+)	–
	HEW_16 (n = 24)							
	% working	92	100	100	100	100	100	100
	A	4	2	2	2	3	4	1
	HWE	0.5195	–	1	1	0.045 (−)	0.0467 (−)	–
	HEW_18 (n = 24)							
	% working	96	100	100	100	100	92	100
	A	3	2	2	2	3	3	1
	HWE	1	1	–	1	0 (+)	0.1372	–
	Sneznik (n = 24)							
	% working	96	100	100	79	100	92	100
	A	4	2	2	1	4	6	2
	HWE	1	0.1241	–	–	0.1881	0.1307	–
	H_O	0.31	0.142	0.108	0.185	0.717	0.44	0.008
	H_E	0.298	0.142	0.103	0.193	0.617	0.457	0.008
A. parallelus	Boc (n = 23)							
	% working	91	100	100	91	100	70	100
	A	2	3	1	2	2	2	1
	HWE	1	1	–	–	0 (+)	0.0952	–
	HEW_16 (n = 24)							
	% working	100	100	100	83	100	100	96
	A	2	3	2	1	2	3	2
	HWE	1	0.0498 (−)	1	–	0 (+)	0.0058 (+)	–
	HEW_18 (n = 24)							
	% working	100	100	100	83	88	100	96
	A	2	3	1	2	2	2	3
	HWE	–	0.0036 (−)	–	–	0 (+)	0.0255 (+)	1
	H_O	0.126	0.268	0.069	0.033	0.984	0.729	0.043
	H_E	0.117	0.364	0.064	0.033	0.511	0.487	0.043

Table 2 continued

Species	Population	apar_25	apar_27	apar_32	apar_34	apar_44	apar_46	apar_50
A. carinatus	Boc (n = 23)							
	% working	91	100	100	100	96	100	91
	A	3	2	2	2	3	2	3
	HWE	0.2229	0.0007 (+)	0.0016 (+)	–	–	0.1775*	1
	H_O	0.667	0.87	0.826	0.043	0.063*	0.235*	0.095
	H_E	0.501	0.51	0.496	0.043	0.063*	0.371*	0.094
A. ovalis	Alb_15 (n = 24)							
	% working	92	100	100	96	92	100	96
	A	4	2	2	4	4	2	2
	HWE	0.0736	0.0022 (+)	0.0068 (+)	0.661	1*	1*	–
	Alb_49 (n = 24)							
	% working	83	100	100	100	100	100	100
	A	3	2	2	2	3	2	1
	HWE	0.5571	0.0185 (+)	0.0002 (+)	1	1*	1*	–
	HEW_16 (n = 24)							
	% working	100	100	100	100	100	100	92
	A	4	3	2	4	3	2	2
	HWE	0.0162 (+)	0.0905	0.0143 (+)	0.515	0.0305 (+)*	1*	1
	HEW_18 (n = 24)							
	% working	92	100	100	88	100	100	100
	A	4	2	2	4	3	2	1
	HWE	0.0059 (+)	0.0066 (+)	0 (+)	0.743	1*	–	–
	Sneznik (n = 24)							
	% working	75	100	92	100	100	100	92
	A	4	2	3	2	3	2	1
	HWE	1	0.0022 (+)	0.0804	0.2007	–	–	–
	H_O	0.624	0.75	0.812	0.439	0.392*	0.246*	0.027
	H_E	0.559	0.484	0.504	0.413	0.329*	0.236*	0.026
A. parallelus	Boc (n = 23)							
	% working	100	100	100	100	96	100	100
	A	3	2	2	3	2	2	2
	HWE	0.4487	0 (+)	0 (+)	1	–	–	–
	HEW_16 (n = 24)							
	% working	96	100	100	100	100	100	100
	A	3	2	2	1	3	2	2
	HWE	0.0168 (−)	0.0008 (+)	0.0158 (+)	–	–	–	1
	HEW_18 (n = 24)							
	% working	83	96	96	100	100	96	100
	A	3	2	2	2	2	2	2
	HWE	0.1425	0.0001 (+)	0.0016 (+)	–	–	0.3056*	–
	H_O	0.469	0.929	0.859	0.043	0.02*	0.229*	0.056
	H_E	0.415	0.509	0.501	0.043	0.02*	0.257*	0.056

For each population we give the percentage of individuals for which a readable result was achieved, the number of alleles found (A), and the *p* value of the HWE test. Mean values of observed (H_O) and expected (H_E) heterozygosity are given for each species. Values marked with asterisks indicate tests which were preformed only on the females due to apar_44 and apar_46 most probably being sex-linked. Non-HWE populations with a heterozygote excess are marked with (+), populations with a heterozygote deficiency are marked (−)

and Largiader 2003). All primer sets were checked to ensure that they are not replicating the same locus using Geneious v5.4. The 54 loci were tested for polymorphism using fluorescent-labeled M13R or CAG tags (Faircloth 2008). We identified a set of 20 polymorphic loci.

We report two multiplex sets (Table 1). The first contains all 20 loci amplified in five multiplex PCRs using CAG/M13R tagged primers and sequenced in two runs. The second contains a subset of 14 loci which are amplified and sequenced in one run using directly labeled primers.

For all amplifications using CAG/M13R tagged primers, amplification was done in 5 µL reactions containing 2.5 µL of 2× Multiplex PCR kit (Qiagen), 0.06 µM CAG/M13R tailed primer, 0.24 µM of the other primer, 0.25 µM of the fluorescent-labeled M13R (GGAAACAGCTATGACCAT) or CAG (CAGTCGGGCGTCATCA) primer, approximately 30 ng of genomic DNA (0.5 µL), and 1 µL water. We ran a touch-down PCR with the following conditions: 1 × 15 min at 95 °C, 20 × [30 s at 94 °C, 30 s at 60 °C (minus 0.5 °C per cycle), 90 s at 72 °C], 20 × (30 s at 94 °C, 30 s at 50 °C, 90 s at 72 °C), 1 × 10 min at 72 °C. PCR products were diluted 1:100 before sequencing. For amplifications using the directly labeled primers, forward and backward primers were combined in equal amounts into a primer working solution (Table 1). Amplification reaction contained 2.5 µL of 2× Multiplex PCR kit (Qiagen), approximately 30 ng of genomic DNA (0.5 µL), 0.5 µL primer mix, and 1.5 µL of water. The amplification conditions remained unchanged. PCR products were diluted 1:20 before sequencing. Fragment size was scanned using either an ABI 3130xl or an ABI 3730 Genetic analyzer (Applied Biosystems). Genotypes were scored automatically by GeneMapper 3.7 and checked manually. Hardy–Weinberg equilibrium (HWE) was tested using Genepop 4.2, linkage disequilibrium (LD) was checked using FSTAT 2.9.3.2, and suspected presence of null alleles was checked using Micro-Checker 2.2.3. We used pop100gene 1.1.03 to find mean observed heterozygosity (H_O) and expected heterozygosity (H_E) values, numbers of alleles per locus (A), and range of allele size per locus. F_{IS} values were calculated with FSTAT 2.9.3.2.

In a scan of 3,432 individuals from 147 populations across Germany using the subset of the 14 directly labeled primers, allele size ranged from 98 to 294 bp and between 3 and 14 alleles were detected across loci (mean: 2.17). Presence of null alleles was indicated in 12 out of 1,716 tested possibilities and deviations from HWE were detected in 72 out of the 1,507 tested combinations. Two loci, apar_44 and apar_46, apparently are sex-linked as testing only the female individuals greatly reduced the number of populations deviating from HWE. For locus apar_44, out of 81 testable populations the number that deviated from HWE was reduced from 46 to 5, and for apar_46, out of 131 testable populations the reduction was from 78 to 2. No significant linkage disequilibrium was found.

Trans-species amplification was tested using the directly labeled multiplex set in *Abax carinatus* (Duftschmid, 1812), *A. parallelus* (Duftschmid, 1812), and *A. ovalis* (Duftschmid, 1812) sampled in Germany and Slovenia (Table 2). The loci apar_2, apar_6, apar_12, apar_23, apar_46, and apar_50 all gave readable results in more than 90 % of the individuals, with no deviations from HWE for any of the tested populations. The primers can probably be used, not only with the three tested species, but with other *Abax* species as well.

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