

Supplementary materials

1 Second sequencing run demultiplexing results

Table 1: Number of demultiplexed reads depending on plate region. We report number of SSR containing loci assembled with HighSSR program for SSRs with consensus size 2-6 nt. In the second sequencing run samples have been multiplexed with standard MIDs described in Roche TCB No. 005-2009 technical bulletin.

Accession #	Taxa	MID	Region	SSR reads	non-SSR reads	Total reads	Loci
5.91	<i>Leucaena lempirana</i>	MID-01	1	4,683	2,499	7,182	174
19.84	<i>Leucaena magnifica</i>	MID-02	1	4,807	2,057	6,864	75
61.88	<i>Leucaena trichodes</i>	MID-03	1	3,129	1,882	5,011	79
135.92	<i>Leucaena shannoni</i>	MID-04	1	5,030	2,102	7,132	162
81.87	<i>Leucaena multicapitula</i>	MID-05	1	2,713	2,023	4,736	112
17.86	<i>Leucaena salvadorensis</i>	MID-06	1	2,995	1,552	4,547	58
134.92	<i>Leucaena lanceolata</i>	MID-07	1	5,877	3,023	8,900	179
128.92	<i>Leucaena trichandra</i>	MID-08	1	4,018	2,019	6,037	62
18.84	<i>Leucaena collinsii zac</i>	MID-10	1	2,231	1,804	4,036	47
55.88	<i>Leucaena macrophylla</i>	MID-11	1	2,304	2,132	4,436	80
90.92	<i>Leucaena lanceolata</i>	MID-13	1	2,046	2,272	4,318	83
GR44531	<i>Bassariscus astutus</i>	MID-14	1	7,131	3,462	10,592	195
GR49586	<i>Bassariscus astutus</i>	MID-15	1	2,786	2,273	5,059	112
AB1	<i>Ariocarpus bravoanus</i>	MID-16	1	553	856	1,409	19
AF1	<i>Ariocarpus fissuratus</i>	MID-17	1	113	88	201	6
AG1	<i>Ariocarpus agavoides</i>	MID-18	1	933	1,063	1,996	17
AT1	<i>Ariocarpus trigonus</i>	MID-19	1	257	404	661	5
AK1	<i>Ariocarpus kotschoubeyanus</i>	MID-20	1	687	768	1,455	15
AM1	<i>Astrophytum caput-medusae</i>	MID-21	1	6,073	1,588	7,662	27
AM2	<i>Astrophytum caput-medusae</i>	MID-22	1	12	4	16	0
121	<i>Arabidopsis thaliana</i>	MID-01	2	2,140	1,871	4,011	65
BB1	<i>Tangara guttata</i>	MID-02	2	1,804	2,047	3,850	77
BB2	<i>Thraupis palmarum</i>	MID-03	2	200	139	340	34
BB3	<i>Tachyphonus rufus</i>	MID-04	2	1,897	2,141	4,038	3
65	<i>Arabidopsis thaliana</i>	MID-05	2	1,897	2,141	4,038	65
5B-10	<i>Ashmunella rhyssa hyporhyssa</i>	MID-06	2	3,568	2,941	6,509	53
9A-3	<i>Ashmunella rhyssa edentata</i>	MID-07	2	5,823	5,769	11,592	88
8B-14	<i>Ashmunella rhyssa edentata</i>	MID-08	2	2,772	2,288	5,060	35
GR33	<i>Urocyon cinereoargenteus</i>	MID-10	2	344	329	673	13
GR28	<i>Urocyon cinereoargenteus</i>	MID-11	2	2,615	2,100	4,715	131
GR378	<i>Dipodomys spectabilis</i>	MID-13	2	2,247	1,707	3,954	39
GR571	<i>Dipodomys spectabilis</i>	MID-14	2	2,826	1,975	4,801	60
GRE3771	<i>Urocyon littoralis</i>	MID-15	2	1,808	2,055	3,863	58
GRP9B	<i>Urocyon littoralis</i>	MID-16	2	3,094	2,304	5,398	126
3	<i>Lepidium meyenii</i>	MID-17	2	7,398	6,654	14,052	268
37	<i>Lepidium meyenii</i>	MID-18	2	2,010	1,973	3,983	65

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Table 1 – continued from previous page

Accession #	Taxa	MID	Region	SSR reads	non-SSR reads	Total reads	Loci
2A	<i>Bouteloua eriopoda</i>	MID-19	2	2,720	2,199	4,922	105
3B	<i>Bouteloua eriopoda</i>	MID-20	2	2,794	2,380	5,172	88
EP155	<i>Cryphonectria parasitica</i>	MID-21	2	3,775	3,376	7,152	141
EP146	<i>Cryphonectria parasitica</i>	MID-22	2	2,241	1,678	3,919	51
81	<i>Aedes aegypti</i>	MID-01	3-4	6,690	4,841	11,531	104
122	<i>Arabidopsis thaliana</i>	MID-02	3-4	5,349	4,758	10,109	257
123	<i>Arabidopsis thaliana</i>	MID-03	3-4	2,655	8,408	11,063	55
124	<i>Arabidopsis thaliana</i>	MID-04	3-4	2,125	1,670	3,795	62
125	<i>Arabidopsis thaliana</i>	MID-05	3-4	63	125	188	3
D4	<i>Euphydryas anicia cloudcrofti</i>	MID-06	3-4	7,396	2,634	10,030	35
D7	<i>Euphydryas anicia cloudcrofti</i>	MID-07	3-4	7,614	7,809	15,423	140
D4	<i>Euphydryas anicia cloudcrofti</i>	MID-08	3-4	5,951	3,128	9,080	36
D7	<i>Euphydryas anicia cloudcrofti</i>	MID-10	3-4	3,039	3,847	6,886	39
D4	<i>Euphydryas anicia cloudcrofti</i>	MID-11	3-4	3,333	3,472	6,805	48
D7	<i>Euphydryas anicia cloudcrofti</i>	MID-13	3-4	7,023	5,124	12,147	138
D4	<i>Euphydryas anicia cloudcrofti</i>	MID-14	3-4	9,166	8,239	17,405	75
D7	<i>Euphydryas anicia cloudcrofti</i>	MID-15	3-4	10,988	14,506	25,494	180
O2	<i>Obregonia denegrii</i>	MID-16	3-4	8	45	53	1
C1	<i>Euphydryas</i>	MID-17	3-4	16,665	24,914	41,580	145
C2	<i>Euphydryas</i>	MID-18	3-4	11,442	11,408	22,847	116
HOP1	<i>Euphydryas anicia eurytion</i>	MID-19	3-4	3,831	5,416	9,247	47
HOP2	<i>Euphydryas anicia eurytion</i>	MID-20	3-4	12,599	18,509	31,108	50
HMT1	<i>Euphydryas anicia eurytion</i>	MID-21	3-4	145	697	842	10
HMT2	<i>Euphydryas anicia eurytion</i>	MID-22	3-4	41	45	86	3

Table 2: Number of sequences decoded with MIDs (Roche TCB No. 005-2009) that have not been used for multiplexing. In this this demultiplexing run we estimated the false positives calling rate.

MID	Plate region	Total reads
MID-23	1	7
MID-24	1	51
MID-25	1	7
MID-26	1	5
MID-27	1	16
MID-28	1	6
MID-29	1	33
MID-30	1	34
MID-31	1	6
MID-32	1	15
MID-33	1	141
MID-34	1	1
MID-35	1	111
MID-36	1	8
MID-37	1	23
MID-38	1	10
MID-39	1	25
MID-40	1	30
MID-41	1	16

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Table 2 – continued from previous page

MID	Plate region	Total reads
MID-42	1	12
MID-23	2	3
MID-24	2	2
MID-25	2	13
MID-26	2	12
MID-27	2	12
MID-28	2	20
MID-29	2	26
MID-30	2	22
MID-31	2	14
MID-32	2	13
MID-33	2	29
MID-34	2	6
MID-35	2	199
MID-36	2	22
MID-37	2	36
MID-38	2	13
MID-39	2	12
MID-40	2	20
MID-41	2	8
MID-42	2	19
MID-23	3-4	4
MID-24	3-4	378
MID-25	3-4	13
MID-26	3-4	80
MID-27	3-4	61
MID-28	3-4	40
MID-29	3-4	163
MID-30	3-4	523
MID-31	3-4	20
MID-32	3-4	30
MID-33	3-4	69
MID-34	3-4	24
MID-35	3-4	337
MID-36	3-4	14
MID-37	3-4	42
MID-38	3-4	30
MID-39	3-4	110
MID-40	3-4	48
MID-41	3-4	40
MID-42	3-4	16

2 Genotyping experiments and PCR protocol

2.1 Collecting and handling biological materials

Samples of *Euphydryas anicia cloudcrofti* [1] (*Lepidoptera: Nymphalidae*) larvae were collected in 2002 from the Sacramento Mountains, Otero County, New Mexico with permits from the U.S. Forest Service. Samples were frozen in -80°C until DNA extraction could be performed.

2.2 PCR protocol

DNA was extracted using a salt-out extraction protocol described by [2]. Polymerase Chain Reaction (PCR) was performed in $20\mu\text{l}$ reaction volume using $1\mu\text{l}$ of DNA extract diluted 1:10, 20pmol concentration of primers, dNTP concentration of $200\mu\text{M}$, 1 unit of Platinum Taq polymerase (Invitrogen), and a MgCl_2 concentration of 1.5mM . The initial PCR cycling profile was 94°C for 2 min, 40 cycles of 94°C for 30s, 58.4°C for 40s, 72°C for 30s, and a final extension period of 72°C for 7 min. Initially PCR was conducted with a temperature titration to determine the proper annealing temperature under 6 temperature conditions 45°C , 51.3°C , 54.6°C , 58.4°C , 61.7°C , 64.3°C , 66.1°C . In the titration experiments, as discussed in Subsection 2.3, primers EC14, EC18, and EC24 showed the best results, and these products were sequenced.

2.3 Genotyping of *Euphydryas anicia cloudcrofti*

For the genotyping experiment primers have been ordered as shown in Table 3. Using the ordered primers the temperature gradient PCR amplifications have been accomplished as shown in Figure 1.

Locus Name	Primer Sequence (5' – 3')	SSR consensus	Annealing T _m (°C)	Target SSR Size (bp)
EC13	F: CCAACCTTGGTATGTGCAAG R: TGTGTAAAATCACCTTATGACGAAA	AAATG		199
EC14	F: AAAAACCCGACTGCGTAAAA R: GTCTCAAACCTTTTGACCGGC	ACAT	58.4°C	188
EC15	F: CCGATTTTCAGGACCACCTA R: AAAAAGTCGTAAGGTGTTGA	ACAT		152
EC16	F: GAATGCCACTCGGCATAAGT R: TGTTCAAGCATTCTATATTTATTTATG	ACACAT		151
EC17	F: CGTACTCACACATTCATAGCAA R: AAGTGTTAGTGTTGATTGTGTGAGTG	ACACAT		115
EC18	F: TCAAACAGCAACAAAGGCAC R: GCTGTTGCTGTTGCATCTGT	AAC	58.4°C	112
EC19	F: CATCATACTATGCCGTGCCA R: CAATCAAGGTTTCAGCTTGTGA	AATGT		196
EC20	F: TATAGAGCGGAGGCTTTTGC R: TCCTCTCAAAGATGATGGGG	ACC		152
EC21	F: TCACACTGGTGAAGGAGCTG R: TGAGTCCTGAGTAAATCATCCTG	AACAGC		149
EC22	F: CATTGTGAGCAAACCTGCAT R: TGTAGACCACATACCGGCAA	ATC		143
EC23	F: TCGTGTATGAGAAAAGGGGG R: ATGGTGATCAGGAAGAAGGC	ACAT		135
EC24	F: TTTATTGGCAATTCCGCTTC R: AGTACGCCCAACTTTCATGC	ACACGC	58.4°C	108
EC25	F: CTGGGCGACTTTTGTGTTGAT R: AGGAGGCCTACGTTCAACAG	ATC		85

Table 3: Primers used

-GC-AACAGC-AGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACGACAACAACAGCATC-AACAACAA
 -GC-AACAGC-AGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGATAGCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAACAA
 -GC-AACAGC-AGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGCAAGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAACAA
 -GC-AACAGC-AGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACGGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AAGGACAA
 -GC-AACAGC-AGCAAC-AGC-AACAGCAACAATAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAACAA
 -GC-AACAGC-AGCAACAAGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAACAAGCAGCAGATGCAACAACAACAACAGCATC-AACAAC-A
 -GC-AACAGCTAGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAACAA
 -GC-AGCAGC-AGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAACAA
 -GCAACAGC-AGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAACAA
 AGC-AACAGC-AGCAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAAC-A
 GAC-AACAGCAGACAAC-AGC-AACAGCAACAACAACAACATCAACAACAAATACAGCAGCAGC-AGAT-GCAACAAC-AGCAGCAGATGCAACAACAACAACAGCATC-AACAACAA

The sequences from locus specific amplifications have been aligned with the 3beb8c97-23da-c2d5-94dc-e7ba7006086d locus sequence (EC18).

D6_EC18 -----tTCAAACAGCAACAAAGGCAGCAGCGTTGCTCAGACAACATCA
 F1_EC18 -----tTCAAACAGCAACAAAGGCAGCAGCGTTGCTCAGACAACATCA
 EC18_Allele_1 -----tcAACAGCAACAAAGGCAGCAGCGTTGCTCAGACAACATCA
 EC18_Allele_2 -----ATCAAACAGCAACAAAGGCAGCAGCGTTGCTCAGACAACATCA
 3beb8c97-23da-c2d5-94dc-e7ba7006086d gatgagtcctgagtaaATCAAACAGCAACAAAGGCAGCAGCGTTGCTCAGACAACATCA

D6_EC18 GATGCAGtTGCAACAGCAGCAACAGCAACAGCAACAACAACAACATCAgCagatgct
 F1_EC18 GATGCAGtTGCAACAGCAGCAACAGCAACAGCAACAACAACAACATCAgCagatgct
 EC18_Allele_1 GATGCAGCTGCAACAGCAGCAACAGCAACAGCAACAACAACAACATCAACAACAAAT
 EC18_Allele_2 GATGCAGCTGCAACAGCAGCAACAGCAACAG---CAACAACAACAACATCAACAACAAAT
 3beb8c97-23da-c2d5-94dc-e7ba7006086d GATGCAGCTGCAACAGCAGCAACAGCAACAGCAACAACAACAACATCAACAACAAAT

D6_EC18 ACAGCAGCAGCAGATGCAACAACAGCAGCAGATGCAAC-----
 F1_EC18 ACAGCAGCAGCAGATGCAACAACAGCAGCAGATGCAAC-----
 EC18_Allele_1 ACAGCAGCAGCAGATGCAACAACAGCAGCAGATGCAACAACAACAACAGCATCAACAACA
 EC18_Allele_2 ACAGCAGCAGCAGAcGCAACAACAGCAGCAGATGCAACAACAACAACAGCATCAACAACA
 3beb8c97-23da-c2d5-94dc-e7ba7006086d ACAGCAGCAGCAGATGCAACAACAGCAGCAGATGCAACAACAACAACAGCATCAACAACA

D6_EC18 -----
 F1_EC18 -----
 EC18_Allele_1 AATACAGATGCAACAGCAACAGCAGCAGCAGCAACAACAGATGCGGCAGAGTCGACAACA
 EC18_Allele_2 AATACAGATGCAACAGCAACAGCAGCAGCAGCAACAACAGATGCGGCAGAGTCGACAACA
 3beb8c97-23da-c2d5-94dc-e7ba7006086d AATACAGATGCAACAGCAACAGCAGCAGCAGCAACAACAGATGCGGCAGAGTCGACAACA

D6_EC18 -----
 F1_EC18 -----
 EC18_Allele_1 ACAGAcACAGTTCGATaATCAAGTAGAGATACAAGTGTTCtcaAACAAGTTCGGAT
 EC18_Allele_2 ACAGAgCAGTTCGAT-ATCAAGTAGAGATACAAGTGTTCtCAACAAGTTCGGAT
 3beb8c97-23da-c2d5-94dc-e7ba7006086d ACAGAcACAGTTCGAT-ATCAAGTAGAGATACAAGTGTTCtCAACAAGTTCGGAT

D6_EC18 -----
 F1_EC18 -----
 EC18_Allele_1 GTTCTGGGTAAGA-TTATAAATTAGC-TTTATAGTTCGGTATACaAAAGTGTGCCATCAG
 EC18_Allele_2 GTTCTGGGTAAGATTTATAAATTAGCTTTTATAGTTCGGTATAC-AAAGTGTGCCATCAG
 3beb8c97-23da-c2d5-94dc-e7ba7006086d GTTCTGGGTAAGATTTATAAATTAGCTTTTATAGTTCGGTATAC-AAAGTGTGCCATCAG

D6_EC18 -----
 F1_EC18 -----

D6_EC24 **nn**ACTAAATAC-TTTTTTCTTACCA---AACACCCACA**a**-----ACGCACACGCACA
EC24_Allele_1 **A**CACTAAATAC-TTTTTTCTTACCA**t**TAAACACCCACAC**a**ACGCACACGCACACGCACA
EC24_Allele_2 **A**CACTAAATAC-TTTTTTCTTACCA-TAAACACCCACAC-ACGCACACGCACACGCACA
b13d51ea-431f-f31a-6d49-b232cef90edb **A**CACTAAATAC-TTTTTTCTTACCA-TAAACACCCACAC-----ACGCACACGCACA
EC24_Allele_3 **A**CACTAAATAC**t**TTTTTTCTTACCA-TAAACACCCACAC-----ACGCACACGCACA

D6_EC24 **CGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACA**
EC24_Allele_1 **CGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACA**
EC24_Allele_2 **CGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACA**
b13d51ea-431f-f31a-6d49-b232cef90edb **CGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACA**
EC24_Allele_3 **CGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACACGCACA**

D6_EC24 **CAAACACGTACACAAACACGTACACGCACACGTCTGT-GTCTAGAAAG**a**G-TTACTTTAC**
EC24_Allele_1 **CAAACACGTACACAAACACGTACACGCACACGTCTGT**a**GTCTAGAAAGTG-TTACTTTAC**
EC24_Allele_2 **CAAACACGTACACAAACACGTACACGCACACGTCTGT-GTCTAGAAAGTG-TTACTTTAC**
b13d51ea-431f-f31a-6d49-b232cef90edb **CAAACACGTACACAAACACGTACACGCACACGTCTGT-GTCTAGAAAGTGTTTACTTTAC**
EC24_Allele_3 **CAAACACGTACACAAACACGTACACGCACACGTCTGT-GTCTAGAAAGTGTTTACTTTAC**

D6_EC24 **AAATACCGGATGTCACTCTATATATTA**t**aCAG**a**A-TCAT**t**AATAT**a**tn**a**TGTAC**n**TATAT**
EC24_Allele_1 **AAATACCGGATGTCACTCTATATATTACTCAGGACTCATC-----**
EC24_Allele_2 **AAATACCGGATGTCACTCTATATATTACTCAGGACTCATC-----**
b13d51ea-431f-f31a-6d49-b232cef90edb **AAATACCGGATGTCACTCTATATATTACTCAGGACTCATCAATAT---ATGTAC**g**TATAT**
EC24_Allele_3 **-AAATACCGGATGTCACTCTATATATTACTCAGGACTCATC-----**

D6_EC24 **GTCGC**n**GAT**n**ATAT**Gn**TGAGTATTTATAT**n**-n**AATGTn**AATAAAATTAGGTTTTGCTTTT**
EC24_Allele_1 **-----**
EC24_Allele_2 **-----**
b13d51ea-431f-f31a-6d49-b232cef90edb **GTCGC**t**GAT**g**ATAT**Gc**TGAGTATTTATAT**gat**AATGT**a**AATAAAATTAGGTTTTGCTTTT**
EC24_Allele_3 **-----**

D6_EC24 **G**c**TCTTAGCATAAAATTTTAA-TTACA**n**ATGTAG**Gn**nt-**
EC24_Allele_1 **-----**
EC24_Allele_2 **-----**
b13d51ea-431f-f31a-6d49-b232cef90edb **G**g**TCTTAGCATAAAATTTTAA**t**TTACA**a**tATGTAG**a**Gtaca**
EC24_Allele_3 **-----**

References

- [1] C.D. Ferris and R.W. Holland. Two new subspecies of *Occidryas anicia* (doubleday) from New Mexico. In *Bulletin fo the Allyn Museum No. 57*. Allyn Museum of Entomology, Sarasota, Florida, 1980.
- [2] J. Sambrook and D.W. Russell. *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Laboratory, 2001.