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DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE LOCI IN THE ENDANGERED CATSPAW, *EPIOBLASMA OBLIQUATA* (BIVALVIA:UNIONIDAE)

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ABSTRACT

The endangered Catspaw, *Epioblasma obliquata*, is restricted to one known reproducing population in Killbuck Creek, Coshocton County, Ohio. Little is known about the genetic diversity of this small population, and such information is needed to help inform recovery planning. We nonlethally sampled 44 individuals of *E. obliquata* using buccal swabs, from which we developed and characterized 14 polymorphic microsatellite loci. Significant deviations from Hardy–Weinberg Equilibrium (HWE), showing deficiencies in heterozygotes, were observed at 6 of the 14 loci, and linkage disequilibrium (LD) was observed at 9 (∼10%) of 91 possible pairwise comparisons among loci. Allelic diversity ranged from 2 to 15 alleles per locus and averaged 7.6 alleles per locus. Observed heterozygosity per locus ranged from 0.091 to 1.000 and averaged 0.674. Possible explanations for deviations from HWE and LD could be from loci located close together on the same chromosome, segregation of null alleles, family structure within the small population, population bottlenecks, inbreeding, hermaphroditic reproduction, or some combination of these factors. Managers can use these microsatellite markers to assess and monitor genetic diversity in the remaining wild population in Killbuck Creek, prospective broodstock, hatchery-reared progeny, and reintroduced populations founded to promote recovery of the species.

KEY WORDS: Catspaw, *Epioblasma obliquata*, freshwater mussel, DNA microsatellite loci, primers, genetic diversity

INTRODUCTION

The Catspaw, *Epioblasma obliquata*, was listed as endangered under the U.S. Endangered Species Act in 1990; at that time, two isolated nonreproducing populations were known, one in the Green River in Kentucky and the other in the Cumberland River in Tennessee (USFWS 1990). These two populations now are considered extirpated. However, in 1994, a population of reproducing *E. obliquata* was discovered in a short reach of Killbuck Creek, a tributary of the Walhonding River in the Muskingum River watershed in Coshocton County, Ohio (Hoggarth et al. 1995). State and federal agencies are using this population as a source of broodstock for captive propagation in an attempt to recover the species.

Given the single-source population, genetic variation in hatchery progeny is a concern. Potential genetic threats to survival of the species include loss of within-population genetic variation from nonrepresentative sampling or low numbers of broodstock and family-size variation in the hatchery (Jones et al. 2006; Cooper et al. 2009). Microsatellites, or simple sequence repeats, are tandemly repeated motifs of multiple bases of nuclear DNA found in all eukaryotic genomes (Zane et al. 2002). Microsatellites are highly polymorphic loci that are ideally suited for genetic monitoring of wild and captive populations. The goal of this study was to develop and evaluate a set of microsatellite DNA PCR primers

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to analyze the genetic variation of the small source population in Killbuck Creek and any progeny produced in hatcheries.

METHODS

We obtained DNA samples from 44 adult *Epioblasma obliquata* that originally were collected from Killbuck Creek, Coshocton County, Ohio. These adults represented all individuals found at multiple sites and during multiple visits to the creek to collect broodstock in 2016–17. Adults were transported to and held at the Kentucky Department of Fish and Wildlife Resources' Minor E. Clark Fish Hatchery as part of the recovery program for the species. We nonlethally sampled these 44 individuals from the hatchery in the fall of 2018 by gently opening each mussel and vigorously swabbing the foot with a buccal swab (Kit DDK-50, Isohelix, Harrietsham, UK). From the buccal swab, DNA was isolated and extracted using an Isohelix DNA isolation kit, and its concentration and purity were assessed by using a μLite PC spectrophotometer (Biodrop, Cambridge, UK). In addition to morphological identification, the identification of all individuals as *E. obliquata* was confirmed using the mitochondrial DNA sequence from the first subunit of NADH dehydrogenase (*ND1*), a protein-encoding gene amplified by PCR using primers and conditions reported by Serb et al. (2003).

The Savannah River Ecology Laboratory at the University of Georgia developed a microsatellite library. Genomic DNA used to isolate the microsatellite loci was extracted from two individuals collected from the wild in 2016, utilizing a DNEasy Blood and Tissue Kit (Qiagen, Germantown, MD, USA). A genomic library was prepared with inserts size-selected to range from 300 to 600 bp. Paired-end reads were sequenced on an Illumina HiSeq sequencer. Using the program MSATCOMMANDER (Faircloth 2008), 463,713 reads containing 3–6 bp repeat motifs were identified. Primer3 (Untergasser et al. 2012) was used for PCR primer design. Initially, we screened 60 primer pairs on a panel of eight *E. obliquata* individuals and narrowed our evaluation to a set of 14 microsatellite polymorphic primer pairs. The criteria used to select these primer pairs were polymorphism of the loci amplified (i.e., observation of more than one allele), tri- or tetranucleotide repeat motif, and annealing temperature close to 59°C for use in subsequent multiplexing. Forward primers were labelled with fluorescent markers as noted in Table 1. Four sets of loci were coamplified in multiplex PCR—*Eoo11* and *Eoo20*; *Eoo16* and *Eoo19*; *Eoo22* and *Eoo24*; *Eoo8*, *Eoo9*, and *Eoo10*; other loci were amplified individually. PCR conditions consisted of H_2O, 5× PCR buffer (Promega, Madison, WI, USA), 2.5 mM $MgCl_2$ (Promega), 2.5 mM deoxynucleotide triphosphate (dNTPs) (ThermoFisher Scientific, Waltham, MA, USA), 1 mg/mL bovine serum albumin (BSA) (ThermoFisher Scientific), 5 μM of each primer, 0.1 μL Go*Taq* Polymerase (New England Biolabs, Ipswich, MA, USA), and 1 μL of genomic DNA at 50 ng/μL, in a total reaction volume of 22 μL. PCR thermal cycling conditions were as follows: 94°C for 3 min, followed by 35 cycles of 94°C for 40 s, 59°C for 40 s, and 72°C for 1 min; a final extension at 72°C for 5 min; and a hold at 4°C. Amplification of PCR products was verified by visualization under ultraviolet light in an ethidium bromide-stained agarose gel. PCR products were sent to the Institute of Biotechnology at Cornell University, Ithaca, New York, for DNA fragment-size analysis. Microsatellites were scored for length using Genemarker (SoftGenetics, State College, PA, USA). Arlequin v3.0 (Excoffier et al. 2005) was used to assess heterozygosity, number of observed alleles per locus, conformance to Hardy–Weinberg equilibrium (HWE), and linkage disequilibrium (LD). Testing for HWE and LD used Arlequin and a critical type I error rate = 0.05. Evidence for a bottleneck at each locus was tested using the Garza–Williamson index (*M*-ratio, the ratio of the number of alleles observed to the number of alleles possible within the observed range in allele sizes) using Arlequin; values of *M* below 0.7 suggest the occurrence of a bottleneck (Garza and Williamson 2001). MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to assess the possibility of segregation of null alleles.

RESULTS AND DISCUSSION

Allelic diversity ranged from 2 to 15 alleles per locus and averaged 7.6 alleles per locus, while observed heterozygosity per locus ranged from 0.091 to 1.000 and averaged 0.674 (Table 1). Significant deviations from HWE, showing deficiencies in heterozygotes, were observed at 6 of the 14 loci, and LD was observed at 9 (∼10%) of the 91 pairwise comparisons among loci and involved 12 of the 14 total loci sampled (*Eoo9* and *Eoo19*; *Eoo11* and *Eoo19*; *Eoo9* and *Eoo22*; *Eoo20* and *Eoo22*; *Eoo16* and *Eoo24*; *Eoo11* and *Eoo31*; *Eoo8* and *Eoo44*; *Eoo31* and *Eoo38*; *Eoo11* and *Eoo60*). The *M*-ratios for six loci were below 0.70, suggesting recent loss of allelic diversity at these loci. Possible segregation of null alleles was detected at loci *Eoo16*, *Eoo20*, *Eoo22*, and *Eoo38*. Because of the small size of the population sampled, deviations from HWE and LD could result from loci being closely located on the same chromosome, segregation of null alleles, family structure, population bottlenecks, inbreeding, hermaphroditic reproduction (van der Schalie 1970), or some combination of these factors. Appendix A1 lists individual genotypes at the 14 loci.

These primer pairs are the third set of microsatellite primers developed for the genus *Epioblasma*. The first set of primers was developed for *Epioblasma capsaeformis* (Jones et al. 2004) and the second for *Epioblasma rangiana* (Zanatta and Murphy 2006). We did not test primers developed for *E. capsaeformis* and *E. rangiana* on *E. obliquata*, but allelic diversity of *E. obliquata* was lower than in those two species. For the 10 loci developed for *E. capsaeformis* ($n = 20$ individuals assessed/locus), allelic diversity ranged from 5 to 17 alleles/locus and averaged 9.7 alleles/locus. For the six loci developed for *E. rangiana* ($n = 73$–86 individuals/locus), allelic diversity ranged from 12 to 28 alleles/locus and averaged 19.3 alleles/locus. After careful screening for null

Table 1. Characteristics of 14 microsatellite loci developed using DNA obtained in 2016 and 2017 from 44 individuals of the Catspaw (*Epioblasma obliquata*) from Killbuck Creek, Coshocton County, Ohio. H_O and H_E are observed and expected heterozygosity, respectively. Statistically significant deviations from Hardy–Weinberg Equilibrium (HWE) are denoted by an asterisk (*). *M*-ratio is the Garza–Williamson index. Individual genotypes at the 14 loci are reported in Appendix A1.

Locus	Primer Sequence (5′–3′) and Fluorescent Label	Melting Temperature (°C)	Repeat Motif	Allele Size Range (bp)	No. of Alleles/ Locus	H_o	H_E	HWE *P* Value	*M*-Ratio
*Eoo8**	F:TATCCCTCCGCTGCTGTAAG – PET	59.7	$ACT_{(16)}$	125–173	5	1.000	0.586	0.000*	0.714
	R:CCCTGGCCTGTAACAATCTTG	59.7							
Eoo9	F:CTCTCCGTGATGTTTGCTCC – VIC	59.3	$AAT_{(29)}$	110–197	4	0.477	0.512	0.081	1.000
	R:TTCCATTCCAAGCACGTACG	59.7							
*Eoo10**	F:CTGGTTGTTCGGTCTTGTGG – NED	59.4	$ATC_{(8)}$	137–161	13	0.864	0.815	0.019*	0.867
	R:ACTTTACATCCTGTCCAACTGC	59.8							
Eoo11	F:GCCGCCATGAATAGCCTATC – 6FAM	59.4	$AAC_{(10)}$	197–227	2	0.455	0.505	0.556	0.667
	R:TCTCCCATCAACCAACATTGTC	59.4							
*Eoo16**	F:TGGGTAGTCTCTGTCGTATGC – NED	59.7	$ACAT_{(11)}$	132–176	8	0.477	0.597	0.021*	0.363
	R:AATGGCGCTAATCCCACAAC	59.7							
Eoo19	F:CCTAGGCAGCAAACAGTTCG – 6FAM	59.8	$AGAT_{(10)}$	109–149	13	0.977	0.900	0.137	0.541
	R:GCGGCCAGTATTAATGGTGG	59.9							
Eoo20	F:ACTACAGTACACGACCAGGC – PET	59.6	$ACAT_{(19)}$	74–150	15	0.786	0.921	0.050	0.789
	R:ACCCATGACCTTCCGTATCC	59.9							
*Eoo22**	F:CAGTCCAAGTCATCTCTCAGG – VIC	58.4	$AGAT_{(15)}$	91–151	12	0.750	0.894	0.003*	0.923
	R:GCATACGTGTAGCTTTATCGTG	58.2							
Eoo24	F:TCACAAGTCCTACACCCTCTC – PET	59.0	$AATC_{(6)}$	169–193	2	0.500	0.471	0.748	1.000
	R:TCTTATCAGTTGGGTTTGGTGG	59.2							
Eoo31	F:CAGTCGGGCGTCATCATTCCCTAGCAA – PET	59.7	$ATC_{(9)}$	205–232	6	0.591	0.651	0.314	0.500
	R:GTTTGGTGTAGTGCTCGGAAAC	58.9							
*Eoo38**	F:CAGTCGGGCGTCATCAGCTAACTCCA – 6FAM	59.4	$AAG_{(11)}$	101–134	13	0.659	0.882	0.000*	0.722
	R:GTTTCGCCACCTGAACAGCATATG	60.1							
*Eoo44**	F:CAGTCGGGCGTCATCACCATTAATACT - VIC	59.8	$AAC_{(8)}$	84–108	2	1.000	0.506	0.000*	0.667
	R:GTTTGGGCATCAACGACTTTCATTC	58.6							
Eoo46	F:CAGTCGGGCGTCATCACTGTAACGAG – NED	58.9	$ATCC_{(6)}$	223–247	2	0.091	0.088	1.000	0.500
	R:GTTTGTTAGTTGGGCGGATGGTTG	59.9							
Eoo60	F:GTTTGCTTGCGGTATGTGCTG – VIC	60.5	$AATC_{(10)}$	167–207	10	0.818	0.845	0.814	0.714
	R:CAGTCGGGCGTCATCACCATCTTCAAG	59.0							
Mean					7.6	0.675	0.655		0.712

alleles, HWE, and LD, some of our microsatellite loci developed for *E. obliquata* may prove useful for cross-species amplification in other species, especially other *Epioblasma*. Likewise, future studies could screen the microsatellite loci developed by Jones et al. (2004) and Zanatta and Murphy (2006) to determine whether additional loci are suitable for cross-species amplification in *E. obliquata*.

Sampling more individuals of *E. obliquata* for further population genetic analysis would benefit conservation management. The screening of more wild individuals and any other populations that may be found could provide insight into the population genetic diversity and natural history of this species. Given the isolation and small size of the remaining known population of *E. obliquata*, these microsatellite loci and other genetic markers will be valuable for monitoring the effects of propagation and management practices seeking to maintain or increase genetic diversity in hatchery stocks and wild populations receiving stocked individuals. For example, if hatchery technology improves to allow for the long-term holding, spawning, and fertilization of broodstock in captivity, the loci developed in this study will be useful for monitoring genetic diversity and inbreeding in parental stocks and progeny, which will be critical for maintaining healthy captive and wild populations of *E. obliquata* (Jones et al. 2020).

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LITERATURE CITED

Cooper, A. M., L. M. Miller, and A. R. Kapuscinski. 2009. Conservation of population structure and genetic diversity under captive breeding of remnant coaster brook trout, *Salvelinus fontinalis*, populations. Conservation Genetics 11:1087–1093.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Faircloth, B. C. 2008. MSATCOMMANDER: Detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources 8:92–94.

Garza, J. C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10:305–318.

Hoggarth, M. A., D. L. Rice, and D. M. Lee. 1995. Discovery of the federally endangered freshwater mussel, *Epioblasma obliquata obliquata* (Rafinesque, 1820) (Unionidae), in Ohio. The Ohio Journal of Science 4:298–299.

Jones, J. W., M. Culver, V. David, J. Struthers, N. A. Johnson, R. J. Neves, S. J. O'Brien, and E. M. Hallerman. 2004. Development and characterization of microsatellite loci in the endangered oyster mussel *Epioblasma capsaeformis* (Bivalvia:Unionidae). Molecular Ecology Notes 4:649–652.

Jones, J. W., E. M. Hallerman, and R. J. Neves. 2006. Genetic management guidelines for captive propagation of freshwater mussels (Unionoidea). Journal of Shellfish Research 25:527–535.

Jones, J. W., W. F. Henley, A. J. Timpano, E. Frimpong, and E. M. Hallerman. 2020. Spawning and gravidity of the endangered freshwater mussel *Epioblasma capsaeformis* (Bivalvia: Unionidae) in captivity for production of glochidia. Invertebrate Development and Reproduction 64:312–325.

Serb, J. M., J. E. Buhay, and C. Lydeard. 2003. Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae: Ambleminae) based on mitochondrial *ND1* sequences. Molecular Phylogenetics and Evolution 28:1–11.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S.G. Rozen. 2012. Primer3–New capabilities and interfaces. Nucleic Acids Research 40(15):e115.

USFWS (U.S. Fish and Wildlife Service). 1990. Purple cat's paw pearlymussel determined to be an endangered species. Federal Register 55:28209–28213.

van der Schalie, H. 1970. Hermaphroditism among North American freshwater mussels. Malacologia 10:93–112.

Van Oosterhout, C., W. F. Hutchinson, D. P. Wills, and P. Shipley. 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Zanatta, D. T., and R. W. Murphy. 2006. Development and characterization of microsatellite markers for the endangered northern riffleshell mussel *Epioblasma torulosa rangiana* (Bivalvia: Unionidae). Molecular Ecology Notes 6:850–852.

Zane, L., L. Bargelloni, and T. Patarnello. 2002. Strategies for microsatellite isolation: a review. Molecular Ecology 11:1–16.

Appendix A1. Scored microsatellite genotypes of 44 individuals of the Catspaw (*Epioblasma obliquata*) from Killbuck Creek, Coshocton County, Ohio, at 14 loci. Microsatellite amplicons were scored for length using Genemarker software (SoftGenetics, State College, PA, USA). Individuals and loci without a scored allele (—) indicate that no allele product was observed at that locus. Columns with either 1 or 2 designate alleles per locus.

Loci	*Eoo8* ACT(16)		*Eoo9* AAT(29)		*Eoo10* ATC(8)		*Eoo11* AAC(10)		*Eoo16* ACAT(11)		*Eoo19* AGAT(10)		*Eoo20* ACAT(19)		*Eoo22* AGAT(15)		*Eoo24* AATC(6)		*Eoo31* ATC(9)		*Eoo38* AAG(11)		*Eoo44* AAC(8)		*Eoo46* ATCC(6)		*Eoo60* AATC(10)	
Individual	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Wildstock1	169	184	182	188	199	211	260	266	224	236	189	201	—	—	205	217	—	—	199	199	289	289	253	259	124	124	242	246
Wildstock2	169	184	182	185	211	217	260	266	240	240	197	213	—	—	209	221	171	175	199	199	253	259	253	259	124	136	234	254
Wildstock3	169	184	179	179	211	220	266	266	240	240	193	197	294	294	185	193	175	175	211	211	256	292	253	259	124	124	242	246
Wildstock4	169	184	182	185	205	205	266	266	228	228	185	201	282	298	213	221	171	175	199	217	253	289	253	259	124	124	234	246
Wildstock5	169	184	182	182	199	199	260	266	240	240	193	201	254	326	221	221	175	175	199	211	241	256	253	259	124	124	242	246
Wildstock6	169	184	182	182	199	211	266	266	240	240	193	197	282	298	185	201	171	175	199	217	253	253	253	259	124	124	246	246
Wildstock7	169	184	182	182	199	220	260	266	240	240	177	205	282	298	213	213	175	175	217	223	250	271	253	259	124	136	242	242
Wildstock8	169	184	179	182	190	211	266	266	240	244	185	225	302	326	201	201	171	175	199	217	277	292	253	259	124	124	202	242
Wildstock9	169	184	185	185	190	199	266	266	240	240	201	217	282	302	201	201	171	171	199	217	286	286	253	259	124	124	218	230
Wildstock10	169	184	182	185	190	199	260	266	240	240	197	205	306	326	209	209	171	175	199	223	271	289	253	259	124	124	246	246
Wildstock11	169	184	182	188	202	211	266	266	228	240	185	189	254	294	173	185	171	171	199	199	289	289	253	259	124	124	234	234
Wildstock12	169	184	182	182	190	208	260	260	240	240	193	205	278	278	193	201	171	175	223	223	256	256	253	259	124	124	242	246
Wildstock13	169	184	182	182	190	199	266	266	228	240	185	201	298	314	201	209	175	175	199	199	265	289	253	259	124	124	218	246
Wildstock14	169	184	182	185	199	199	260	260	240	240	185	205	282	314	185	213	175	175	199	199	259	259	253	259	124	124	230	246
Wildstock15	169	181	182	182	193	211	266	266	232	240	177	205	278	298	205	221	171	175	199	217	250	256	253	259	124	124	234	242
Wildstock16	169	184	182	182	187	208	260	266	240	240	193	201	310	326	185	193	171	175	217	217	241	277	253	259	124	124	242	242
Wildstock17	169	184	179	185	190	202	260	260	232	232	193	213	254	314	213	213	175	175	199	199	256	289	253	259	124	124	218	226
Wildstock18	169	181	182	182	199	220	260	266	240	240	133	189	262	286	205	205	171	175	199	226	259	292	253	259	124	124	218	234
Wildstock19	169	184	182	188	199	199	260	260	240	240	193	213	286	326	173	221	175	175	199	211	256	256	253	259	124	124	242	242
Wildstock20	169	184	182	185	202	211	260	266	240	240	185	201	282	306	213	221	171	175	199	199	256	265	253	259	124	124	218	234
Wildstock21	169	184	182	182	190	190	260	266	232	240	177	181	282	318	181	213	175	175	211	211	256	292	253	259	124	124	218	242
Wildstock22	169	187	179	179	199	211	260	266	240	240	197	201	254	294	205	205	171	175	199	211	256	256	253	259	124	124	234	242
Wildstock23	169	184	182	185	190	199	260	266	228	228	133	201	314	314	185	197	171	171	211	211	241	253	253	259	124	124	202	246
Wildstock24	169	184	182	185	196	220	260	260	232	236	181	205	298	310	213	217	—	—	211	217	250	262	253	259	124	124	242	246
Wildstock25	169	184	182	185	190	199	260	266	232	240	133	201	278	314	201	209	171	175	199	199	259	259	253	259	124	124	218	246
Wildstock26	169	184	182	185	190	199	260	260	240	240	181	205	302	322	193	201	171	175	211	211	256	265	253	259	124	124	206	226
Wildstock27	169	184	182	185	190	199	260	260	240	240	189	197	254	254	193	201	171	175	199	211	256	265	253	259	124	124	226	246
Wildstock28	169	181	179	185	190	199	266	266	240	240	209	209	314	322	201	213	175	175	199	211	256	289	253	259	124	124	218	218
Wildstock29	169	184	182	188	199	211	260	266	228	240	185	189	254	314	173	185	171	171	199	217	289	289	253	259	124	124	234	242
Wildstock30	169	184	182	182	190	199	266	266	168	240	197	201	306	306	221	221	171	175	199	199	256	289	253	259	124	124	234	246
Wildstock31	169	181	179	182	208	217	260	266	232	240	189	201	278	290	193	213	175	175	199	211	253	253	253	259	124	124	226	254
Wildstock32	169	184	182	188	196	220	260	260	240	240	133	193	282	290	209	217	175	175	199	217	259	259	253	259	124	124	218	242
Wildstock33	169	184	182	182	190	208	260	266	168	240	133	197	282	302	209	209	171	175	211	217	292	292	253	259	124	124	202	234
Wildstock34	169	184	182	182	199	211	260	266	168	240	133	197	282	294	201	209	175	175	199	217	241	259	253	259	124	124	206	238
Wildstock35	169	184	182	182	211	220	260	260	168	240	185	193	278	298	181	193	175	175	199	211	262	262	253	259	124	124	218	246

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Loci	*Eoo8* ACT(16)		*Eoo9* AAT(29)		*Eoo10* ATC(8)		*Eoo11* AAC(10)		*Eoo16* ACAT(11)		*Eoo19* AGAT(10)		*Eoo20* ACAT(19)		*Eoo22* AGAT(15)		*Eoo24* AATC(6)		*Eoo31* ATC(9)		*Eoo38* AAG(11)		*Eoo44* AAC(8)		*Eoo46* ATCC(6)		*Eoo60* AATC(10)	
Wildstock36	169	184	182	185	199	205	260	266	168	168	197	217	302	302	185	201	171	175	199	232	256	289	253	259	124	136	202	218
Wildstock37	169	178	182	182	199	214	260	266	168	240	133	193	310	310	185	221	175	175	199	217	253	292	253	259	124	124	246	254
Wildstock38	169	178	182	182	199	211	260	266	232	240	133	193	282	282	189	193	175	175	199	199	259	265	253	259	124	136	234	246
Wildstock39	169	178	182	182	199	199	266	266	240	240	185	201	302	314	185	201	171	175	199	217	277	289	253	259	124	124	202	218
Wildstock40	169	184	182	185	202	214	260	266	236	240	133	185	294	302	185	205	171	175	199	211	256	289	253	259	124	124	218	234
Wildstock41	169	184	182	182	199	211	266	266	160	232	189	201	278	306	209	221	175	175	199	199	241	259	253	259	124	124	234	234
Wildstock42	169	184	182	182	199	211	260	260	168	232	193	205	310	310	189	193	171	171	199	211	256	256	253	259	124	124	218	238
Wildstock43	169	184	182	182	193	199	260	260	168	240	197	205	294	302	201	201	171	175	211	232	265	280	253	259	124	124	218	226
Wildstock44	169	184	182	182	178	199	266	266	160	228	133	197	254	298	205	217	171	175	199	199	262	289	253	259	124	124	234	246