Using nuclear microsatellite loci to characterize genetic variation within and among populations of the federally endangered *Astragalus bibullatus* (Pyne's ground plum; Fabaceae)

by
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A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

Spring 2019

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Abstract

Astragalus bibullatus is a federally endangered plant endemic to the cedar glades of Middle Tennessee. To aid in conservation efforts, genetic data have been generated using microsatellite markers. These markers were developed in a previous study, Morris et al. (2016), in which seven loci were genotyped for 361 individuals across nine populations. This provided the mean number of alleles, mean number of effective alleles, observed heterozygosity, and unbiased expected heterozygosity across all nine populations. In the present study, one additional locus, Abib152, was used to genotype the same individuals as the previous study, evaluated using the same metrics, and combined with the data from Morris et al. (2016) to determine how the new locus impacted the overall results. Adding Abib152 resulted in lower values for all calculated metrics, which was unexpected.

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Introduction

Astragalus bibullatus is a federally endangered plant, more commonly known as Pyne's ground plum. *A. bibullatus* is a perennial herb that is endemic to the limestone cedar glades of Rutherford County, Tennessee (Barneby and Bridges, 1987). According to the Tennessee Department of Environment and Conservation, there are 22 Element Occurrences (EOs) (TDEC, pers. comm., A.B. Morris). However, of the 22 EOs, only 11 are extant, seven of which are found on protected lands managed by TDEC. The remaining four EOs reside on private property. With the exception of one of the populations (Rockvale), all of the EOs are found within a five-mile radius of Flat Rock Cedar Glades and Barrens State Natural Area.

A. bibullatus usually flowers in April or May, and fruits mature in late May to early June (U.S. Fish and Wildlife Service 2009). The habitat of the species is limited to a narrow zone along the open cedar glades and the forest edge. Flowering is restricted to occurring under the shade, and woody encroachment of the surrounding cedars has been proposed as a major threat to the success of the species (Albrecht et al. 2016). In efforts to understand the current condition of *A. bibullatus*, studies using allozymes and amplified fragment length polymorphism (AFLP) have been conducted in order to determine the genetic diversity among the populations.

Previous studies using allozymes (Baskauf and Snapp 1998, Morris et al. 2002) and AFLPs (Baskauf and Burke 2009) showed limited genetic variation within and among populations of *A. bibullatus*. However, each study used samples taken from different populations with little overlap, and some of the published population names did not correspond to the designated TDEC EO definitions. Additionally, all three studies

predate reintroduction efforts. Morris et al. (2002) found more genetic variation in the soil seed bank than in the above ground plants from six sampled populations. Baskauf and Burke (2009) found evidence for two genetic clusters among seven sampled populations. Since the time that these previous studies were conducted, sequencing cost has been reduced such that genotyping several loci is now a cost-effective method that yields more data than previously used methods. The current project is the first study to use sequencing of microsatellite loci to characterize the genetic variation of *A. bibullatus* in both natural and reintroduced populations. This combined data set of this study and the Morris et al. 2016 study has made it the most comprehensive genetic study to date for the species.

Microsatellite loci are among the most common tools used to document genetic structure within and among populations (Selkoe and Toonen 2006). A microsatellite is a tandemly repeating region of DNA (e.g., CACACA), and these regions occur throughout the genomes of most organisms. The loci are amplified using a fluorescently-tagged polymerase chain reaction (PCR), and the products can be identified as fragment data on a capillary sequencer. The different length fragments are recorded as different alleles. Multilocus genotypes are then used to calculate allele frequencies within and among populations. In a previous study (Morris et al. 2016), 12 nuclear microsatellite loci were developed and characterized for a subset of *A. bibullatus* individuals. Seven loci were genotyped for 361 individuals from six naturally occurring populations and three reintroduced populations (Morris et al., unpublished data). The objective of this current project was to determine the genotype for these same 361 individuals of *Astragalus bibullatus* (Fabaceae) using a previously published microsatellite locus (Abib152), and to

determine how that locus influenced common measures of genetic diversity previously calculated for a seven-locus data set generated by students in the Morris Lab.

Thesis Statement

Genetic diversity of the sampled populations and the species as a whole is expected to be relatively low based on limited numbers of populations and small population sizes. Additionally, this study was conducted with the following expectation: the eight-locus data set is expected to result in increased measures of the mean number of alleles, mean number of effective alleles, observed heterozygosity, and unbiased expected heterozygosity across sampled populations relative to the seven-locus data set.

Methods

Field Sampling

Individual plants were previously sampled from both the naturally occurring populations and reintroduced populations of *A. bibullatus.* In this study, there are six naturally occurring "populations" and three reintroduced "populations," which combined are recognized as seven EOs by TDEC. Naturally occurring populations included: Davenport West and Davenport East (considered a single EO, both at Flat Rock State Natural Area [SNA]), Airport, Davis, Manus Road SNA, and Rockvale. The reintroduced populations included Stones River National Battlefield glades 7 (SRNB07) and 54 (SRNB54), and Couchville Cedar Glade SNA. All populations except Rockvale were chosen because they are the subjects of a long-term demographic study led by Albrecht and Long (2018). For the Rockvale population, an attempt to sample all existing plants

was made except for those that were considered too small, with the potential to have their growth and development inhibited by the procedure. Leaf material was collected and preserved in silica gel desiccant. No vouchers were collected due to the extreme rarity of the species and the well-documented localities on record with TDEC. All sampling was previously completed (Morris et al. 2016).

Extraction of total genomic DNA from silica-dried leaves using the Qiagen Dneasy Plant Mini Kit (Qiagen, Valencia, California) following the manufacturer's protocols with slight modifications. The microsatellite locus used in this study was previously developed for *A. bibullatus* by Morris et al. (2016). The 12 nuclear microsatellite loci were previously published by Morris et al. (2016) (Table 1). Previously genotyped loci include Abib059, Abib113, Abib093, Abib051, Abib170, Abib095, Abib083. The remaining five loci (Abib028, Abb094, Abib120 Abib152, and Abib156) were targeted for this study. Due to challenges associated with the amplification of these loci (see Discussion), one locus, Abib152, was selected and used to genotype the 361 individuals of this study.

Amplification followed the protocols described in Morris et al. (2016) using the three-primer approach of Schuelke (2000), with a 17-base tail (5'-GTAAAACGACGGCCAGT-3') added to the 5' end of each forward primer and a 7-base 'pigtail' (5'-GTTTCTT-3') added to the 5' end of each reverse primer. The third primer in each reaction was identical to the forward tail and was fluorescently labeled with a Fluorescein amidite (FAM) tag. The final reaction had the following concentrations: Taq buffer (Life Technologies), 2 mM $MgCl_2$, 0.05 μM forward primer with the 5'- M13 tail, 0.15 μM fluorescently-labeled M13 primer, 0.2 μM pig-tailed reverse primer, 0.2 mM

dNTPs, 0.5 U Platinum Taq (Life Technologies), and 1 μL DNA. Amplified products were then combined with LIZ-500 size standard and HiDi Formamide and shipped to the Cornell University Institute of Biotechnology for genotyping on an ABI 3730 (Life Technologies, Foster City, CA USA). Results were typically available for download from the Cornell website within 48 hours of shipping. Alleles were scored using GeneMarker MTP software (SoftGenetics, State College, Pennsylvania).

Data Analysis

Analysis of genetic diversity was performed on two sets of data: 1) the seven-locus data set previously generated by Morris et al. (unpublished), and 2) the eight-locus data set consisting of the seven-locus data set combined with the newly generated Abib152 locus data. Measures of genetic diversity were calculated using GenAlEx 6.5 (Peakall and Smouse 2012). Summary statistics were calculated for each locus over all populations, as well as for each population over all loci. These statistics included the mean number of alleles (N_a), mean effective number of alleles (N_e), mean observed heterozygosity (H_o), and mean unbiased expected heterozygosity (H_e).

Results

Locus Abib152 was successfully amplified and genotyped for 209 out of 361 individuals across nine populations. Of the remaining 152 individuals, the percentage of missing data varied by population (Table 2): SRNB07 = 81.8%; SRNB54 = 81.3%; Manus = 69.2%; Couchville = 67.6%; Airport = 47.1%; Davenport East = 13.3%; Davis = 13.0%; and Rockvale = 10.2%. The only population to have complete recovery of data for Abib152 was Davenport West.

Metrics by locus across populations

Abib152 exhibited fewer alleles than any other locus in the combined eight-locus data set and expressed the lowest values for the mean number of alleles, mean number of effective alleles, observed heterozygosity, and unbiased expected heterozygosity across all populations (Table 3). Abib093 expressed the next lowest values for the metrics tested in the eight-locus data set, and the differences in the values of the remaining six loci and Abib152 were even greater. The mean number of alleles across populations for Abib152 was 1.56, compared to 2.56 from Abib093. Across all populations, the mean number of effective alleles in Abib152 was 1.07 and 1.60 for Abib093. The observed heterozygosity for Abib152 was 0.05 and was 0.31 for Abib93. The unbiased heterozygosity was 0.06 for Abib152 and 0.36 for Abib093.

Metrics by population across all loci

The addition of locus Abib152 to the seven-locus data set resulted in a lower mean number of alleles, mean number of effective alleles, observed heterozygosity, and unbiased expected heterozygosity across all populations. This decrease can be seen across all populations for the eight-locus data set compared to the seven-locus data (Table 4). This change is best demonstrated in the Davenport West population due to it being the only population to have full data recovery for Abib152. The mean number of alleles in the seven-locus data set decreased from 5.14 to 4.75 for the eight-locus data set. The

mean number of effective alleles in the seven-locus data set decreased from 2.94 to 2.69 for the eight-locus data set. The observed heterozygosity of the seven-locus data decreased from 0.59 to 0.52 for the eight-locus data set. The unbiased observed heterozygosity for the seven-locus data set decreased from 0.64 to 0.56 in the eight-locus data set. Differences in the values between the seven-locus and eight-locus data sets, however, were all within the ranges of standard error.

Impacts of missing data

Abib152 exhibited the greatest percentage of missing data across populations of any locus within the eight-locus data set (data not shown). While Abib152 did exhibit lower values compared to the original seven-locus data set, this does not seem to be a result of missing data. The two populations with the greatest data recovery for Abib152 were Davenport West (100%) and Rockvale (90%), and the two populations with the greatest amount of missing data for Abib152 were SRNB07 (81.8% missing) and SRNB54 (81.3% missing) (Table 2). Despite the differences in the amount of data recovered demonstrated by these four populations, the metrics calculated for them was similar across all populations (Tables 4 and 5). Davenport West and Rockvale had a mean number of alleles of 1.03 and 1.0, respectively. Alternatively, populations with the greatest amount of missing data for Abib152, SRNB07, and SRNB54, had a mean number of alleles of 1.00 and 2.00, respectively. Davenport West and Rockvale had a mean number of effective alleles of 0.72 and 1.00. SNRB07 and SNRB54 had a mean number of effective alleles of 1.00 and 1.28. For Davenport West and Rockvale, the observed heterozygosity was 0.27 and 0.00 In the SNRB07 and SNRB54 populations, the

observed heterozygosity was 0.00 and 0.25. The unbiased expected heterozygosity for Davenport West and Rockvale was 0.00 and 0.25. For SNRB07 and SNRB54 populations, the unbiased observed heterozygosity was 0.25 and 0.00.

Discussion

The addition of Abib152 to the seven-locus data set was done to determine the influence of an additional locus to the seven-locus data set for *A. bibullatus*. The eight-locus data set expands the scope of the genetic information for *A. bibullatus* necessary for future genetic monitoring. The addition of an eighth locus was expected to result in increased measures of the mean number of alleles, mean number of effective alleles, observed heterozygosity, and unbiased expected heterozygosity across sampled populations relative to the seven-locus data set. The increase of the values tested was expected to increase due to the number of loci evaluated increasing, which in turn increases the potential of finding additional alleles within a particular locus. However, the expected outcomes were not observed as both the number of alleles and the frequency of the alleles within Abib152 were lower than that of any other locus within the previous 7-locus data set. As a result, there was a decrease in the values for all metrics tested within the eight-locus data set from the original seven-locus data set. This highlights the importance of marker selection in differentiation between populations. It is important that the markers used are neutral, and that a large number of markers are used to provide a baseline that can be used to safeguard against loci that do not accurately represent the condition of the population as a whole. While the addition of Abib152 did not produce the expected results, the data generated are still useful and provide insight into the

importance of marker selection for conservation efforts. Challenges in genotyping all 361 of the previously genotyped individuals were responsible for the lower number of individuals genotyped for Abib152 and are discussed below.

Implications of observed data

Abib152 lowered the overall values of the metrics tested within and among all populations across all loci, if only slightly, as the values for the eight-locus data set remained within the standard error. This can be an indication that Abib152 is not particularly biologically informative as a whole, as it contributed little information that could be used to differentiate populations. This assumption is reinforced when evaluating the eight-locus data set on an individual locus basis. For the values of the metrics tested across all loci, Abib152 had lower values compared to the loci of the seven-locus data set. It is not likely that the low recovery of data for Abib152 is a contributing factor to the lower values of all metrics tested. As previously stated, Davenport West and Rockvale, which were the populations with the highest amounts of data recovery, had similar results to the two lowest scoring populations, SNRB07 and SNRB54. This indicates that Abib152 has an inherently low number of alleles and that the amount of data retrieved does not appear to affect the locus' lower values for metrics tested. In order to prevent a locus such as Abib152 from mischaracterizing the genetic makeup of the species as a whole, as many proper markers should be used as possible

Importance of marker selection

Larger sample size can better represent the condition of a population. It is possible for one locus to demonstrate an uncharacteristically high number of alleles or allelic frequency or conversely particularly low allele frequency or the low number of alleles. Increasing the number of markers used can help eliminate sampling error, in which a few loci containing an uncharacteristic locus, is used to inadequately represent the allelic frequency or number of alleles for a population as a whole (Selkoe and Toonen 2006). To generate data that can be used for conservation, it is important to select markers that are neutral. Neutral markers exhibit changes as a result of random mutations which will develop independently in other populations. This provides greater insight into the differences between populations when evaluating the number of alleles and the allelic frequency within a population (Hendrik 2001). The assumption for this study was that none of the loci tested contain alleles that were under selection. Selection can result in particularly low allelic frequency if the allele is detrimental to an organism or if the allele is advantageous, that particular allele frequency will be high (Hendrik 2001). It is possible that Abib152 is under selection, given the lower values compared to the rest of the loci in the seven-locus data set. In the event that there was an external pressure that selected against Abib152, all populations would more than likely be affected, given the close proximity of the populations to each other. This would lower the number and frequency of alleles across all populations. In order to confirm that Abib152 is in fact under selection as opposed to having an inherently low number of alleles, more data would need to be retrieved. This would require using additional parameters that were not

included in this study as well as retrieving as much of the missing data as possible, which in spite of many efforts to reduce the amount of missing data in this study, remained high.

Challenges of genotyping samples

As previously stated, 42% of the individuals from the original 361 members of the seven-locus data set were not successfully genotyped for Abib152. Several attempts were made to fill in the gaps, but these were unsuccessful. The problems encountered in genotyping these samples arose from several factors. The majority of the DNA samples that were used for the original seven-locus data set were depleted, and as a result, new extractions for the same individuals had to be carried out. At this point, it was discovered that some of the leaf material for these individuals had been depleted. An attempt was made to retrieve new leaf material from these individuals which had been individually tagged. Upon returning to these individuals it was discovered that many of the plants had died in the interim from when they were originally sampled in 2014.

Additional complications in genotyping were experienced that may have been related to the fluorescent tags used. DNA samples that were amplified through PCR did not produce a clean signal that was strong enough for the fluorescent tag to be detected. In many cases, the fluorescent tags would not appear, and only the size standard could be seen after being genotyped. For some of the samples, the fluorescently tagged PCR products would be visible, but very weak. To rule out human error, the same sample would be reamplified through PCR and then genotyped again. In an effort to troubleshoot the exact problem, some of the samples were genotyped multiple times and, in some cases, were genotyped both in-house using MTSU equipment and also through Cornell,

with varying success. As previously discussed, the data did not appear to be adversely affected by the 42% missing data, and observing how other similar species behave can help determine whether or not the data received is completely uncharacteristic.

Astragalus bibullatus in relation to similar species

In a similar study by Wall et al. (2013), microsatellites were used to characterize individuals from *Astragalus michauxii*, a rare plant that can be found in small pockets in North Carolina, South Carolina, and Georgia. This is one of only a few *Astragalus* studies that use microsatellites for population genetics, making it a suitable comparison to the work completed here for *A. bibullatus.* For *A. michauxii*, the mean number of alleles ranged from 2.86 to 7.75, and the observed heterozygosity ranged from 0.47 to 0.69 (Wall et al. 2013). In comparison, in *A. bibullatus*, a range of 3.63 to 4.75 for the mean number of alleles was observed (Table 4), and a range of 0.40 to 0.54 was seen for observed heterozygosity. The larger differences in *A. michauxii* are to be expected given its increased range and larger population sizes over *A. bibullatus*. For *A. bibullatus*, fragmentation of habitat as has occurred in Rutherford County has isolated populations from one another, likely limiting gene flow across populations. However, limited variation among populations suggests that there may not have been enough time for populations to diverge. This indicates that this plant, which can live for many years only to produce few offspring, is more resilient to change than might be expected, given its rarity.

Conclusions

The inclusion of Abib152 to the seven-locus data set did not yield the results that were predicted. While the presence of Abib152 lowered the calculated metrics in terms of the mean number of alleles, the mean number of effective alleles, observed heterozygosity, and unbiased expected heterozygosity across sampled populations, there was not a significant decrease from the previous results of the seven-locus data set. Abib152 was not particularly informative, and while it did not add to the genetic resolution of A. *bibullatus*, it provided insight into the impact that a single locus can have. The populations with the most data recovered demonstrated similar values in the metrics tested compared to the populations with the least amount of data recovered for Abib152. This indicated that missing data for the added locus is not solely responsible for lowering the values for the metrics tested across all loci within all populations, but rather Abib152 had inherently low values. Increasing the number of markers in a data set should provide an accurate depiction of the genetic makeup of the populations so that the effects of sampling error are minimized. This provides all the more reason for adding the remaining four loci, which have not been genotyped, to the eight-locus data set. This would enhance the knowledge of how an individual locus affects the data set as a whole, over the current eight-locus dataset. With the 12-locus data set completed, greater protection against abnormal makers can be gained, providing a more accurate baseline. This is important for conservation, in which the smaller number of markers used has a greater potential of mischaracterizing the condition of *A. bibullatus* as a whole. These additional loci will expand genetic sampling, providing a better understanding of the structure within and among populations as a whole. In doing this, these data can be

combined with long-term demographic studies to aid in conservation efforts of this rare species.

Table 1. Microsatellite loci developed for *Astragalus bibullatus* (Morris et al. 2016).

Locus	Primer Sequence	Repeat motif	Allele size (bp)	GenBank accession no.
Abib028	F:ACCAGCGAATAGTGCTTACGTG R:CTGTAGCCATTGAAGGAACCAC	$(AAC)_5$	212	KT905411
Abib051	F:AGTCTGTACATTGCGAACTCAAC R:AGTCTGTACATTGCGAACTCAAC	$(AG)_6$	170	KT905412
Abib059	F:CATTTCTTGTAACGCCAGAAACG R:GATGAGTTGTGAAGAAGACTGGG	$(AAC)_7$	342	KT905413
Abib083	F:AATCTCAGAGGCATAGAGGGTAG R:TGAAATAGGAGATGATTATGGCCC	$(AC)_9$	197	KT905414
Abib093	F:ACTTATCCTCTCACTCCAACCTC R:CTCAACTCTCTGCCACTTGAATC	$(AAG)_5$	343	KT905415
Abib094	F: AGATCCAAAGTTTGCCATCCATC R:TTCCTTCACTTCCGCCATTAATG	$(AG)_8$	186	KT905416
Abib095	F: CAGGCATGCAAATGGGATAATTG R: TAATCACATTCTCTCATGCACGC	$(AG)_9$	215	KT905417
Abib113	F: CTCTTCTCTCGAATCATCATCCC R: CTCTATCCTCTACCACCGCTTC	$(AG)_{11}$	228	KT905418
Abib120	F:TTCTTCATCAGTTGCTAAGCCAC R:TATACTTCAGAACGGTGAGGAGG	$(AGG)_6$	267	KT905419
Abib152	F:TGCTACCTACAATGCCACTATTG R:TGCTTTAACTAGTGCTTTGTCAC	$(AG)_{10}$	235	KT905420
Abib156	F:AAGTGTGTGCGGTGATTAGAAAG R:AAGTGTGTGCGGTGATTAGAAAG	$(AAG)_6$	212	KT905421
Abib170	F:ATTTGTCACCTTTCTCCACATGC R:ATTTGTCACCTTTCTCCACATGC	$(AAC)_5$	350	KT905422

Table 2. Amount of missing data by population for locus Abib152.

Population	*n*	*152 N*	*% Missing*
Airport	51	27	47.06
Davenport East	30	26	13.33
Davenport West	37	37	0.00
Davis	23	20	13.04
Manus	52	16	69.23
Rockvale	59	53	10.17
SRNB54	16	3	81.25
SRNB07	22	4	81.82
Couchville	71	23	67.61

N is the number of individuals sampled by population; *152 N* is the number of individuals successfully genotyped by population.

Table 3. Comparison of genetic diversity by locus over all populations for each locus with the mean and standard error for *A. bibullatus*.

Locus	*n*	N_a	N_e	H_o	uH_e
Abib059	38.889 (5.945)	5.000 (0.167)	3.158 (0.280)	0.671 (0.054)	0.670 (0.035)
Abib113	39.778 (6.224)	5.444 (0.294)	2.624 (0.102)	0.678 (0.030)	0.624 (0.015)
Abib083	38.222 (5.899)	4.556 (0.242)	3.370 (0.197)	0.700 (0.028)	0.707 (0.017)
Abib051	37.222 (6.260)	10.111 (0.873)	3.808 (0.442)	0.668 (0.046)	0.727 (0.028)
Abib170	38.889 (6.183)	2.889 (0.111)	1.933 (0.098)	0.446 (0.036)	0.481 (0.023)
Abib095	38.667 (5.942)	4.333 (0.236)	1.852 (0.148)	0.405 (0.041)	0.441 (0.042)
Abib093	39.333 (6.591)	2.556 (0.176)	1.598 (0.098)	0.308 (0.053)	0.360 (0.043)
Abib152	23.222 5.190	1.556 0.176	1.067 0.032	0.053 0.030	0.060 0.029

N_a is the mean number of alleles across all loci; N_e is the mean number of effective alleles across all loci, H_o is mean observed heterozygosity across all loci; uH_e is mean unbiased expected heterozygosity across all loci; and standard error for each metric

Table 4. Comparison of genetic diversity for the seven-locus and eight-locus data sets with the mean and standard error over loci for each population for *A. bibullatus*.

Population	*n*	N_a		N_e		H_o		uH_e	
		7 loci	8 loci	7 loci	8 loci	7 loci	8 loci	7 loci	8 loci
Airport	51	5.000 (1.113)	4.625 (1.034)	2.231 (0.299)	2.087 (0.296)	0.459 (0.079)	0.402 (0.089)	0.494 (0.080)	0.441 (0.087)
Davenport East	30	4.857 (0.829)	4.500 (0.802)	2.917 (0.416)	2.698 (0.422)	0.655 (0.052)	0.592 (0.077)	0.622 (0.058)	0.562 (0.078)
Davenport West	37	5.14 (0.88)	4.75 (0.86)	2.935 (0.36)	2.69 (0.86)	0.59 (0.07)	0.52 (0.093)	0.638 (0.043)	0.561 (0.085)
Davis	23	4.714 (0.837)	4.375 (0.800)	2.679 (0.408)	2.475 (0.408)	0.574 (0.058)	0.508 (0.082)	0.590 (0.057)	0.522 (0.084)
Manus	52	5.571 (1.462)	5.000 (1.389)	3.071 (0.719	2.812 (0.674)	0.599 (0.094)	0.525 (0.111)	0.583 (0.079)	0.510 (0.100)
Rockvale	59	4.000 (0.488)	3.625 (0.565)	2.452 (0.205)	2.270 (0.254)	0.537 (0.054)	0.470 (0.082)	0.576 (0.042)	0.504 (0.081)
SRNB 54	16	4.429 (0.685)	4.000 (0.732)	2.475 (0.359)	2.291 (0.362)	0.540 (0.113)	0.473 (0.119)	0.558 (0.069)	0.488 (0.092)
SRNB 07	22	4.857 (0.986)	4.500 (0.926)	2.441 (0.388)	2.296 (0.366)	0.517 (0.084)	0.483 (0.080)	0.537 (0.077)	0.501 (0.075)
Couchville	71	6.286 (1.409)	5.625 (1.388)	2.383 (0.223)	2.210 (0.259)	0.514 (0.047)	0.450 (0.076)	0.559 (0.048)	0.489 (0.081)

N_a is the mean number of alleles across all loci; N_e is the mean number of effective alleles across all loci, H_o is mean observed heterozygosity across all loci; uH_e is mean unbiased expected heterozygosity across all loci; and standard error for each metric

Table 5. Comprehensive comparison of genetic diversity by population, by locus for *A. bibullatus*.

Population	Locus	*n*	N_a	N_e	H_o	uH_e
Airport	AB59	50	5.000	3.021	0.580	0.676
	AB113	51	6.000	2.461	0.569	0.599
	AB83	50	4.000	2.755	0.640	0.643
	AB51	49	11.000	3.078	0.694	0.682
	AB170	51	3.000	1.768	0.294	0.439
	AB95	46	4.000	1.341	0.261	0.257
	AB93	51	2.000	1.192	0.176	0.162
	ABIB152	27	2.000	1.077	0.000	0.073
Davenport East	AB59	29	5.000	4.195	0.724	0.775
	AB113	30	5.000	2.862	0.800	0.662
	AB83	25	5.000	4.252	0.680	0.780
	AB51	18	9.000	3.560	0.778	0.740
	AB170	30	3.000	1.972	0.667	0.501
	AB95	30	5.000	1.777	0.467	0.445
	AB93	30	2.000	1.800	0.467	0.452
	ABIB152	26	2.000	1.166	0.154	0.145
Davenport West	AB59	36	5.000	4.095	0.889	0.766
	AB113	37	5.000	2.573	0.703	0.620
	AB83	37	5.000	4.305	0.703	0.778
	AB51	36	10.000	3.232	0.583	0.700
	AB170	37	3.000	2.136	0.432	0.539
	AB95	34	5.000	2.242	0.441	0.562
	AB93	37	3.000	1.960	0.378	0.496
	ABIB152	37	2.000	1.027	0.027	0.027

Table 5. Continued

Population	Locus	*n*	N_a	N_e	H_o	uH_e
Davis	AB59	23	5.000	1.766	0.435	0.443
	AB113	23	5.000	3.158	0.696	0.699
	AB83	23	5.000	3.806	0.696	0.754
	AB51	21	9.000	4.345	0.762	0.789
	AB170	21	3.000	1.789	0.429	0.452
	AB95	23	4.000	1.920	0.391	0.490
	AB93	23	2.000	1.967	0.609	0.502
	ABIB152	20	2.000	1.051	0.050	0.050
Manus	AB59	52	5.000	4.024	0.808	0.759
	AB113	51	6.000	2.153	0.510	0.541
	AB83	51	4.000	2.937	0.784	0.666
	AB51	50	14.000	6.878	0.940	0.863
	AB170	50	3.000	2.589	0.520	0.620
	AB95	52	4.000	1.516	0.365	0.344
	AB93	52	3.000	1.398	0.269	0.287
	ABIB152	16	1.000	1.000	0.000	0.000
Rockvale	AB59	59	4.000	2.787	0.525	0.647
	AB113	57	4.000	2.582	0.719	0.618
	AB83	58	4.000	3.109	0.672	0.684
	AB51	58	6.000	2.536	0.552	0.611
	AB170	56	2.000	1.711	0.375	0.419
	AB95	58	5.000	2.738	0.586	0.640
	AB93	58	3.000	1.700	0.328	0.415
	ABIB152	53	1.000	1.000	0.000	0.000

Table 5. Continued

Population	Locus	*n*	N_a	N_e	H_o	uH_e
SNRB54	AB59	15	5.000	3.462	0.867	0.736
	AB113	16	5.000	2.349	0.750	0.593
	AB83	16	4.000	2.926	0.875	0.679
	AB51	16	8.000	3.821	0.500	0.762
	AB170	16	3.000	1.724	0.438	0.433
	AB95	16	3.000	1.373	0.188	0.280
	AB93	12	3.000	1.674	0.167	0.420
	ABIB152	3	1.000	1.000	0.000	0.000
SNRB07	AB59	22	5.000	2.501	0.682	0.614
	AB113	22	6.000	2.581	0.682	0.627
	AB83	21	4.000	3.052	0.667	0.689
	AB51	22	10.000	4.283	0.636	0.784
	AB170	20	3.000	1.670	0.350	0.412
	AB95	22	4.000	1.783	0.500	0.449
	AB93	20	2.000	1.220	0.100	0.185
	ABIB152	4	2.000	1.280	0.250	0.250
Couchville	AB59	64	6.000	2.572	0.531	0.616
	AB113	71	7.000	2.894	0.676	0.659
	AB83	63	6.000	3.188	0.587	0.692
	AB51	65	14.000	2.539	0.569	0.611
	AB170	69	3.000	2.041	0.507	0.514
	AB95	67	5.000	1.979	0.448	0.498
	AB93	71	3.000	1.469	0.282	0.322
	ABIB152	23	1.000	1.000	0.000	0.000

N_a is the mean number of alleles across all loci; N_e is the mean number of effective alleles across all loci, H_o is mean observed heterozygosity across all loci; uH_e is mean unbiased expected heterozygosity across all loci.

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