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# Genetic Analysis of the Federally Endangered Winged Mapleleaf Mussel to Aid Proposed Re-introduction Efforts

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# Report: Genetic Analysis of the Federally Endangered Winged Mapleleaf Mussel to Aid Proposed Re-introduction Efforts.

**Final Report**
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**Background:**

The winged mapleleaf, *Quadrula fragosa*, historically occurred in the Mississippi, Tennessee, Ohio, and Cumberland river drainages, but has suffered severe population and range reductions. At the time that the species was federally listed as endangered, its range was thought to have been reduced to a stretch of the St. Croix River between northwestern Wisconsin and east-central Minnesota (USFWS 1991, 1997). Recently, morphologically "*Q. fragosa*-like" specimens were discovered at sites in Arkansas (Ouachita River and Saline River), Missouri (Bourbeuse River), and Oklahoma (Little River) (Hemmingsen 2008). These specimens were genetically determined to be *Q. fragosa* with mitochondrial DNA sequence Hemmingsen 2008), suggesting that two additional populations of *Q. fragosa* exist in addition to the known population in the St. Croix River. In response to this recent information, genetic variation among all hypothesized populations of *Q. fragosa* was compared using microsatellites and construction of a DNA genomic library (Hemmingsen et al. 2009). These preliminary results were confirmed with a subsequent investigation using 9 species-specific microsatellite markers developed for this purpose (Hemmingsen et al. 2009).

Subsequently, a plan was proposed to re-introduce *Q. fragosa* into portions of its historic range where its been extirpated from within the upper Mississippi River basin. The plan calls for introducing individual *Q. fragosa* through propagation efforts using the St. Croix *Q. fragosa* as the source population in an effort to augment the natural population (supplementation propagation). Ideally, when attempting this and other types of propagation, several criteria should be met. The source population should occur in the same geographic region as the proposed stocking or reintroduction event to minimize the risk of outbreeding depression (Ryman and Laikre, 1991). The number of individuals to be used as broodstock for the propagation effort should be reflective of the genetic diversity of the source population as a whole. This concept reflects a fundamental principal of all species reintroduction programs, which is to provide populations with the genetic potential to successfully establish and grow in size. High levels of genetic diversity provide reintroduced populations with the capacity to adapt to environmental changes over time (Hughes and Sawby 2004). Selecting broodstock with high levels of genetic diversity is a concern for mussel reintroduction programs, where vulnerability of juvenile mussels to predation and other sources of mortality may result in demographic bottlenecks due to high post-stocking mortality (Einum and Fleming 2001).

Several project objectives were developed, with the ultimate goal being the establishment of an additional self-sustaining population of *Quadrula fragosa*. The project objectives were 1.) Recommend the number of "founder" individuals required to generate the same level of genetic diversity in a newly established population as seen in the original population; 2.) In addition, allow for the ability to identify newly recruited juvenile mussels using microsatellite genotyping, and link individuals from the founded population back to the St. Croix River source population.

To accomplish these objectives the effective population size of the St. Croix River *Q. fragosa* population needed to be accurately estimated. Due to small sample size and the limited number of polymorphic microsatellite loci, reported in Hemmingsen et al. (2009) I was unable to

confidently estimate the "effective population size" of the St. Croix *Q. fragosa* population based on those data. There was therefore a need to increase the sample size to between 30 and 50 individual *Q. fragosa* for statistical power and robust analyses.

**Methods:**

Additional *Q. fragosa* specimens were collected with the assistance of the USFWS and when combined with the 13 St. Croix River specimens used in the Hemmingsen et al. (2009) resulted in 52 unique samples (Table 1). Samples for DNA extraction were collected using a non-destructive method (Henley et al. 2006) and the DNA was extracted in laboratory of the PI. Additional microsatellite loci were developed by screening other *Q. fragosa* loci that were not previously used by Hemmingsen et al. (2009) as well as testing non-species specific markers that were also being used for analyses of other species in the lab of the PI. A total of 53 microsatellite markers were screened for polymorphism in *Q. fragosa* resulting in 20 polymorphic loci for use in the study (Table 2). The multilocus genotypes were generated for the *Q. fragosa* samples using developed PCR primers in conjunction with the BIOLASE™ PCR kit (Bioline, Boston, MA) and approximately 2 ng of genomic DNA. The resulting PCR products (genotypes) were sized at the ISU DNA Facility using an Applied Biosystems 3730 DNA Analyzer. Raw data output was visualized and alleles called using GeneMarker® software (Softgenetics, State College, PA). The genotype data was checked for the presence of null alleles, linkage disequilibrium, and deviation from Hardy-Weinberg equilibrium using the program GENEPOP (Raymond & Rousset, 1995). Gene diversity and the inbreeding coefficient were calculated in GENEPOP (Raymond & Rousset, 1995) for each locus.

Typically, estimators of effective population size ($N_e$) require two temporally spaced genetic samples from different generations (i.e., temporal method) (Waples and Do 2008) in order to provide an accurate estimate. Due to the long-lived nature of freshwater mussels and their long generation time, utilizing the temporal method for calculating $N_e$ is not practical over the course of this relatively short study. Instead, a contemporary estimate of $N_e$ was obtained from the microsatellite genotypes generated in this project using an approach that is based on a single temporal population sample. The method used in this study is based on gametic disequilibrium, and is implemented in the program $LDN_E$ (Waples and Do 2008). For this estimate we used the lowest allele frequency observed from the data (0.01). Confidence intervals were estimated using the standard parametric method and the jackknife.

Once an estimate of $N_e$ is obtained it can be used to determine how many individual animals should be contributing gametes or juvenile mussels to establish a new population in order to maximize genetic diversity.

**Results & Discussion:**

Preliminary analyses of the of the data for deviation from Hardy-Weinberg (H-W) equilibrium indicated that three of the 20 loci examined deviated from H-W expectations by exhibiting significant excess homozygosity (Table 2), after correcting for multiple comparisons. Excess homozygosity (a deficit of heterozygotes) is one expected outcome of mating between relatives (inbreeding), examination of the data indicate a reduction in heterozygosity (Weir and Cockerham, 1984) at 16 of the 20 loci ranging from 2% to 46% (mean [all loci] = 8.6%) relative to a randomly mating population. Examination of the data for evidence of linkage disequilibrium indicated 12 instances of potential non-independence between loci out of 210

comparisons. Analysis for the presence of null alleles indicates that of seven out of 20 loci may include a null allele at a frequency >10%.

The effective population size for the St. Croix *Q. fragosa* population was estimated as $N_e = 149.2$ individuals. The 95% CI reported using the jackknife approach ranged from 112.1 – 217.3, and was slightly less narrow than the 95% CI estimated using the parametric approach (120.7-193.1) as reported by Waples (2006). The relationship between the census population size ($N_c$) and $N_e$ is often expressed as a ratio of $N_e/N_c$. This ratio has been shown to range between 0.5–0.8, which if applied to the estimated $N_e$ for *Q. fragosa* would predict a census population size between 186-298 individuals. More recently, Frankham (1995) calculated the average ratio of $N_e/N_c = .11$, in a meta-analysis of 38 cases. Using the $N_e$ values estimated for the St. Croix population this would result in a $N_c = 1356$ individuals. Even more recently, Frankham et al. (2010) summarized $N_e/N_c$ ratios for highly fecund species of fishes and invertebrates and found even lower ratios (0.01 – 0.00001).

It is considered that species with $N_e < 500$ individuals are at a heightened risk for extinction due to erosion of genetic diversity via genetic drift and inbreeding (Frankham et al. 2010). This decrease in genetic variation can leave them vulnerable to and unable to cope with environmental change and other threats. One recommendation for such populations is managing them to increase reproduction and long-term survival.

Captive propagation and the founding of additional populations can be a way to insure the survival of a species. Propagation is one way to ensure a larger proportion of individuals survive, especially when early life stages are fraught with high mortality/predations rates, although care should be exercised to avoid any negative impact associated with propagation (Jones et al. 2006). One such negative effect is the creation of an artificial bottleneck and loss of additional genetic diversity by using only a small number of individuals as broodstock. The relationship between the number of population founders and the proportion of the genetic diversity they represent in the original population is expressed by the following equation

$$[1-(1/2N)]$$

where $N$ is the number of individuals used to found the new population. Using as few as 10 founders will thus retain 95% of the variation in the original population. It has been stated that in the initial phase of population founding or recovery the priority should be placed on producing a large number of individuals to avoid the additional loss of alleles which would happen if the population was kept small (<500 N*e*) over multiple generations (Frankham et al. 2004). Avoidance of the loss of rare alleles via a protracted bottleneck event then should be the first priority for recovering the St. Croix *Q. fragosa* population.

The genotype data generated in this study also allows for the estimation of the relatedness of the individuals included in it. Because age data is not known potential parentage cannot be assed directly but by using a maximum likelihood approach as employed in the program ML-Relate (Kalinowski et al. 2006). ML-Relate calculates an estimate of the relatedness ($r$) of individuals in a population using co-dominant data (microsatellites). The output is in the form of likelihood values for each of four possible outcomes: unrelated, half-siblings, full-siblings, or parent-offspring. The estimates for the St. Croix *Q. fragosa* population are presented in Table 1. Summarizing the results, ML-Relate estimates there are nine pairs of individuals that related at the same level as full siblings, and seventy-four pairs of individuals that are related at the same level as half-siblings. The remaining pairs of individuals (606) have values for $r$ that indicate

they are "unrelated." Since the gender of any individuals included in this study are unknown, the information on level of relatedness in this report could be applied in the propagation of St. Croix *Q. fragosa* by preferentially placing "unrelated" individuals in close proximity to each other so as to enhance the likelihood that unrelated males and females will reproduce. Similarly, and perhaps more importantly, individuals that are full or half siblings (Table 1) should be placed in separate locations so as to avoid mating between close relatives. A possible management scenario would be similar to the low intensity genetic management approach outlined by Princée (1995). In such a scenario species that typically exist in multi-male and/or multi-female groups are managed to minimize inbreeding by moving individuals between groups of unrelated individuals on a schedule. Even in such a scenario, knowing the gender of the individuals would be advantageous, as it would allow related males and females to be placed in different groups to avoid inbreeding.

When propagation is used to augment the same population that the broodstock was drawn from (supportive breeding) caution should be exercised so that the large numbers of introduced offspring do not result in a reduction of the $N_e$ of the target population. Large contributions of offspring by a small number of parents will result in a change in the variance of family size that in turn will reduce the $N_e$ dramatically (Ryman and Laikre, 1991). For example, in a population, the total effective population size was 200 and the offspring of ten "effective parents" were reared in captivity for introduction back in to wild population. If these captive offspring composed more than ~15% of the total (wild and captive) offspring produced that generation, they would effectively decrease the $N_e$ for the population. If the captive offspring composed 20% of the total offspring, the new $N_e$ would be ~150 (a decrease of 50). The effect become larger the larger the proportion of the offspring is from a smaller number of effective parents. In and effort to avoid drastic reductions in Ne, one alternative to avoid differential reproductive rates is to attempt to capture all of the wild population for a single generation enhancement (Ryman et al. 1995).

The existing St. Croix *Q. fragosa* populations should be monitored for any evidence of natural recruitment. Lack of recruitment over multiple years would possibly indicate deleterious effects resulting from inbreeding, or environmental effects such a predation or poor habitat for juveniles. Several species of freshwater mussels are known to capable of self-fertilization (Burch, 1975), and the reproductive strategy could be more wide spread (Kat, 1983). If self-fertilization occurs in the St. Croix *Q. fragosa*, it could explain the level of heterozygote deficiency observed. Self-fertilization is considered to be the most extreme form of inbreeding, but if it is a "normal" aspect of the reproductive biology of a species, then decreased levels of heterozygosity may not themselves be cause for alarm. A comparison to a related sympatric species (*Q. pustulosa*) that is not endangered might reveal whether *Q. fragosa* is exhibiting low or normal genetic diversity.

The genotype data collected for this project theoretically would allow the identification of the progeny of these 52 freshwater mussels. Since the paternal contribution is usually not known in freshwater mussels, if juveniles were propagated from one or more of these adult females, the glochidia and juveniles of different females should be kept separated and a subsample of each should be genotyped to determine the paternal contribution(s). Freshwater mussels have been shown to exhibit multiple paternities (Christian et al. 2007), and such information would assist in identifying male *Q. fragosa* in the St. Croix River and aid in the design of a plan to minimize inbreeding such as the one briefly outlined above.

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Table 1. Matrix of relarionships.

This table shows the relationship between each pair of individuals
that has the highest likelihood among the four following relationships:

U = Unrelated
HS = Half Sibs
FS = Full Sibs
PO = Parent / Offspring

| | 94 | 308 | 318 | 340 | 349 | 393 | A021 | A028 | A030 | C001 | C002 | C003 | C004 | C005 | C006 | C007 | C008 | C009 | C010 | C011 | C012 | C014 | C015 | C016 | C598 | C821 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 94 | - | | | | | | | | | | | | | | | | | | | | | | | | | |
| 308 | U | - | | | | | | | | | | | | | | | | | | | | | | | | |
| 318 | U | U | - | | | | | | | | | | | | | | | | | | | | | | | |
| 340 | U | U | U | - | | | | | | | | | | | | | | | | | | | | | | |
| 349 | U | U | U | U | - | | | | | | | | | | | | | | | | | | | | | |
| 393 | U | U | U | U | HS | - | | | | | | | | | | | | | | | | | | | | |
| A021 | U | U | U | U | U | U | - | | | | | | | | | | | | | | | | | | | |
| A028 | U | U | HS | U | U | U | U | - | | | | | | | | | | | | | | | | | | |
| A030 | U | U | U | U | U | U | U | U | - | | | | | | | | | | | | | | | | | |
| C001 | U | U | U | FS | U | U | U | U | U | - | | | | | | | | | | | | | | | | |
| C002 | U | U | HS | HS | U | U | U | HS | U | U | - | | | | | | | | | | | | | | | |
| C003 | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | | | | | | |
| C004 | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | | | | | |
| C005 | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | | | | |
| C006 | U | U | U | HS | U | U | U | U | U | HS | HS | HS | U | U | - | | | | | | | | | | | |
| C007 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | | |
| C008 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | |
| C009 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | - | | | | | | | | |
| C010 | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | |
| C011 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | |
| C012 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | HS | U | U | - | | | | | |
| C014 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | |
| C015 | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | - | | | |
| C016 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | |
| C598 | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | |
| C821 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | - |
| C881 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C882 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | FS | U | U | U |
| C883 | U | HS | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U |
| C884 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C887 | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C889 | U | U | U | HS | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C890 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U |
| C891 | U | U | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U |
| C892 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U |
| C893 | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C894 | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C895 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C896 | U | U | U | HS | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| C897 | HS | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| 30124 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| 30125.1 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U |
| 30126.8 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U |
| 30126.9 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | HS | U | U | U | U | HS |
| 30128.1 | U | U | HS | U | U | U | U | HS | U | U | HS | U | U | U | U | U | U | U | U | HS | U | U | U | U | HS | U |
| SC57 | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | FS | U | U | U |
| SC95 | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| SC341 | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | HS | U | U |
| SC342 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| SCA031 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| SCA032 | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| SCA033 | U | U | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U |

Table 1. cont.

| | C881 | C882 | C883 | C884 | C887 | C889 | C890 | C891 | C892 | C893 | C894 | C895 | C896 | C897 | 30124 | 30125.1 | 30126.8 | 30126.9 | 30128.1 | SC57 | SC95 | SC341 | SC342 | SCA031 | SCA032 | SCA033 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 94 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 308 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 318 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 340 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 349 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 393 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A021 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A028 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A030 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C001 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C002 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C003 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C004 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C005 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C006 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C007 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C008 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C009 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C010 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C011 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C012 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C014 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C015 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C016 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C598 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C821 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C881 | - | | | | | | | | | | | | | | | | | | | | | | | | | |
| C882 | U | - | | | | | | | | | | | | | | | | | | | | | | | | |
| C883 | U | U | - | | | | | | | | | | | | | | | | | | | | | | | |
| C884 | U | U | HS | - | | | | | | | | | | | | | | | | | | | | | | |
| C887 | U | U | U | U | - | | | | | | | | | | | | | | | | | | | | | |
| C889 | U | U | U | U | U | - | | | | | | | | | | | | | | | | | | | | |
| C890 | U | U | U | U | U | U | - | | | | | | | | | | | | | | | | | | | |
| C891 | U | U | U | U | U | HS | U | - | | | | | | | | | | | | | | | | | | |
| C892 | U | U | U | U | U | U | HS | U | - | | | | | | | | | | | | | | | | | |
| C893 | U | U | U | U | U | U | U | U | U | - | | | | | | | | | | | | | | | | |
| C894 | U | U | U | HS | U | U | U | U | U | HS | - | | | | | | | | | | | | | | | |
| C895 | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | | | | | | |
| C896 | U | U | U | U | U | HS | U | U | U | U | U | U | - | | | | | | | | | | | | | |
| C897 | U | U | U | U | U | U | U | U | HS | U | U | U | FS | - | | | | | | | | | | | | |
| 30124 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | | | |
| 30125.1 | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | - | | | | | | | | | | |
| 30126.8 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | | | | | | |
| 30126.9 | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | - | | | | | | | | |
| 30128.1 | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | HS | U | U | U | - | | | | | | | |
| SC57 | U | HS | U | U | U | U | U | U | U | U | U | HS | U | U | U | HS | U | U | U | - | | | | | | |
| SC95 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | HS | U | - | | | | | |
| SC341 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | | | |
| SC342 | U | U | U | U | U | U | U | U | FS | U | U | U | U | HS | U | HS | U | U | U | U | U | U | - | | | |
| SCA031 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | | |
| SCA032 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | - | |
| SCA033 | U | U | U | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | HS | - |

Table 2. Loci used, pcr annealing temperature, repeat motif, estimates of heterozygote deficiciency and inbreeding coefficient, and number of alleles per locus. * indicates significant value equvalent to 0.05 level after correcting for multiple comparrisons.

| Locus | Annealing Temp°C | Repeat | Heterozygote Deficiency (P-value) | Inbreeding (Fis) Weir & Cockerham | Number of Alleles |
|---|---|---|---|---|---|
| Pc C105 | 49 | Trinucleotide | 0.7692 | -0.0495 | 9 |
| Pc C125 | 55 | Trinucleotide | 0.0534 | 0.0376 | 13 |
| Pc C6 | 55 | Trinucleotide | 0.24 | 0.0562 | 12 |
| Pc D113 | 49 | Tetranucleotide | 0.0181 | 0.0917 | 7 |
| Qf A103 | 53 | Dinucleotide | 0.009 | 0.1997 | 8 |
| Qf A112 | 56 | Dinucleotide | 0.1767 | 0.0762 | 9 |
| Qf A130 | 58 | Dinucleotide | 0.0728 | 0.1102 | 11 |
| Qf C102 | 56 | Trinucleotide | 0.0083 | 0.0328 | 12 |
| Qf C109 | 55 | Trinucleotide | 0.3581 | 0.0678 | 8 |
| Qf C114 | 58 | Trinucleotide | 0.0504 | 0.13 | 6 |
| Qf C12 | 58 | Trinucleotide | 0.5 | -0.0374 | 14 |
| Qf C2 | 54 | Trinucleotide | 0.0993 | 0.0412 | 15 |
| Qf C4 | 58 | Trinucleotide | 0* | 0.0562 | 21 |
| Qf C6 | 58 | Trinucleotide | 0.24 | -0.0427 | 13 |
| Qf D102 | 53 | Tetranucleotide | 0.2124 | 0.0588 | 14 |
| Qf D103 | 59 | Tetranucleotide | 0.0037 | 0.2788 | 2 |
| Qf D11 | 58 | Tetranucleotide | 1 | -0.0942 | 17 |
| Qf D110 | 58 | Tetranucleotide | 0.4809 | 0.0199 | 10 |
| Qf D116 | 55 | Tetranucleotide | 0* | 0.4617 | 6 |
| Qf D5 | 56 | Tetranucleotide | 0* | 0.2355 | 6 |