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REGULAR ARTICLE

MUSSELS PROPAGATED FROM A SINGLE BROODSTOCK FEMALE RETAIN MOST POPULATION-LEVEL GENETIC VARIATION BUT HAVE ALTERED GENETIC STRUCTURE

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ABSTRACT

Captive propagation followed by release to natural habitats has become a common conservation practice to restore and augment mussel populations, but the genetic effects of these efforts remain poorly studied. We examined genetic variation and genetic structure in 2- to 3-yr-old subadults of *Lampsilis cardium* and *L. siliquoidea* that each was propagated from a single broodstock female and subsequently used to augment existing wild populations. We compared genetic variation and structure of the propagated individuals to that of the wild population, including the broodstock females. Using microsatellite markers, we found that propagated subadults retained levels of heterozygosity comparable to the wild population and showed no sign of genetic bottlenecks. This is likely due to high levels of multiple paternity in both species, with the single broodstock females of *L. cardium* and *L. siliquoidea* mating with an estimated 13 and 25 sires, respectively. However, propagated subadults had significantly fewer alleles and lower allelic richness and altered allele frequencies compared with wild adults, and genetic structure of propagated individuals was distinct from the wild population. Our results show that propagation from even a single broodstock female can result in offspring that retain most population-level genetic variation. However, the reduced allelic richness and altered genetic structure we observed in propagated individuals underscore the need for future studies to investigate the ecological and evolutionary impacts of propagated individuals on wild populations.

KEY WORDS: captive breeding, multiple paternity, microsatellites, parentage analyses, propagation, augmentation, genetic variability

INTRODUCTION

Freshwater mussels (Bivalvia: Unionida) are among the most endangered organisms on Earth (Haag and Williams 2014; Pereira et al. 2014; Lopes-Lima et al. 2021). Captive propagation followed by release to natural habitats has become a common conservation practice to restore and augment mussel populations (McMurray and Roe 2017; Patterson et al. 2018; Rytwinski et al. 2021). Mussels typically are propagated by harvesting parasitic larvae from gravid female broodstock, inoculating host fishes with larvae, and harvesting juveniles that metamorphose on fishes (Patterson et al. 2018). Captively propagated juveniles often are raised to the subadult stage before release, which can reduce the high mortality characteristic of the juvenile stage in the wild (McMurray and Roe 2017). Captive propagation initially was used mainly for imperiled species, but it is now used widely for a variety of species and conservation goals (Patterson et al. 2018; Strayer et al. 2019).

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Despite the benefits of captive propagation, it has potential negative ecological and genetic consequences (McMurray and Roe 2017; Strayer et al. 2019; Rytwinski et al. 2021). One of the primary concerns is preservation of maximum genetic variability within species and populations, which is important for maximizing evolutionary potential and the ability to adapt to environmental change (Pelletier et al. 2009). In some cases, mussel propagation programs use only one or a few broodstock females to produce large numbers of juveniles, which has the potential to capture only a small portion of genetic variation present in the wild population (Hoftyzer et al. 2008). However, freshwater mussels are spermcasters (Bishop and Pemberton 2006), in which spermatozoa are released into the water column by males and captured by females to fertilize their eggs. Consequently, the brood of individual females can be fertilized by multiple males, resulting in multiple paternity within the brood (Christian et al. 2007; Wacker et al. 2018; Garrison et al. 2021). Multiple paternity can increase genetic diversity within the brood of a single female, thereby reducing chances for potential negative effects from using a small number of broodstock females (Jennions and Petrie 2000). Nevertheless, few programs currently quantify genetic diversity of propagated mussels or compare it to that of source or recipient populations (Rytwinski et al. 2021). A better understanding of genetic characteristics of captively propagated mussels is needed to avoid negative consequences potentially associated with stocking those animals into the wild.

We examined genetic variation and genetic structure in 2- to 3-yr-old subadults of *Lampsilis cardium* and *L. siliquoidea* that each was propagated from a single broodstock female and subsequently used to augment existing wild populations. We compared genetic variation of the propagated individuals to that of the wild population, including the broodstock females. We also estimated the number of paternal contributions present within each brood used to produce the propagated individuals. We discuss how our results inform the development of captive propagation programs that can reduce the potential for negative genetic effects.

METHODS

Propagation of *L. cardium* and *L. siliquoidea* was conducted by the Forest Preserve District of DuPage County at the Urban Stream Research Center in Warrenville, Illinois, USA, as part of a mussel conservation program in the West Branch DuPage River. A single broodstock female of each species was collected from the West Branch DuPage River in January 2016 for *L. cardium* and February 2017 for *L. siliquoidea*. Glochidia were extracted from the marsupial gills of the broodstock, and their viability was checked by exposing them to a droplet of saturated NaCl solution. The viable glochidia were inoculated on Largemouth Bass (*Micropterus nigricans*). The infested fish were held in flow-through tanks until the encysted glochidia metamorphosed into juveniles and dropped off the hosts. Tissue-swab genetic samples from each broodstock female were taken before releasing them into the natural population. Genetic samples were preserved in 95% ethanol and stored at −20°C. However, the *L. siliquoidea* sample became desiccated and thus unusable for DNA extraction; therefore, we estimated microsatellite genotypes of the *L. siliquoidea* broodstock from the offspring genotypes (see below).

Juveniles were reared in the laboratory until they reached approximately 3.5 mm shell length, after which they were moved to floating baskets in a pond on the Forest Preserve District property and reared for 9 mo (*L. siliquoidea*) or 21 mo (*L. cardium*) until they reached the subadult stage (25–40 mm shell length). Subadults were tagged with passive integrated transponders (PIT tags), vinyl shellfish tags, or glitter dots. The tagged subadults were released at multiple sites in the West Branch DuPage River in July 2017 (*L. cardium*) and October 2017 (*L. siliquoidea*).

In summer 2019, we conducted postrelease monitoring for the propagated subadults at all sites. During monitoring, we collected tissue-swab genetic samples from 18 subadults for *L. cardium* and 37 subadults for *L. siliquoidea*, preserved them in 95% ethanol, then stored them at −20°C. In summer 2020, we collected tissue-swab genetic samples of 31 wild adult *L. cardium* and 24 wild adult *L. siliquoidea* at a location near where the broodstock females were collected previously.

We extracted total DNA from all samples using cetyltrimethylammonium bromide (CTAB)-chloroform extraction followed by ethanol precipitation. We diluted the extracted DNA to a concentration of 10 ng/μL and used it as a template in polymerase chain reaction (PCR) amplification of microsatellite loci. For amplification, we used primers developed for *Lampsilis abrupta* (Eackles and King 2002) and *Venustaconcha ellipsiformis* (Inoue et al. 2021). Prior to genotyping, we screened a subset of microsatellite loci for each species for PCR success and polymorphisms. We selected a total of 10 loci for *L. cardium* and 11 loci for *L. siliquoidea* for study (Table 1). We performed PCR reactions in 10 μL volume, including 5 μL of GoTaq® G2 Master Mix (Promega Corp., Madison, WI, USA), 0.25 μM of universal fluorescently labeled primer and nontailed primer, 0.05 μM of tailed primer, and 10 ng of DNA. We used the following PCR conditions: initial denaturing at 95°C for 2 min, followed by 40 cycles at 95°C for 30 s, annealing at 61°C for 45 s, extension at 72°C for 45 s, and final extension at 72°C for 30 min (Inoue et al. 2021). We conducted fragment analyses on a 3730xl DNA Analyzer (ThermoFisher Scientific, Inc., Waltham, MA, USA) at the Field Museum (Chicago, IL, USA) with Orange DNA Size Standard (MCLAB, South San Francisco, CA, USA). We verified peak calling using Geneious Prime v2020.1.2 (https://www.geneious.com, accessed September 16, 2023) with the microsatellite plugin based on the microsatellite motifs, and we assigned integer numbers to DNA fragment sizes. Briefly, in Geneious, we created locus information for each locus (e.g., diploid, repeat unit, expected range of fragment sizes) and used Third-Order Least Squares as a sizing method. For each species, we included all individuals to verify size standard and microsatellite peaks, create fragment size bins based on

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Table 1. Descriptive statistics of 10 microsatellite loci for *Lampsilis cardium* and 11 loci for *L. siliquoidea* from the West Branch DuPage River, Illinois, USA. Propagated subadults were collected 2 yr after release in the stream. Wild adults were collected from the same sites where subadults were released and include a single broodstock female for each species.

Lampsilis cardium

	Propagated subadults						Wild adults					
Locus	N_A	A_R	P_A	H_O	H_E	F_{IS}	N_A	A_R	P_A	H_O	H_E	F_{IS}
LabC2	3	3.0	0	0.89	0.61	−0.45	4	3.8	1	0.59	0.61	0.02
LabC23	3	3.0	0	0.94	0.52‡	−0.81	4	3.5	1	0.28	0.30	0.06
LabC24	2	2.0	0	0.72	0.46	−0.57	2	2.0	0	0.34	0.39	0.12
LabD213	7	7.0	1	0.67	0.53	−0.25	21	16.3	15	0.91	0.91	0.00
Ve008	5	5.0	1	0.61	0.48	−0.27	4	3.8	0	0.44	0.52	0.16
Ve010	5	5.0	0	0.39	0.34	−0.15	7	6.5	2	0.59	0.79†	0.25
Ve015	4	4.0	0	0.56	0.61	0.09	4	4.0	0	0.59	0.60	0.01
Ve025	6	6.0	0	0.56	0.66	0.16	8	7.7	2	0.63	0.82†	0.24
Ve078	10	10.0	1	0.94	0.79	−0.19	13	11.8	4	0.88	0.90	0.03
Ve169	6	6.0	0	0.61	0.49	−0.25	11	9.0	5	0.69	0.78	0.11
Mean values	5.1	5.1	0.3	0.69	0.55	−0.27	7.8	6.8	3.0	0.59	0.66	0.10

Lampsilis siliquoidea

	Propagated subadults						Wild adults					
Locus	N_A	A_R	P_A	H_O	H_E	F_{IS}	N_A	A_R	P_A	H_O	H_E	F_{IS}
LabC23	3	3.0	0	0.38	0.32	−0.17	4	4.0	1	0.64	0.53	−0.21
LabD187	13	11.3	1	0.89	0.80	−0.11	18	18.0	6	0.76	0.93†‡	0.19
LabD213	13	10.8	2	0.68	0.70	0.04	17	17.0	6	0.80	0.87	0.08
Ve001	4	3.4	1	0.22	0.47†‡	0.54	6	6.0	3	0.40	0.64†	0.37
Ve008	9	7.7	0	0.73	0.68	−0.07	9	9.0	0	0.76	0.80	0.06
Ve015	4	3.6	2	0.51	0.45	−0.14	7	7.0	5	0.44	0.46	0.05
Ve025	7	6.8	0	0.73	0.73	0.00	10	10.0	3	0.72	0.87†	0.17
Ve050	7	6.8	1	0.78	0.73	−0.08	7	7.0	1	0.84	0.83	−0.01
Ve058	5	4.8	0	0.32	0.58†‡	0.44	7	7.0	2	0.44	0.68†‡	0.35
Ve138	9	8.0	1	0.51	0.68†	0.25	11	11.0	3	0.88	0.86	−0.02
Ve169	7	6.4	0	0.76	0.70	−0.09	9	9.0	2	0.72	0.69	−0.05
Mean values	7.4	6.6	0.7	0.59	0.62	0.06	9.5	9.5	2.9	0.67	0.74	0.09

N_A, number of alleles; A_R, rarefied allelic richness; P_A, number of private alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. † indicates potential null allele presence. ‡ indicates deviation from Hardy-Weinberg proportion.

the size of the observed peaks, and assign fragment sizes. When there were no peaks, or when the observed peaks were weak, we repeated PCR amplifications to ensure the correct peak calling.

The sample from the broodstock female *L. siliquoidea* became desiccated and was unusable for DNA extraction. Consequently, we estimated maternal microsatellite genotypes of the *L. siliquoidea* broodstock female COLONY v2.0.6.5 (Jones and Wang 2010) based on the offspring genotypes. We used default input parameters except that the mating system was set to female polygamy (i.e., maternal half-sibs exist) and male monogamy (i.e., no paternal half-sibs exist because the offspring was derived from a single female), and the length of run was set to "long." We assigned all propagated subadults of *L. siliquoidea* as the offspring of the same female. We included all microsatellite loci in the analyses with an allele dropout rate of 0 and a genotyping error of 0.0001. The genotype of the *L. siliquoidea* broodstock was confirmed with 100% probability at all loci, except for the locus Ve015, which had 99.7% probability. Therefore, we included the estimated genotype of the broodstock female in subsequent analyses.

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For all subsequent analyses, we included observed or estimated genetic data for the two broodstock females within the wild individuals for each species. We did this because we were interested mainly in the proportion of genetic variation present in the entire wild population that was preserved in propagated subadults; we were less interested in the proportion of genetic variation in the individual broodstock females that was preserved in their offspring. Consequently, we evaluated genetic variation and structure in two sample groups: propagated subadults and wild individuals (including broodstock).

We assessed the utility of each locus by testing for the presence of null alleles using Micro-Checker v2.2.3 (van Oosterhout et al. 2004). We performed exact tests of pairwise linkage disequilibrium (LD) and deviation from Hardy-Weinberg proportion (HWP) for each sample group within each species (i.e., propagated subadults and wild adults) using GenePop v4.7 (Raymond and Rousset 1995; Rousset 2008). We applied sequential-comparison Bonferroni correction for multiple comparisons of LD and HWP (i.e., locus-by-group) (Lessios 1992). We estimated population genetic indices (number of alleles, N_A; observed and expected heterozygosity, H_O and H_E; and inbreeding coefficient, F_{IS}) for each locus and sample group using GenAlEx v6.5 (Peakall and Smouse 2006, 2012). We estimated rarefied allelic richness (A_R) using FSTAT v2.9.4 (Goudet 1995) to correct for sample-size biases. We used Wilcoxon signed-rank tests to assess statistical differences in the genetic indices between propagated subadults and wild adults for each species.

Based on the allele frequencies calculated by GenAlEx, we calculated the proportion of alleles retained in the propagated subadults relative to the wild adults in both species. We categorized each allele as a rare allele (allele frequency < 0.05), intermediate frequency allele ($0.05 <$ allele frequency < 0.25), or high-frequency allele (allele frequency > 0.25) based on the wild populations. Additionally, we counted the number of private alleles/locus (i.e., alleles observed in only one group) in GenAlEx.

To assess population genetic structure, we estimated Weir and Cockerham's θ (Weir and Cockerham 1984) (equivalent to Wright's F_{ST}) between propagated subadults and wild adults for each species using GENETIX v4.05 (Belkhir et al. 2004). To test whether θ differed significantly from zero (i.e., no population genetic substructure), we calculated 95% confidence intervals around the estimate of θ based on 1,000 bootstraps. Additionally, we used a non-negative matrix factorization algorithm using the *snmf* function implemented in the R package LEA v.3.10.2 (Frichot et al. 2015) to estimate the optimal number of genetic clusters within the samples. Unlike the ΔK method used for STRUCTURE analysis (Pritchard et al. 2000; Evanno et al. 2005), the entropy criterion method allows evaluating $K = 1$ (Frichot et al. 2014). We ran the *snmf* function for $K = 1$–10 with 100 replicates for each value of K, and we estimated the optimal number of genetic populations based on the cross-entropy criterion.

To detect recent population bottlenecks within groups, we tested for deviations from mutation-drift equilibrium with BOTTLENECK v1.2.02 (Piry et al. 1999). This method assumes that recently bottlenecked population would exhibit reductions in allelic diversity faster than reductions in heterozygosity, resulting in heterozygote excess expected under mutation-drift equilibrium. We evaluated three mutation models: infinite allele (IAM), two-phase mutation (TPM), and stepwise mutation (SMM). We modeled TPM with a combination of 70% single-step mutations and 30% multistep mutations. We used Wilcoxon tests to test for significant heterozygote excess.

We estimated the most likely number of sires/brood and potential family structure within offspring for each species using COLONY v2.0.6.5 (Jones and Wang 2010). We used the same input parameters described previously.

RESULTS

Ten loci for *L. cardium* and 11 loci for *L. siliquoidea* were successfully amplified, and all showed polymorphism (Table 1). After Bonferroni correction, we found no evidence of LD in 200 locus-by-group pairs. However, deviations from HWP and potential null alleles were found in some loci (Table 1). The number of alleles ranged from two to 21/locus (a total of 81 different alleles over 10 loci for *L. cardium* and 113 alleles over 11 loci for *L. siliquoidea*). Mean rarefied allelic richness ranged from 5.1 alleles/locus for the propagated subadults of *L. cardium* to 9.5 alleles/locus for the wild adults of *L. siliquoidea*. Observed and expected heterozygosity values ranged from 0.55 for the propagated subadults of *L. cardium* to 0.74 for the wild adults of *L. siliquoidea*.

Propagated subadults had significantly fewer alleles and lower rarefied allelic richness than wild adults in both species (Fig. 1; Table 1). Observed heterozygosity did not differ between propagated and wild individuals for either species; expected heterozygosity differed between the groups for *L. siliquoidea* but not for *L. cardium*. The inbreeding coefficient was significantly lower in propagated subadults than wild adults for *L. cardium*, but it did not differ between groups for *L. siliquoidea*.

The propagated subadults of both species retained over half of the alleles present in the wild adults (*L. cardium*, 61.5%; *L. siliquoidea*, 69.5% (Fig. 2). However, the retention rates decreased as the alleles became rarer. While the propagated subadults retained all high-frequency alleles, they retained, on average, 82.6% of intermediate-frequency alleles and only 37.2% of rare-frequency alleles (intermediate alleles: 80.0% in *L. cardium*, 85.2% in *L. siliquoidea*; rare alleles: 30.3% in *L. cardium*, 44.2% in *L. siliquoidea*). Additionally, in both species, a higher number of private alleles were found in the wild adults (P_A: 30 in *L. cardium*; 32 in *L. siliquoidea*) than in the propagated subadults (P_A: three in *L. cardium*; eight in *L. siliquoidea*; Table 1).

The mean pairwise θ values between propagated subadults and wild adults were 0.097 for *L. cardium* and 0.071 for *L. siliquoidea*. The 95% confidence intervals did

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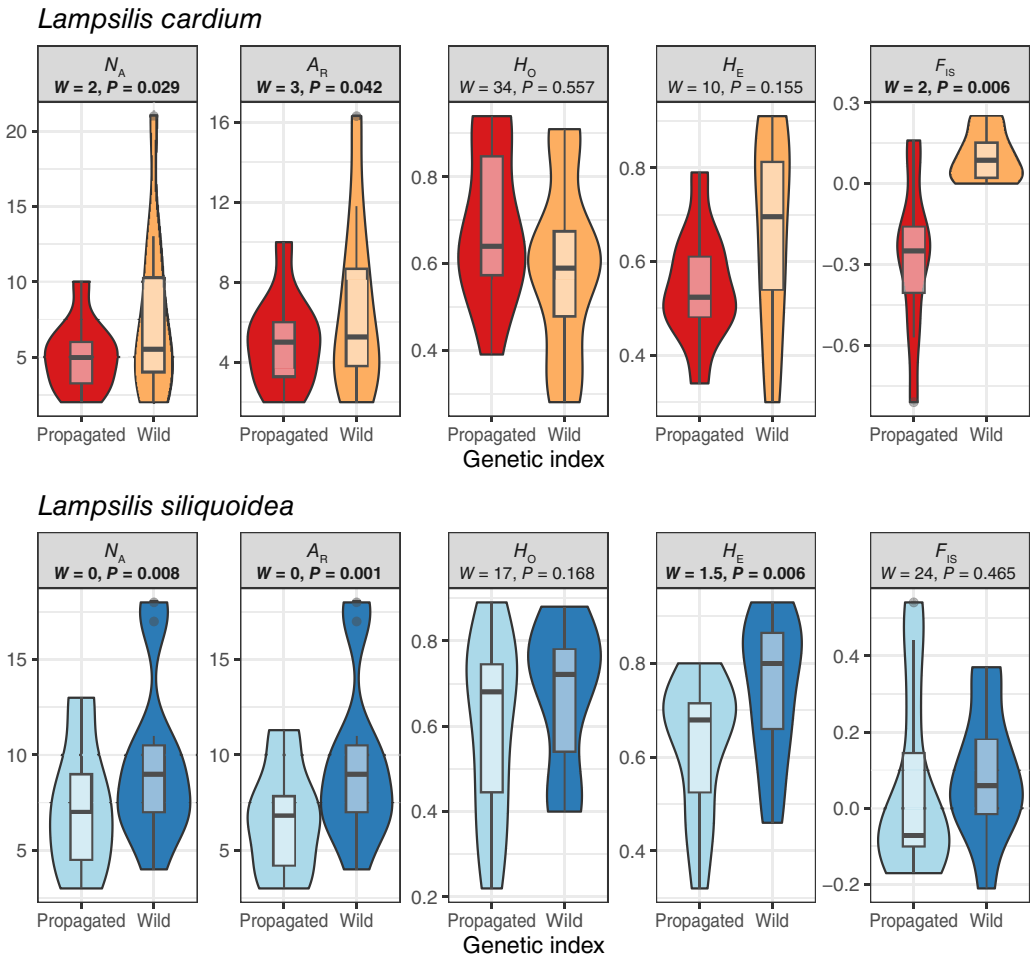

Figure 1. Violin plots of genetic diversity measures for propagated subadult and wild adult *Lampsilis cardium* and *L. siliquoidea* from the West Branch DuPage River, Illinois, USA. The bold horizontal line is the median value, boxes are the interquartile range, vertical lines are 1.5 × interquartile range and violin shapes indicate kernel density, representing the probability of observations for a given value. Results of Wilcoxon signed-rank tests for differences between subadults and wild adults are given for each measure. *Lampsilis cardium* and *L. siliquoidea*. Significant test results are bolded. N_A, number of alleles; A_R, rarefied allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient.

not include zero for either species (0.043–0.152 for *L. cardium*; 0.046–0.102 for *L. siliquoidea*), indicating significant genetic differentiation between groups. Furthermore, the LEA analysis recovered two distinct genetic clusters for both species ($K = 2$; Fig. 3), and the clusters generally were segregated between the propagated subadults and wild adults (Fig. 4). In *L. cardium*, the propagated subadults grouped exclusively into cluster 1 with the broodstock female, while most of the wild adults grouped into cluster 2 with some admixture with cluster 1. In *L. siliquoidea*, the broodstock female was assigned to both clusters 1 and 2, and a majority of the propagated subadults grouped into cluster 1.

None of the population groups exhibited heterozygote excess (IAM: $P = 0.139$–0.652; SMM: $P = 0.688$–1.000; TPM: $P = 0.246$–0.997), except for the wild adults of *L. cardium* under the IAM ($P = 0.009$). These results indicate no recent population bottlenecks in most groups and only a small population bottleneck within the wild adults of *L. cardium*.

A high level of multiple paternity was estimated for both species. The COLONY analyses showed that the most likely number of sires/brood was 13 for *L. cardium* and 25 for *L. siliquoidea*, indicating that most of the propagated subadults were half-siblings. Among the 13 families in *L. cardium*, five contained two full siblings. While most families had high probabilities of being true families (0.75 to 0.94), one family had a probability of 0.27, meaning that the family can be split into two families. Similarly, among the 25 families in *L. siliquoidea*, four families contained two full siblings and four families contained three full siblings. However, the probabilities were rather low in seven families (<0.01 to 0.60), indicating that the sibship family structure was statistically unresolved within *L. siliquoidea*.

DISCUSSION

Propagated subadults of *L. cardium* and *L. siliquoidea* derived from a single broodstock female and released into the

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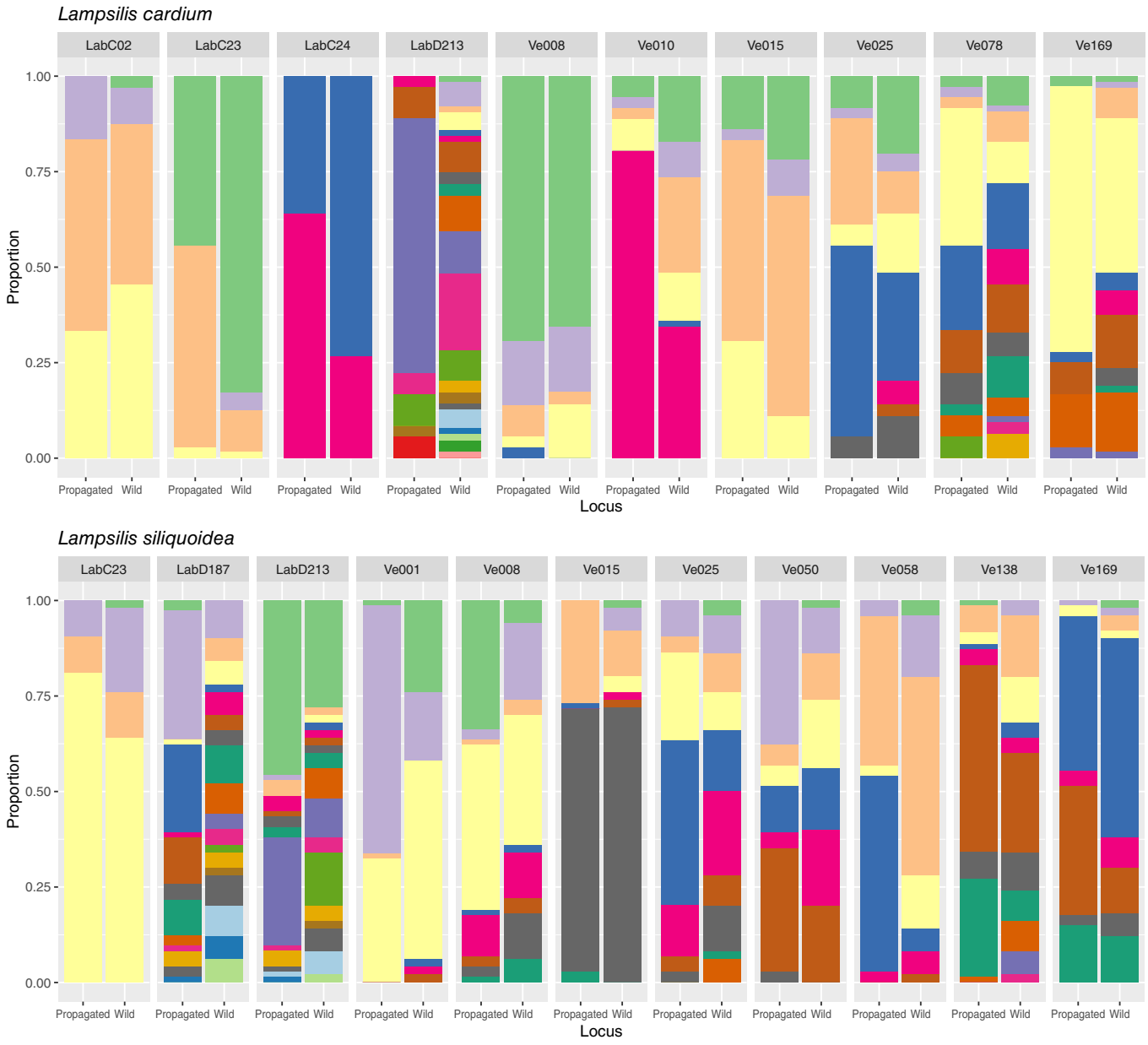

Figure 2. Stacked bar plots of allele frequencies for microsatellite loci in propagated subadult and wild adult *Lampsilis cardium* and *L. siliquoidea* from the West Branch DuPage River, Illinois, USA. Colors represent unique alleles at each locus.

wild retained levels of heterozygosity comparable to the wild source and recipient populations. The propagated subadults maintained all the common alleles that were present in the wild populations and even possessed a few private alleles not observed in the wild. High heterozygosity and allele retention in the propagated subadults likely were facilitated by multiple paternity within broods, with less than one-third of the offspring sharing the same father.

The degree of multiple paternity in mussels can vary widely. Our estimates of 13 and 25 sires in each brood are similar to those observed on *Margaritifera margaritifera* in Norway, where up to 32 sires contributed to a single brood (Wacker et al. 2018). However, other studies reported only two to six sires/brood, including three sires in broods of *L. cardium* in Ohio (Bai et al. 2011; Ferguson et al. 2013). Factors such as the abundance and density of reproductively active males, their position relative to females, timing of sperm release, and the females' ability to capture sperm can influence the levels of multiple paternity. Environmental factors, including flow velocity and hydrodynamics, also may play a role, but the influence of such factors in multiple paternity has not been examined. Further research is needed to investigate variability in multiple paternity among species and to determine the optimal number of offspring required to estimate total paternal contributions within a single female.

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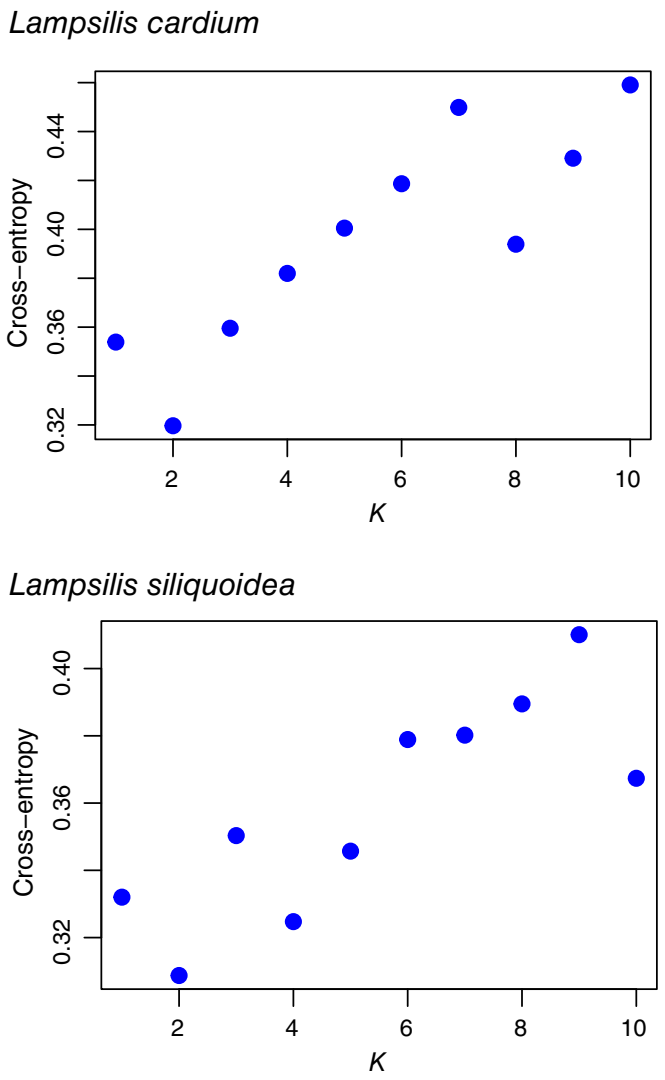

Figure 3. Cross-entropy plots for estimating the optimal number of genetic clusters (*K*) within samples of propagated subadult and wild adult *Lampsilis cardium* and *L. siliquoidea* from the West Branch DuPage River, Illinois, USA, based on the R package LEA (see text). Each value of *K* was assessed for 100 replicates. Lower values of cross-entropy represent greater support for a specific value of *K*.

While the propagated subadults retained all the common alleles, we observed a significant loss of alleles with rare and intermediate frequencies as well as a higher number of private alleles in the wild adults. This led to reductions in overall allelic richness and changes in allele frequencies within the subadult gene pool. Notably, some alleles that were rare in the wild populations became more common among the propagated subadults, likely due to the over-representation of maternal (broodstock female) genotypes within the brood. These changes in allele frequencies resulted in altered population genetic structures of propagated subadults compared to wild population in both species. Although the current study focused on neutral genetic markers, the observed alterations in allele frequencies and population genetic structures have potential implications for genes under selection. Captive breeding programs can affect genes under selection by relaxing selection pressures found in the wild or artificially selecting traits that are advantageous in the captive environment (i.e., domestication) (Frankham 2008; Christie et al. 2012). Modification of genetic structure within and among populations are documented in other species, such as salmonid fishes (Perrier et al. 2013). Because mussel populations often are locally adapted and genetically structured (Riusech and Barnhart 2000; Barnhart et al. 2008; Inoue et al. 2015), altering genetic diversity and genetic admixture between wild and captive-reared individuals may lead to a loss of local adaptation and reduced fitness in wild populations (Araki et al. 2007).

The alteration of genetic diversity and structure that we observed probably was largely due to the production of subadults from a single broodstock female, which underscores the importance of using multiple females in propagation programs (Jones et al. 2006). A previous study found no significant alternation of population genetic structure when juveniles were propagated from multiple broodstock females (VanTassel et al. 2021), but that study evaluated only three to six juveniles/female. Future research is needed to better understand the effect of the number of broodstock females on population genetic structure of propagated juveniles.

The subadults we studied were released into the wild 2 yr prior to genetic sampling, and we were unable to sample the individuals after metamorphosis or prior to release. During 2 yr in the wild, genetic structure of the subadults may have been influenced by natural selection or stochastic factors, and it would be informative to study how genetic structure changes after release to the wild. However, our results depict the functional genetic variability and structure of propagated cohorts near the time they may begin to interbreed with and influence the genetic structure of natural populations.

As captive propagation techniques for freshwater mussels have advanced, captive propagation and release programs have become widely used in conservation and restoration projects (Patterson et al. 2018). Although previous studies have provided guidance for genetic management in propagation programs (Jones et al. 2006; Hoftyzer et al. 2008; McMurray and Roe 2017), many programs still do not evaluate the genetic characteristics of broodstock, propagated individuals, or recipient populations, and they lack postrelease genetic monitoring (Rytwinski et al. 2021). Given that large numbers of propagated mussels often are released to natural habitats (>10,000 propagated individuals; Bishop et al. 2006), captive propagation and release programs have the potential to significantly alter existing genetic variability and disrupt evolutionary processes necessary for species' adaptation to environmental changes. It is crucial to incorporate strategic genetic management and monitoring into captive propagation and release programs to maximize species recovery success while minimizing negative genetic impact on natural populations.

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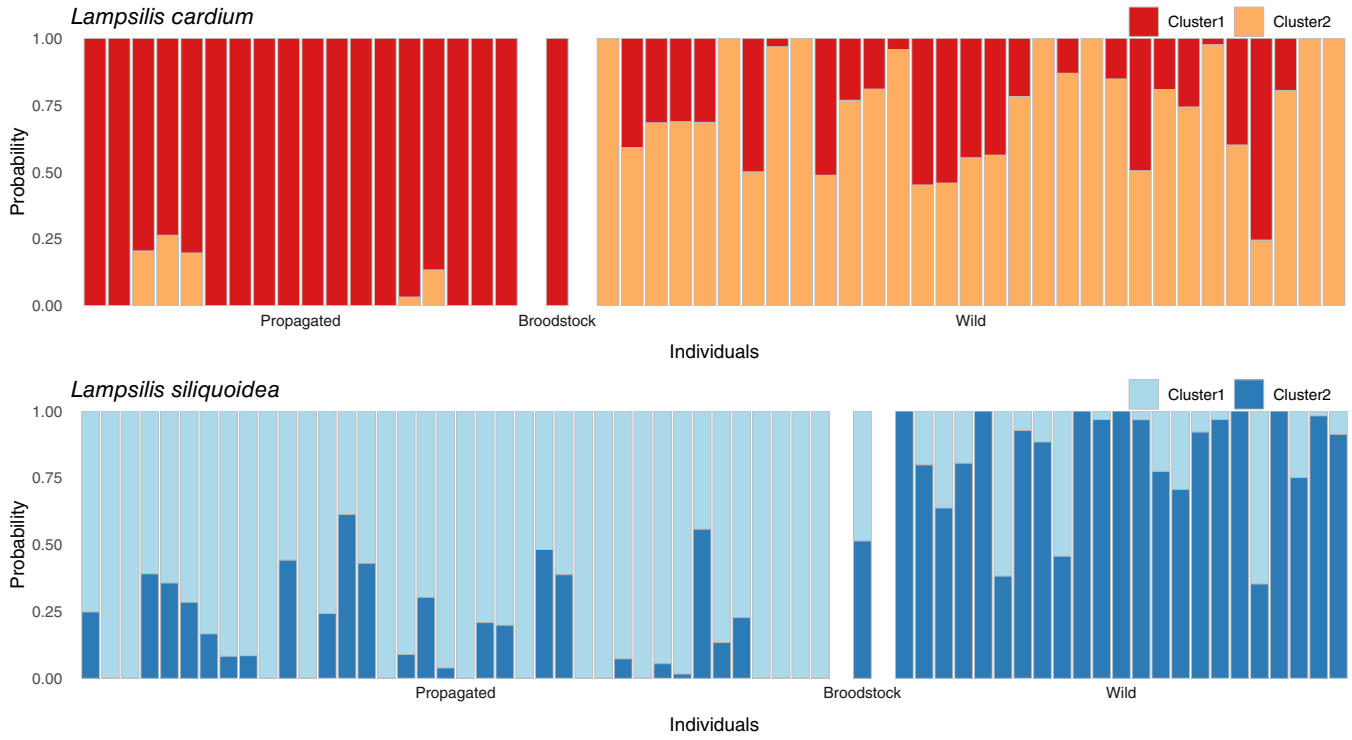

Figure 4. Stacked bar plots showing the probability of individuals belonging to two genetic clusters identified within propagated subadult and wild adult *Lampsilis cardium* and *L. siliquoidea* from the West Branch DuPage River, Illinois, USA, based on the R package LEA (see text).

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