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Reproductive success of captive-reared Allegheny Woodrats (*Neotoma magister*) released into genetically depauperate populations

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Abstract

Habitat fragmentation and degradation have led to a proliferation of small and isolated populations that are vulnerable to genetic erosion. Reduction in habitat and concomitant declines in population connectivity can expedite the collapse of species that exist as natural metapopulations. In recent years, Allegheny woodrats (*Neotoma magister*) have experienced local extirpations and declines in genetic diversity across their range due to disease-related mortality, reduced food availability, and disruption of connectivity among subpopulations. In response, we developed a captive-breeding program to facilitate genetic management of isolated woodrat populations in Indiana. Between 2010 and 2012, 27 captive-reared individuals were released to four sites with declining woodrat abundance. We used 99 single nucleotide polymorphisms and 11 microsatellite loci to genotype sympatric woodrats from the four sites, sampled before and after the release of captive-reared individuals, to: (1) evaluate the post-release reproductive success of captive-reared individuals and (2) characterize changes in genetic diversity. A minimum of five of the 27 captive-reared woodrats produced at least 20 offspring, 11 of which were sired by a single male. One site exhibited increased abundance by the end of the monitoring period, although genetic variability decreased. Two sites experienced population declines in concert with fluctuating genetic variability. The last site showed comparable abundance at the beginning and end of the monitoring period, with an increase in observed heterozygosity. Our results indicate that captive-reared woodrats were, in some cases, able to reproduce in the wild, interbreed with residents and increase genetic diversity and abundance among small, isolated subpopulations.

Keywords Genetic drift · Inbreeding · Fitness · Metapopulations · Genetic diversity · Captive-reared

Introduction

Anthropogenic habitat fragmentation has led to a proliferation of small and isolated populations (Spielman et al. 2004; Ralls et al. 2017). As population size declines, genetic drift and increasing consanguinity among isolated populations can result in the loss of genetic variability and subsequent decreases in individual fitness (Ellstrand and Elam 1993; Reed 2005; Freeland 2011). Lack of genetic variability results in population-wide homogeneity that limits most species' capacity to adapt to environmental pressures and increases the likelihood of local extinction (Spielman et al. 2004; Frankham 2005).

To combat losses of genetic diversity, agency biologists and their conservation partners may translocate individuals from genetically diverse populations to isolated, genetically depauperate populations to increase genetic variability and fitness (Madsen et al. 1996; Zajitschek et al. 2009; Smyser

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et al. 2013; Whiteley et al. 2015). Translocations represent human-mediated gene flow, often enriching genetic diversity within the recipient population (Freeland et al. 2011; Whiteley et al. 2015). In recent years, there has been a rapid increase in conservation-based translocations worldwide (Seddon et al. 2007; Ralls et al. 2017).

Translocations have become an increasingly important recovery tool for threatened Allegheny woodrat (*Neotoma magister*) populations in Indiana (Smyser et al. 2013; Blythe et al. 2015), New Jersey (G. Fowles, personal communication), Ohio (C. Molloyhan, personal communication) and Pennsylvania (G. Turner, personal communication). Allegheny woodrats are native to the Appalachian Mountains and the Highland regions of USA (Poole 1940) where they occupy isolated rocky habitats in forested landscapes. The scattered distribution of habitat, even in the most intact landscapes, results in woodrat populations being structured as metapopulations, characterized by small subpopulations connected by dispersal (Castleberry et al. 2002; Wood 2008). The threats to woodrat persistence are numerous and include loss of hard mast food resources, increased predation, forest loss and fragmentation, mortality related to raccoon roundworm (*Baylisascaris procyonis*) infection, disrupted functional connectivity, and a loss of genetic diversity (LoGiudice 2006; Smyser et al. 2012b). Although the combined effects of these factors vary throughout the species range (Kanine et al. 2018), they have resulted in subpopulation declines and extirpations from entire regions of its historic range (Wright 2008; Smyser et al. 2012b).

Previous translocations of Allegheny woodrats from stable populations in Kentucky and Tennessee to declining populations in Indiana restored genetic diversity and elicited a genetic rescue effect (Smyser et al. 2013, 2016). However, wild-to-wild translocations may not be sustainable for long-term conservation of declining populations if the repeated removal of individuals jeopardizes the persistence of source populations (Janz et al. 2000; Smyser et al. 2013; Landa et al. 2017). Captive rearing may mitigate the risk of genetic decline among isolated populations while minimizing or eliminating the cost to source populations. This approach involves maximizing the reproductive output of wild-caught individuals and subsequently releasing their offspring, with the intent to reinforce declining populations (Ebenhard 1995; McCleery et al. 2014). Captive breeding programs have facilitated the recovery of the California condor (*Gymnogyps californianus*) (Snyder and Snyder 1989), American bison (*Bison bison*) (Biggins et al. 1998), black-foot ferret (*Mustela nigripes*) (Conway 1980), shark bay mice (*Pseudomys fieldi*) (Lambert et al. 2016) and Eastern barred bandicoots (*Perameles gunnii*) (Parrott et al. 2017).

In 2009, a captive breeding program was established with 12 Allegheny woodrats collected from extant populations in Indiana and Pennsylvania (Smyser and Swihart

2014). Between 2010 and 2012, captive-reared woodrats (i.e., offspring of wild-caught founders) were released into four declining populations in Indiana. Survival analyses of radio-marked captive-reared and sympatric, wild-born young of the year woodrats demonstrated that survival rates were lower for captive-reared woodrats (Blythe et al. 2015). However, captive-reared individuals introduced to the wild with soft-release techniques had higher initial survival rates than those introduced through hard releases (Blythe et al. 2015). Soft-released woodrats underwent a transitional period in a provisioned enclosure adjacent to potential dens, while hard-released individuals were released directly into the wild (Blythe et al. 2015). Even short-term survival allows individuals to potentially contribute to a population genetically and demographically through reproduction, however, the effects of these releases on the genetic diversity of the four recipient populations has not been assessed. Herein, we compare genetic data from woodrats sampled from recipient populations pre- and post-reinforcement. Our objectives were to evaluate whether captive-reared individuals successfully reproduced following release (effective gene flow) and to characterize any changes in genetic variation associated with the introduction of captive-reared individuals.

Methods

Sample collection

Allegheny woodrats were reared in captivity as described in Smyser and Swihart (2014). Between 2010 and 2012, 27 (17M; 10F) captive-reared individuals were released into four sites in Indiana: Cold Friday (CF; $n = 11$), Shelterhouse (SH2; $n = 6$), South Harrison Crawford (SHCF; $n = 7$) and Tobacco Landing (TL; $n = 3$) (Table 1). These four subpopulations were previously known to suffer from declining genetic diversity due to the loss of metapopulation function and connectivity among subpopulations (Smyser et al. 2012a, 2013). Woodrats were 59 to 334 days old at release, making 75% of females and 81% of males already sexually mature (i.e., > 3 months, although woodrats may delay reproducing until 10+ months; Poole 1940; Castleberry et al. 2006; LoGiudice 2008). Woodrats were monitored at all four sites between 2007 and 2013 (i.e., before and after release) using standard live-trapping protocols (Johnson 2002; Mengak et al. 2008; Smyser et al. 2013). Ear tissue samples (2-mm biopsy punches) were collected from each captured individual and preserved in 100 percent ethanol. We extracted DNA from tissue samples using an ammonium acetate extraction protocol (as in Smyser et al. 2012a, 2012b) or the Zymo quick DNA miniprep kit.

Table 1 Resident population sex ratios and the number of captive-reared woodrats released between 2010 and 2012

Site	2010 Releases	2010 Population sex ratio (F:M) ^a	2011 Releases	2011 Population sex ratio (F:M)	2012 Releases	2012 Population sex ratio (F:M)
CF	1M ^b	8:5	2M, 1F ^c	7:14	4M, 3F	4:6
SH2	N/A	6:4	3M, 1F	5:6	1M, 1F	1:2
SHCF	2M	7:3	1F, 1M	8:11	2F, 1M	7:8
TL	1F	8:7	2M	19:10	N/A	24:8

^aF: M, sex ratios at each site^bM, male woodrat^cF, female woodrat

Single nucleotide polymorphism and microsatellite genotyping

We used the Fluidigm® BioMark HD™ Genotyping System to genotype 333 woodrats with 190 single nucleotide polymorphism (SNP) markers. The Fluidigm SNPtype assay includes both putatively neutral and gene-associated SNPs and was designed following the methods described in Doyle et al. (2016) and Doyle et al. (2018). We edited SNP calls using the Fluidigm Genotyping Analysis Software.

We excluded markers from downstream analyses if data did not cluster into distinct homozygous and heterozygous states or if minor allele frequencies were less than 0.025 (Doyle et al. 2016, 2018). We excluded individuals from analysis if five or more loci were not successfully genotyped. We identified a number of markers in linkage disequilibrium using the program snpStats (Clayton 2015) but presumed that, in many cases, this was due to relatedness among sampled individuals, rather than two markers being in close proximity within the genome. As such, we generated two data sets using stringent ($r^2 > 0.20$) or relaxed ($r^2 > 0.40$) benchmarks to identify linkage disequilibrium. After identifying two markers in linkage disequilibrium, one of the two markers was removed.

We used single locus PCR amplification protocols to genotype 333 woodrats at 11 microsatellite markers. The microsatellite DNA amplification reaction and annealing temperatures were optimized as described in Smyser et al. (2012a). Amplified fragments were analyzed and sized using an ABI 3730. Genotypes were assigned using GeneMapper v3.7.

Genetic variability and parentage analyses

We used GenAlEx 6.501 (Peakall and Smouse 2012) to calculate patterns of genetic diversity (allelic richness, observed and expected heterozygosity) for each year and site combination (Table 2). We additionally used GenAlEx to track the number of loci with novel alleles present in 2013 (following releases) but not in 2009 or 2010 (prior to and concurrent with initial releases) at each site. To evaluate whether changes in genetic diversity across time were statistically significant at each site, we calculated the mean difference in the number of heterozygous loci for each year $\times$ year comparison, generating 1000 bootstrap replicates where the 2.5th and 97.5th percentiles represent the 95% confidence intervals (Payton et al. 2003).

Table 2 Measures of SNP genetic diversity for Allegheny woodrat populations at four sites in Indiana augmented with captive-bred individuals, 2009–2013

Site	2009				2010				2011				2012				2013			
	n ^a	A ^b	H _o ^c	H _e ^d	n	A	H _o	H _e	n	A	H _o	H _e	n	A	H _o	H _e	n	A	H _o	H _e
CF	19	1.51	0.09	0.08	13	1.26	0.08	0.07	22	1.70	0.12	0.12	11	1.59	0.18	0.17	4	1.32	0.12	0.11
SH2	12	1.23	0.09	0.08	10	1.22	0.10	0.08	11	1.21	0.08	0.08	3	1.15	0.05	0.06	1	1.2	—	—
SHCF	16	1.22	0.08	0.07	11	1.20	0.08	0.07	20	1.48	0.10	0.10	15	1.68	0.15	0.16	14	1.70	0.22	0.21
TL	14	1.74	0.29	0.24	16	1.74	0.26	0.23	33	1.81	0.24	0.23	32	1.88	0.23	0.22	25	1.92	0.25	0.24

^an, individuals genotyped^bA, average number of alleles per locus across SNP loci^cH_o, observed heterozygosity^dH_e, expected heterozygosity

We used Cervus 3.0.7 (Kalinowski et al. 2007) to assign juveniles sampled following release to resident and captive-reared parents, treating all woodrats trapped at each site as candidate parents. Simulations included 100,000 replicate cycles, with the candidate dams and sires changing per year and site. The proportion of candidate dams and sires sampled was assumed to be 0.94 and 0.76, respectively, based on the probability of capture estimated from the long-term monitoring of these populations (Smyser et al. 2016). The proportion of typed loci was 0.995 for all sites and years, and the proportion of loci mistyped was set to 0.04 (Doyle et al. 2018). The minimum confidence level for parentage assignment was 95%.

Results

SNP and microsatellite heterozygosity

After locus pruning, we retained 99 SNP loci that had minor allele frequencies greater than 0.025 and were in linkage equilibrium ($r^2 < 0.40$) for all subsequent analyses. Of these 99 SNP loci, 52 loci were in neutral regions, whereas 47 were in the coding regions of genes. To explore the potential effect of linkage disequilibrium on our results, we generated a dataset in which markers with an $r^2 > 0.20$ were assumed to be in linkage disequilibrium. One of the two markers in linkage disequilibrium was removed, and the remaining 63 markers were used for downstream analyses. The results of these analyses were qualitatively the same as when an r^2 of 0.40 was utilized, although average observed heterozygosity increased (Supplementary File 1).

In 2009 (i.e., one year before releases), observed heterozygosity (H_O), expected heterozygosity (H_E) and average number of alleles (A) at SNP loci ranged from 0.08 to 0.29, 0.07 to 0.24 and 1.22 to 1.74, respectively, across sites (Table 2; Fig. 1). H_O , H_E and A at SNP loci ranged from 0.12 to 0.25, 0.11 to 0.24 and 1.24 to 1.92, respectively, across sites in 2013 (i.e., year after releases; Table 2; Fig. 1). Estimates of genetic variability were comparable for neutral and gene-associated markers (Supplementary File 1). We identified 7, 13, 46 and 18 SNP loci with novel alleles (i.e., alleles present in 2013 but not 2009 or 2010) at CF, SH2, SHCF and TL; respectively. At microsatellite loci, H_O , H_E and A ranged from 0.43 to 0.68, 0.37 to 0.58 and 2.18 to 4.18, respectively, across sites in 2009 (i.e., prior to releases; Table 3; Fig. 2). In 2013, H_O , H_E and A at microsatellite loci ranged from 0.43 to 0.59, 0.43 to 0.58 and 1.45 to 4.82, respectively, across sites (Table 3; Fig. 2). We identified 2, 0, 10 and 8 microsatellite loci with novel alleles (i.e., alleles present in 2013 but not 2009 or 2010) at CF, SH2, SHCF and TL; respectively.

The mean differences in the number of heterozygous loci for each year $\times$ year comparison are presented with bootstrapped 95% confidence intervals in Table 4. There were significant increases in the number of heterozygous loci from 2009 to 2012, 2010 to 2011 and from 2010 to 2012 at CF (i.e., the 95% CI associated with the distribution of differences in number of heterozygous loci for two years did not overlap 0; Payton et al. 2003). There were no statistically significant temporal changes in genetic diversity at SH2. At SHCF, there were significant increases in the number of heterozygous loci in 2012 and 2013 when compared to 2009 and 2010 (i.e., 95% CIs did not overlap 0; Table 4). The number of heterozygous loci was also greater in 2013 when compared to 2011. The number of heterozygous loci at TL decreased in 2011, 2012 and 2013 when compared to 2009.

Parentage analyses

Reproductive success by captive-reared woodrats was documented at all sites except SH2. In total, captive-reared woodrats produced a minimum of 20 offspring (9M; 11F) across all sites. Eleven woodrats (7M; 4F) were released to CF in 2010, 2011 and 2012. A single female woodrat, released in 2011, reproduced to have four offspring (3M; 1F; Supplementary File 1). At SHCF, three of seven released woodrats (2F; 1M) successfully reproduced to have a total of five offspring (2M; 3F; Supplementary File 1). These three individuals each had two offspring, including one that was parented by two captive-reared woodrats. Additionally, we found evidence of two generations of descendants from a captive-reared woodrat (Supplementary File 1). SHCF was the only site where woodrats released in 2010 and 2012 successfully reproduced and where two captive-reared woodrats released in the same year successfully reproduced. At TL, three captive-reared woodrats (1F; 2M) were released. No reproduction was documented for the female released in 2010, nor one of males released in 2011. The other male woodrat released in 2011 reproduced with at least six different females to produce 11 offspring (4M; 7F; Supplementary File 1). One of this woodrat's six mates was his daughter; the remaining five mates were resident females. We did not document any reproductive success for any of the six captive-reared woodrats released in 2011 and 2012 to SH2.

Population abundance and sex ratios

The minimum number of woodrats known to be alive was determined for each year and site during the monitoring period (i.e., 2009–2013; Fig. 3) using standard live-trapping methods and was assumed to reflect population sizes. At CF, population size fluctuated until 2011 and then continuously decreased until the end of the monitoring period in 2013 (Fig. 3). At SH2, abundance steadily decreased from

Fig. 1 Mean observed heterozygosity (H_o) ± SE for Allegheny woodrats (*Neotoma magister*) captured between 2007 and 2013 in **a** Cold Friday (CF), **b** Shelterhouse (SH2), **c** South Harrison Crawford (SHCF) and **d** Tobacco Landing (TL) and genotyped at SNP loci. Black bars indicate the years that augmentation occurred

Table 3 Measures of microsatellite genetic diversity for translocated Allegheny woodrat populations at four sites in Indiana augmented with captive-reared individuals, 2009–2013

Site	2009				2010				2011				2012				2013			
	n ^a	A ^b	H _o ^c	H _e ^d	n	A	H _o	H _e	n	A	H _o	H _e	n	A	H _o	H _e	n	A	H _o	H _e
CF	19	2.91	0.50	0.45	13	2.18	0.43	0.42	22	3.45	0.46	0.47	11	3.18	0.58	0.49	4	2.45	0.43	0.43
SH2	12	2.18	0.45	0.42	10	2.18	0.48	0.42	11	2.36	0.37	0.34	3	1.81	0.45	0.30	1	1.45	—	—
SHCF	16	2.27	0.43	0.37	11	2.00	0.47	0.37	20	2.72	0.43	0.41	15	3.54	0.58	0.52	14	3.54	0.56	0.56
TL	14	4.18	0.68	0.58	16	3.91	0.66	0.58	33	4.64	0.60	0.57	32	4.09	0.60	0.57	25	4.82	0.59	0.58

^an, individuals genotyped

^bA, average number of alleles per locus across microsatellite loci

^cH_o, observed heterozygosity

^dH_e, expected heterozygosity

Fig. 2 Mean observed heterozygosity (H_O) $\pm$ SE for Allegheny woodrats (*Neotoma magister*) captured between 2007 and 2013 in **a** Cold Friday (CF), **b** Shelterhouse (SH2), **c** South Harrison Crawford (SHCF) and **d** Tobacco Landing (TL) at 11 microsatellite loci. Black bars indicate the years that augmentation occurred

Table 4 The mean difference in the number of heterozygous loci and 95% CI for each year x year comparison at each site

Years	Sites			
	CF	SH2	SHCF	TL
2009–2010	– 1.54 (– 5.59, 0.83)	0.87 (– 1.13, 2.72)	0.22 (– 1.06, 1.45)	– 3.36 (– 7.60, 1.08) ^a
2009–2011	2.91 (– 2.25, 7.67)	– 0.50 (– 2.21, 1.22)	2.16 (– 0.38, 5.66)	– 4.83 (– 8.56, – 0.74) ^c
2009–2012 ^b	9.23 (2.28, 16.72)		6.99 (1.54, 14.20)	– 5.38 (– 8.69, – 1.89)
2009–2013			14.19 (7.33, 21.52)	– 4.54 (– 8.44, – 0.54)
2010–2011	4.29 (0.77, 8.73)	– 1.23 (– 3.39, 0.80)	1.81 (– 0.75, 5.29)	– 1.63 (– 5.71, 2.18)
2010–2012	10.58 (4.03, 17.92)		6.85 (1.55, 13.40)	– 2.13 (– 6.25, 1.59)
2010–2013			13.74 (7.08, 21.06)	– 1.24 (– 5.41, 2.86)
2011–2012	6.73 (– 1.41, 14.36)		5.03 (– 0.90, 12.32)	– 0.44 (– 3.54, 2.85)
2011–2013			11.80 (4.33, 19.60)	0.29 (– 3.27, 3.75)
2012–2013			6.76 (– 2.99, 16.14)	0.91 (– 2.36, 4.27)

^aThe first number under each site is the mean difference in heterozygous SNP loci, followed by the 95% confidence interval in parentheses for each year

^bSH2 in 2012–2013 and CF in 2013 were not included in calculations due to small sample sizes

^cResults in bold indicate statistically significant mean differences in number of heterozygous loci

Fig. 3 Number of Allegheny woodrats captured at reinforcement sites in Indiana (CF: solid light grey line, SH2: solid dark grey line, SHCF: solid black line and TL: dashed black line), 2009–2013. Augmentations occurred between 2010 and 2012

12 woodrats to one by the end of the monitoring period in 2013. At SHCF, population numbers remained reasonably stable during the monitoring period (e.g., population size in 2009: 16; 2013: 14). Following the first two years of releases at TL, observed population size more than doubled from 14 to 33. By 2013, twenty-five individuals were present at TL.

We examined annual sex ratios of resident populations for each year during which releases occurred (Table 1). At TL, there was an increasing female bias across all three years whereas sex ratios remained relatively even at SH2 throughout the monitoring period. Population numbers were initially female-biased at CF and SHCF (2010 population sex ratio (F:M): CF (8:5); SHCF (7:3)) but shifted toward a male bias in subsequent years (2011 population sex ratio (F:M): CF (7:14); SHCF (8:11)).

Discussion

The viability of remnant Allegheny woodrat populations in Indiana is adversely affected by spatial isolation and lack of gene flow (Smyser et al. 2016). Our study provides an example of how reinforcement of wild populations through the release of captive-reared individuals can be useful in conservation and genetic maintenance of isolated populations. The reproductive success of the captive-reared woodrats resulted in a minimum of 20 offspring, which contributed to relatively stable abundance and increased genetic variation at one of four sites. Our results imply that among the varying demographic pressures and increasing isolation that threaten Allegheny woodrat populations, captive breeding has the potential to increase abundance and genetic diversity in isolated subpopulations.

Genetic diversity and reproductive success

The mortality rate of captive-reared individuals at our study sites was relatively high (60% mortality in the 5 weeks following release) and may have been influenced by a drought that occurred between 2010 and 2012 (Wood et al. 2008; Blythe et al. 2015). Yet, our analyses revealed that several captive-reared individuals successfully contributed to the infusion of new alleles at three of our four sites. At both CF and TL, the high fecundity of a single individual coincided with population growth within a year following release. At SHCF, increases in genetic variability were sustained and accompanied by comparable abundance at the beginning and end of the monitoring period.

Reproductive success among sites may have been affected by sex biases within recipient populations, which has been documented in similar efforts for other species (Letty et al. 2007; Lambertucci et al. 2013). A skewed male bias in 2011–2012 in CF and 2011 in SHCF may have precipitated the reproductive success of the first captive-reared female woodrats released to both sites. The exceptionally high breeding success of a captive-reared male in TL was linked to a deficit in resident male woodrats. Thus, sex biases within populations could have limited mate competition for these captive-reared woodrats and contributed to their reproductive success.

Successful reproduction by captive-reared individuals was not always correlated with increased genetic diversity. Despite successful reproduction by a captive-reared male at TL, both population numbers and genetic variability ultimately decreased by the end of the monitoring period. Prior to reinforcement, genetic variability in the resident population was relatively high and comparable to levels found

throughout more robust populations in Pennsylvania (as estimated from microsatellites, Smyser et al. 2012a; SNPs, data not shown). Genetic variability was also comparable to outbred populations of other species genotyped using the same technology (e.g., golden eagles, Doyle et al. 2016; gray whales, DeWoody et al. 2017; Tule elk, Bali 2018). The remarkably high reproductive success of a single male may have subsequently contributed to inbreeding, as demonstrated by subsequent reproduction with one of his offspring. Although one individual can increase the fitness of a population, benefits can be temporary and insufficient for sustaining the genetic variability in future generations (Hedrick et al. 2019; Robinson et al. 2019). The introduction of one male gray wolf (*Canis lupus*) to a small inbred population on the island of Isle Royale initially increased population fitness and growth. In later generations, however, the populations experienced a decrease in fitness and genetic variation due to increased relatedness and inbreeding (Hedrick et al. 2014, 2019). Management approaches that employ the release of captive-reared individuals should be mindful that initial increases in population fitness and genetic variability may be temporary and plan accordingly to mitigate this outcome.

Management implications

Translocation programs for herbivores and omnivores are more successful than those of carnivores (Griffith et al. 1989; Wolf et al. 1996), with predator and prey species differing in the challenges encountered following translocations (Griffith et al. 1989; Wear et al. 2005; Aaltonen et al. 2009; Ceballos et al. 2017). For prey species like woodrats, predation is often the greatest challenge to successful transition of captive-reared individuals to the wild (Short et al. 1992; Letty et al. 2007; Tetzlaff et al. 2019) and high mortality as a result has been the cause of several failed translocation efforts (Short et al. 1992; Grey-Ross et al. 2009; Moseby et al. 2011; White et al. 2012; Tetzlaff et al. 2019).

In our study, mortality rates of captive-reared woodrats were high (Blythe et al. 2015), however we documented reproduction by ~36% of those individuals that survived at least 5 weeks. Therefore, future work on the recovery of Allegheny woodrats and other small-bodied prey species should focus on maximizing survival during the transition to the wild. Anti-predator training, in addition to using soft-release techniques to ease the transition of captive-reared woodrats into the wild, may improve survival rates and increase reproductive opportunities (Blythe et al. 2015; Salehi et al. 2019).

Sex-skewed demographics in pre-augmented populations may have facilitated the reproductive success of captive-reared woodrats and demonstrates the importance of characterizing demographics of recipient populations to

maximize the success of releases (Sarrazin and Legendre 2000; Wedekind 2002). Monitoring efforts to quantify woodrat population demographics (i.e., sex ratios) prior to augmentation could reveal which sex would be most beneficial for release into different populations (Lambertucci et al. 2013). This strategy would make efficient use of limited individuals available for release, could limit mate competition and increase captive-reared woodrat fecundity, and thus improve the likelihood of infusing long-term genetic diversity into the population.

Conclusions

Our study provides an assessment of the reproductive success of the captive-reared individuals and subsequent changes in genetic variation to recipient populations. We determined that admixture occurred at three of the four sites. Reproductive success of the captive-reared individuals had positive effects on population growth and genetic diversity in some cases. Our results suggest that future conservation efforts utilize captive-reared individuals, in concert with continued efforts to preserve habitat and reduce fragmentation, to further promote the genetic diversity and persistence of Allegheny woodrats and other metapopulations.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10592-021-01372-z>.

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Author contributions TJS, SAJ and RKS conceived and designed the research. TJS, SAJ, JD and JLL maintained the captive-breeding program, secured permits and collected field data and samples. TJS and JMD performed DNA extractions and associated laboratory procedures. MMD and JMD performed all analyses. MMD, JMD and TJS led the writing effort and all authors (MMD, TJS, SAJ, JD, JLL, RKS and JMD) reviewed, revised, commented on and approved the final version of the manuscript.

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Data availability Microsatellite and single nucleotide polymorphism data sets are archived with Dryad digital repository (doi:<https://doi.org/10.5061/dryad.66t1g1k25>).

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval All animal handling procedures were approved by the Purdue Animal Care and Use Committee Protocol #1111000236 and 1201000596.

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