Comparison of three techniques for genetic estimation of effective population size in a critically endangered parrot

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Abstract

Understanding the current population size of small, spatially aggregating populations of species is essential for their conservation. Reliable estimates of the effective population size (N_e) can be used to provide an early warning for conservation managers of the risks to genetic viability of small populations. Critically endangered, migratory swift parrots *Lathamus discolor* exist in a single panmictic population in Australia. In their Tasmanian breeding range, they are at severe risk of predation by introduced sugar gliders, exacerbated by deforestation. We used three genetic approaches to estimate N_e using DNA samples genotyped by microsatellite markers and existing life-history data of swift parrots. Based on all samples, we revealed small contemporary N_e estimates across methods (range: 44–140), supporting the need to urgently address threatening processes. Using the 0.5 N_e/N ratio calculated from demographic data suggests that the minimum potential contemporary population size is below 300 individual swift parrots. This is considerably lower than the published estimates derived from expert elicitation, and accords with modeled estimates of extinction risk in this species. Our study has important implications for other threatened species with unknown population sizes and demonstrates that by utilizing available genetic data, reasonable estimates of N_e can be derived.

Introduction

Conservation management of declining populations is heavily reliant on estimating population size. Genetic techniques may be used with high reliability to detect small population sizes if enough samples can be collected (Frankham, Briscoe, & Ballou, 2010). If detected early, intervention to increase population size may be implemented successfully (Legault *et al*., 2013). Reliable estimates of the effective population size (N_e) may be used to provide an early warning for conservation managers of the risks to genetic viability of small populations (Luikart *et al*., 2010). If intervention is not possible or is implemented too late, then Allee effects (inverse density dependence) may arise and exacerbate extinction risk in some small populations (Stephens & Sutherland, 1999). However, in some cases, populations have survived at small size with low genetic diversity, demonstrating that genetic factors are not the sole reasons leading to extinction (Reed, 2010). Still, for species with small, spatially aggregating populations, estimating N_e may be especially important because once genetic diversity is lost, it may be impossible to restore.

There are multiple approaches for estimating N_e using genetic techniques; however, the underlying assumptions and data requirements for these approaches can sometimes be challenging to meet. For example, species that are cryptic, or occur at low densities or in challenging environments may severely curtail opportunities to collect genetic material. Furthermore, collection of samples from within overlapping generations may be unavoidable (e.g., in long-lived species), which violates key assumptions of some genetic approaches for estimating population size (Luikart *et al*., 2010). Similar challenges exist when siblings are overrepresented within a batch of genetic samples (which may occur in species where nests/litters are more easily sampled than free-ranging adults). However, recent advances in analytical techniques have aimed to overcome some of these challenges, opening the opportunity for estimating N_e of age-structured, long-lived populations (Waples, Antao, & Luikart, 2014). Evaluating the most appropriate analytical approach for the available genetic data is not straightforward, and conservation scientists may be faced with multiple analytical options for estimating N_e from opportunistically collected genetic samples. Given this uncertainty, comparing different approaches for

estimating N_e of imperfect genetic data is of high value to conservation practitioners.

Swift parrots Lathamus discolor are a good model to evaluate different approaches for estimating N_e because they exist as a single, nomadic, panmictic population (Stojanovic et al., 2018). Threatened by an introduced predator (Stojanovic et al., 2014) and severe deforestation (Webb, Stojanovic, & Heinsohn, 2018), swift parrots are predicted to decline by 95% within three generations, making them critically endangered (Heinsohn et al., 2015). Estimates of swift parrot population size are mostly based on expert opinion (Garnett, Szabo, & Dutson, 2011) and the true contemporary population size is unknown. Genetic samples have been collected from swift parrots to address other research questions (Stojanovic et al., 2018; Heinsohn et al., 2019). However, these were taken primarily from siblings within nests found during routine monitoring and thus present analytical challenges for the estimation of N_e. In this study, we (1) employ three widely used genetic techniques to compare N_e estimates; (2) consider the ecology of our model species by factoring in available information about the mating system and predation rates; and (3) predict that the three methods should yield comparably low estimates of N_e given predicted population declines (Heinsohn et al., 2015) and severe habitat limitation due to deforestation (Webb, Stojanovic, & Heinsohn, 2018; Vergara-Tabares et al., 2020).

Materials and methods

Sample collection and DNA extraction followed Stojanovic et al. (2018) and the samples from that study are included here. Nestlings ($n = 310$) from 93 nests were sampled between 2010 and 2015. On average, 17 (range = 9–32) nests were sampled per year (mean brood size = 3.3). At nests found during standardized monitoring (Webb et al., 2014), we collected blood, feathers, or tissue (from dead birds) from 165 male, 142 female, and three unknown sex nestlings, plus 31 additional adults (22 males and 9 females).

We extracted DNA from blood following standard procedure for nucleated erythrocytes (Smith & Burgoyne, 2004), and from feather/tissue using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) with some protocol modifications (Olah et al., 2016). We used seven microsatellite loci (Stojanovic et al., 2018) to construct full genotypes of 341 samples and the 2550F/2718R primers (Fridolfsson & Ellegren, 1999) for the molecular sexing. The seven microsatellite loci used in this study were exhaustively tested with POWSIM power analysis previously, demonstrating the risk of type I errors to be below 5% and almost complete power (96%) for detecting genetic differentiation at $F_{ST} = 0.01$ (Stojanovic et al., 2018). We have also shown that these same markers have enough power to recover all unique genotypes even among siblings ($PI_{sibs(7)} = 0.002$) over a large sample of individuals (see details in Table 1 of Heinsohn et al., 2019).

We used three different methods to estimate the effective population size and we calculated genetic estimates of N_e ($\hat{N}_e$) or both $\hat{N}_e$ and the effective number of breeders ($\hat{N}_b$) depending on the method (Table 1). We first estimated N_e using the modified temporal method based on variance (Jorde, 2012) and the linkage disequilibrium (LD) method based on linkage (Waples, 2006). N_e was first estimated using each method individually and then we combined different estimates of N_e within and across methods (Waples & Do, 2010). Since our study system contained overlapping generations, we also estimated N_b each year using the LD method and the sibship assignment method (Wang, 2009). We caution that our estimates of N_b are biased by the number of nests accessible for DNA sampling in any given year (due to challenges in accessing nests in structurally unsound trees, more nests are detected than can be sampled for genetic material).

The modified temporal method (Table 1) applies the theory of standard temporal method of discrete generations (Jorde & Ryman, 1995), but with modifications to account for overlapping generations (Jorde, 2012). We used the software GONe (Coombs, Letcher, & Nislow, 2012) with all

Table 1 Methods used to estimate the effective populations size (N_e) and the effective number of breeders (N_b)

Method	Estimates	Software	Specifications and key assumptions	Demographic information used	References
Modified temporal	$\hat{N}_e$	GONe	Accounts for overlapping generations using life tables; closed populations.	Demographic data from Heinsohn et al. (2015) for equilibrium and predation scenarios.	Jorde (2012); Coombs, Letcher & Nislow (2012)
Linkage			disequilibrium (LD)	$\hat{N}_e$ & $\hat{N}_b$	NeEstimator 2.1
Selective neutrality; discrete generations; closed populations.	Adjusted	estimates by AgeNe using	demographic parameters from Heinsohn et al. (2015).	Waples (2006); Do et al. (2014); Waples, Do & Chopelet (2011)	
Sibship assignment	$\hat{N}_b$	COLONY 2	Calculates maximum likelihoods by the frequencies of full- and half-sib dyads; discrete generations; closed populations.	Estimated demographic parameters from the genotypes of a single cohort.	Wang (2009); Jones & Wang (2010)

341 samples divided into five spatially discrete subpopulations by breeding season. Plan 1 of the software was used (i.e., samples taken after reproduction or non-lethally before reproduction) and an estimated maximum census size of 2,158 (Garnett, Szabo, & Dutson, 2011) was used for the calculations. We ran two models using life tables according to two different scenarios: (1) equilibrium and (2) predation by sugar gliders. For these scenarios, we used previously published demographic values (Heinsohn *et al.*, 2015): (i) nine age classes, (ii) age at first reproduction is 2 years old, (iii) average clutch size of 3.14 in equilibrium and 1.87 in predation scenario, (iv) 45% juvenile mortality, and (v) 29.4% adult mortality (for both males and females) in equilibrium while 29.4% adult male and 56.4% adult female mortalities in predation scenario. Clutch sizes were automatically rescaled to result in population lambda = 1 (Coombs, Letcher, & Nislow, 2012). GONe computed a correction factor "C" and generation times for each model. We report the mean genetic drift estimator Fs (Jorde & Ryman, 2007) over all intervals per model with 95% confidence intervals (CIs).

For the LD method (Table 1), we used the software NeEstimator v2.1 (Do *et al.*, 2014) with a threshold frequency of 0.01 for screening out rare alleles, assumed random mating (Heinsohn *et al.*, 2019), and calculated 95% CIs for $\hat{N}_e$ by a jackknife-across-samples method (Jones, Ovenden, & Wang, 2016). To estimate N_e, we used adult samples ($n = 31$) and the pooled juvenile samples ($n = 310$). We also calculated $\hat{N}_b$ using juvenile samples from each year ($n_{2010} = 54$, $n_{2011} = 24$, $n_{2012} = 57$, $n_{2013} = 72$, $n_{2014} = 21$, $n_{2015} = 82$). We calculated the relationships among the total census population size (N), adult census size (N_A), N_e, and N_b with the software AgeNe (Waples, Do, & Chopelet, 2011), using the same demographic parameters and scenarios as in the previous model, with Poisson factor = 1 and the number of

newborns each year (N_1) = 93 in equilibrium and 23 in predation scenario estimated from empirical data (Stojanovic, unpublished data). In AgeNe, we used the following demographic information (Heinsohn *et al.*, 2015): AL (adult life span) = 8, α (age at maturity) = 2, and CVf (coefficient of variation of mean number of offspring for adult life span) = 0. We calculated three adjusted values (following Table 3 of Waples, Antao, & Luikart, 2014) using (1) true N_b/N_e from AgeNe; (2) AL and α; and (3) AL, α, and CVf. $\hat{N}_e$ of this method is most strongly affected by N_e in parental generation but also influenced by N_e in the previous three generations.

The sibship assignment method is a hybrid approach (Table 1), estimating demographic parameters from the genotypes of a single cohort, which are then used to estimate N_b based on the frequencies of full- and half-sib dyads in the sample (Wang, 2009). We used the software COLONY 2 (Jones & Wang, 2010) with the following parameters based on default options of the manual and our previously published findings: (i) no inbreeding (Stojanovic *et al.*, 2018), (ii) social monogamy (monogamous males and polygamous females; Heinsohn *et al.*, 2019), (iii) full sibship size scaled down by the full likelihood method (default), (iv) one "very long" run (recommended), and (v) "high" precision for full likelihood (default). We provided the known maternal sibships (siblings from a single nest were recorded during sampling) and ran the estimations using the same juvenile sample set described for the LD method to estimate N_b.

Results

Genetic estimates of N_e derived from the modified temporal and LD methods are presented in Table 2A. Using the modified temporal method, the equilibrium scenario produced a
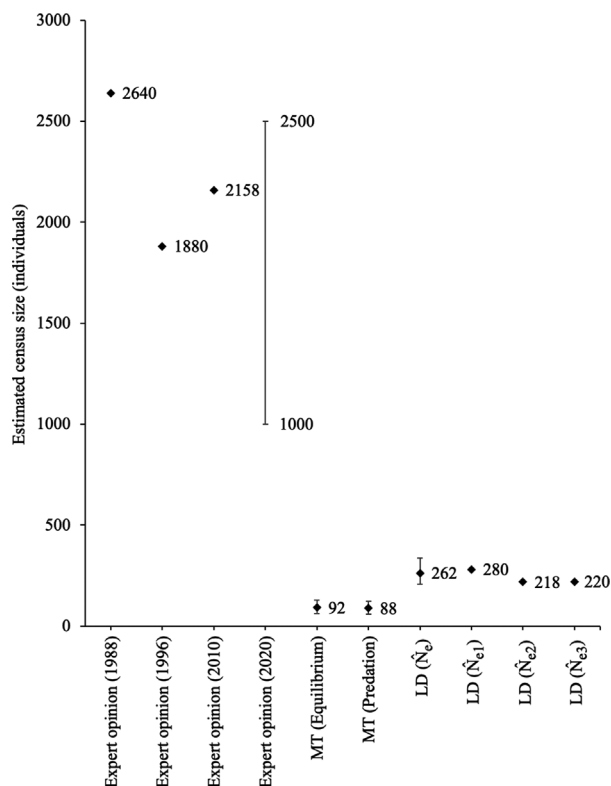
Table 2 Estimates of (A) effective population size (N_e); and (B) effective number of breeders (N_b) of swift parrot (*Lathamus discolor*) with 95% confidence intervals (in parentheses). Methods used are the modified temporal, linkage disequilibrium (LD), and sibship assignment (SA) methods. We include adjusted LD estimates using the following adjustments: $\hat{N}_{e1\ and\ b1}$ = true N_b/N_e from AgeNe; $\hat{N}_{e2\ and\ b2}$ = AL and α; $\hat{N}_{e3\ and\ b3}$ = AL, α, and CVf (see methods). Unweighted harmonic means (HM) were calculated for N_b estimates using the SA results and the corresponding adjusted LD estimates.

(A)

Samples	Modified temporal method $\hat{N}_e$		Linkage disequilibrium method			
	Equilibrium	Predation	$\hat{N}_e$	Adjusted LD estimates		
				$\hat{N}_{e1}$	$\hat{N}_{e2}$	$\hat{N}_{e3}$
Adults	-	-	471 (25–∞)	502	392	396
Nestlings	-	-	126 (86–195)	135	105	106
All	46 (31–64)	44 (30–62)	131 (104–169)	140	109	110

(B)

Year	Sample size	LD method $\hat{N}_b$	Adjusted LD estimates			SA method $\hat{N}_b$	HM of LD and SA methods		
			$\hat{N}_{b1}$	$\hat{N}_{b2}$	$\hat{N}_{b3}$		$\hat{N}_{b1}$	$\hat{N}_{b2}$	$\hat{N}_{b3}$
2010	54	34 (19–72)	36	44	45	37 (23–59)	36	40	41
2011	24	10 (5–20)	10	13	13	30 (18–56)	15	18	18
2012	57	28 (19–42)	30	36	38	34 (22–55)	32	35	36
2013	72	44 (30–72)	47	58	60	55 (36–82)	51	56	57
2014	21	37 (12–∞)	39	48	50	29 (17–61)	33	36	37
2015	82	37 (24–59)	39	48	50	54 (36–80)	45	51	52

N_e estimate only slightly higher than the scenario that included predation by sugar gliders (Table 2A).

Demographic estimates for the equilibrium and predation scenarios in AgeNe were very similar in some respects but not others. Using equal number of newborns (N_1) for both scenarios, in predation the total census population size (N) was reduced by 14%, adult census size (N_A) by 22%, N_e by 13%, and N_b by 29%. When we used empirical N_1 values (75% reduction under predation), the N was reduced by 79%, N_A by 81%, N_e by 78%, and N_b by 83% under the predation scenario. The N_e/N_A ratio increased by 13% under predation. The N_e/N ratio was 0.52 in equilibrium scenario and increased only by 2% (to 0.53) in predation. We used $N_e/N = 0.5$ to calculate total census population size from the different estimates (Fig. 1).

The LD method calculated the average $\hat{N}_e$ of previous generations for adults and pooled juveniles (Table 2A), and $\hat{N}_b$ when calculated for each year using juvenile samples only (Table 2B). The adjusted LD estimates of N_e fall into the range of 392–502 for adult samples, and into the range

of 105–135 for nestling samples (Table 2A). The adjusted N_b estimates were much lower, ranging from 10 to 60 across years, showing some fluctuations among the years (Table 2B). Following calculation of Waples & Do (2010), the weighted harmonic mean of $\hat{N}_b$ was 32 over the study period (although we caution that this is an underestimate because not all nests were accessible for sampling each year).

The sibship assignment method calculated $\hat{N}_b$ as ranging from 29 to 55 over the study period (Table 2B). We also calculated the unweighted harmonic means of the adjusted LD and sibship assignment methods (Table 2B), as they have roughly comparable precision (Waples & Do, 2010).

Discussion

Our results reveal that swift parrots have a worryingly small $\hat{N}_e$ and support the critically endangered status of the species. These findings highlight the need for urgent conservation action to halt further population decline. Cumulative impacts of severe deforestation (Webb, Stojanovic, & Heinsohn, 2018) and predation (Heinsohn *et al.*, 2015) likely contributed to a small contemporary N_e, which results in loss of evolutionary potential and increased vulnerability to genetic stochasticity (Frankham, Briscoe, & Ballou, 2010). The "50/500 rule" in conservation genetics says that N_e should not fall below 50 to minimize short-term problems related to inbreeding and should remain above 500 in the long term to maintain sufficient evolutionary potential (Franklin & Frankham, 1998). With some qualifications, this rule still has a useful place in conservation biology (Jamieson & Allendorf, 2012), while others even recommended that this rule should be changed to 100/1,000 (Frankham, Bradshaw, & Brook, 2014). In either case, our results indicate that low population size may be an emerging threat for swift parrots. Using the most realistic predation scenario for the study species, the modified temporal method estimated N_e of swift parrots at 44, which indicates that the species might not be able to maintain sufficient evolutionary potential even in the short term. Unfortunately, the species is a monotypic genus (Provost, Joseph, & Smith, 2018), and options like genetic rescue via hybridization with related species are not available to conservation managers. The small population sizes we found may make swift parrots particularly vulnerable to the emergence of Allee effects, i.e. the decrease of their population growth rate in parallel to the decline of their population size (Crates *et al.*, 2017). In the case of the critically endangered orange-bellied parrot *Neophema chrysogaster*, establishment of acaptive population temporarily decreased genetic diversity of the wild population (Morrison *et al.*, 2020), and release of captive bred birds to the wild has not corrected their population decline (Stojanovic *et al.*, 2020). For swift parrots, cessation of deforestation of breeding and foraging habitat may stop exacerbating habitat limitation and predation (Stojanovic *et al.* 2014), which is an important step for halting further reduction of the wild population size.

Figure 1 Total census population size estimates of the swift parrot (*Lathamus discolor*) based on previous expert opinions (Brown, 1989; Brereton, 1996; Garnett, Szabo, & Dutson, 2011; BirdLife International, 2020) and the current study. Expert opinions from 1988 and 1996 were multiplied by 2 as they reported breeding pairs originally. Genetic estimates of census size are based on results in Table 1A (MT = modified temporal, LD = linkage disequilibrium methods) and $N_e/N = 0.5$ calculated by AgeNe. Confidence intervals are displayed where available.

The timespan of estimates

It is important to note that different methods provide complementary information and one is not necessarily better than others. We estimate that N_e of earlier generations than those sampled (i.e., before 2010) may have reached 500 for adults and 105–135 for combined data from nestlings (Table 2A). Since our adult swift parrot samples reflect N_e of earlier generations than those of nestlings, the drop in estimated N_e between adult and juvenile samples may be evidence for steep population decline (Heinsohn *et al.*, 2015). Temporal methods estimate the harmonic mean of $\hat{N}_e$ between years sampled. In the current study, this is between 2010 and 2015 and of a magnitude well below 100 (Table 2A). A study on the endangered black-fronted tern *Chlidonias albostriatus* showed that a combination of $\hat{N}_e$ and $\hat{N}_b$ is more appropriate assessing the status of threatened mobile and migratory species than solely $\hat{N}_e$ when using mixed-age samples (Schlesselmann & Robertson, 2020).

N_b estimates the effective number of adults that bred each year to produce the cohorts of nestlings sampled (Waples *et al.*, 2018). We found substantial overlap among the annual estimates of N_b and their associated confidence intervals using the LD and sibship assignment methods with even lower values (all < 60; Table 2B). The number of nests accessible for sample collection varied each year depending on local habitat availability (Webb *et al.*, 2014, 2019), thus fluctuation in $\hat{N}_b$ was likely an artifact of sampling effort and the fraction of nests that could be reached by a climber in a given year. These considerations, and that all siblings per nest were included in the analysis, influenced the random sampling assumption of the estimators (Waples & Anderson, 2017). We suspect that this downwardly biased $\hat{N}_b$ and $\hat{N}_e$ (Jorde, 2012).

Equilibrium versus predation scenarios

Demographic $\hat{N}_e$ and $\hat{N}_b$ both decreased in the predation scenario, but N also decreased under predation modulating the change in the N_e/N ratio. If mortality was increased uniformly across both sexes, the expectation is that both N_e and N would decline but N_e/N would increase because N decreased more than N_e (Kuparinen, Hutchings, & Waples, 2016). In our predation model, however, mortality only increased for adult females, which skewed the sex ratio and reduced the N_e/N ratio. In the modeled scenario, the two factors approximately canceled each other out, so there was only a 2% change in the N_e/N ratio, when N is the total population. However, because only adult females had a reduction in N, adult census size (N_A) declined by a greater percentage. As a consequence, the reduction in abundance was proportionally larger for adults (81% reduction of N_A compared to 79% reduction of N when using empirical data), and the N_e/N_A ratio increased under predation. Shortage of females may curb the number of males that breed. However, female swift parrots often mate with more than one male (Heinsohn *et al.*, 2019), so we predicted that this is a case of "social monogamy," where one male helps raise the chicks, but more than one male contributes genetically.

Effects of genetic markers and source of samples

We previously demonstrated that our handful of microsatellite loci had adequate power to differentiate populations (Stojanovic *et al.*, 2018) and even individual swift parrots (Heinsohn *et al.*, 2019). Using these seven loci for genotyping our samples, the probability that we find two identical genotypes in a population including siblings is 1 in 500 (Heinsohn *et al.*, 2019). Hence, our presented estimates can be considered as reliable and more molecular data would have not necessarily enabled better estimates of N_e. A recent study on the population dynamics of a critically endangered Australian songbird used over 500 SNP loci (Crates *et al.*, 2019), without being able to obtain a more precise N_e estimate with the same methods tested here. Although microsatellites are sometimes considered outdated markers in modern genomics (Allendorf, Hohenlohe, & Luikart, 2010), if they are carefully selected and tested for the species of interest, even a small number of them can provide reliable population size estimations.

Our source data (comprising few adults but many juvenile siblings) are typical of many conservation projects, where opportunistic collection of DNA samples during monitoring may violate some assumptions of some analytical approaches. However, as for many species, detailed demographic data derived from ecological studies were available to provide important context for our genetic analyses. By employing three commonly used analytical procedures, and using available samples (and subsets), we provide an important comparison of the range of estimates that may be derived using imperfect but commonly available genetic data. Combining multiple consecutive cohort samples in the LD method was shown to downwardly bias $\hat{N}_e$ and $\hat{N}_b$ in the great tit (Fig. S9 in Waples, Antao, & Luikart, 2014). Although the modified temporal method accounted for overlapping generations, we believe (based on assumptions of short generation time in our model species) that the generational overlaps led to underestimation of the real N_e. Samples from more widely spaced generations could provide much more precise estimates for the temporal method (Jorde & Ryman, 1995). However, achieving such a sampling scheme is unrealistic for this and many threatened species (Grimm *et al.*, 2016), particularly if ongoing declines in population size and available habitats limit opportunities to collect genetic material from increasingly scarce animals.

Importantly, we show that reliable population estimates can be derived from juvenile samples only. This is important because juveniles may sometimes be easier to sample than adults. For studies where samples from adults are unavailable, we recommend that the LD or the sibship assignment method be utilized (Waples & Anderson, 2017). The results of this approach can then be treated as minimum estimates of N_e, and still yield important population data for conservation management.

Expert opinions versus genetic estimators

In conservation management, knowing the census population size (N) of a species or population may be more practical

for implementing conservation action, than simply using $\hat{N}_e$ alone. Since the habitat use of the swift parrot varies over time (Webb *et al.*, 2014), it is hard to produce a reliable population size estimate based on traditional census methods (Fig. 1). Breeding population size was estimated at 1,320 breeding pairs in 1988 (Brown, 1989) and 940 in 1996 (Brereton, 1996). In 2010, a survey estimated a total of 2,158 birds, including immatures, and based on this information, the total population was assessed to comprise 2,000 mature birds (Garnett, Szabo, & Dutson, 2011). Today, BirdLife estimates the population size as between 1,000 and 2,500 individuals (BirdLife International, 2020). According to our population viability analysis (Heinsohn *et al.*, 2015), which was based on these probably optimistic assumptions, the swift parrot population size in 2020 was predicted to be around 1,000 birds or below. Estimates of the N_e/N ratio in other species (Frankham, 1995; Luikart *et al.*, 2010) showed that N_e is often 10–20% of the census population size, while our study estimated that N_e is much closer to the census N ($\sim$ 50%). The mean $\hat{N}_e$ of the adjusted LD estimates (based on all samples) was 120 in the current study (Table 2A). Hence, we can interpolate a minimum potential contemporary population size between 60 and 338 individual swift parrots across methods (Fig. 1). This estimated N is much lower than previously published estimates derived from expert elicitations.

Expert opinions are often used in conservation prioritizations (Carter *et al.*, 2000; Keith *et al.*, 2011; Martin *et al.*, 2012) but they can also fail to account for the true conservation status of species (Charney, 2012; Reed *et al.*, 2017). If opinions overestimate the true population size of a species, as shown in this study, they can be detrimental to species conservation. When assumptions are met, genetic methods can provide a more objective method to estimate N_e and interpolate N across taxa. The stark contrast between our results and previous expert estimates of population size underscores the risk to effective conservation when lack of empirical evidence results in over-reliance on opinion for deciding the status of species perceived to be at risk. Our study shows that experts can make overly conservative population estimates based on weak evidence and that this may create a false sense of security about a species' conservation status for decades. This may be especially apparrent for species like the swift parrots that are iconic and often observed by the public. We hope our study encourages others to more carefully scrutinize population estimates (and conservation status listings) of species where empirical data (either direct counts or population genetics) are lacking.

Conclusion

We show that it is possible to detect a small population size despite occurrence of siblings and overlapping generations of a critically endangered parrot species. Our study has important implications for other threatened species with unknown population sizes and demonstrates that even a few microsatellite markers can be enough to derive reasonable estimates of effective population sizes if they have adequate resolution. However, the resolution of markers should be tested beforehand. Based on our results, we recommend using the adjusted LD method on pooled juvenile samples to estimate contemporary N_e and the harmonic mean of the LD and sibship assignment methods to estimate N_b. Conservation planning depends on reliable estimates of population size, and we caution against over-reliance on expert opinions of population size to assess the conservation status of a species when it is feasible to obtain empirical estimates of key population parameters.

Acknowledgments

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