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TECHNICAL NOTES

The use of microsatellite loci for accurate hybrid detection in a recent contact zone between an endangered and a recently-arrived hummingbird

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Abstract Interspecies hybridisation frequently occurs when the distributional ranges of two closely-related species overlap after a period of geographic isolation. From a conservation perspective, such hybridisation events can incur detrimental effects on the viability of each species involved, especially for species which are already threatened by other ecological processes, such as human-induced declines in population size. The early and accurate detection of hybrids within recent contact zones is therefore of crucial importance for conservation strategies. A recent contact zone occurs in the north of Chile between the endangered Chilean Woodstar (*Eulidia yarrellii*) and the non-native and recently-arrived Peruvian Sheartail (*Thaumastura cora*), which expanded its range from Peru into Chile during the 1970s. Several factors suggest that these species may be hybridising. We here describe a set of microsatellite loci which prove to be a powerful tool in detecting F1 hybrids and backcrosses between the two species. These loci will be an invaluable tool for future research to ascertain the degree of hybridisation that is occurring between the two species and to devise appropriate conservation strategies.

Keywords *Eulidia yarrellii* · Hummingbirds · Hybridisation · Microsatellites · *Thaumastura cora*

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Zusammenfassung

Die Anwendbarkeit von Microsatelliten Loci für exakte Hybrid Identifizierung einer neuen Überlappungszone zwischen einer bedrohten und einer eingewanderten Kolibri-Art

Hybridisierung zwischen zwei Arten kommt häufig dann vor, wenn sich die Verbreitungsgebiete zweier nah verwandter Arten nach einer Periode geografischer Isolation überlappen. Aus der Sicht des Artenschutzes kann das Auftreten solcher Hybridisierungen die Überlebensfähigkeit besonders jener der beteiligten Arten vermindern, die bereits wegen anderer Vorgänge, wie menschlich bedingter Populationsrückgang, bedroht sind. Hybride rechtzeitig aufzuspüren und genau zu identifizieren, ist daher für Schutzstrategien entscheidend. Im Norden von Chile entstand eine Kontaktzone zwischen der gefährdeten Yarrellelfe (*Eulidia yarrellii*) und dem bis dahin nicht vorkommenden und jüngst angekommenen Corakolibri (*Thaumastura cora*), der sein Verbreitungsgebiet von Peru in den 70er Jahren des vorigen Jahrhunderts nach Chile

ausdehnte. Einige Faktoren sprechen für eine mögliche Hybridisierung der beiden Arten. Wir beschreiben hier einen Satz von Mikrosatelliten-Loci und belegen, dass diese ein mächtiges Werkzeug für die Feststellung von F1-Hybriden und Rückkreuzungen darstellen. These Loci werden ein unschätzbares Hilfsmittel für die künftige Forschungsarbeit sein, um festzustellen in welchem Ausmaß die beiden Kolibriarten hybridisieren und um geeignete Schutzstrategien zu entwickeln.

Introduction

The distributional ranges of species constantly change over ecological and evolutionary time (e.g. Graham et al. 1996; Root et al. 2003; Hadlya et al. 2009). A direct consequence of the dynamic nature of species boundaries is that closely-related species often come into contact after a period of geographic isolation. When insufficient time has passed to promote pre-zygotic isolation between the species, cross-species matings and hybridisation can occur. The effect of hybridisation on species viability is variable, and can promote either reproductive isolation or speciation (Barton 2001). For example, benefits accrued from hybridisation can include higher fitness of hybrids (e.g. Grant and Grant 1992; Veen et al. 2001), or a superior immune system (e.g. Tompkins et al. 2006). Conversely, costs of hybridisation include lower fitness of hybrids (e.g. Lancaster et al. 2007; Muhlfeld et al. 2009).

In recent times, the frequency of recent contact zones between closely-related species has increased dramatically due to direct and indirect human-induced disturbances, including habitat modification, climate change and the deliberate introduction of species outside their natural distributional range (Strayer et al. 2006). Such hybridisation events can incur detrimental effects on the species viability, especially when those species are already threatened by other human-induced activities. Much research in conservation biology has therefore targeted hybridisation between non-native species and threatened native species (Huxel 1999; Fitzpatrick et al. 2010; Maschinski et al. 2010; Steeves et al. 2010). As the conservation of genetic diversity is one of the principal goals of conservation biology, the early detection of hybrids is crucial in maintaining the genetic integrity of endangered species.

Due to molecular advances, the genetic detection of hybrids has become relatively straightforward, relying on interspecies variation in nuclear loci. Many molecular techniques have been developed to detect hybrids, including the use of single nucleotide polymorphisms (e.g. Chatfield et al. 2010; Väli et al. 2010), amplified fragment length polymorphisms (e.g. Vallender et al. 2007; Sternkopf et al. 2010) and restriction fragment length polymorphisms (e.g. Gill 1997; Koutsogiannouli et al. 2010). By far the most common technique for hybrid detection is microsatellite genotyping, in part because appropriate microsatellite loci can typically be found with relative ease for any particular species. Most importantly, however, the high mutation rate of microsatellites results in often pronounced differences in allele frequencies between closely-related species. F1 and later generation hybrids can then be detected with a high degree of power using various admixture modelling techniques (e.g. Pritchard et al. 2000; Anderson and Thompson 2002).

The Chilean Woodstar (*Eulidia yarrellii*) is a bee hummingbird species endemic to northern Chile and, formerly, southern Peru. Once locally very common, the range and abundance of this species has diminished dramatically since the 1970s and is now restricted to a few fertile valleys within the Atacama Desert (refer to Estades et al. 2007 for map of the species' distribution). A recent census of all known populations in 2009 indicated that less than 400 individuals remain (Estades and Aguirre 2009). Along with increased agricultural activity in the region (which results in more intense habitat destruction and pesticide use), this drastic decline in population size corresponded with the arrival of the closely-related Peruvian Sheartail (*Thaumastura cora*), a bee hummingbird absent from Chile before the 1970s, but which is now rapidly expanding its population across northern Chile (Estades et al. 2007). The two species are morphologically very similar, with males differing predominantly in their tail morphology (as well as in song structure). Plumage differences between the females of the two species are even more subtle (Jaramillo et al. 2003). The possibility therefore exists that the Sheartails are negatively impacting on the viability of the Woodstar population either via resource competition or reproductive interference. Reproductive interference involves any interactions between species associated with their mating system that is caused by incomplete species recognition systems that can ultimately lead to species hybridisation (Seehausen 2004; Hochkirch et al. 2007). The likelihood that these two species are hybridising is increased due to (1) the close-relatedness of the two species (median raw distance amongst hummingbirds in the Bee clade based on NADH dehydrogenase subunit 2 gene = 0.085, IQR = 0.044–0.102; observed difference between Chilean Woodstar and Peruvian Sheartail = 0.0355; van Dongen, unpublished data), (2) the relatively high prevalence of interspecies matings amongst hummingbirds, even across different clades (e.g. Graves 2004, 2006, 2007a, b), and (3) the observation that hybridisation is more common in disturbed habitats and when one species is rare (Mayr 1963), as occurs in this system. The accurate detection of hybrids between the two species therefore forms a crucial component of future conservation management strategies. We here identify and characterise a set of ten microsatellite loci, isolated from other species, that

differ in allele frequencies between the Woodstars and Sheartails. We show that these loci are a powerful tool in the detection of F1 and later generation hybrids between the Woodstars and Sheartails.

Methods

Sample collection

We collected five pin feather samples from the head, back or breast of individuals of both species that were captured using mistnets in the fertile valleys of the Atacama Desert in northern Chile, during August 2008, and August to October 2010. For the present study, we aimed to only use samples collected from allopatric populations of both species to ensure that we had known pure individuals of both species. All the known populations of Chilean woodstars occur in Azapa valley (18°32′S, 70°10′W), Vitor valley (18°49′S, 70°08′W), Codpa valley (18°50′S, 69°45′W) and Camarones valley (19°01′S, 69°52′W). With the exception of Azapa valley, Peruvian Sheartails were very rare or absent from these valleys at the time of the study. Both species are, however, present in Azapa valley, although the distribution of the Chilean Woodstar is very patchy, being largely absent from the lower section of the valley (Estades and Aguirre 2009). Sheartail samples used for the present study were therefore collected from those areas in Azapa valley where Woodstars had not been sighted for several years ($n = 44$). Overall, Chilean Woodstars were captured in Vitor valley ($n = 35$), Codpa valley ($n = 6$) and Camarones valley ($n = 4$). The identity of the individuals used in this study as pure species was later confirmed via admixture analyses (see below). We additionally obtained samples from a museum collection, consisting of muscle samples from six Peruvian Sheartails captured in Peru, where the Chilean Woodstars do not occur (Supplementary Table 1).

Laboratory analyses

DNA extraction of pin feather samples was conducted using a QIAGEN QIAamp DNA Micro Kit, following the protocol for extracting DNA from nail clippings. DNA from tissue samples was isolated using a QIAGEN DNeasy Blood and Tissue Kit. The DNA concentration of all samples was estimated using a Nanodrop 2000c spectrophotometer (Thermo Scientific), and diluted to approximately 25 ng/µl. We tested microsatellite markers previously developed for the Red-billed Streamertail (*Trochilus polytmus*; Tro2, Tro3, Tro4, Tro5, Tro6, Tro10, Tro11, Tro13, Tro15, Tro17, Tro18, Tro19, Tro20, Tro21, Tro23; Lance et al. 2009), as well as a range of other microsatellite markers developed for other non-hummingbird species: Hru 3, 5, 6, 7 and 8 (Primmer et al. 1995), Hru10 (Primmer et al. 1996), Escμ6 (Hanotte et al. 1994), Mcyμ4 (Double et al. 1997), Ltr6 (McDonald and Potts 1994), Aar4 (Hansson et al. 2000), Ase18 (Richardson et al. 2000) and Rri2, 3, 4, 5, 8 and 9 (van Dongen et al. 2010). PCR was performed in 10.0-µl reaction volumes containing a forward primer (labelled with a Beckman Coulter dye: D2, D3 or D4) and reverse primer (0.2 mM each), 0.04 units of AmpliTaq DNA polymerase (Applied Biosystems), 1× reaction buffer (Applied Biosystems), 2.5 mM $MgCl_2$ (Applied Biosystems), 0.2 mM dNTPs and approximately 25 ng of genomic DNA. PCRs were run on a Biometra T1 thermocycler. An initial denaturation step (94°C, 5 min) was followed by 36 cycles of 30 s at 94°C, 30 s at the locus specific annealing temperature (Table 1), 60 s at 72°C, and a final extension step for 10 min at 72°C. We assessed the allele frequencies of the loci that consistently produced strong single PCR products. PCR products were electrophoresed on a Beckman Coulter CEQ 8000 automated sequencer and fragment sizes were estimated using the Beckman Coulter CEQ 8000 fragment analysis software. All polymorphic loci were used in downstream analyses, as were monomorphic loci where allele sizes differed between the two species.

Data analysis

We implemented Cervus 3.0.3 (Kalinowski et al. 2007) to characterise the loci, including number of alleles and observed and expected heterozygosity. Homoplasy in the microsatellite alleles may occur when comparing allelic variation between distantly-related species. However, given the close relatedness of the Woodstar and Sheartail (see above) and the relatively high number of loci used, the probability of homoplasy in our set of loci is reduced. To assess the assignment power of our set of microsatellite loci, we first generated various hybrid categories using HybridLab 1.0 (Nielsen et al. 2006), which simulates interspecific hybrids based on the genotypes of known pure species individuals. We were interested in identifying five categories of species affiliation: pure Sheartails, pure Woodstars, F1 hybrids, Sheartail backcrosses (F1 × Sheartail) and Woodstar backcrosses (F1 × Woodstar). We did not test the assignment power of other hybrid categories, such as F2 or other later-generation crosses as, given the probable scarcity of hybrids in the wild (van Dongen, unpublished data), the probability of such matings occurring is likely to be very low. We therefore simulated 50 individuals of each of the three hybrid categories.

NewHybrids (Anderson and Thompson 2002) was used to assess the assignment power of our microsatellite loci. NewHybrids calculates Bayesian posterior probabilities (q_n) that individuals fall within particular user-defined hybrid categories. In addition to NewHybrids, Structure (Pritchard et al. 2000) is also commonly used to detect hybrids, which differs

Table 1 Characterisation of microsatellite loci amplified in Chilean Woodstars (*Eulidia yarrellii*) and Peruvian Sheartails (*Thaumastura cora*)

Locus	Repeat motif in clone	Primer sequence (5′–3′)	Species	T_a	n	n_a	bp	H_o	H_e	KLD	Reference
Tro2	$(AAC)_7$	F: AGTCTGAGCCCAATACTGCC	Woodstar	55	45	1	171	0.00	0.00	7.4	Lance et al. (2009)
		R: CGAGGAATGGAGTAGGCG	Sheartail	55	48	3	161–171	0.40	0.35		
Tro5	$(AGAT)_{13}$	F: CCACCTGTTCTCTGTTGGC	Woodstar	60	45	4	198–206	0.60	0.51	4.5	Lance et al. (2009)
		R: TGGACCTGTCAGTTGAGGC	Sheartail	60	48	6	184–214	0.58	0.68		
Tro6	$(AAAC)_5$	F: TGAACTCACAGCATCTTTGCTC	Woodstar	60	45	1	423	0.00	0.00	17.4	Lance et al. (2009)
		R: TTTGCACTGGAAGAAACACC	Sheartail	60	50	4	437–443	0.52	0.51		
Tro11	$(AC)_{12}$	F: TTTGCATGGCGTCTCGTG	Woodstar	55	45	1	263	0.00	0.00	10.4	Lance et al. (2009)
		R: AGACAGGAACATAAACATCTCTGC	Sheartail	55	48	3	261–265	0.50	0.54		
Tro13	$(AC)_4CG(AC)_8$	F: CACTGACCCAAGACATCAAAGG	Woodstar	55	45	1	289	0.00	0.00	15.4	Lance et al. (2009)
		R: GTCCTGGGCATCTGCAAAC	Sheartail	55	50	2	283–287	0.20	0.20		
Tro15	$(AGAT)_7(AGAC)_8$	F: GGTTCCTGCAAGCACAAGG	Woodstar	55	45	1	267	0.00	0.00	9.8	Lance et al. (2009)
		R: CCGCTGCTTCTGAATGGTG	Sheartail	55	48	10	267–303	0.85	0.77		
Tro21	$(ATCT)_{10}$	F: AGAGGCTTAGGAGGGAGGG	Woodstar	60	44	5	348–364	0.30	0.37	13.3	Lance et al. (2009)
		R: CCCAGATCCTTTGCTGTGC	Sheartail	60	50	7	344–372	0.82	0.78		
Tro23	$(AAAC)_5$	F: TGTGCATATACAAGCAAGCAC	Woodstar	55	45	1	323	0.00	0.00	3.9	Lance et al. (2009)
		R: GACTGCACTGGAAATCTCACG	Sheartail	55	50	3	315–327	0.64	0.64		
Hru3	$(CA)_{13}$	F: CACTGGCTCTAGGCTGTCATC	Woodstar	55	44	6	221–246	0.73	0.78	53.9	Primmer et al. (1995)
		R: CTGTCCCATGTCAGGCCAGTC	Sheartail	56	47	27	195–291	0.82	0.77		
Hru6	$(AAAG)_{17}(AG)_2(AAAG)_2$	F: GCTGTGTCATTTCTACATGAG	Woodstar	50	44	1	345	0.00	0.00	12.9	Primmer et al. (1995)
		R: ACAGGGCAGTGTTACTCTGC	Sheartail	50	48	1	348	0.00	0.00		

The repeat motif of each locus is that as quoted in the original publication. In addition, the following variables are listed for each locus and species: the primer annealing temperature (T_a), number of individuals tested (n), number of alleles (n_a), allele size range in base pairs (bp), observed (H_o) and expected (H_e) heterozygosity, the Kullback–Leibler divergence (KLD) and the original publication where the loci were described

from NewHybrids in its analytical methods, thus potentially providing conflicting results. In practice, however, the two programmes typically provide very similar results, and appropriate marker selection appears to be a lot more important than analysis method (Vähä and Primmer 2006; Gomes et al. 2009; Neaves et al. 2010; Väli et al. 2010). We therefore only use NewHybrids here. Following Vähä and Primmer (2006), we described the hybrid detection power of our set of loci using detection efficiency, accuracy and overall performance. *Efficiency* describes the proportion of individuals in a group that were correctly identified (i.e. the number of individuals correctly identified within a group divided by the true number of individuals within that group). *Accuracy* describes the proportion of individuals assigned to a particular group that actually belong to that group (i.e. number of individuals assigned to a group divided by number of individuals that actually belong to that group). As efficiency often declines as accuracy increases, a trade-off between the two is often required when selecting an appropriate minimal q_n value. The overall performance of a set of markers can therefore be informative, calculated as the efficiency multiplied by the accuracy for each particular group. We also calculated efficiency and accuracy for individuals that were of general hybrid ancestry (i.e. F1 and both backcrosses grouped). Finally, NewHybrids also calculates Kullback–Leibler divergence values for each locus, which describes the information content of the locus (i.e. the higher the divergence value, the more informative the locus in the identification of hybrids; Kullback and Leibler 1951; Anderson and Thompson 2002).

Our principal aim was to gain a comprehensive overview of the strength of our loci. We therefore tested the efficiency and accuracy of the loci over five values of q_n (0.5, 0.6, 0.7, 0.8 and 0.9). Individuals with q_n values less than the threshold remain unassigned. Testing over a wide range of q_n values was additionally useful to compare our results with that in published literature, as past studies have used a wide range of minimum q_n values (see, for example, Gomes et al. 2009; Colliard et al. 2010; Coscia et al. 2010; Hird et al. 2010; Huff et al. 2010; Neaves et al. 2010). We conducted 10 independent runs using the newly generated genotypes for the three hybrid categories for each run (i.e. 500 individuals within each hybrid category over the 10 runs). Each run lasted 500,000 sweeps after a burn-in of 50,000 using Jeffreys priors. Conducting runs using uniform priors instead of Jeffreys priors did not greatly vary the results.

Results

Microsatellite marker characteristics

We identified ten microsatellite markers that differed between the pure species in allele frequencies and were therefore appropriate for the detection of hybrids (Table 1; Supplementary Figure 1). No locus differed significantly from Hardy–Weinberg Equilibrium (all $P > 0.05$ after Bonferonni corrections). Using Genepop (Rousset 2008), we found no evidence of linkage disequilibrium between the loci (all $P > 0.05$). Based on the Kullback–Leibler divergence values, the most informative locus for detecting hybrids was Hru3 (KLD = 53.9), while the least informative was Tro23 (KLD = 3.9; Table 1).

We tested whether DNA samples extracted from feathers had higher rates of allelic drop out than those extracted from fresh tissue samples, using individual heterozygosity as an estimate of drop out rates (i.e. the number of heterozygous loci divided by total number of loci typed for a given individual). We found no evidence for higher allelic drop-out in feather samples, for both woodstars across three variable loci (mean heterozygosity for feather samples: 0.56 ± 0.23 SD, $n = 39$; for tissue samples: 0.44 ± 0.18 SD, $n = 6$; Mann–Whitney U test: $U = 83.0$, $P = 0.24$) and sheartails across eight variable loci (mean heterozygosity for feather samples: 0.64 ± 0.14 SD, $n = 34$; for tissue samples: 0.65 ± 0.17 SD, $n = 16$; ANOVA: $F_{1,48} = 0.092$, $P = 0.76$).

Hybrid detection performance

As expected, the assignment efficiency and accuracy of the ten microsatellite loci by NewHybrids depended on which threshold q_n value was used, with higher q_n thresholds decreasing the assignment efficiency, while simultaneously increasing the assignment accuracy (Table 2). Between 83 and 100% of all individuals had q_n values greater than the threshold and were therefore successfully assigned to a specific group, regardless of whether or not this assignment was correct. Overall, the detection efficiency of the ten microsatellites was very high, ranging from 77 to 100%, while accuracy estimates ranged from 92 to 100%.

The detection of pure Woodstars was the most reliable (efficiency = 96.4–100%), followed by pure Sheartails (efficiency = 87.8–100%), while Sheartail backcrosses were the most difficult to accurately categorise (efficiency = 77.0–89.0%). Importantly, when all three hybrid categories were combined, the accuracy of detection of hybrids was always 100% (i.e. pure species were never incorrectly assigned as hybrids), although hybrids were occasionally incorrectly classified as pure species (e.g. in 6.1% of cases for $q_n = 0.5$). Overall therefore, based on our ten microsatellite loci, when NewHybrids assigns an individual as a hybrid, this is very likely to be a correct assignment, although the true number of hybrids within a population may be slightly underestimated due to some hybrids being classified as pure species.

Table 2 Characteristics of the hybrid detection power of the ten microsatellite loci under various minimum q_n values in NewHybrids

Class	$q_n = 0.50$				$q_n = 0.60$				$q_n = 0.70$				$q_n = 0.80$				$q_n = 0.90$			
	ASS	EFF	ACC	PERF	ASS	EFF	ACC	PERF	ASS	EFF	ACC	PERF	ASS	EFF	ACC	PERF	ASS	EFF	ACC	PERF
Woodstar	1.000	1.000	0.962	0.962	1.000	1.000	0.968	0.968	0.996	0.996	0.972	**0.972**	0.964	0.964	0.975	0.940	0.964	0.964	0.980	0.945
Sheartail	1.000	1.000	0.924	0.924	1.000	1.000	0.933	0.933	0.994	0.994	0.941	**0.935**	0.972	0.972	0.957	0.930	0.878	0.878	0.952	0.834
F1	1.000	0.992	0.947	0.939	1.000	0.992	0.950	0.942	0.994	0.986	0.959	**0.945**	0.964	0.962	0.966	0.929	0.910	0.908	0.962	0.873
Woodstar backcross	1.000	0.936	0.992	**0.928**	0.972	0.916	0.991	0.908	0.952	0.904	0.991	0.895	0.934	0.894	0.998	0.892	0.902	0.868	0.998	0.866
Sheartail backcross	1.000	0.890	1.000	**0.890**	0.968	0.870	1.000	0.870	0.926	0.844	1.000	0.844	0.890	0.830	1.000	0.830	0.834	0.770	1.000	0.770
Hybrid	1.000	0.939	1.000	**0.939**	0.980	0.926	1.000	0.926	0.957	0.911	1.000	0.911	0.929	0.895	1.000	0.895	0.882	0.849	1.000	0.849

Variables are: percentage of individuals assigned to a group regardless of whether this assignment was correct (*ASS*), detection efficiency (*EFF*), detection accuracy (*ACC*) and overall performance of the loci (*PERF*). Refer to main text for a detailed explanation of each variable. The 'hybrid' class represents all hybrid individuals grouped together (i.e. F1 plus backcrosses). Performance values in bold represent the q_n value for which performance is maximum for each class

Discussion

We have identified a set of ten microsatellite loci that detect hybrids between the endangered Chilean Woodstar and the non-native recently-arrived Peruvian Sheartail. Using these loci, we were able to correctly assign all pure species and a very high proportion of F1 and Woodstar backcrosses. The only hybrid category where assignment confidence was lower was for Sheartail backcrosses. For this group, a relatively high proportion of individuals were misclassified as pure Sheartails (e.g. at $q_n = 0.5$, 8% of Sheartail backcrosses were incorrectly assigned as pure Sheartails, while at $q_n = 0.9$, 5% were assigned as pure species). However, after combining all hybrids categories (F1 and both backcrosses), we could always detect hybrids with an accuracy of 100% and an efficiency typically higher than 90%. Therefore, when a hybrid is detected in a real population, we can know with certainty that it is indeed a hybrid (i.e. high accuracy), although in some cases a backcross may be assigned as a F1 hybrid and vice versa. Of principal importance, however, is the detection of hybrids within a given population, while the exact identification of the hybrids (F1 or later generation) is of secondary importance (to ascertain, for example, whether hybrids are fertile and can successfully reproduce).

The efficiency and accuracy of our microsatellite loci depended on the q_n threshold used in the NewHybrids analysis. There appears to be no consensus in which threshold value to use and q_n values used in past studies vary widely, ranging from 0.5 (e.g. Bittner et al. 2010; Colliard et al. 2010; Väli et al. 2010) to 0.8 (e.g. Hird et al. 2010). In order to select the appropriate q_n threshold, it is necessary to document the performance of the loci over a range of threshold values. The selection of an appropriate q_n threshold typically represents a trade-off between efficiency and accuracy—increasing q_n increases detection accuracy but decreases efficiency. Vähä and Primmer (2006) suggest that the threshold selected should depend on the purpose of the study and also on the characteristics of the markers and samples. They state that, for conservation studies detecting hybrids, a high hybrid detection efficiency is preferable at the expense of misclassifying some pure individuals. However, this approach may not be entirely optimal due to the strong implications of hybrid detection on conservation management strategies. If accuracy is sacrificed for efficiency, the risk of committing a Type I error increases. In an extreme case scenario, hybrids may therefore be 'detected' in population containing only pure species, thus erroneously leading to the conclusion that a hybrid prevention strategy be implemented. In our samples, increasing q_n from 0.5 to 0.9 had little or no effect on the accuracy of detection of all hybrid categories (increases in accuracy ranged from 0 to 1.5%). The increase in accuracy

of detection of pure Woodstars (1.8%) and Sheartails (2.8%) were slightly higher, but still low. Based on these data and the purposes of this study, using a q_n of 0.5 is optimal, therefore increasing detection efficiency with little effect on overall accuracy. Overall, however, for maximum accuracy, genetic data should be combined with morphological data, at least to aid in the differentiation between pure species and F1 hybrids (e.g. Graves 2004, 2006, 2007a, b). These analyses can additionally be combined with the sequencing of mitochondrial genes of identified hybrids to infer the species identification of the parents (see, for example, McGuire et al. 2007 for information on mitochondrial genes in hummingbirds) and hence contribute to the conservation of the endangered Chilean Woodstar.

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