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# Behavioural and genetic interactions between an endangered and a recently-arrived hummingbird

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**Abstract** The invasion or expansion of non-native species into new geographic areas can pose a major threat to the conservation of biodiversity. These threats are augmented when the newly-arrived species interacts with native species that are already threatened by other ecological or anthropogenic processes. Potential interactions can include both competition for scarce resources and reproductive interference, including hybridisation. Understanding the dynamics of these interactions forms a crucial component of conservation management strategies. A recent contact zone occurs in the north of Chile between the endangered Chilean woodstar (*Eulidia yarrellii*) and the closely-related and recently-arrived Peruvian sheartail (*Thaumastura cora*), which expanded its range from Peru into Chile during the 1970s. We characterised the interactions between the species by combining population size estimates with molecular, morphological and behavioural data. We show that a low degree of hybridisation, but not introgression, is occurring between the two species. Despite interspecific morphological similarities, behavioural observations indicate that food niche overlap between the species is relatively low, and that the dietary breadth of sheartails is larger, which may have aided the species' range expansion. Finally, woodstars dominate the sheartails in male–male territorial interactions. However, potentially increased energetic costs for woodstars associated with frequent territorial chases and courtship displaying with sheartails may exacerbate the effects of other threats on woodstar viability, such as human-induced habitat modification. This study highlights the value of implementing multidisciplinary approaches in conservation biology to gain a more complete understanding of interactions between recently-arrived and endangered species.

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## Introduction

A major threat to the conservation of biodiversity is the invasion or expansion of non-native species into areas inhabited by endangered species. Recently-arrived species can threaten native species in areas of sympatry (known as contact zones) via various processes, including competition for scarce resources or reproductive interference and hybridisation (Allen et al. 2004; Strayer et al. 2006; Muñoz-Fuentes et al. 2007; Steeves et al. 2010). In recent times, the frequency of such contact zones 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). The likelihood that a recently-arrived species has a negative impact on a native species (and vice versa) is increased when the species are closely-related or occupy similar ecological niches (e.g. Edmands 2002; MacDougall et al. 2009). Moreover the negative effects are exacerbated when the native species is already threatened by other human-induced activities (Allendorf et al. 2001). In the current study, we examine the interactions between a recently-arrived hummingbird and an endangered native hummingbird in a recent contact zone.

The processes driving dominance hierarchies among species have long been of interest to conservation biologists and ecologists (e.g. Preston 1948; McNaughton and Wolf 1970; MacDougall et al. 2009). Theory suggests that species that are able to establish in new environments may dominate native species via three mechanisms, which need to be disentangled to formulate appropriate conservation strategies involving recently-arrived or invasive species. First, the apparent dominance of one species over another may be unrelated to direct interactions between the species, but instead be due to an environmental change which favours the expansion of one species and suppresses the abundance of the second (e.g. anthropogenic habitat modifications, MacDougall and Turkington 2005; King and Tschinkel 2006).

Alternatively, the recently-arrived species may directly suppress the abundance of the native species

via either resource competition or reproductive interference (MacDougall and Turkington 2005; Hochkirch et al. 2007; Sol et al. 2012). Resource competition can occur when there is high niche overlap between the species, for example when similar food resources are exploited that are not abundant in the environment (Petren and Case 1996; Byers 2000; Fox 2002; Vogel and Pechmann 2010). In contrast, reproductive interference involves any interactions between species associated with their mating system that are caused by incomplete species recognition systems (Seehausen 2004; Hochkirch et al. 2007). It can result in wasted energy and gametes courting and mating with heterospecifics, and can ultimately lead to hybridisation. These hybridisation events can have diverse effects, either promoting reproductive isolation between parental species or promoting speciation of admixed individuals (Barton 2001). If hybrids are fertile and do not have lower fitness, then introgression can occur and lead to hybrid swarms (Allendorf et al. 2001). From a conservation perspective, as hybridisation is more common in disturbed habitats and when one species is rare (Mayr 1963), it is especially problematic for endangered species and can ultimately result in species declines (Allendorf et al. 2001). Therefore, the early detection and characterisation of reproductive interference and hybridisation is crucial in maintaining the genetic integrity of endangered species.

The Chilean woodstar (*Eulidia yarrellii*) is a 'bee' hummingbird species endemic to northern Chile and southern Peru (Jaramillo et al. 2003; Schulenberg et al. 2007). The species was first sighted in Peru in the early 1980 s (Parker 1982), but has not been recorded in this region in more recent times and is now considered as officially extinct in this country (Cruz 2006). In Chile, the Chilean woodstar was once locally very common, but its range and abundance has diminished dramatically since the 1970s and the species is now listed as Endangered on the IUCN Red List of Threatened Species (Estades et al. 2007). Along with increased agricultural activity throughout its Chilean distribution, this drastic decline coincided with the arrival of the Peruvian sheartail (*Thaumastura cora*), a closely-related bee hummingbird (McGuire et al. 2009). The sheartail was absent from Chile before the 1970s, but is now rapidly expanding its population across northern Chile (Estades et al. 2007). The two species are morphologically very similar, with males differing

predominantly and conspicuously in their tail morphology (as well as in song structure: C. Clark, unpublished data). Plumage differences between females of the two species are even more subtle (Jaramillo et al. 2003). Sheartails may therefore be reducing the viability of the woodstar population either via resource competition (for example, if they share a similar foraging niche) or reproductive interference (if mistakes in species recognition occur). We therefore integrate information on abundance, genetics, morphology and behaviour of both hummingbirds to quantify the nature of the interactions between the two species and to ascertain whether the population crash in woodstars could indeed be caused by the sheartails. Specifically, we first present abundance estimates of both species spanning an 8-year period. Then, using molecular methods, we quantify the degree of genetic similarity between the species and test for hybridisation and introgression. We next document morphological differences between the species and finally quantify the degree of niche overlap and territorial aggression between the species. We hope that these data will form the basis for future conservation management plans involving the Chilean woodstar and Peruvian sheartail in Chile.

## Materials and methods

Field-based work

*Study sites*

All known populations of Chilean woodstars occur in the Atacama Desert in northern Chile and are restricted to four fertile valleys—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: refer to Estades et al. 2007 for a map of the region). Azapa valley experiences heavy agricultural practices, with olive and tomato plantations dominating and relatively little native vegetation remaining. Fewer anthropogenic disturbances have occurred in Vitor valley, resulting in larger tracts of native vegetation remaining, although olive plantations are still common. Codpa valley is a high-altitude extension of Vitor valley (1,800 m above sea level) and contains a mix of native vegetation and fruit trees. Finally, Camarones valley experiences high levels of

salinity resulting in few crops or plantations, but more cattle grazing.

*Abundance estimates*

We estimated abundance of both species using fixed-radius point-counts as outlined in detail in Estades et al. (2007). Briefly, at each point we recorded all birds seen and heard within and outside a 30 m radius during a three-minute period. Following Bibby et al. (1992) records outside the 30 m radius were used to correct density estimates for the effect of detectability using the equation:

$$Density = \left(Ln\left(\frac{n}{n_2}\right)\right)\left(\frac{n}{m\pi r^2}\right),$$

where $n$ is the total number of birds counted, $n_1$ is the number of birds counted within the radius, $n_2$ is the number of birds counted beyond the radius (such that $n_1 + n_2 = n$), $m$ is the total number of point counts and $r$ is the fixed radius. Counts were conducted at 'sampling stations', which comprised a 200 m radius circle within which six points were randomly placed. A team of two people sampled all six points within a sampling station within 20 min. We conducted counts at a total of 201 sampling stations (1,206 point-counts) spread throughout Azapa (110 stations), Vitor (35 stations) and Camarones (22 stations) valleys, as well as two neighbouring valleys where woodstars and sheartails have not been observed in recent times (Lluta valley: 20 stations; Camiña valley: 14 stations). As Codpa valley is a high altitude extension of Vitor valley, we considered these two areas to constitute one valley which we here refer to as Vitor valley. The first census was conducted in 2003 after which censuses were conducted annually between 2006 and 2011. All censuses occurred before 1200 during September or October of each year, which corresponds to the breeding period for both species (C. Estades, unpublished data).

Population size estimates of both species were conducted separately for each valley. Due to the relative scarcity of both species, we could not normalise our data (i.e. at many stations no individuals were detected). We therefore estimated population size using a Monte Carlo resampling approach (Manly 1997) based on a spatially explicit simulation model. In this model we replicated the same sampling scheme

used in the field (i.e. the exact location of sampling stations in a GIS map) and simulated our real bird counting procedure (i.e. six sampling plots within each 200 m radius station). We then simulated a series of scenarios in which we varied the number of virtual individuals of each species of hummingbird randomly allocated to each valley. We assumed that birds were detected with certainty within the 30 m radius, with probability 0.2 between 31 and 70 m, and that no birds were recorded beyond 70 m (C. Estades, unpublished data). We simulated 10,000 replicates for each scenario (i.e. for a total population size of 50, 100, 150 etc. individuals.). Finally, for each scenario we recorded the frequency of simulations that produced the same result as the real sampling and plotted these frequencies against the population number in each scenario to produce a probability distribution for the total population size (for an example see Estades et al. 2007). Confidence intervals were calculated based on the values falling within the 5th and 95th percentiles of the data. The mean of the distribution was used as the estimate of the total population size. Refer to Estades et al. (2007) for full details.

To test whether woodstar and sheartail abundances were associated between years, we regressed abundance estimates for each species within a year, including valley identity ($n = 3$ valleys) as a covariable. As it is possible that an increase in abundance of one species has a negative impact on the other species only in the subsequent year, we also correlated woodstar abundances with sheartail abundances of the previous year, and vice versa. Our sample sizes depended on how many years of population estimates we had for both species in each valley. For example, for the abundance correlations using data for the same year for each species, we had data for seven years for both Azapa and Vitor valleys and five years for Camarones valley. For the analyses involving abundances in subsequent years, our sample sizes were lower because we could not include our estimates from 2003.

### Hummingbird capture and morphometric measures

We captured woodstars and sheartails during August 2008, and from August to October 2010. Hummingbirds were passively captured in fine-meshed mistnets erected in close proximity to flowering plants where they were observed to be feeding. Captured individuals were fitted with an aluminium ring containing a unique identification number. Morphological measurements included mass (g), bill length (mm), folded wing length (mm) and lengths of the five tail feathers from R1 (the innermost feather) to R5 (the outermost feather). We also collected up to five pin feathers for subsequent genetic analyses.

We used these data to test whether (1) morphological differences existed between woodstars and sheartails and (2) these morphological differences could be used to discriminate species and identify potential hybrids. Analyses of variance were used to test for interspecies differences in morphology, unless Shapiro–Wilk tests revealed that the data were non-normal (e.g. R1 and R2 for females). In these cases, we tested for differences using non-parametric Mann–Whitney $U$ tests. Secondly, discriminant function analyses (DFA) were used to identify the combination of morphological variables best separating the species and to identify potential hybrid individuals. All morphological measures outlined above were incorporated into the DFA. Discriminant scores for the extracted functions were then saved and the scores of the first two functions for all species and sexes were displayed on the same plot to visualise group differences. All non-genetic statistical analyses were conducted using SPSS 17.0 (SPSS, Chicago, Illinois, USA).

### Behavioural observations

In order to determine niche sharing and dominance hierarchies between the two species, we conducted behavioural observations at patches of flowering vegetation. Flower patches were identified where at least one species was observed to be feeding. Observations occurred at 23 patches including 11 in Azapa valley and 12 in Vitor valley (83 observations in total; mean distance between each patch and nearest patch = $648 \pm 1,646$ SD m, range = 45–7,371 m). We also included the sympatric oasis hummingbird, *Rhodopis vesper*, in our observations as this species is the most common hummingbird in the study areas.

Observations were carried out between September and October 2009. For each flower patch we conducted two 30-min observations in the morning (between 0700 and 1000) and two in the afternoon (between 1600 and 1900) and then combined the data. Depending on the number of sexes and species

observed at the patch (minimum: 1 and maximum: 6), between one and three observers conducted the observation. An observation consisted of an observer dictating activities of the focal species onto a digital recorder including (1) number of feeds (one feed was defined as the action of inserting and removing the bill from a flower), (2) species of flower at which the focal species fed, (3) number of times the focal species chased or was chased by another hummingbird and (4) the sex and species of the chasing/chased humming-bird. These latter two variables were used to elucidate dominance hierarchies between the various species and sexes (following Altshuler et al. 2004), by using Chi square tests to elucidate whether the direction of chases between each dyad differed significantly from an even ratio. When more than one individual of a species/sex category was simultaneously present on the flower patch, we followed the first one that we sighted. These observations are assumed to approximate the true feeding patterns of both species, although some variation was not captured by our data (for example, sheartails were observed to feed on additional floral species outside the observation period).

To assess the degree of dietary overlap between the two species, we calculated Morisita's index of similarity (Horn 1966):

$$C = \frac{2 \sum x_i y_i}{\sum x_i^2 + y_i^2},$$

where $x_i$ and $y_i$ are the proportional use of each nectar source, $i$, by hummingbird species $x$ and $y$, respectively. Compared to other indices, Morisita's index provides an accurate and unbiased estimate of overlap, especially for low samples sizes (Smith and Thomas 1982). Morisita values above 0.6 are considered to represent significant niche overlap (Zaret and Rand 1971). Interspecific differences in diet breadth were estimated using Levins (1968) modification of the Simpson index, whereby Food Niche Breadth (FNB) $= \frac{1}{\sum p_i^2}$, where $p_i =$ the proportion of prey type $i$ in the diet. The index ranges from 1 to N (the number of flower species included in the analysis) and higher values correspond to a broader diet. As different areas where we sampled may vary in the number of nectar sources available, we calculated a standardised index of diet breadth that is independent of the number of flower species included i.e. $FNB_{ST} = \frac{B_{obs} - B_{min}}{B_{max} - B_{min}}$, where

$B_{obs} =$ observed FNB, $B_{min} = 1$, and $B_{max} =$ the number of prey types used in computing $B_{obs}$ (Marti et al. 1993).

### Genetics work

For hybrid detection, we only used samples obtained from within the Chilean study areas because, due to apparent low sheartail immigration rates from Peru to Chile (W. van Dongen, unpublished data), hybrid individuals would unlikely have parents from the Peruvian population. In contrast, to avoid underestimation of introgression levels if hybridisation has already been occurring for many years within the Chilean sites, we obtained museum tissue samples from 18 Peruvian sheartails captured in Peru, where the Chilean woodstars do not occur (Supporting Information, Table S1). These individuals are assumed not to have been subject to introgression with woodstars.

#### Microsatellite analyses

In order to detect hybrid individuals within our study population, we genotyped all woodstars and sheartails at 10 microsatellite loci as outlined in van Dongen et al. (2012). All loci were in Hardy–Weinberg equilibrium and we detected no evidence of linkage disequilibrium. van Dongen et al. (2012) have shown that these loci represent a powerful tool for the detection of F1 hybrids and woodstar and sheartail backcrosses.

#### DNA sequencing

To investigate introgression, we amplified a total of 3141 base pairs from the two mitochondrial genes, NADH dehydrogenase subunit 2 (ND2: 1041 bp) and cytochrome oxidase I (COI: 694 bp), and one nuclear gene, intron 7 of beta fibrinogen (βFib: 1,406 bp). In addition, we amplified an additional nuclear gene (intron 5 of adenylate kinase: AK1) for our estimates of genetic distances between the species (see below). Laboratory protocols for the amplification and sequencing of these genes are available as Supporting Information (S2). All unique sequences were deposited in GenBank under Accession numbers JQ025410–JQ025435.

## Genetic analyses

To estimate relatedness between the woodstars and sheartails, we calculated the genetic distance between woodstars and sheartails for each gene, and compared these distances to mean distances calculated for North and South American bee species. Choice of species used in the analysis was based on the availability of gene sequences on GenBank (refer to Supporting Information, S3, for species used). Genetic distances among sequences were calculated according to the Jukes-Cantor Model, the simplest way to correct for multiple substitutions at the same site (Jukes and Cantor 1969). To estimate the extent of genetic differentiation between the two species, we calculated $F_{ST}$ based on the ten microsatellite loci and tested for significance using an analysis of molecular variance in GENALEX 6.4 (Peakall and Smouse 2006).

Using the data from the ten microsatellite loci, NewHybrids (Anderson and Thompson 2002) and Structure (Pritchard et al. 2000) were implemented to determine the likely species affiliation of our samples. We were interested in identifying five categories of species affiliation: pure sheartails, pure woodstars, F1 hybrids, sheartail backcrosses (F1 $\times$ sheartail) and woodstar backcrosses (F1 $\times$ woodstar). Protocols for NewHybrids are outlined in van Dongen et al. (2012), while those for Structure are outlined in the Supporting Information (S4). In order to ascertain the degree of introgression that is occurring between the woodstars and sheartails, we created haplotypes networks to determine whether haplotype sharing occurs between the species. Networks were created using TCS 1.2 (Clement et al. 2000) which implements statistical parsimony and 95 % confidence intervals.

# Results

## Abundance estimates

Figure 1 outlines population trends of both species in the three valleys. Sheartails were very common in Azapa valley, with the population appearing to be stable. Woodstars experienced a population crash in Azapa in 2009 and only approximately 250 individuals remained in 2011. Sheartails were absent from Vitor valley until as recently as 2007, and have never been recorded in this valley in large numbers. Woodstars are much more abundant in this valley than sheartails.

Lastly, both species were absent in Camarones valley in 2003 and were first detected there in 2008. The woodstars appear to have established a stable population in this valley. In contrast, sheartail abundances appeared to have diminished after 2009. Between valleys, we found no correlation between woodstar and sheartail abundances across years (Effect on woodstar abundance: valley identity: $F_{2,15} = 1.71$, $p = 0.215$, sheartail abundance: $F_{1,15} = 0.39$, $p = 0.542$). We also detected no relationship between abundances of woodstars with sheartail abundances of the previous year and vice versa (Effect on woodstar abundance: valley identity: $F_{2,9} = 1.24$, $p = 0.334$, sheartail abundance in previous year: $F_{1,9} = 1.01$, $p = 0.341$; Effect on sheartail abundance: valley identity: $F_{2,9} = 186.9$, $p < 0.001$, woodstar abundance in previous year: $F_{1,9} = 0.45$, $p = 0.518$).

## Species relatedness

Based on two mitochondrial and two nuclear genes, the genetic distance between the woodstar and sheartail was closer than the average for bee hummingbirds (Table 1).

However, genetic differentiation between the two species based on 10 microsatellite loci was still high ($F_{ST} = 0.57$, $p < 0.0001$).

## Hybrid identification

NewHybrids assigned 100 % of putative pure woodstars (n = 52) as pure woodstars and 98.2 % (55/56) of putative sheartails as pure sheartails (Fig. 2a). One putative sheartail male was classified as a F1 hybrid (mean $q_n$ of individual over five independent runs = $0.981 \pm 0.000$). In agreement with these data, the analysis with Structure assigned all putative woodstars a probability of greater than 0.9, and 98.2 % (55/56) of putative sheartails a probability of less than 0.1. Again, the same putative sheartail male was assigned as a F1 hybrid (Fig. 2b). Based on mitochondrial ND2 and COI sequences, the mother of F1 hybrid male was a woodstar and the father a sheartail.

## Introgression

If introgression between the woodstars and sheartails is occurring, then haplotype mixing of both nuclear and mitochondrial genes is expected. However, we found no sharing of haplotypes between species
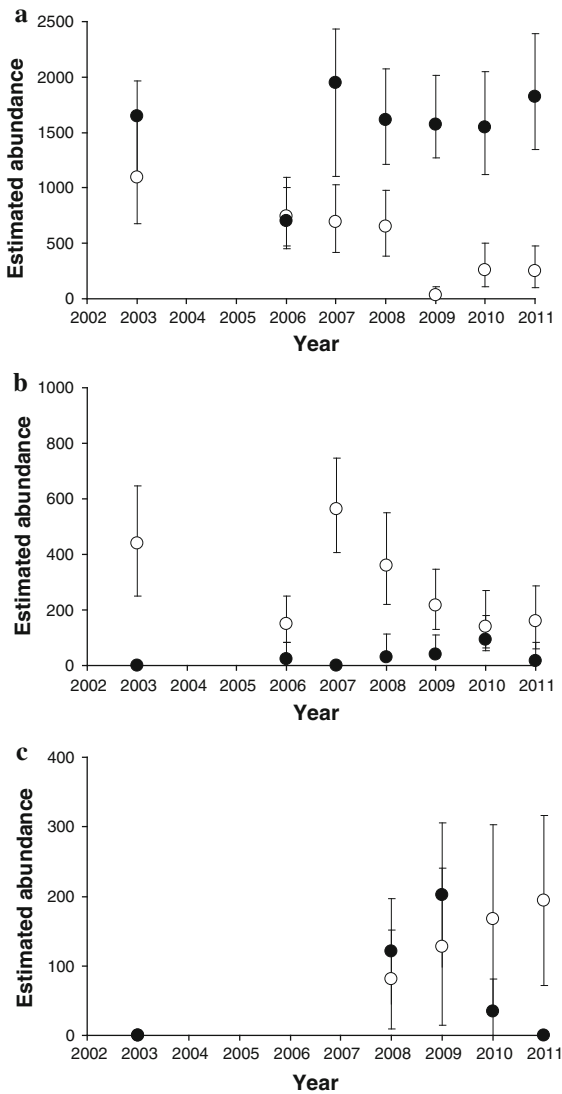
**Fig. 1** Population estimates (and 90 % confidence intervals) across years of Chilean woodstars (*open circles*) and Peruvian sheartails (*closed circles*) in **a** Azapa valley, **b** Vitor valley and **c** Camarones valley

**Table 1** Genetic distances amongst bee hummingbirds and between Chilean woodstars and Peruvian sheartails based on two mitochondrial (NADH dehydrogenase subunit 2 and cytochrome oxidase I) and two nuclear (intron 5 of adenylate kinase and intron 7 of beta fibrinogen) loci

| Gene | Mean distance (±SD) amongst bee hummingbirds | Range | $n$ | Distance between woodstar and sheartail |
| --- | --- | --- | --- | --- |
| ND2 | 0.074 | 0.018–0.121 | 16 | 0.035 |
| COI | 0.084 | 0.016–0.145 | 9 | 0.040 |
| AK1 | 0.016 | 0.000–0.034 | 16 | 0.002 |
| βFib | 0.007 | 0.000–0.015 | 16 | 0.003 |

also differed significantly amongst both males and females. We reduced the seven morphological traits outlined in Table 2 into three explanatory functions using discriminant function analysis (DFA). The three functions reliably differentiated among the groups ($F_1$: $\lambda = 0.001$, $X^2(21) = 358.46$, $p < 0.001$, $r^2$-canonical $= 0.96$; $F_2$: $\lambda = 0.032$, $X^2(12) = 184.0$, $p < 0.001$, $r^2$-canonical $= 0.91$; $F_3$: $\lambda = 0.3771$, $X^2(5) = 52.16$, $p < 0.001$, $r^2$-canonical $= 0.62$). The $F_1$ axis explained 66.9 % of the variation between species and sexes and was positively correlated with R1 length and negatively with R2 and R3. The $F_2$ axis explained 28.7 % of the variation and was positively correlated with wing and bill length, and R2 and R3, while $F_3$ explained 4.4 % of the variation and was positively correlated R5 and bill length (Table 2). The DFA correctly assigned 100 % individuals to their respective groups, except for the F1 hybrid which had intermediate values to the sheartail and woodstar males (Fig. 4).

Niche use and behavioural interactions

Males of both species were highly territorial during the breeding period (September–October), surveying their territories from a number of perches and chasing any intruding males. The principal activity of males was territory surveillance, with only occasional feeding from flowers either within or outside their territories. In areas where both woodstars and sheartails occur, males often defended a territory immediately adjacent to a heterospecific male. Interspecies chases between neighbours regularly occurred when one species
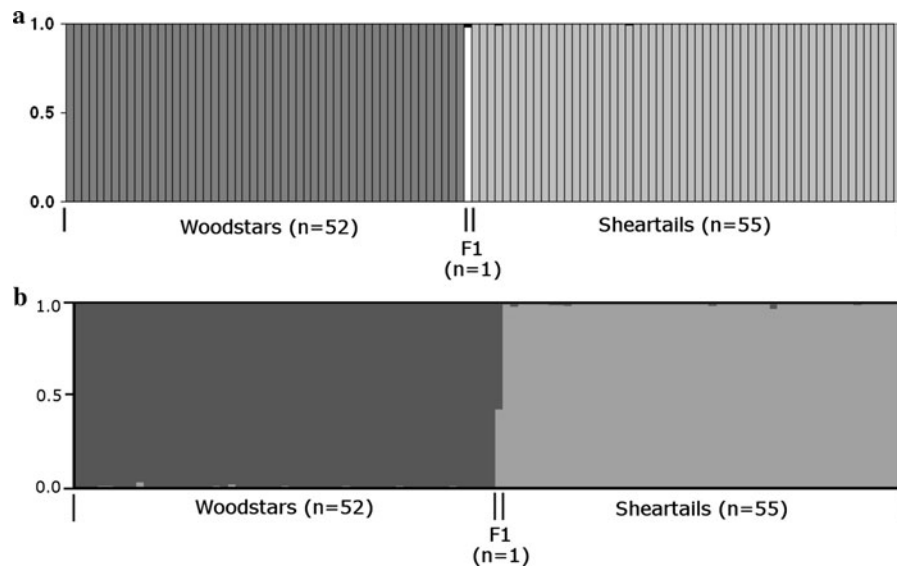
(Fig. 3). Indeed, we detected almost no variation among woodstar individuals in the three genes sampled. In contrast, sheartails displayed a greater degree of haplotypic variation, due partly to the fact that samples originating from both Chile and Peru were used.

Morphological characteristics

Both sexes of the sheartails had longer bills and wings than woodstars (Table 2). In addition, tail morphologies

**Fig. 2 a** Probabilities that individuals belong to woodstars (*dark grey*), sheartails (*light grey*), F1 hybrid (*white*), woodstar backcross (not visible in figure) or sheartail backcross (*black*), as calculated in NewHybrids. Each individual is assigned to a particular category when the assignment probability ($q_n$) is greater than 0.5. **b** Probabilities that each individual belongs either to woodstars (*dark grey*) or sheartails (*light grey*) based on Structure output. Individuals are assigned as pure woodstars when q = 0.9–1.0, pure sheartails when q = 0.0–0.1, F1 hybrids when q = 0.4–0.6, woodstar backcrosses when q = 0.65–0.85 and sheartail backcrosses when q = 0.15–0.35

invaded an adjacent territory. During the feeding observations and casual sightings, we recorded 173 intraspecific chases and 105 interspecific chases between woodstars, sheartails and the oasis hummingbird. Woodstar males were dominant not only to sheartail males, but also to male oasis hummingbirds, while both male and female oasis hummingbirds were dominant to sheartail males (Table 3). For several dyads, we observed very few chases, suggesting that little competition exists between these species or sexes.

Both hummingbird species fed on a variety of native and non-native flowering species (see Supporting Information, S5). Although we conducted 31 of 83 observations in areas where both species were present in that year, we recorded both species feeding within the same flower patch during only one observation period. In addition, although some flowering species were a very important nectar source for woodstars in the absence of sheartails in Vitor (e.g. *Geoffroea decorticans*), we did not observe woodstars feeding on these species in Azapa, where sheartails dominate.

We detected relatively low overlap in the diets of the two species (Morisita's index = 0.355, *n* = 23 patches). This estimate of dietary overlap includes several flower patches in areas where only one species

was observed during that year. Including only those patches in areas where both species were present (*n* = 10 flower patches and 31 observations), there was slightly more overlap in the flower species which were fed upon (Morisita's index = 0.426). Finally, the diet breadth of sheartails was 29 % larger than that of the woodstars (standardised Food Niche Breadth index: sheartails = 0.412, woodstars = 0.320).

## Discussion

The abundances of the Chilean woodstar and Peruvian sheartail do not appear to be negatively correlated across valley and years, as would be expected if a short-term increase in sheartail numbers resulted in a decline in woodstar abundance (although Estades et al. 2007 detected possible spatial segregation between the two species within Azapa valley). In addition, although the two species are closely-related and are morphologically similar, hybridisation and introgression does not appear to have occurred to a great extent and we detected no evidence of a hybrid swarm. Lastly, despite predictions, woodstars were territorially more aggressive than sheartails and niche overlap between the species was relatively low. Although
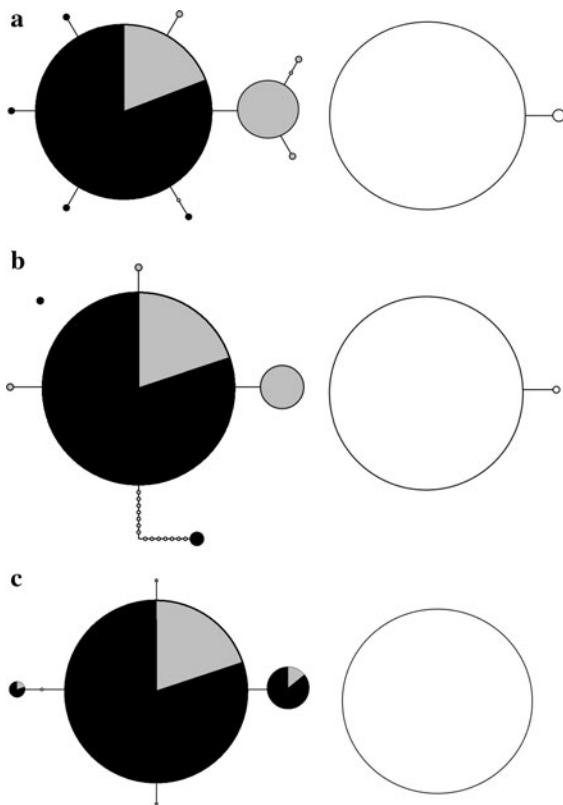
**Fig. 3** Parsimony haplotype networks of two mitochondrial genes and one nuclear gene, where *grey circles* correspond to Peruvian sheartails capture in Peru, *black circles* correspond to Peruvian sheartails capture in Chile and white circles correspond to Chilean woodstars captured in Chile. Circle diameter is proportional to the overall abundance of each haplotype and dots on branches correspond to the divergence between haplotypes. **a** ND2 (sheartails—Peru: $n = 17$; sheartails—Chile: $n = 30$; woodstars: $n = 31$), **b** COI (sheartails—Peru: $n = 17$; sheartails—Chile: $n = 30$; woodstars: $n = 31$) and **c** ßFib (sheartails—Peru: $n = 36$; sheartails—Chile: $n = 44$; woodstars: $n = 62$). Note that one COI haplotype could not be joined to the network assuming 95 % confidence intervals

these data suggest that sheartails only have a minimal impact on woodstars, the possibility remains that the invader impacts on the resident species in more subtle ways. For example, woodstars may experience increased energetic costs associated with constantly displaying to heterospecific females and expelling the more abundant sheartail males from their territories. In addition, our feeding observations show that *Geoffroea decorticans* is a major nectar source for woodstars in Vitor but not in Azapa. As sheartails are rare in Vitor but common in Azapa, this finding suggests that some competitive exclusion by the sheartails may be

occurring. These negative effects may exacerbate other threats on woodstars survival, such as ongoing habitat destruction.

Interspecies hybridisation

Our molecular analyses within the bee hummingbird clade on the genetic distances of two mitochondrial and two nuclear genes suggest that the woodstars and sheartails are indeed closely-related. As hybridisation events are more common between closely-related species (Edmands 2002; Price and Bouvier 2002), a tendency of the two species to hybridise in areas of sympatry is therefore expected, especially since hybridisation is relatively common in bee hummingbirds (e.g. Graves 2004, 2007a, b) and, more generally, in disturbed habitats where one species is rare (Mayr 1963). We found that hybrids are rare between the species, with only one F1 hybrid identified out of 108 woodstars and sheartails sampled, although it is possible that we underestimated hybrid frequency due to lower hybrid survivorship (Price and Bouvier 2002; Casas et al. 2012). We also found no woodstar or sheartail backcrosses in our samples. Since F1 hybrids are rare, backcross hybrids are expected to be even rarer. For these analyses, 52 woodstars and 56 sheartails were sampled representing approximately 10 and 3 % of the estimated Chilean population size for each species, respectively. Although these sample sizes are relatively low, they are far from trivial, especially in the case of the woodstars. Increasing our sampling effort would undoubtedly improve the precision of our estimation of hybridisation rates but, with the existing data, we have still been able to show that a hybrid swarm is very likely not to exist and that a very large proportion of woodstars and sheartails remain pure species.

The only hybrid individual that was identified was an offspring of a woodstar mother and sheartail father in Azapa valley, where woodstars are rare. This suggests that females of the rarer species may mate with heterospecific males as a last resort when unable to find conspecific mates. Even in the absence of hybridisation events, reproductive interference may still suppress fitness in each species. For example, mistakes in species recognition can result in wasted time and gametes spent courting and mating with heterospecific mates. In support of this, we observed several heterospecific courtship displays including one

**Table 2** Morphological comparison between woodstar and sheartail males and females

| | Females | | | | Males | | | | | DFA | | |
| | Woodstars ($n = 19$) | Sheartails ($n = 35$) | $F$ or $Z$ | $p$ | Woodstars ($n = 9$) | Sheartails ($n = 19$) | F1 Hybrid | $F$ or $Z$ | $p$ | $F_1$ | $F_2$ | $F_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bill length | $15.7 \pm 0.6$ | $17.6 \pm 0.6$ | 122.8 | <0.001 | $14.8 \pm 0.4$ | $16.4 \pm 0.5$ | 15.2 | 67.0 | <0.001 | 0.15 | 0.39 | 0.44 |
| Wing length | $36.7 \pm 1.0$ | $41.1 \pm 1.0$ | 213.8 | <0.001 | $31.5 \pm 1.1$ | $39.1 \pm 0.9$ | 34.7 | 358.7 | <0.001 | 0.24 | 0.76 | 0.08 |
| R1 | $23.7 \pm 0.9$ | $24.8 \pm 1.9$ | $-2.4^a$ | 0.018 | $14.6 \pm 0.5$ | $12.7 \pm 1.2$ | 16 | 17.0 | <0.001 | 0.62 | −0.11 | −0.23 |
| R2 | $25.8 \pm 0.9$ | $31.1 \pm 1.8$ | $-5.5^a$ | <0.001 | $24.6 \pm 1.0$ | $84.2 \pm 14.3$ | 40 | $-3.8^a$ | <0.001 | −0.43 | 0.63 | −0.37 |
| R3 | $25.1 \pm 1.3$ | $28.5 \pm 1.4$ | 66.9 | <0.001 | $30.5 \pm 1.0$ | $43.3 \pm 5.1$ | 42 | $-3.8^a$ | <0.001 | −0.41 | 0.43 | 0.09 |
| R4 | $23.9 \pm 1.2$ | $24.8 \pm 3.0$ | 2.1 | 0.156 | $30.6 \pm 1.0$ | $31.3 \pm 4.2$ | 39 | $-0.4^a$ | 0.735 | −0.32 | 0.10 | 0.19 |
| R5 | $21.2 \pm 1.7$ | $21.1 \pm 1.6$ | 0.025 | 0.875 | $30.0 \pm 1.0$ | $23.4 \pm 2.7$ | 33 | 33.3 | <0.001 | −0.22 | −0.16 | 0.48 |

Sample sizes within each group are shown in parentheses. R1–R5 refer to the 5 tail feathers from the innermost (R1) to outermost (R5) and $F_1$–$F_3$ corresponds to the three functions extracted from a discriminant function analysis incorporating the 7 morphological variables. The correlation between each function and morphological trait is displayed

[a] A Mann–Whitney $U$ test was used due to non-normally distributed data
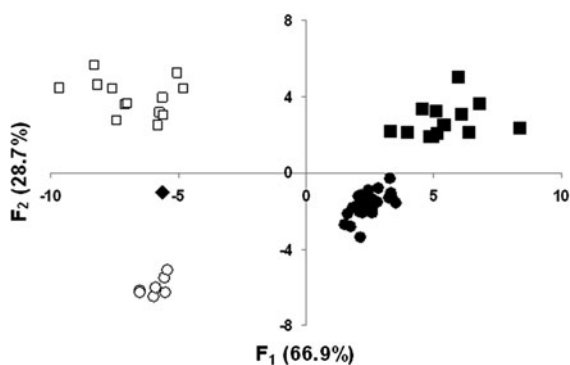


**Fig. 4** Plot between Function 1 ($F_1$) and Function 2 ($F_2$) extracted from a discriminant function analysis incorporating bill length, wing length and length of the R1–R5 tail feathers of woodstars and sheartails. Symbols represent sheartail males (*open squares*), sheartail females (*black squares*), woodstar males (*white circles*), woodstar females (*black circles*) and an F1 male hybrid (*black diamond*)

female that simultaneously received courtship displays from both a male woodstar and sheartail (W. van Dongen, personal observation).

Despite the ability of the two species to hybridise, based on two mitochondrial and one nuclear gene, plus 10 microsatellite markers, we found no evidence of introgression, suggesting that hybrids are either too rare to have an impact on the species' genome, are not fertile or have lower fitness. For example, the song and courtship display of the parental species are very different to each other, but that of the F1 hybrid was intermediate (C. Clark, unpublished data). Similarly, both male woodstars and sheartails produce sounds with their tail feathers during courtship displays (C. Clark, unpublished data), and in other species, a precise tail feather morphology is needed to produce sounds during courtship displays (Clark and Feo 2008). Therefore, hybrid males may be unable to attract matings due to their unique tail morphology and behaviour. Our findings are similar to a recent study in the endangered black stilt (*Himantopus novaezelandiae*) from New Zealand which is at risk of hybridisation with the recently-arrived pied stilt (*H. himantopus*) from Australia (Steeves et al. 2010). Although extensive hybridisation was detected between the species the authors reported almost no evidence of introgression, which was attributed to both a reduced reproductive success of hybrids and stochastic population sizes.

Interspecies resource competition

In contrast to earlier suggestions that sheartails are more aggressive than woodstars and may usurp them from high quality habitat (Estades et al. 2007), we found that male woodstars were more aggressive than not only sheartails but also the much larger oasis hummingbird. Although chases between females are common at nectar sources (W. van Dongen, personal observation), we do not have dominance data for female woodstars and sheartails due to their morphological similarity. In areas where sheartails and woodstars occurred in sympatry, males created a mosaic of heterospecific territories with frequent male–male aggressive interactions, as also reported in other hummingbird species (Pitelka 1951; Clark

**Table 3** Direction and frequency of interspecific chases between woodstars, sheartails and the oasis hummingbird

| Dyad | | Dominant species | | $\chi^2$ | $p$ |
|---|---|---|---|---|---|
| Species A | Species B | Species A | Species B | | |
| Woodstar male | Sheartail male | 22 | 1 | 19.2 | >0.001 |
| Woodstar male | Oasis male | 15 | 4 | 6.4 | 0.012 |
| Woodstar female | Oasis male | 1 | 7 | 4.5 | 0.034 |
| Sheartail male | Oasis male | 6 | 19 | 6.8 | 0.009 |
| Sheartail male | Oasis female | 6 | 19 | 6.8 | 0.009 |
| Woodstar male | Oasis female | 0 | 0 | | |
| Woodstar female | Oasis female | 0 | 2 | | |
| Oasis male | Sheartail female | 3 | 0 | | |
| Oasis female | Sheartail female | 2 | 0 | | |

No data were available for the woodstar male/sheartail female, woodstar female/sheartail male and woodstar female/sheartail female dyads due to the difficulty in distinguishing between female sheartails and woodstars during chases where the two species co-occur. Statistical analyses were not performed for dyads containing less than eight observations

et al. 2011). Despite their lower dominance status, sheartails may still affect woodstar settlement patterns and viability via a number of mechanisms. For example, sheartails may be highly persistent in their intrusions into woodstars territories, such that woodstars eventually relinquish their territories (e.g. Switzer et al. 2001). In support of this, from several years of observations at the study sites, we have recorded at least ten cases in which areas once inhabited by woodstars are now only occupied by sheartails (C. Estades, personal observation). Secondly, the density of woodstars may be depressed by the mere presence of the sheartails. For example, Pitelka (1951) showed that Allen's hummingbird (*Selasphorus sasin*) was subordinate to Anna's hummingbird (*Calypte anna*) where they occur in sympatry. However, Allen's hummingbird was still able to control habitat that was less favourable to Anna's hummingbird, thereby reducing the total area available to the latter species for territories. The potential number of territories occupied by each species was therefore depressed by the presence of the other species. Finally, territorial behaviour such as frequent chasing is known to be costly in hummingbirds (Powers and Conley 1994; Powers and McKee 1994). The presence of sheartails may therefore increase the energetic output of woodstars via frequent chases. However, it is yet to be established whether chase frequency, and hence energetic output, is indeed higher when woodstars defend territories in a mosaic of sheartail/woodstar territories compared to in areas where only woodstars occur.

Our estimate of food niche overlap between woodstars and sheartails suggest that interspecific competition for nectar sources exists, but only at moderate levels, at least during the Austral spring period. Nevertheless, the fact that some plant species, such as *Geoffroea decorticans*, are a very important nectar source for woodstars in the absence of sheartails, but are not fed upon by woodstars in the presence of sheartails, suggests that some competitive exclusion may be occurring. Interestingly, sheartails had a substantially broader diet than woodstars, which should reduce competitive interference. One reason for the broader diets of the sheartails may be their slightly longer bills. Hummingbird species with relatively long bills typically have a generalist diet due to their ability to exploit flowers with longer corollas (Feinsinger and Colwell 1978). For example, *Tecoma fulva*, a very common floral species in Azapa valley with a long corolla, formed a major component of the sheartail diet. During our study, we never observed woodstars feeding on this species, perhaps because they could not access the nectar (but see Estades et al. 2007). Indeed, the broader diet of the sheartails may be one of the reasons for their successful spread throughout the north of Chile. Species with broader diets are predicted to be more successful in the colonisation of new areas (e.g. Duncan et al. 2001).

Conservation implications

We found little evidence that the population crash of the woodstars over the last decades was principally

due to the arrival of the Peruvian sheartail. However, although our results showed that woodstars were more aggressive than sheartails, over eight years of work, we have never observed a sheartail territory being taken by a woodstar (C. Estades, personal observation). This latter finding, coupled with the apparently higher tolerance of sheartails to human disturbance, may pose an important limitation for woodstar population recovery efforts. In addition, any negative effects of the sheartails on the viability of the woodstar population are likely to be exacerbated by other threats within the valleys. In particular, human-induced habitat modification and inappropriate agricultural practices have likely played a prominent role in the decline in woodstar abundance. All valleys where the woodstars occur are subject to at least some, and often very intensive, agriculture. During the current two-year study period we observed several woodstar territories being destroyed either by deliberately lit fires or vegetation removal. In contrast to woodstars, sheartails occur in high densities in areas with high human disturbance, and are frequently observed in gardens with nectar sources. Indeed, our population size estimates over multiple years suggest that woodstar abundance is steadily declining in Azapa, the most heavily disturbed valley, while sheartails may be increasing in abundance. The continual degradation of suitable woodstar habitat in Azapa valley and concurrent increase in sheartail abundance could result in elevated energetic costs associated with territory maintenance by woodstars against sheartails (e.g. Carrete et al. 2010). Habitat restoration trials are currently being conducted in Vitor valley by the Chilean Ornithologists' Union (Aves Chile) and the Ministry of the Environment as a response to the rapid degradation of woodstar habitat.

It remains unclear whether or not the expansion of sheartails into Chile was natural or anthropogenic, although it is possible that the contemporary conversion of desert environments into agricultural land in the south of Peru (Velazco 2001) may have created a corridor for the southern migration of the sheartail. Given the uncertainty in the causes of the sheartail arrival, it is unclear whether the prevention of hybridisation between the two species as a conservation plan is justified (Allendorf et al. 2001). In addition, as sheartails are well established in north of Chile, programs aiming at the complete eradication of sheartails in Chile are unlikely to be feasible. Despite the fact that no hybrid swarm was detected, long-term genetic monitoring of the populations is warranted given that species dynamics can change over time (Strayer et al. 2006).

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