

# Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*

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## Abstract

Geographic variation in microsatellite allele frequencies was assessed at nine sites in two regional vocal dialects of the parrot *Amazona auropalliata* (yellow-naped amazon) to test for correspondence between dialects and population structure. There was no relationship between the genetic distances between individuals and their dialect membership. High rates of gene flow were estimated between vocal dialects based on genetic differentiation. In addition, 5.5% of pairs of individuals compared across the dialect boundary were estimated to be related at the level of half siblings, indicating that dispersal is ongoing. The number of effective migrants per generation between dialects estimated with the microsatellite data was roughly one-seventh the number estimated with mitochondrial control region sequence data from the same individuals, suggesting that gene flow may be female-biased. Together, these results suggest that the observed mosaic pattern of geographic variation in vocalizations is maintained by learning of local call types by immigrant birds after dispersal. We found no evidence that ongoing habitat fragmentation has contributed to cryptic population structure.

*Keywords:* *Amazona auropalliata*, gene flow, microsatellite, parrot, sex-biased dispersal, vocal dialect

Received 5 July 2004; revision received 19 November 2004; accepted 10 December 2004

## Introduction

Vocal dialects have been documented in a range of bird species, including members of the Passeriformes (Krebs & Kroodsma 1980; Catchpole & Slater 1995), Apodiformes (Gaunt *et al.* 1994), and Psittaciformes (Wright 1996; Baker 2000). Several hypotheses have been proposed to explain how this patchwork variation in learned acoustic signals is maintained through time (reviewed in Payne 1981; Rothstein & Fleischer 1987; Slabbekoorn & Smith 2002). One long-standing hypothesis is that vocal differences among dialects are associated with reduced dispersal of individuals from one dialect to another. Dispersal could be limited because immigrants to a new dialect experience reduced survival due to ecological specializations to habitats corresponding to particular dialects, or because they have reduced success in finding a mate, establishing a territory, or gaining access to social groups (Marler &

Tamura 1962; Nottebohm 1969; Slabbekoorn & Smith 2002). The reduced dispersal hypothesis predicts that gene flow will also be reduced between dialects. If dialects are sufficiently long-standing, then reduced gene flow would lead to structuring of neutral genetic variation along dialect lines.

An alternative hypothesis suggests that dialects are maintained when dispersing individuals learn local vocalizations following dispersal, perhaps to enhance success at obtaining a mate, establishing a territory, or gaining access to a social group (Payne 1981; Feeles 1982; Rothstein & Fleischer 1987; Beecher *et al.* 1994). In this case there would be no expectation of genetic structuring along dialect boundaries no matter how long these boundaries have been present. A recent theoretical analysis of song variation and dispersal found that song divergence could evolve at the population level under a range of conditions and that the degree of postdispersal learning was critical in determining the degree of genetic divergence between populations with divergent songs (Ellers & Slabbekoorn 2003).

While a number of empirical studies have examined genetic structure of vocal dialects, at present most have

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found limited evidence for a general concordance between genetic variation and vocal dialects. In some cases there is little indication of genetic differentiation across any dialect boundaries (Fleischer & Rothstein 1988; Payne & Westneat 1988; Loughheed & Handford 1992; Wright & Wilkinson 2001), while in others genetic discontinuities are apparent at some, but not all, dialect boundaries (Kroodsma *et al.* 1985; Balaban 1988). In the latter case, these genetic discontinuities may correspond to areas of secondary contact between different subspecies (Kroodsma *et al.* 1985; Balaban 1988). Perhaps the strongest evidence for an isolating effect of vocal dialects comes from recent work on the mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*), in which a significant relationship was found between vocal dialect membership and genetic variation as assayed using nuclear microsatellite loci (MacDougall-Shackleton & MacDougall-Shackleton 2001). This finding suggests that rapidly evolving microsatellites may be a good marker for detecting population divergence spurred by recently arisen vocal variation.

The yellow-naped amazon (*Amazona auropalliata*) has large regional dialects throughout its range in Central America. Dialects in this parrot species are defined by acoustic shifts in multiple classes of the vocal repertoire (Wright 1996, 1997), and birds respond differently to playbacks of local dialects than to foreign dialects (Wright & Dorin 2001). Some elements of this vocal repertoire are sex-specific, in particular the notes used by mated males and females during pair duets (Wright 1997). Evidence from recordings deposited in sound libraries suggest that dialects are present throughout the Central American range of this species and are stable over at least 10- to 20-year spans (Wright & Wilkinson 2001). Furthermore, the occurrence of dialects in the sister species *Amazona ochrocephala* and *Amazona oratrix* imply that the propensity to form dialects exists in the common ancestor of this clade (Wright & Wilkinson 2001). A previous study of population variation in this species by Wright & Wilkinson (2001) using mitochondrial (mtDNA) control region sequences found extensive variation (19 haplotypes among 41 individuals), but dialect populations were not genetically distinct ( $N_{ST}$  was effectively zero) and gene flow between them was high ( $N_j m = 16.8$ ).

Mitochondrial DNA is maternally inherited as a single locus, and thus may not generally represent population dispersal patterns and history. Direct comparison of results from mtDNA with those from multiple nuclear markers such as microsatellites could provide a more detailed picture of these processes. For example, if dialects do isolate populations but these vocal differences have only recently become established, then the high rates of mutation experienced by microsatellites could allow a more rapid accumulation of novel alleles in different populations than would occur for mtDNA (Hedrick 1999; Waits *et al.* 2000;

Parker *et al.* 2004), even with the larger effective population size of the nuclear genome (Birky *et al.* 1983). Rapidly evolving microsatellites will also have higher polymorphism than mtDNA resulting in improved power on population differentiation tests (Estoup *et al.* 2002) and assignment tests (Hedrick 1999), although this effect may be counteracted by an increased likelihood of allelic size homoplasy for loci with extremely high mutation rates (Estoup *et al.* 2002; O'Reilly *et al.* 2004). On the other hand, if dispersal between dialects does occur, then the high degree of resolution provided by multilocus microsatellite genotypes could be used to identify close relatives sampled in different dialects, one of which would presumably represent a recent migration event (Hedrick 1999; Flagstad *et al.* 2003; Hansson *et al.* 2003).

Perhaps most importantly, direct comparisons of mtDNA and microsatellite patterns can give insight into the biology of vocal dialects not available from studies using only a single marker type. Of particular interest is how males and females may differ in the acquisition of the sex-specific portions of their repertoire and how dispersal patterns might affect this acquisition. If sex-biased dispersal occurs across dialect boundaries, then estimates of gene flow will differ between maternally inherited mtDNA and biparentally inherited microsatellites (e.g. Lyrholm *et al.* 1999; Escorza-Treviño & Dizon 2000; Piertney *et al.* 2000a,b). Maximum-likelihood methods based on the coalescence (Beerli & Felsenstein 1999) can estimate gene flow based on a variety of data and thus provide values that can be directly compared between microsatellites and mtDNA.

Finally, the assessment of microsatellite variation could also provide valuable information for conservation of the yellow-naped amazon. The dry-forest habitat of this species has become highly fragmented over the last 200 years because of deforestation to give way for cattle ranching and, more recently, large-scale agriculture (Janzen 1983). The popularity of this species in the pet trade has also led to high rates of nest poaching, with over 60% of nests at some sites in Costa Rica and Guatemala losing chicks to poaching (Wright *et al.* 2001). Because of these threats the species was listed as 'vulnerable' in the International Union for Conservation of Nature and Natural Resources (IUCN) Parrot Conservation Action Plan (Snyder *et al.* 1999) and continued concerns prompted the IUCN to move this species from a CITES Appendix II listing to Appendix I in 2003 (Inskipp & Gillett 2003). The habitat fragmentation experienced by this species could potentially have reduced gene flow between populations in ways that are not associated with vocal dialects. Microsatellites can be used to test for cryptic population structure that may result from habitat fragmentation; these data would be of value to *in situ* efforts to preserve this species and numerous other parrot species that face similar threats.

In this study, we employ nuclear microsatellites to test for reduced gene flow between two vocal dialects of the yellow-naped amazon in Costa Rica and to search for evidence of recent migrants between these dialects. We evaluate the degree of sex-biased dispersal across dialects by directly comparing estimates of migration rates from microsatellites to those from mtDNA control region sequences of the same individuals using a maximum-likelihood estimator of migration rates. We also use data from microsatellites to examine the possibility of cryptic population structure in this species that could result from habitat fragmentation.

## Materials and methods

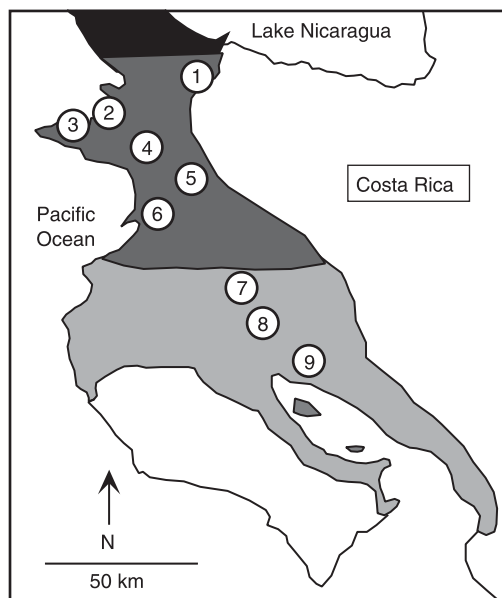
### Genetic sampling

We collected genetic samples from 75 individuals at nine sites between 1993 and 2003 (Fig. 1). Sites were defined as collections of nests within a 5-km radius. Sampling sites (with number of samples obtained) were: (i) Inocentes (2); (ii) Cuaniquil/Juanquillal (7); (iii) Bahia Santa Elena/

Murcielago (5); (iv) Santa Rosa/Pelon Altura (11); (v) Ahogados (15); (vi) Playa Cabuyal/Horizontes (7); (vii) Pelon Bajura (19); (viii) Llano Cortes/Lomas Barbudal (7); (ix) Tamarindo (2). These sites were previously sampled in Wright & Wilkinson (2001) and provide overlapping ranges of intersite distances for within- and between-dialect comparisons. Sixty-six of our 75 samples were obtained from nestlings; in 41 nests that contained more than one chick, we randomly selected one chick for genotyping. Pairs will reuse the same nesting cavities in subsequent years (T.F. Wright, unpublished) so cavities were sampled once over the course of the study. Of the remaining nine birds in our sample, six were nesting females (at nests from which no chicks were sampled) and three were non-nesting birds.

We extracted DNA from either feather tips or blood samples preserved in lysis buffer (Longmire *et al.* 1992) using DNeasy kits (QIAGEN). We amplified eight unlinked polymorphic loci using primers originally designed for the congener *Amazona guildingii* (Russello *et al.* 2001). Magnesium concentration (1–4 mM MgCl<sub>2</sub>), annealing temperature (50–58 °C) and polymerase type (AmpliTaq or AmpliTaq Gold, Applied Biosystems) were optimized for each locus; conditions are available upon request. Loci were amplified with polymerase chain reaction (PCR) in 15-μL volumes with a final concentration of 1X PCR buffer, 0.8 mM of dNTP's, 1–4 mM of MgCl<sub>2</sub>, 0.4 μM each of fluorescently labelled forward and unlabelled reverse primers, 3 μL of dilute template, 0.75 U of *Taq* and ultrapure water to volume. PCR was performed on PTC-100 Thermocycler (MJ Research) with a profile of 95 °C for 5 min (12 min for AmpliTaq Gold), 30 cycles of 95 °C for 30 s, 50–58 °C for 45 s, and 72 °C for 60 s, and 10 min of extension at 72 °C. Products were sized on 3100 Genetic Analyser (Applied Biosystems) with GENESCAN version 3.7 software (Applied Biosystems). Genotypes were scored using GENOTYPER version 2.5 (Applied Biosystems). Positive controls were included to ensure consistent size scoring across all runs. All loci showed Mendelian inheritance indicating autosomal linkage.

We also sequenced 680 bp of the first domain of the mitochondrial control region and flanking region. This species has duplicate mtDNA control regions; we amplified from the first control region using primers LThr and CR522Rb (Eberhard *et al.* 2001). Products were PCR-amplified in 20-μL volumes with final concentrations of 1X PCR buffer, 0.8 mM of dNTP's, 2.5 mM of MgCl<sub>2</sub>, 1 μM of each primer, 2 μL of dilute template, 0.8 U of AmpliTaq and ultrapure water to volume. PCR was performed on PTC-200 Thermocycler with a profile of 95 °C for 5 min followed by 35 cycles of 95 °C for 25 s, 54 °C for 45 s, and 72 °C for 90 s, and 10 min of extension at 72 °C. We sequenced both strands of the product using BigDye Terminator version 3.1 (Applied Biosystems) and a 3100 Genetic Analyser running SEQUENCING ANALYSIS 3.7 (Applied Biosystems).



**Fig. 1** A map of northwestern Costa Rica indicating the nine sites at which parrots were sampled within two vocal dialects. Dark grey shading indicates the extent of the North dialect, light grey indicates the extent of the South dialect, and black shading indicates a third dialect (Nicaraguan) that was not sampled in this study. Sites are: (1) Inocentes (11°01'N, 85°30'W) (2) Cuaniquil/Juanquillal (10°58'N, 85°40'W) (3) Bahia Santa Elena/Murcielago (10°55'N, 85°44'W) (4) Santa Rosa/Pelon Altura (10°51'N, 85°35'W) (5) Ahogados (10°46'N, 85°31'W) (6) Playa Cabuyal/Horizontes (10°41'N, 85°36'W) (7) Pelon Bajura (10°27'N, 85°24'W) (8) Llano Cortes/Lomas Barbudal (10°27'N, 85°22'W) (9) Tamarindo (10°22'N, 85°18'W).

Sequences were edited and aligned using SEQUENCHER 4.1.2 (Gene Codes).

### Analysis

We calculated observed and expected heterozygosities for each locus across the entire population and tested for deviations from Hardy–Weinberg equilibrium (HWE) using GENEPOP version 3.3, updated from Raymond & Rousset (1995). We calculated  $F_{ST}$  between the two dialects (Weir & Cockerham 1984) and derived a matrix of pairwise genetic distances between all individuals based on  $D_{ps}$ , the proportion of shared alleles (Bowcock *et al.* 1994), using MICROSATELLITE ANALYSER (MSA) version 3.01 (Dieringer & Schlötterer 2003). Correspondence between the genetic distance matrix and matrices of geographic distances between sampling sites and dialect membership was tested using Mantel tests (Mantel 1967) implemented in the program R version 4.0 (Casgrain & Legendre 2001). We used matrices of interindividual distances rather than intersite distances because of unequal sample sizes among sites.

We used a number of methods to estimate migration rates between vocal dialects using the microsatellite data. We used the maximum-likelihood coalescence approach implemented in MIGRATE version 1.7.6.1 (Beerli & Felsenstein 1999) to estimate the effective number of migrants per generation ( $N_e m$ ) between the two vocal dialects. We ran 10 replicate runs to simultaneously estimate  $4N_e m$ , the product of effective population size and migration rate, and theta ( $4N_e \mu$ ), the product of effective population size and mutation rate. We used default parameters for the stepwise mutation microsatellite data model with constant mutation rates among loci, starting estimates based on  $F_{ST}$  calculations: burn-in = 10 000 trees; short chains = 10 000 trees; and long chains = 100 000 trees. We report the estimates for  $4N_e m$  in each direction between the two dialects and the values for the lower and higher profile likelihood percentiles (0.025 and 0.975, respectively) that approximate the 95% confidence interval for the estimate (Beerli & Felsenstein 1999). We then calculated a single value for overall number of migrants exchanged between dialects ( $N_e m$ ) by summing the estimates for  $4N_e m$  in each direction and dividing by 4. For comparison, we estimated  $N_e m$  using the private alleles method (Slatkin 1985) implemented in GENEPOP.

We used KINSHIP version 1.3.1 (Queller & Goodnight 1989) to identify pairs of individuals sampled in different dialects who were related to each other at the half-sibling level or higher (relatedness  $\geq 0.25$ ). Pairs with this level of relatedness could result from several scenarios, including comparisons between the offspring of successive matings by one individual that changed mates (and nesting sites), or comparisons between a nestling and the offspring of an older full sibling that had dispersed from an earlier nest in that location. We tested the significance of pair relatedness

by comparing log-likelihoods of a primary hypothesis of  $R = 0.25$  vs. a null hypothesis of  $R = 0$  (over 100 000 simulated pairs). Pairs of individuals in different dialects with significant log-likelihood ratios at  $P < 0.05$  were considered to have resulted from a dispersal event from one dialect to another.

To evaluate the extent of sex-biased gene flow across vocal dialect boundaries, we also estimated gene flow using the mitochondrial control region sequence data and MIGRATE and compared these values to those obtained for microsatellite data. When estimating migration rates using mtDNA, MIGRATE calculates the effective number of female migrants per generation in the form of  $2N_f m$ , where  $N_f$  is the effective population size of females. In the case of a 1:1 sex ratio and low variance in male reproductive success,  $N_f$  would be equivalent to half of the  $N_e$  calculated for microsatellite data (P. Beerli, personal communication). It is thus theoretically possible to detect a sex-bias in gene flow if the estimated migration rates for the maternally inherited mtDNA differ from those of the biparentally inherited microsatellites. We ran 10 replicate runs for the mtDNA sequences using the default parameters for the sequence data model, with the exception of an empirically based transition:transversion rate of 12.7 (Wright & Wilkinson 2001), starting estimates based on  $F_{ST}$  calculations (burn-in = 100 000 trees; short chains = 100 000 trees; and long chains = 1 000 000 trees). As with the microsatellite estimates, we report the estimates for  $2N_f m$  in each direction and the profile likelihood percentiles approximating the 95% confidence interval and then calculate a single value for overall number of migrants exchanged between dialects ( $N_e m$ ) assuming an equal sex ratio and no variance in reproductive success.

To examine the possibility of cryptic population structure among the sampling sites, we used the model-based clustering method implemented in STRUCTURE version 2.1 (Pritchard *et al.* 2000) to test for the most probable number of populations represented in our sample and assign individuals to these populations. We ran STRUCTURE for 10 replicate runs at  $K = 2-9$  populations to obtain an average log-likelihood of the model at each number of populations ( $K$ ), then computed the posterior probability of each  $K$  following Pritchard *et al.* (2000). We used default parameters for the admixture model with no sampling site information, correlated allele frequencies between populations, a burn-in chain length = 100 000, and a Markov chain Monte Carlo chain length = 100 000.

## Results

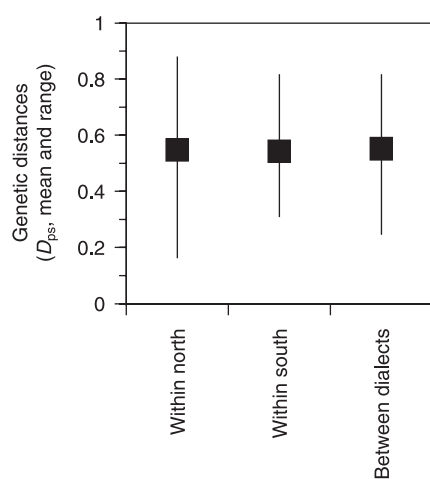
### Microsatellite amplification

Mean amplification success across all microsatellite loci was 99% for the 75 samples (Table 1). The number of alleles amplified per locus ranged from three to 25 (mean = 9.6).

**Table 1** Amplification success, number of alleles, expected heterozygosity and observed heterozygosity for microsatellite loci used in this study

Locus	Amplification success (%)	No. of alleles	Expected heterozygosity	Observed heterozygosity*
AgGT81	96	25	0.89	0.79
AgGT83	100	16	0.89	0.85
AgGT72	99	12	0.87	0.82
AgGT04	93	7	0.80	0.67
AgGT90	100	6	0.67	0.65
AgGT21	100	5	0.67	0.68
AgGT22	100	3	0.08	0.08
AgGT29	100	3	0.14	0.15
Mean $\pm$ SE over eight loci	98.5 $\pm$ 0.9	9.6 $\pm$ 2.7	0.62 $\pm$ 0.12	0.59 $\pm$ 0.11

\*All loci are in Hardy–Weinberg equilibrium when tested across the entire sample.



**Fig. 2** The mean (and range) of genetic distances for pairwise comparisons of individuals within the North dialect, within the South dialect, and between the two dialects. Genetic distances are measured as  $D_{ps}$ , the proportion of shared alleles.

Mean expected heterozygosity across all loci was 0.62 and mean observed heterozygosity was 0.59. All loci were in HWE when tested across the entire sample, and no loci were in linkage disequilibrium.

#### Microsatellite differentiation by dialect

There was little evidence of population structure between the two vocal dialects. Genetic distances between individuals sampled in different dialects were on average no greater than those between individuals from the same dialect (Fig. 2). There was no association between a matrix of genetic distances between individuals (measured as the proportion of alleles shared) and a matrix of dialect membership (Mantel  $r = -0.026$ ,  $P = 0.17$ ) nor between the genetic distance matrix and a matrix of geographic distances between sampling sites (Mantel  $r = 0.016$ ,  $P = 0.33$ ). A partial Mantel test for association between genetic

distance and dialect membership while controlling for the effect of geographic distance also was not significant (Mantel  $r = -0.021$ ,  $P = 0.34$ ). Similar results (not shown) were obtained using  $\delta\mu^2$ , a genetic distance based on the stepwise mutation model (Goldstein *et al.* 1995). The degree of population subdivision along dialect lines as measured by  $F_{ST}$  was low and nonsignificant ( $F_{ST} = 0.006$ ,  $P > 0.05$ ).

Several lines of evidence indicated substantial ongoing gene flow between vocal dialects. The 10 replicate runs of the program MIGRATE gave an estimate across all eight microsatellite loci of  $4N_e m = 3.2$  (95th percentiles = 2.7–3.8) from the south to the north dialect and  $4N_e m = 5.3$  (95th percentiles = 3.4–6.6) from the north to the south dialect. Adding these two estimates for  $4N_e m$  and dividing by four gives an overall estimate of  $N_e m = 2.1$  effective migrants exchanged per generation between the two dialects. The private alleles method gave an estimate of  $N_e m = 4.7$  between the two dialects.

Analysis of relatedness in KINSHIP identified a substantial number of cross-dialect pairs of relatives at the half-sibling level or closer. Of 1316 pairs of individuals examined across dialect boundaries, 72 pairs (5.5%) were identified as related at the  $R = 0.25$  with significant likelihood scores at  $P < 0.05$  (corresponding to a type II error rate of 0.51). By comparison, 13% of pairs within the north dialect were related at  $R = 0.25$  with  $P < 0.05$ , while 5.3% of pairs within the south dialect were significantly related at this level. When the alpha level was reduced to  $P < 0.001$  (corresponding to a type II error of 0.91) there were four pairs of individuals located in different dialects that were identified as related at  $R = 0.25$ .

#### Sex-biased gene flow

The 10 replicate runs of the program MIGRATE gave an estimate for mtDNA of  $2N_e m = 7.1$  (95th percentiles = 3.3–13.3) from the south to the north dialect and  $2N_e m = 8.0$  (95th percentiles = 0.4–65.0) from the north to the south dialect.

Adding these two estimates and dividing by 2 gives an overall estimate of  $N_e m = 7.55$ , which in a population with equal sex ratio would be equivalent to  $N_e m = 15.1$  migrants exchanged per generation between the two dialects.

#### Cryptic population structure

There was little evidence of cryptic population structure in these populations. Replicate runs of the Bayesian model-based clustering program STRUCTURE with the number of populations ( $K$ ) ranging from 1 to 9 indicated that the most likely number of populations from which our sample was drawn was a single population. Models with one population had an average posterior probability of 1, while all models with  $K > 1$  population had probabilities effectively equal to zero. Population membership coefficients for individuals tended to be divided evenly between the number of populations for that run; for example in runs with  $K = 2$  populations, most individuals were assigned to each population with a 50% probability (Fig. 3), while in runs with  $K = 9$  populations, most individuals were assigned to each population with an 11% probability. Thus, in all runs with more than one population, most individuals were considered equally admixed between the hypothesized populations.

#### Discussion

We found strong concordance between all measures of population structure in the yellow-naped amazon based on variation in nuclear microsatellites. These measures were uniform in their indication of high levels of gene flow among sampling sites in Costa Rica and low levels of population structure both within and between the two vocal dialects. In the succeeding discussion, we tackle the implications of these results for the maintenance of vocal dialect variation, for sex-biased dispersal and for the conservation of the yellow-naped amazon.

#### Implications for the maintenance of vocal dialects

We found no evidence that vocal dialect boundaries act as a barrier to gene flow in the yellow-naped amazon. There was no relationship between genetic variation and dialect membership based on a Mantel test, and  $F_{ST}$  between dialects was 0.006. The maximum-likelihood estimate from microsatellite data of the effective number of migrants per generation ( $N_e m$ ) between the two dialects was 2.1. The numerous cases in which relatedness values identified pairs of close relatives living in different dialects further suggest that migration between dialects is both substantial and ongoing.

The lack of concordance between vocal dialects and population genetic structure in the yellow-naped amazon mirrors that found in a range of oscine songbird species with vocal dialects. Table 2 summarizes the results for  $F_{ST}$  and analogous measures of population subdivision among vocal dialects assessed with three genetic marker types for seven bird species or subspecies. These measures range from below 0.01 in five studies to a maximum of 0.06 for allozymes in the rufous-collared sparrow (*Zonotrichia capensis*), which implies that variation among dialects never accounts for more than 6% of overall genetic variation found in a species. Included in this table is a recent study by MacDougall-Shackleton & MacDougall-Shackleton (2001) that found a significant relationship between dialect membership and genetic variation in the mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*) using a partial Mantel test that removed the effect of geographic distance between populations. Their result, however, may have been influenced by the fact that their measure of dialect membership was a continuous measure based on a continuous index of song similarity rather than a binary measure. If the songs of neighbouring dialects tend to be more similar to each other than the songs of distant dialects in this species, then the significant relationship between dialect membership and genetic similarity would remain confounded by the geographical proximity between

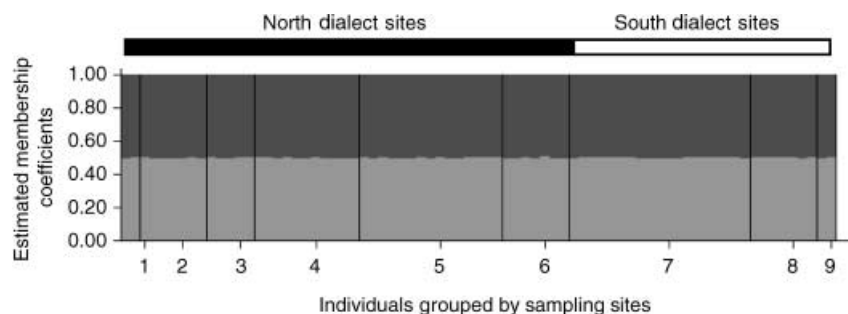


Fig. 3 Individual population membership coefficients estimated by the program STRUCTURE when  $K = 2$  populations (the number of vocal dialects). The membership for each individual in each hypothesized population is represented by the two shades of grey; the lack of variation among individuals indicates that all individuals are equally admixed between both populations. Site labels correspond to those in Fig. 1 and are arranged on a gradient from north to south. The vocal dialects corresponding to the sites are indicated above the graph.

**Table 2** Measures of population subdivision among vocal dialects from published studies

Species	Vocal learning mode*	Genetic marker	Dialects sampled	Measure of subdivision	Reference
<i>Amazona auropalliata</i>	open-ended	microsatellites	2	$F_{ST} = 0.006$	present study
<i>Zonotrichia leucophrys oriantha</i>	temporally restricted	microsatellites	8	$F_{ST} = 0.01†$	(MacDougall-Shackleton & MacDougall-Shackleton 2001)
<i>Zonotrichia leucophrys pugetensis</i>	temporally restricted	microsatellites	5	$F_{ST} = 0.005$	(Soha <i>et al.</i> 2004)
<i>Amazona auropalliata</i>	open-ended	mtDNA sequence	2	$F_{ST} = -0.003$	(Wright & Wilkinson 2001)
<i>Molothrus ater</i>	temporally restricted	morphometrics, mtDNA restriction fragments	4	not calculated, evidence of gene flow between dialects	(Fleischer & Rothstein 1988; Fleischer <i>et al.</i> 1991)
<i>Zonotrichia leucophrys nuttalli</i>	temporally restricted	allozymes	4	$F_{ST} = 0.03$	(Zink & Barrowclough 1984)
<i>Zonotrichia capensis</i>	temporally restricted	allozymes	6	$F_{ST} = 0.06$	(Loughheed & Handford 1992)
<i>Passerina cyanea</i>	temporally restricted	allozymes	3	$F_{ST} = 0.004$	(Payne & Westneat 1988)

\*Open-ended species are typically able to acquire new vocal variants throughout life, while temporally restricted species generally acquire vocalizations only during a limited sensitive period, usually early in life (Catchpole & Slater 1995).

†Did find significant effect of dialect boundaries on genetic distance with Mantel test (but see Discussion).

neighbouring populations, despite the effort to remove this effect with a partial Mantel test. Thus, their Mantel test results may overestimate the isolating effect of dialect boundaries in *Z. leucophrys oriantha*. A second recent study on the *pugetensis* subspecies of *Z. leucophrys* which also used microsatellite markers but employed a binary measure of dialect membership found no correlation between genetic distance and dialect membership using the Mantel test (Soha *et al.* 2004).

The robust pattern across these studies provides strong support for a general hypothesis that dialect boundaries do not limit the movement of individuals from one dialect to another. There is, however, evidence from several species that the mosaic pattern of vocal variation characteristic of vocal dialects can be maintained over long periods (Trainer 1983; Harbison *et al.* 1999; Wright & Wilkinson 2001). The persistence of such mosaic patterns in the face of continued dispersal is most likely explained by learning of new dialects by immigrants after dispersal so as to match local song types. Such learning has been reported in a number of studies (Baptista & Morton 1988; Whitney 1992; Beecher *et al.* 1994; Nelson 2000; O'Loughlen & Rothstein 2002). A recent spatial model of song divergence found that the inclusion of postdispersal learning in the model could lead to strong patterns of acoustic divergence in song without significant divergence in the genetic component of song learning (Ellers & Slabbekoorn 2003). Such learning is presumably driven by some form of selective advantage for individuals who can match local vocalizations, such as the ability to establish territories or attract mates. More systematic studies of the timing and of the costs and benefits of such learning by immigrants would enhance our understanding of the nature of these selective advantages and how they promote the maintenance of dialects through time.

### Sex-biased dispersal

The estimate for migration rates using maternally inherited mtDNA was  $N_e m = 15.1$ , roughly seven times higher than the estimate of  $N_e m = 2.1$  found using biparentally inherited nuclear microsatellites. The higher value for the maternally inherited mtDNA data suggests that dispersal may be biased towards females, as is common in many avian groups (Greenwood 1980). However, this result should be viewed with caution given the relatively large 95% likelihood percentiles around our mtDNA estimates. A recent evaluation of MIGRATE using a simulated data set similar in composition to our mtDNA data set found that it did not always accurately estimate migration rates, in part because of the use of a single locus rather than multiple loci (Abdo *et al.* 2004). This result also depends on the simplifying assumptions of an equal sex ratio and low variance in male reproductive success. Although we have no direct data on either parameter, these assumptions may well be reasonable for the yellow-naped amazon, given that nestling sex ratios show no departure from equality (South & Wright 2002) and it exhibits long-term monogamous pair bonds with low rates of extra-pair paternity (T.F. Wright *et al.*, unpublished). Direct studies of dispersal are required to better evaluate the extent of sex-biased dispersal between dialects in the yellow-naped amazon and examine whether it is accompanied by differences in learning patterns between the sexes.

### Implications for conservation of the yellow-naped amazon

Costa Rican populations of the yellow-naped amazon have experienced both habitat fragmentation and high levels of nest poaching. These threats are of concern from

a conservation standpoint because they could potentially reduce both gene flow and overall genetic diversity. Our results suggest that these threats have not yet had a strong impact on genetic structure and diversity. We found no evidence of cryptic population structure among sampling sites, which suggests ongoing gene flow between sites despite the highly fragmented habitat. Such gene flow is perhaps not surprising, given that both nests and communal night roosts of this species are often found in highly disturbed habitat such as cattle pastures and remnant riparian forests (T.F. Wright, unpublished). Overall, our findings give hope that, if the current high levels of illegal nest poaching can be reduced, these parrots may be able to maintain sufficient genetic diversity and dispersal capacity to repopulate suitable habitat.

### Acknowledgements

We thank Santos Estrada, Kevin Matson, Hugo Guadamuz, Roger Blanco and the staff of Area de Conservación Guanacaste for vital assistance in the field. Michael Russello generously shared unpublished microsatellite primers, and he and the members of the Smithsonian National Museum of Natural History's Genetics Program were a source of valuable advice on lab techniques. Research permits were granted by the Sistema Nacional de Areas de Conservación in Costa Rica and importation authorized by the Convention on the International Trade of Endangered Species, U.S. Department of Agriculture, and U.S. Fish and Wildlife Service. The research was conducted while TFW was a Smithsonian postdoctoral fellow (Office of Fellowships and Grants) and a Robinson postdoctoral fellow (Friends of the National Zoo). Research funding was provided by a Franklin Research Grant from the American Philosophical Society, the Friends of the National Zoo (to Eugene Morton) and the NMNH Genetics Program.

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