Journal of Avian Biology

JAV-00545
Ribeiro, R. D., McCormack, J. E., Álvarez, H. G., Carrasco, L., Grether, G., Mena, P., Sedano, R., Smith, T. B. and Karubian, J. 2015. Loss of sexual dimorphism is associated with loss of lekking behavior in the green manakin X*enopipo holochora*. – J. Avian Biol. doi: 10.1111/jav.00545

Supplementary material

APPENDIX 1

Molecular sexing methods

Molecular sexing was based on DNA extracted from blood or feather samples collected in the field and followed a standardized protocol, the QIAamp DNA Mini kit (QIAgen Technologies). DNA was amplified in 50μl of PCR reaction mixture. Each amplification mixture contained 30μl H_2O, 5μl buffer, 5μl $MgCl_2$, 5μl dNTPs, 2.5μl 2550F primer (Fridolfsson and Ellegren 1999), 2.5μl MSZ1R primer (Sehgal et al. 2005) and 0.25μl Taq polymerase. The amplification reaction was developed with the following PCR profile program: denaturation, 95°C for 30s; annealing, 56°C for 45s and extension, 72°C for 45s. The profile is repeated in 45 cycles and then sits at 72°C for 10min and storage at 4°C. Amplified DNA fragments were separated using electrophoresis in 2% agarose gel with TBE buffer. The gel was stained with SYBR© Safe (Life Technologies) to visualize PCR products for CDHZ and CDHW genes. Males are identified by having only the CDHZ band, while females present both CDHZ and CDHW bands. One band of CDHZ and CDHW were cut out of the agarose gel and put into 400μl of ABD buffer and left under 55°C for 10 min to dissolve the DNA. The following mixture was prepared for the two bands to confirm gene sequence: 2μl H_2O, 2μl Seq SYBR buffer, 3μl DNA, 1μl *sex 2550F*/*sex M5Z1R* primers and 1μl BigDye© (Life Technologies). Basic Local Alignment Search Tool (BLAST) was used to identify of the amplified DNA fragments in the Genebank.

References

Fridolfsson, A.K. and Ellegren, H. 1999. A simple and universal method formolecular sexing of non-ratite birds. J. Avian Biol. 30:116–121.

Sehgal, R.N.M., H.I. Jones and T.B Smith. 2005. Molecular evidence for host specificity of parasitic nematode microfilariae in some African rainforest birds. Mol. Ecol. 14:3988–3997.

APPENDIX 2

Table A1. Plumage differences by sex and subspecies for *Xenopipo holochlora* specimens measured at two different institutions. Non-significant differences are denoted by an X. Differences that remained significant after Bonferroni correction are shown in bold.

Test	**LSU by sex *(both subspecies)***	**LSU by sex (*litae*)**	**LSU by sex (*holochlora*)**	**UCLA by sex *(both subspecies)***	**UCLA by sex (*litae*)**	**UCLA by sex (*holochlora*)**	**LSU by subspecies**	**UCLA by subspecies**
Sample Size[1]	**43 males, 23 females**	**5 males, 3 females**	**38 males, 20 females**	**30 males, 16 females**	**10 males, 7 females**	**20 males, 9 females**	**58 *holochlora*, 9 *litae***	**32 *holochlora*, 17 *litae***
Crown								
Hue	X	X	X	X	X	X	0.009	**<0.0001**
Chroma	X	X	X	X	X	X	0.02	**<0.0001**
Brightness	X	X	X	X	0.01	X	0.007	**<0.0001**
Back								
Hue	X	X	X	X	X	X	**<0.0001**	**<0.0001**
Chroma	0.05	X	X	X	X	X	**<0.0001**	**<0.0001**
Brightness	X	X	X	X	X	X	**<0.0001**	0.004
Tail								
Hue	X	X	X	X	X	X	0.004	0.002
Chroma	X	X	X	X	X	X	0.05	**<0.0001**
Brightness	X	X	X	X	0.05	X	X	X
Wing Coverts								
Hue	X	X	X	X	X	0.05	0.004	0.0005
Chroma	X	X	X	X	X	0.02	**<0.0001**	**<0.0001**
Brightness	X	X	X	X	X	X	**0.0001**	X
Wing Edging[2]								
Hue	X	X	X	-	-	-	X	-
Chroma	X	X	0.03	-	-	-	0.03	-
Brightness	X	X	X	-	-	-	X	-

Breast									
	Hue	X	X	X	X	X	X	0.1	**<0.0001**
	Chroma	X	X	X	X	X	X	0.02	X
	Brightness	X	X	X	X	X	0.03	**<0.0001**	**<0.0001**
Belly									
	Hue	X	X	X	0.05	X	0.03	0.01	0.004
	Chroma	X	X	X	X	X	X	0.04	0.01
	Brightness	X	X	0.0003	X	X	X	X	X
Bill									
	Hue	X	X	X	X	X	X	**0.0001**	X
	Chroma	X	X	X	X	X	0.04	0.0006	X
	Brightness	X	X	X	X	X	X	0.004	0.02
Chin									
	Hue	X	X	X	0.01	X	0.1	**0.0001**	X
	Chroma	X	X	X	X	X	X	**<0.0001**	0.02
	Brightness	X	X	X	X	X	X	**<0.0001**	**<0.0001**
PC1		X	X	X	X	X	X	**<0.0001**	**<0.0001**
PC2		X	X	X	X	X	X	**<0.0001**	X
PC3		X	X	X	X	X	X	X	X
PC4		X	X	X	X	0.05	X	0.001	X
PC5		X	X	0.006	X	X	0.01	X	X
PC6		0.02	X	0.04	X	X	X	0.05	X
PC7		X	X	0.01	X	X	X	X	X
PC8		0.007	X	0.004	X	X	X	X	X

TABLE A2: PC loadings for plumage reflectance data of *Xenopipo holochlora* specimens measured at UCLA.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Crown Hue	0.285	0.176	-0.165	0.194	0.086	0.129	-0.030
Crown Chroma	-0.301	0.135	-0.002	0.147	0.136	0.189	0.219
Crown Brightness	0.242	-0.016	0.338	0.084	-0.227	-0.081	0.092
Back Hue	0.245	0.129	-0.336	0.049	-0.191	0.149	0.037
Back Chroma	-0.311	0.082	0.015	0.162	-0.174	0.186	0.235
Back Brightness	0.180	0.098	0.194	-0.179	0.270	-0.219	0.271
Tail Hue	0.172	0.007	0.035	0.349	0.120	-0.493	-0.017
Tail Chroma	-0.280	0.058	0.216	0.209	0.168	-0.340	-0.029
Tail Brightness	0.123	-0.134	-0.054	0.300	-0.379	0.013	0.288
Wing Hue	0.172	0.086	0.042	0.316	0.415	0.261	-0.064
Wing Chroma	-0.260	0.055	0.154	0.116	0.438	0.288	0.064
Wing Brightness	0.019	0.009	0.513	0.111	-0.252	0.173	-0.180
Breast Hue	0.232	0.339	0.032	-0.043	-0.098	0.143	-0.215
Breast Chroma	0.101	0.299	0.309	0.127	-0.133	0.266	-0.057
Breast Brightness	0.303	0.079	0.197	-0.114	0.071	0.010	0.051
Belly Hue	-0.212	0.334	-0.015	-0.077	-0.094	0.133	0.066
Belly Chroma	-0.216	0.157	-0.098	0.123	-0.258	-0.163	0.348
Belly Brightness	0.036	-0.224	0.414	0.030	0.000	-0.052	0.250
Bill Hue	-0.116	0.228	0.104	-0.415	-0.082	-0.094	0.138
Bill Chroma	-0.016	0.309	0.100	-0.392	0.031	-0.153	-0.117
Bill Brightness	0.164	-0.188	-0.074	-0.260	0.156	0.178	0.372
Chin Hue	0.004	0.451	-0.007	0.105	-0.025	-0.134	0.155
Chin Chroma	0.086	0.315	-0.161	0.183	0.127	-0.255	0.039
Chin Brightness	0.253	0.057	0.013	-0.047	0.114	0.107	0.502
% Variation Explained	0.258	0.155	0.082	0.073	0.063	0.056	0.049

TABLE A3: PC loadings for plumage reflectance data of *Xenopipo holochlora* specimens measured at LSU.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Crown Hue	-0.068	0.331	0.165	-0.199	-0.209	-0.414	0.101	0.089
Crown Chroma	0.209	-0.117	-0.248	0.021	0.281	0.416	-0.026	-0.140
Crown Brightness	-0.282	0.057	0.141	0.230	-0.121	-0.039	-0.034	0.024
Back Hue	0.032	0.439	-0.104	-0.250	0.040	0.117	0.147	-0.153
Back Chroma	0.243	-0.180	-0.154	-0.119	-0.048	0.218	0.190	0.081
Back Brightness	-0.217	0.195	0.113	0.219	0.126	-0.014	-0.227	0.008
Tail Hue	0.030	0.331	0.028	0.042	0.207	0.256	0.170	-0.184
Tail Chroma	0.045	0.042	-0.398	-0.032	-0.216	-0.265	0.210	0.002
Tail Brightness	-0.128	0.040	0.379	0.279	0.245	0.012	-0.150	0.047
Wing Hue	0.113	0.417	-0.032	-0.195	-0.028	0.015	-0.058	-0.195
Wing Chroma	0.242	-0.099	-0.024	-0.059	-0.210	-0.142	-0.273	0.118
Wing Brightness	-0.219	0.138	-0.006	0.133	0.215	0.121	0.287	-0.004
Wing Edging Hue	0.259	0.243	0.051	0.053	-0.183	0.083	-0.018	0.098
Wing Edging Chroma	0.188	-0.055	0.363	-0.124	-0.223	0.268	0.177	0.213
Wing Edging Brightness	-0.075	0.056	-0.459	0.234	0.139	-0.300	-0.015	0.075
Breast Hue	0.261	0.246	0.196	0.047	0.123	-0.080	-0.018	-0.023
Breast Chroma	0.253	0.001	0.129	-0.072	0.303	-0.198	-0.017	-0.038
Breast Brightness	-0.223	0.109	-0.173	0.029	-0.117	0.289	-0.302	0.213
Belly Hue	0.208	0.199	-0.084	0.266	-0.105	0.076	-0.164	0.230
Belly Chroma	0.137	-0.149	0.235	-0.293	0.177	-0.137	-0.131	0.155
Belly Brightness	0.008	-0.027	0.082	0.335	-0.187	0.086	0.506	0.355
Bill Hue	0.273	0.152	-0.108	0.194	-0.087	0.042	-0.140	0.114
Bill Chroma	0.047	-0.159	0.151	0.179	-0.325	0.012	0.091	-0.589
Bill Brightness	-0.108	0.028	-0.030	-0.242	0.299	-0.034	0.248	0.383
Chin Hue	0.278	0.153	0.046	0.301	0.000	-0.066	0.037	0.038
Chin Chroma	0.264	-0.090	-0.045	0.140	0.202	-0.070	-0.163	0.094
Chin Brightness	-0.199	0.120	-0.033	-0.234	-0.243	0.284	-0.287	0.166
% Variation Explained	0.294	0.134	0.083	0.067	0.059	0.051	0.048	0.040