Journal of Avian Biology

Supplementary material

JAV-01263
Maher, K. H., Eberhart-Phillips, L. J., Kosztolányi, A., dos Remedios, N., Carmona-Isunza, M. C., Cruz-López, M., Zefania, S., St Clair, J., AlRashidi, M., Weston, M. A., Serrano-Meneses, M. A., Krüger, O., Hoffman, J. I., Székely, T., Burke, T. and Küpper, C. 2017. High fidelity: extra-pair fertilisations in eight *Charadrius* plover species are not associated with parental relatedness or social mating system. – J. Avian Biol. doi: 10.1111/jav.01263

Appendix 1.

Table A1. Multiplex design and multiplex specific annealing temperatures (T_a) in *Charadrius* plovers.

Species	Multiplex	T_a (°C)	Locus	Dye	Reference
Snowy plover	SP1	57	Calex-02	PET	Küpper et al. 2007
			Calex-04	NED	Küpper et al. 2007
			Calex-05	6-FAM	Küpper et al. 2007
			Calex-10	6-FAM	Küpper et al. 2007
			Calex-18	6-FAM	Küpper et al. 2007
			Calex-19	VIC	Küpper et al. 2007
			Calex-23	PET	Küpper et al. 2007
			Calex-39	VIC	Küpper et al. 2007
			Calex-43	6-FAM	Küpper et al. 2007
			CAM-18	6-FAM	Dawson et al. 2013
			P2/P8	NED	Griffiths et al. 1998
	SP2	60	Calex-12	6-FAM	Küpper et al. 2007
			Calex-14	VIC	Küpper et al. 2007
			Calex-31	NED	Küpper et al. 2007
			Calex-32	6-FAM	Küpper et al. 2007
			Calex-35	6-FAM	Küpper et al. 2007
			HrU2	NED	Primmer et al. 1995
Kittlitz's plover	KiP1	56	Calex-01	6-FAM	Küpper et al. 2007
			Calex-18	NED	Küpper et al. 2007
			Calex-19	NED	Küpper et al. 2007
			Calex-33	HEX	Küpper et al. 2007
			Calex-45	HEX	Küpper et al. 2007
			BmaTATC371	6-FAM	Küpper et al. 2008
		65	Calex-16*	NED	Küpper et al. 2007
	KiP2	56	Calex-36	HEX	Küpper et al. 2007
			Mopl 15	6-FAM	Küpper et al. 2008
			P2/P8	HEX	Griffiths et al. 1998
		48	HrU2*	NED	Primmer et al. 1995
	KiP3	56	Calex-15	6-FAM	Küpper et al. 2007
			Tgu06	HEX	Dawson et al. 2010
Kentish plover	KP1	57	Calex-02	PET	Küpper et al. 2007
White-fronted plover			Calex-04	NED	Küpper et al. 2007
Red-capped plover			Calex-05	6-FAM	Küpper et al. 2007
			Calex-08	VIC	Küpper et al. 2007
			Calex-18	NED	Küpper et al. 2007
			Calex-19	VIC	Küpper et al. 2007
			Calex-23	PET	Küpper et al. 2007
			Calex-24	6-FAM	Küpper et al. 2007
			Calex-39	VIC	Küpper et al. 2007
			Calex-43	6-FAM	Küpper et al. 2007
			Calex-45	6-FAM	Küpper et al. 2007
			P2/P8	NED	Griffiths et al. 1998
	KP2	62	Calex-01	6-FAM	Küpper et al. 2007
			Calex-11	NED	Küpper et al. 2007
			Calex-12	6-FAM	Küpper et al. 2007
			Calex-14	VIC	Küpper et al. 2007
			Calex-22	VIC	Küpper et al. 2007
			Calex-26	VIC	Küpper et al. 2007

			Calex-28	NED	Küpper et al. 2007
			Calex-31	NED	Küpper et al. 2007
			Calex-37	PET	Küpper et al. 2007
	KP3	60	Calex-10	HEX	Küpper et al. 2007
			Calex-32	6-FAM	Küpper et al. 2007
			Calex-35	HEX	Küpper et al. 2007
			C201	6-FAM	Funk et al. 2007
			C203	NED	Funk et al. 2007
	KP4	60	C204	6-FAM	Funk et al. 2007
			C205	HEX	Funk et al. 2007
			HrU2	NED	Primmer et al. 1995
Two-banded plover	TbP1	57	Calex-02	PET	Küpper et al. 2007
			Calex-05	6-FAM	Küpper et al. 2007
			Calex-14	VIC	Küpper et al. 2007
			Calex-17	6-FAM	Küpper et al. 2007
			Calex-39	VIC	Küpper et al. 2007
	TbP2	56	Man 13	HEX	Piertney et al. 2002
			Tgu04_004	HEX	Dawson et al. 2010
			C201	6-FAM	Funk et al. 2007
	TbP3	57	Calex-07	6-FAM	Küpper et al. 2007
			BmaTATC444	HEX	Küpper et al. 2008
			Tgu06	HEX	Dawson et al. 2010
	TbP4	62	Calex-33	HEX	Küpper et al. 2007
			Calex-40	6-FAM	Küpper et al. 2007
			P2/P8	NED	Griffiths et al. 1998
Rufous-chested dotterel	RcD1	56	Calex-35	HEX	Küpper et al. 2007
			Tgu01_040	6-FAM	Dawson et al. 2010
			Tgu03_098	HEX	Dawson et al. 2010
			Mopl 21	6-FAM	Küpper et al. 2008
			Man 13	HEX	Piertney et al. 2002
		48	HrU2*	NED	Primmer et al. 1995
		65	Calex-16*	6-FAM	Küpper et al. 2007
	RcD2	56	Calex-11	NED	Küpper et al. 2007
			Calex-45	6-FAM	Küpper et al. 2007
			Tgu06	HEX	Dawson et al. 2010
			Mopl 15	6-FAM	Küpper et al. 2008
	RcD3	56	Tgu04_004	HEX	Dawson et al. 2010
			SNIPE B2	6-FAM	Küpper et al. 2008
			P2/P8	NED	Griffiths et al. 1998
Madagascar plover	MP1	56	Calex-01	6-FAM	Küpper et al. 2007
			Calex-07	6-FAM	Küpper et al. 2007
			Calex-10	HEX	Küpper et al. 2007
			Calex-18	NED	Küpper et al. 2007
			Calex-19	NED	Küpper et al. 2007
			Calex-45	HEX	Küpper et al. 2007
			Tgu04_004	HEX	Dawson et al. 2010
			Mopl 6	6-FAM	Küpper et al. 2008
	MP2	56	Calex-11	NED	Küpper et al. 2007
			Calex-35	HEX	Küpper et al. 2007
			Tgu05_053	6-FAM	Dawson et al. 2010
			P2/P8	NED	Griffiths et al. 1998

*Primers were run using a different annealing temperature and then combined within a single multiplex post PCR.

Table A2. Combined non-exclusion probabilities for plover populations, first parent, second parent and parent pair. Calculated in Cervus 3.0.3 (Kalinowski et al. 2007), using adults only.

Species	**Population**	**Combined non-exclusion probability**		
		1st parent	**2nd parent**	**Both parents**
Snowy plover	Mexico	0.11956226	0.01419684	0.00077796
Kittlitz's plover	Madagascar	0.01117399	0.00036923	0.00000183
Kentish plover	Turkey	0.0000022	1.47E-09	8.60E-16
Kentish plover	Cape Verde	0.00075197	0.00000289	3.52E-10
Kentish plover	UAE	0.000021	0.00000004	2.17E-13
Kentish plover	Azores	0.0053364	0.00008139	0.00000013
Kentish plover	Saudi Arabia	0.00152764	0.00002575	0.00000002
Two-banded plover	Falklands Islands	0.02532726	0.00192364	0.00002864
Rufous-chested dotterel	Falklands Islands	0.21518926	0.04244281	0.00435115
White-fronted plover	Madagascar	0.03382107	0.00100349	0.0000064
Madagascar plover	Madagascar	0.3154022	0.09064241	0.01784802
Red-capped plover	Australia	0.00204499	0.0000278	0.00000002

Table A3. Details of model simplification for Generalized Linear Mixed Models (GLMMs with a cloglog error) to test for the presence of extra-pair paternity (EPP) and extra-pair fertilisations (EPF) within plover broods. Population nested within species was fitted as a random factor. All factors included in the full model are reported. $\chi^2$and *p* values from model simplification are reported for each variable after removal from the full model. Only back-transformed estimates and standard errors are reported for variables kept in the final model (in italics). The variance and standard deviation are reported for random effects of population nested within species.

	Order of removal	Variables	df	Strict (n=257)		Random effect Variance, SD Population:Species (Species)	Relaxed (n=338)		Random effect Variance, SD Population:Species (Species)
				Estimate ± SE	χ^2 (*p*)		Estimate ± SE	χ^2 (*p*)	
i) EPP		*Intercept*		*0.0098 ± 0.92*		0.38, 0.62 (<0.001, <0.001)	*0.015 ± 0.87*		0.79, 0.89 (<0.001, <0.001)
	3	Standardised hatch date	1		3.81 (0.051)	0.58, 0.76 (<0.001, <0.001)		2.68 (0.10)	0.80, 0.89 (<0.001, <0.001)
	2	Relatedness	1		0.33 (0.56)	0.61, 0.78 (<0.001, <0.001)		0.15 (0.70)	0.79, 0.89 (<0.001, <0.001)
	1	Relatedness x standardised hatch date	1		0.0025 (0.96)	0.62, 0.79 (<0.001, <0.001)		0.25 (0.62)	0.87, 0.93 (<0.001, <0.001)
ii) EPF		*Intercept*		*0.0098 ± 0.92*		0.38, 0.62 (<0.001, <0.001)	*0.025 ± 0.81*		0.69, 0.83 (<0.001, <0.001)
	3	Standardised hatch date	1		3.81 (0.051)	0.58, 0.76 (<0.001, <0.001)		1.20 (0.27)	0.69, 0.83 (<0.001, <0.001)
	2	Relatedness	1		0.33 (0.56)	0.61, 0.78 (<0.001, <0.001)		0.39 (0.53)	0.71, 0.84 (<0.001, <0.001)
	1	Relatedness x standardised hatch date	1		0.0025 (0.96)	0.62, 0.79 (<0.001, <0.001)		0.42 (0.52)	0.74, 0.86 (<0.001, <0.001)

References

Dawson, D. A., Horsburgh, G. J., Küpper, C., Stewart, I. R. K., Ball, A. D., Durrant, K. L., Hansson, B., Bacon, I., Bird, S., Klein, Á., Krupa, A. P., Lee, J. W., Martín-Gálvez, D., Simeoni, M., Smith, G., Spurgin, L. G. and Burke, T. 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility - as demonstrated for birds. - Mol. Ecol. Resour. 10: 475–494.

Dawson, D. A., Ball, A. D., Spurgin, L. G., Martín-Gálvez, D., Stewart, I. R. K., Horsburgh, G. J., Potter, J., Molina-Morales, M., Bicknell, A. W. J., Preston, S. A. J., Ekblom, R., Slate, J. and Burke, T. 2013. High-utility conserved avian microsatellite markers enable parentage and population studies across a wide range of species. - BMC Genomics 14: 176.

Funk, W. C., Mullins, T. D. and Haig, S. M. 2007. Conservation genetics of snowy plovers (*Charadrius alexandrinus*) in the western hemisphere: population genetic structure and delineation of subspecies. - Conserv. Genet. 8: 1287–1309.

Griffiths, R., Double, M. C., Orr, K. and Dawson, R. J. G. 1998. A DNA test to sex most birds. - Mol. Ecol. 7: 1071–1075.

Kalinowski, S. T., Taper, M. L. and Marshall, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. - Mol. Ecol. 16: 1099–1106.

Küpper, C., Horsburgh, G. J., Dawson, D. A., Ffrench-Constant, R., Székely, T. and Burke, T. 2007. Characterization of 36 polymorphic microsatellite loci in the Kentish plover (*Charadrius alexandrinus*) including two sex-linked loci and their amplification in four other Charadrius species. - Mol. Ecol. Notes 7: 35–39.

Küpper, C., Burke, T., Székely, T. and Dawson, D. A. 2008. Enhanced cross-species utility of conserved microsatellite markers in shorebirds. - BMC Genomics 9: 502.

Piertney, S. B., Shorey, L. and Höglund, J. 2002. Characterization of microsatellite DNA markers in the white-bearded manakin (*Manacus manacus*). - Mol. Ecol. Notes 2: 504–505.

Primmer, C. R., Møller, A. P. and Ellegren, H. 1995. Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. - Mol. Ecol. 4: 493–498.