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Supplementary material

Appendix 1

This file is the Supplementary Material to the article: Tomotani, B.M., Caglar, E., de la Hera, I., Mateman, C., Visser, M.E. Early arrival is not associated with more extra-pair fertilizations in a long-distance migratory bird. Journal of Avian Biology.

1) Details on the paternity assignment

We used the program Cervus (Kalinowski et al. 2007) to analyze the paternity of the offspring. The results obtained from Cervus are summarized in Table A1. Our analysis started with a total of 691 samples (488 eggs, 110 males and 93 females), but not all loci were recognizable in all cases.

Table A1: Summary statistics for the five microsatellite loci used in this study. Number of birds screened (*n*), number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), Weir and Cockerham's (1984) within-population inbreeding coefficient (F_{IS}), frequency of null alleles (F_{Null}), exclusion probability of the locus for the first parent (P_{Ex1}), exclusion probability of the locus for the second parent with the first assigned (P_{Ex2}), and the exact probability for deviation from Hardy-Weinberg equilibrium ($P_{H\text{–}W}$).

Locus	n	NA	H_O	H_E	F_{IS}	F_{Null}	P_{Ex1}	P_{Ex2}	P_{HW}
Fhyu234	688	29	0.93	0.92	0.92	0.005	0.27	0.16	0.95
Fhyu304	691	10	0.83	0.83	0.81	0.001	0.5	0.33	0.001
Fhyu336	691	19	0.87	0.87	0.86	0.002	0.4	0.25	0.29
Fhyu448	691	23	0.92	0.88	0.87	0.02	0.37	0.23	0.0002
Fhyu453	687	24	0.89	0.91	0.9	0.009	0.32	0.19	0.003

We had three loci that deviated from the Hardy-Weinberg equilibrium, therefore we verified the accuracy of these by re-visualizing all mismatches between known pairs of parent and offspring to obtain the final and accurate paternity data (Marshall et al. 1998, Hoffman and Amos 2005, Casey et al. 2011).

We also calculated the combined exclusion power of our five loci with the program Cervus, obtaining the following results:

- Combined exclusion probability (first parent): 0.9936;
- Combined exclusion probability (second parent): 0.99945;
- Combined exclusion probability (parent pair): 0.9999976.

2) Contingency tables for Fisher's exact test

Table A2: Contingency tables used for the Fisher's exact test described in the Methods section. **a)** Table containing all eggs in the (experiment described above) and used to test for differences in assignment between eggs with lay order 1 to 6 from eggs with lay order 7. **b)** Table containing all assigned eggs from Table A2a and used to determine whether lay order could affect proportion of EPP.

a.	Eggs 7	Eggs 1 to 6	row total
Assigned	10	314	324
Not assigned	14	35	49
column total	24	349	373

b.	Eggs 7	Eggs 1 to 6	row total
EPP	2	21	23
not EPP	8	293	301
column total	10	314	324

3) Sample sizes

Table A3: Sample sizes at different moments of the data collection from the estimated number of birds based on the number of broods (94) to the actual sampled and assigned birds.

	Detected (brood)	Sampled (incl. bachelors)	Assigned	With Arrival date	With laying date	With arrival and laying date
Males	94	110	109	87	87	72
Females	94	93	93	n.a.	n.a.	n.a.
Eggs	555	488	481	n.a.	n.a.	n.a.
Total	743	691	683	87	87	72

4) Additional tables

Table A4: Model results of individual simple and multiple logistic regression analysis, explaining the probability of gain extra-pair paternity, lose paternity or father an egg. Statistics are given at the point of exclusion from the model. In the case of significant quadratic terms, statistics for the other terms are given in the presence of the quadratic term. The initial sample size was two overlapping (but not the same) sets of 87 birds with known arrival and/or laying date of the female (and, from these, 72 had both arrival and laying date known). Estimates and standard errors are only given in case of significant terms.

Response variable	Explanatory variables	Estimates	s.e.	df	Sample size	χ^2	*p* -value
Probability to gain extra-pair paternity	(Arrival date of male)^2			1	72	0.10	0.75
	Arrival date of male			1	72	0.51	0.47
	Lay date of female	-0.07	0.04	1	87	4.39	**0.04 ***
Probability to gain extra-pair paternity (simple regressions)	Arrival date of all males			1	87	0.58	0.45
	Arrival date of males (excl. bachelors)			1	72	0.01	0.94
	Lay date of female	-0.07	0.04	1	87	4.39	**0.04 ***
Probability to lose paternity	(Arrival date of male)^2	0.03	0.01	1	72	5.27	**0.02 ***
	Arrival date of male	-1.12	0.48	1	72	5.84	**0.02 ***
	Lay date of female			1	72	1.11	0.29
Probability to father an egg (simple regressions)	Arrival date of male	-0.19	0.08	1	87	6.90	**0.01***
	Lay date of female			1	87	0.11	0.75

Table A5: Model results of individual simple and multiple regression analysis, explaining the laying date of the female, the number of fathered eggs and the difference in laying date of social and extra-pair female. Statistics are given at the point of exclusion from the model. Estimates and standard errors are only given in case of significant terms.

Response variable	Explanatory variables	Estimate	s.e.	ndf	ddf	R^2 (adjusted)	*F*-test	*p*-value
Lay date of the female	Arrival date of male	0.81	0.20	1	70	0.18	16.37	**< 0.01***
Number of fathered eggs (with EPP)	(Arrival date of male)^2			1	68	0.18	3.58	0.06
	Arrival date of male			1	69	0.15	0.48	0.49
	Lay date of female	-0.09	0.02	1	85	0.14	14.75	**< 0.01***
Number of fathered eggs (with EPP) (simple regressions)	Arrival date of male			1	70	0.00	0.88	0.35
	Lay date of female	-0.09	0.02	1	85	0.14	14.75	**< 0.01***
Number of fathered eggs (only EPP lost)	(Arrival date of male)^2			1	68	0.18	4.07	0.05
	Arrival date of male			1	69	0.14	0.02	0.90
	Lay date of female	-0.07	0.02	1	85	0.13	13.69	**< 0.01***
Number of fathered eggs (only EPP lost) (simple regressions)	Arrival date of male			1	70	0.01	1.91	0.17
	Lay date of female	-0.07	0.02	1	85	0.13	13.69	**< 0.01***
Number of social eggs	(Arrival date of male)^2			1	68	0.04	0.57	0.45
	Arrival date of male			1	69	0.04	1.29	0.26
	Lay date of female	-0.05	0.02	1	85	0.09	9.71	**< 0.01***
Number of social eggs (simple regressions)	Arrival date of male			1	70	-0.01	0.02	0.88
	Lay date of female	-0.05	0.02	1	85	0.09	9.71	**< 0.01***
Number of fathered eggs (with EPP)	Number of social eggs	0.84	0.12	1	85	0.35	47.87	**< 0.01***
Number of fathered day 12 chicks (with EPP)	(Arrival date of male)^2			1	68	0.05	0.97	0.33
	Arrival date of male			1	69	0.05	2.38	0.13
	Lay date of female	-0.06	0.02	1	85	0.05	5.31	**0.02 ***
Number of fathered day 12 chicks (with EPP) (simple regressions)	Arrival date of male			1	70	-0.01	0.37	0.55
	Lay date of female	-0.06	0.02	1	85	0.05	5.31	**0.02 ***
Number of fathered day 12 chicks (no gained EPP)	(Arrival date of male)^2			1	67	0.04	1.05	0.31
	Arrival date of male			1	68	0.04	1.64	0.20
	Lay date of female	-0.05	0.02	1	84	0.04	4.39	**0.04 ***
Number of fathered day 12 chicks (no gained EPP) (simple regressions)	Arrival date of male			1	69	-0.01	0.16	0.69
	Lay date of female	-0.05	0.02	1	84	0.04	4.39	**0.04 ***
Difference (lay date social - EP nest)	(Arrival date of male)^2			1	9	0.51	0.02	0.89
	Arrival date of male			1	10	0.56	1.59	0.24
	Lay date of female	-1.36	0.32	1	13	0.54	17.18	**< 0.01***
Difference (lay date social -	Arrival date of male			1	11	-0.08	0.11	0.75

EP nest) (simple regressions)	Lay date of female	-1.36	0.32	1	13	0.54	17.18	**< 0.01***

5) Arrival and breeding synchrony

Because breeding synchrony could be an important factor to determine the amount of extra pair paternity in a given year (Canal et al. 2012), we calculated the breeding synchrony for all years we had laying date data in our population using the formula from Kempenaers (1993). We assumed that the fertile period of the female ranged from 5 days before the first egg was laid until the egg laying date of the penultimate egg. The higher the index, the more synchronous the population. The year 2014 had one of the lowest values of breeding synchrony in our population, which would predict a large amount of extra pair paternity (Fig. A1a). For comparison, we also calculated the synchrony in arrival dates showing that 2014 was an average year in terms of spread of arrival dates (Fig. A1b).

Observed low breeding synchrony in 2014 not only predicts a large amount of EPP but also a weaker relationship between EPP and female laying date (Canal et al. 2012). Thus, sampling in years with higher breeding synchrony could lead to a stronger relationship between EPP and laying dates. A similar effect on male arrival date, however, would only happen if the association between arrival and laying dates is strong (i.e. large R-squared). Or if the strength of this association increases in a context of high breeding synchrony. Since neither one of these scenarios was supported by our data (Fig. A1c), it is unlikely that sampling another year with a higher breeding synchrony would have led to a stronger/significant relationship between arrival date and EPP. Likewise, a decrease in the standard deviation in arrival dates does not lead to a stronger association between arrival and laying dates (Fig. A1d).

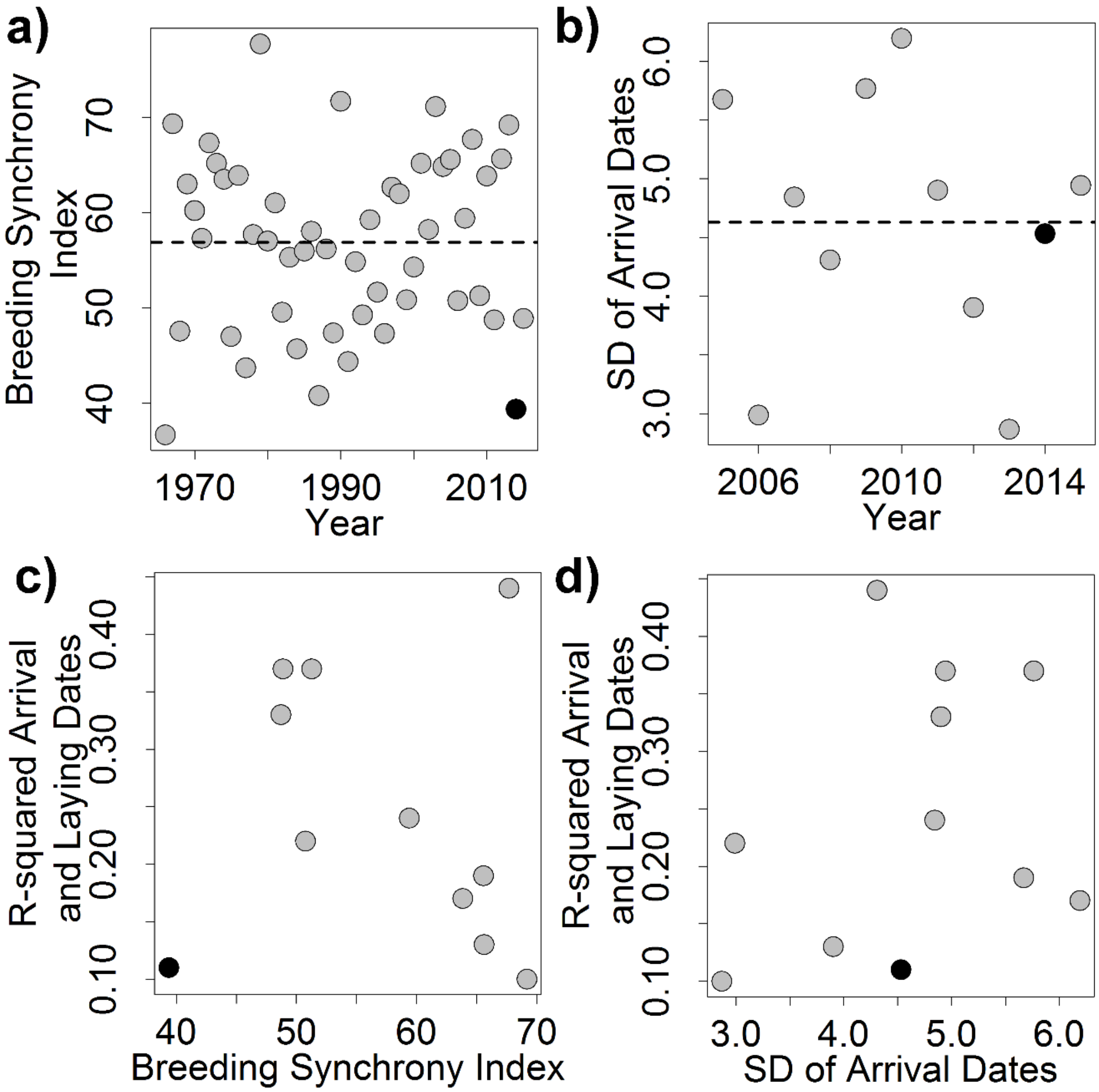

Figure A1: a) Breeding synchrony index from 1964 to 2015; **b)** Standard deviation of arrival dates from 2005 to 2015 **c)** R-squared of the relation between arrival and laying dates in relation to the breeding synchrony index from 2005 to 2015 (*p*-value = 0.69); **d)** R-squared of the relation between arrival and laying dates in relation to the standard deviation of arrival dates from 2005 to 2015 (*p*-value = 0.39). The black dot represents 2014, the year in which this data collection took place. The dashed line represent the average value for the breeding synchrony index and standard deviation of arrival dates in plots **a** and **b**, respectively.

6) Extra-pair paternity and male characteristics

Since other characteristics could also be correlated to EPP, we tested using our dataset whether male blackness and front patch size could be correlated to probability of gain and lose paternity, father eggs and also on the number of fathered eggs and chicks. In 2014, there was no effect of male characteristics on these components for our population (Table A6).

Table A6: Model results of individual multiple (logistic) regression analysis explaining the probability of gain extra-pair paternity and lose paternity and the number of fathered eggs and chicks in relation to male blackness and front patch size. Statistics are given at the point of exclusion of the term from the model. Estimates and standard errors are only given in case of significant terms.

Probability to gain extra-pair paternity	**Estimates**	**s.e.**	**df**	**Sample size**	**χ^2**	***p*-value**
Arrival date of the male : Blackness			1	97	0.30	0.58
Arrival date of the male : Front patch size			1	97	0.12	0.73
Lay date of the female : Blackness			1	97	0.23	0.63
Lay date of the female : Front patch size			1	97	0.92	0.34
Arrival date of the male			1	97	0.41	0.52
Lay date of the female	-0.07	0.04	1	97	4.39	**0.04***
Blackness			1	97	0.73	0.39
Front patch size			1	97	0.04	0.84
Probability to gain lose paternity	**Estimates**	**s.e.**	**df**	**Sample size**	**χ^2**	***p*-value**
Arrival date of the male : Blackness			1	97	0.12	0.73
Arrival date of the male : Front patch size			1	97	0.55	0.46
Lay date of the female : Blackness			1	97	0.99	0.32
Lay date of the female : Front patch size			1	97	0.02	0.88
Arrival date of the male			1	97	0.64	0.43
Lay date of the female			1	97	0.54	0.46
Blackness			1	97	2.03	0.15
Front patch size			1	97	1.39	0.24
Probability to father an egg	**Estimates**	**s.e.**	**df**	**Sample size**	**χ^2**	***p*-value**
Arrival date of the male : Blackness			1	97	0.04	0.84
Arrival date of the male : Front patch size			1	97	0.06	0.80
Arrival date of the male	-0.19	0.08	1	97	6.90	**0.01***
Blackness			1	97	0.10	0.76
Front patch size			1	97	0.12	0.73
Number of fathered eggs (with EPP)	**Estimates**	**s.e.**	**ndf**	**ddf**	***F*-test**	***p*-value**
Arrival date of the male : Blackness			1	56	0.67	0.42
Arrival date of the male : Front patch size			1	54	0.01	0.94
Lay date of the female : Blackness			1	55	0.51	0.48
Lay date of the female : Front patch size			1	73	1.31	0.26
Arrival date of the male			1	57	0.33	0.57
Lay date of the female	-0.09	0.02	1	87	-3.84	**< 0.01***
Blackness			1	74	0.02	0.88
Front patch size			1	75	0.81	0.37

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