**APPENDIX**

**A1. Materials and methods**

Genomic DNA was extracted from blood samples from 103 individuals (7 sampled in 2015, 33 in 2018, and 63 in 2019) using a commercial kit (High Pure PCR Template Preparation Kit, Roche) following the manufacturer’s instructions. All individuals were sexed by determining the size of two different introns of the *CHD* gene. For this, the following primer pairs were used: 3007/3112 (Ellegren & Fridofsson, 1997) and sex1'/sexmix (Wang et al., 2010). PCR conditions available under request.

We screened the 103 samples with a set of 16 polymorphic microsatellite loci (Tables A1 and A2). In order to determine whether part or the whole S brood was sired by the subordinate immigrant male we needed a reference population, i.e. a gene pool to calculate the allele frequencies of the alleged father's own population. Because samples from 2015 were limited in number, we used individuals sampled in 2018 and 2019 as reference population. Given the lifespan of the species and the absence of genetic differentiation between samples from 2018 and 2019, the obtained dataset can be considered as a suitable reference population (see below). Eleven loci had previously proved polymorphic in *Corvus corone* (Baglione et al.; 2002, Haas & Hansson, 2008), whereas markers CoBr19 (Schoenle et al., 2007), Cb06, Cb20 (Verdugo et al., 2012), and ApCo30, ApCo31 (Stenzler & Fitzpatrick 2002) were tested in carrion crow for the first time in the present study. PCR products (1.2 μl) were mixed with 16 ul formamide containing GENESCAN-500 (ROX) Size Standard (Applied Biosystems, ABI) and the allele size of PCR products was determined on a 96-capillary 3730xl DNA Analyzer (ABI). Allele size was scored with GENEIOUS 8.1.9 (www.geneious.com).

Number of alleles observed, observed and expected heterozygosities, as well as *F*IS values and their significance after 10 000 permutations were calculated with GENODIVE 3.03 (Meirmans, 2020). Deviations from Hardy-Weinberg equilibrium (HWE) were tested using the web version of GENEPOP 4.7 (Rousset, 2008) by applying the probability (exact) test for each locus and the following Markov chain parameters: Dememorization number = 1000, Number of batches = 200, Number of iterations per batch = 1000. Deviations from gametic equilibrium between pairs of loci (LD) were tested with the loglikelihood ratio test (G-test) implemented in GENEPOP and using the aforementioned Markov chain parameter. We estimated the frequency of null alleles using ML-NULLFREQ (Kalinowski & Taper, 2006). For this, we firstly tested each locus for heterozygote deficiency using 100 000 randomisations and then calculated the frequency of null alleles for any marker with *p* < 0.05. To correct for multiple comparisons in analyses that we repeated 16 times, we adjusted alpha = 0.05 by applying the Holm-Bonferroni correction (a.k.a. sequential Bonferroni). The gametic equilibrium results (120 tests) were corrected controlling the False Discovery Rate (FDR) according to Benjamini & Yekutieli (2001) as implemented in MYRIADS 1.2 (Carvajal-Rodríguez 2018).

We calculated the mean polymorphic information content (PIC) of these 16 markers and their nonexclusion probabilities with CERVUS 3.0.7 (Kalinowski et al., 2007).

We used COLONY 2.0.6.6 (Jones & Wang 2010), which implements a full-pedigree likelihood approach to simultaneously infer sibship and parentage among a group of individuals provided that multilocus genotype data are available. We performed ten replicate runs for all samples, each of them with a different random number seed, excluding individuals 1933 and 1808 (see Results). All replicates were run using the following setting: dioecious and diploid species, female polygamy, male monogamy, outbreeding model, without clones, medium run length, full-likelihood estimation, medium length of run, allele frequencies estimated from the dataset (no updating), no sibship size scaling, and no sibship prior. Following Baglione et al. (2017) and Bolopo et al. (2017) dropout rate was set as the null alleles frequency estimated from the dataset. Error rate per locus was calculated as the proportion of mismatches between the different valid electropherograms obtained for the same individual and locus and the reference genotype (Pompanon et al., 2005). For this, an average of 15.69% of the genotypes per locus were repeated. Error rate per locus was zero for ten of the surveyed loci, but it ranged between 0.008 (L18) to 0.063 (L8) (average = 0.021, Table A1) for the remaining loci. Therefore, we conservatively applied a genotyping error rate of 0.021 for all markers, but for L5, L8, L9, L19, and L20. These loci were analysed using the estimated error rate indicated in Table A1. COLONY requires the probability of the genotype of a real parent to be present in the multilocus datafiles of candidate adults. We estimated this value as the average proportion of genotyped adults considering the 19 territories surveyed in 2018–2019. This calculation resulted in 0.816 for females and 0.614 for males. Number of known paternal sibships, known maternal sibships, offspring with excluded fathers, offspring with excluded mothers, excluded paternal sibships and excluded maternal sibships were all set to zero. Only those cases of parentage or sibship assignment that appeared in at least 7 of the 10 replicates with probabilities ≥0.95 were considered.

We ran ten replicate simulations in COLONY to evaluate the information sufficiency of our set of markers to correctly assign parentage/sibship in the studied scenario (Wang, 2013). The simulated genotypes for offspring, mothers and fathers were generated based on the observed allele frequencies for the 16 markers. The mating matrix was generated based on the BESTCONFIG file obtained in the aforementioned empirical runs. With regard to the remaining parameters, we used identical options/values to the empirical runs. The result of this simulation analysis showed that the probability to correctly assign two individuals as full sibs was 96.0 ± 5.1% (mean ± SD); the probability to correctly assign two individuals as half sibs was 83.3 ± 14.8%; the probability to correctly assign two individuals as non-sibs was 99.8 ± 0.2%, the probability to correctly leave unassigned parentage when the parent has not been sampled was 98.1 ± 0.2%; and the probability to correctly assign parentage when the parent has been sampled was 97.3 ± 1.9%.

**A2. Results**

Only loci L2 and L13 deviated from HWE after sequential Bonferroni correction probability (exact) test, *p* = 0.0001 and 0.002, respectively). The studied dataset conformed, however, to the Hardy-Weinberg expectations for a single panmictic population (global test for heterozygote deficiency and excess, *p* = 0.919 and 0.081, respectively). With regard to tests for gametic equilibrium, 15 locus × locus out of 120 combinations were significant after FDR correction (L4×L8, L12×L13, L8×L12, L11×L14, L4×L19, L7×L14, L13×L19, L4×L13, L9×L20, L2×L7, L7×L11, L9×L12, L4×L9, L3×L5, L12×L18). However, this result should be hardly noticeable on sibship and parentage analysis using COLONY. LD effect is detectable only when all of the markers are taken from a very small chromosomal fragment (approx. 1MB), as checked by simulations (Wang, 2013), which does not occur here (Table A1).

Locus L19 was the only one showing a significant *F*IS value after sequential Bonferroni correction (*p* = 0.002, Table A1). Locus L3 was the only one showing a significant deficiency of heterozygotes (*p* = 0.0401), likely caused by null alleles. The frequency of the putative null variant was 0.042. This null-allele did not substantially affect the observed allele frequencies (average of the differences for the six alleles = 0.007, SD = 0.010).

PIC value was 0.563 and the combined non-exclusion probabilities for the first, second parent, and parent pair were 4.359×10–3, 8.070×10–5 and 1.2×10–5, respectively. The combined non-exclusion probabilities for identity and sib identity were 2.44×10–13 and 9.68×10–6, respectively.

Two chicks (codes 1923 and 1933) sampled in 2019 in different territories showed identical genotypes for the 16 markers. We are certain this is not a lab error, as these two individuals show a different genotype for locus Ck.5A5F (Tarr & Fleisher, 1998) (data not shown; this locus and three other markers had been tested during the optimization process but finally discarded). We removed individual 1933PM from subsequent analyses, as COLONY assumes all multilocus genotypes to be different.

The percentage of missing data was 1.88. This value decreased to 0.67% after removal of individual 1808PF, which produced failed electropherograms in nine out of the 16 genotyped loci. Following the COLONY user guide, we removed this specimen from subsequent analyses.

With regard to the nest where copulations were reported (coded S, see main text), all replicates of the paternity test ran in COLONY under the assumption of female monogamy ruled out the immigrant male A6 (helper, laboratory code 1523) as the biological father of the five nestlings (probability < 0.001 in the ten replicates). The adult female A2 (laboratory code 1522) was assigned as the biological mother with probability > 0.994 in eight out of ten replicates (and probabilities of 0.625 in the remaining two replicates). The five nestlings (codes 1524–1528) were full-sibs, (full sib dyad analysis, probability ≥ 0.999 for all combinations of five nestlings taken two at a time; best full sib family analysis, probabilities > 0.922 for the ten replicates). Lastly, these five chicks, the adult female and the same unsampled male were the best family cluster (probabilities > 0.954). These results did not change when allowing both male and female polygamy. In conclusion, the whole S brood was sired by a unique unsampled male that was not the subordinate immigrant male. Considering that: (i) full-brood paternity by an extra-group male is, at best, very rare in this population, with only one suspected case out of 38 analyzed broods (2.6%; Baglione et al., 2002, unpublished data); (ii) the unbanded group member behaved as a typical male breeder, being the dominant in its group and contributing substantially to chick provisioning (3.1 feeds per hour, as compared to the average provisioning rate of male breeders 3.2 feeds per hour, SE = 0.4; see Canestrari et al., 2005), we concluded that the observed copulations most likely occurred between a father and his son.

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**Table A2**. Laboratory information needed to replicate the laboratory work described in this work. All PCR reactions were prepared with the Type-it Microsatellite PCR Kit (Qiagen) following the manufacturer instructions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Locus | Alias | Annealing temperature (ºC) | MULTIPLEX | Dye | Allele range |
| Ck.5A4B | L2 | 60 | M4 | NED | 112–128 |
| Ck.5A4D | L3 | 57 | M6 | NED | 106–122 |
| Ck.B6D | L4 | 57 | M1 | HEX | 146–164 |
| Ck.5A5G | L5 | 57 | M2 | FAM | 189–195 |
| Ck.1B5D | L7 | 57 | M1 | NED | 84–96 |
| CmeG9 | L8 | 60–50\* | M3 | HEX | 221–237 |
| CmeH9 | L9 | 60–50\* | M3 | FAM | 193–201 |
| CmeH2 | L11 | 60–50\* | M3 | NED | 146–172 |
| Ck.1B6G | L12 | 60 | M4 | FAM | 108–126 |
| CoBr19 | L13 | 60 | M4 | HEX | 363–468 |
| MJG1 | L14 | 57 | M2 | NED | 182–242 |
| Cb06 | L15 | 57 | M6 | HEX | 121–141 |
| Cb20 | L16 | 57 | M6 | FAM | 111–115 |
| Cb05 | L18 | 57 | M5 | FAM | 92–98 |
| ApCo30 | L19 | 57 | M5 | NED | 197–209 |
| ApCo31 | L20 | 57 | M5 | HEX | 109–155 |

\* Touchdown program as described in Haas & Hansson (2008).