

Extra-pair paternity as a strategy to reduce the costs of heterospecific reproduction? Insights from the crow hybrid zone

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Abstract

Within hybrid zones of socially monogamous species, the number of mating opportunities with a conspecific can be limited. As a consequence, individuals may mate with a heterospecific (social) partner despite possible fitness costs to their hybrid offspring. Extra-pair copulations with a conspecific may thus arise as a possible post hoc strategy to reduce the costs of hybridization. We here assessed the rate of extra-pair paternity in the hybrid zone between all-black carrion crows (*Corvus (corone) corone*) and grey hooded crows (*C. (c.) cornix*) and tested whether extra-pair paternity (EPP) was more likely in broods where parents differed in plumage colour. The proportion of broods with at least one extra-pair offspring and the proportion of extra-pair offspring were low overall (6.98% and 2.90%, respectively) with no evidence of hybrid broods having higher EPP rates than purebred nests.

KEYWORDS

extra-pair paternity, hybridization, reinforcement, reproductive character displacement

1 | INTRODUCTION

In stable zones of secondary contact and hybridization, species have diverged to a degree that they still interbreed but do not collapse back into one (Price, 2008). Reproductive isolation between hybridizing taxa can be maintained through pre- and post-zygotic barriers to gene flow (Irwin, 2019), such that heterospecific pairings are either largely avoided by assortative mating (prezygotic barriers; Mayr, 1942) or hybrid offspring suffer a reduction in fitness (post-zygotic barriers; Barton & Hewitt, 1989; Hatfield & Schluter, 1999). Both processes are likely to interact: assortative mating may

induce (post-zygotic) mating disadvantages to minority phenotypes (Irwin, 2019; Londei, 2013) or decreased fitness because of behavioural incompatibilities or reduced investment in heterospecific broods (e.g. depositing fewer nutrients to eggs or allocating less sperm; Ihle, Kempnaers, & Forstmeier, 2015). Similarly, post-zygotic costs of hybridization could promote the establishment of prezygotic barriers to gene flow inducing divergence in mating signals and preference functions (termed reinforcement; Servedio & Noor, 2003).

In the absence of strong post-zygotic costs of hybridization, the mere existence of a stable hybrid zone demonstrates that individuals

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mate preferentially with conspecifics (Brodin & Haas, 2009; Price, 2008). Assortative mating, however, need not be absolute and some heterospecific pairing may still exist (Yang, Servedio, & Richards-Zawacki, 2019), partly because the benefits of evolving stronger assortative mating mechanisms become smaller as the preference for conspecifics increases (Moore, 1957; Yeh, Boughman, Sætre, & Servedio, 2018) and partly to secure breeding potential if the number of conspecifics is low (Irwin & Price, 1999; Yeh et al., 2018). Nonetheless, having a heterospecific partner should not be the preferred option. In socially monogamous species, the costs of nonpreferred heterospecific pairings could be reduced by seeking copulations with a preferred conspecific outside the social pair bond (extra-pair copulations; Veen et al., 2001).

The Eurasian crow hybrid zone is a well-documented example of an avian contact zone that is believed to be (near)-exclusively maintained by assortative mating (Brodin & Haas, 2009; Knief et al., 2019; Meise, 1928; Randler, 2007). Common ancestors of all-black carrion (*Corvus (corone) corone*) and grey hooded crows (*C. (c.) cornix*) likely survived the last glacial period in two separate refugia in southern Europe or the Middle East (Mayr, 1942). After the ice had retreated around 10,000 years ago, the ancestors of remnant carrion and hooded crow populations met in secondary contact in Central Europe, forming a narrow and stable hybrid zone (Meise, 1928). Mate choice appears to be assortative according to plumage colouration (summarized in Randler, 2007), a trait that is likely derived in hooded crows and encoded by two unlinked, epistatically interacting genetic loci (Knief et al., 2019). However, mixed pairings occur regularly within the hybrid zone (Meise, 1928; Randler, 2007) and hybrids seem to suffer from some reduction in fitness, which may merely be due to frequency-dependent sexual selection (Irwin, 2019; Metzler, Knief, Peñalba, & Wolf, in press; Saino & Villa, 1992). Additional potential post-zygotic effects, such as reduced egg size or hatching success of hybrid pairs, have also been discussed (Saino, 1990; Saino & Bolzern, 1992; Saino & Villa, 1992). It may thus be advantageous for members of hybrid pairs to seek extra-pair copulations with conspecifics. Yet, it is not known whether there is any extra-pair behaviour in unassisted breeding crows (for crows breeding in cooperative groups see Baglione, Marcos, Canestrari, & Ekman, 2002), and whether it is used in the hybrid zone to reduce the fitness costs associated with hybridization. In this study, we first quantify the number of extra-pair young in nests from the hybrid zone and then test whether extra-pair behaviour occurs more frequently in heterospecific pairings.

2 | METHODS

2.1 | Individuals and sampling

In May–June 2007, 2008, 2013 and 2014, we sampled carrion and hooded crows (*Corvus (corone) corone* and *C. (c.) cornix*) and their hybrids across the European hybrid zone in eastern Germany. The transect was chosen such that it included phenotypically pure

populations at the endpoints resembling the parental allopatric populations, and several geographically spaced populations with mixed hybrid phenotypes in between. In total, we collected 152 nestlings from 55 nests. We included allopatric individuals from north-western Germany (adult carrion crows, $N = 45$), Poland and Sweden (adult hooded crows, $N = 30$) for genealogical class assignments and the spatial distribution of colour differences. For all individuals, blood samples were taken from the brachial vein and stored either in Queen's lysis buffer, EDTA- or heparin-coated tubes.

2.2 | Sample preparation and SNP genotyping

We extracted DNA using a standard phenol–chloroform assay and assessed DNA quantity and quality with the SYBR green fluorescence assay (Invitrogen) and the NanoDrop™ 2000 spectrophotometer, respectively. We selected 1,152 SNPs spread across the whole genome for genotyping with the GoldenGate assay (Illumina). A detailed description of the assay design, SNP calling and quality control procedure is given in Knief et al. (2019). Our final data set comprised all 152 individuals in the hybrid zone that were genotyped at 1,111 polymorphic loci (average call rate of 99.48%).

We included 65 individuals of the allopatric populations in the GoldenGate genotyping and added 10 hooded crows that had been sequenced on the HiSeq2000 (Illumina) platform (paired-end libraries; coverage ranged from 7.12 × to 13.28×, average = 9.77×, median = 9.83×) and genotyped using the HaplotypeCaller in GATK (v3.3.0; DePristo et al., 2011; Vijay et al., 2016).

2.3 | Analyses

All individuals were sexed based on their heterozygosity for 114 SNPs located on the sex chromosome Z (excluding the pseudo-autosomal region located at $chrZ \leq 2.56$ Mb, $N = 15$ SNPs).

Although most of the genome shows very low levels of genetic differentiation between European carrion and hooded crows, a region of low recombination on chromosome 18 (*chr18*) clearly distinguishes between the two (Poelstra et al., 2014; Vijay et al., 2016). The genetic ancestry for this region can be well described by its diplotype. Together with an unlinked locus on chromosome 1 (*NDP*), it explains the vast majority of plumage colour variation in the hybrid zone (Knief et al., 2019). Based on the assumption that assortative mate choice in crows is largely determined by plumage colouration (Brodin & Haas, 2006; Londei, 2013; Randler, 2007), these two interacting loci constitute the genetic basis of the major mating cue. Using the SNPs on chromosome 18 ($N = 230$ SNPs), we assigned all individuals from the hybrid zone to their diplotype using the NewHybrids software (v2.0+Developmental. July/August 2007; Anderson & Thompson, 2002) called from within the parallelnewhybrid package in R (v0.0.0.9002; Wringe, Stanley, Jeffery, Anderson, & Bradbury, 2017). This analysis separated individuals into six distinct genealogical classes (purebred carrion or hooded crows, F1 or F2 hybrids, and

backcrosses to carrion or hooded crows). F2 hybrids and backcrosses could only be assigned in case of a rare (interspecific) recombination event between the carrion and hooded crow *chr18* haplotype (Knief et al., 2019). The allopatric individuals were set as being of pure origin not influencing the mixing proportions of genotype frequency classes (π). We used uninformative Jeffreys-type priors for the estimation of allele frequencies (θ) and π , discarded the first 20,000 generations as burn-in and estimated parameters from the following 200,000 MCMC algorithm iterations. Details on how we assessed NewHybrids' assignment efficiency, accuracy and overall performance (Vähä & Primmer, 2006) are provided in Knief et al. (2019). In the end, all individuals were separated into *chr18* diplotypes with a posterior probability of 1. We used the SNP showing the strongest association with plumage colour on chromosome 1 as our genotype for the *NDP* locus, which is fixed in pure hooded crows but polymorphic (without phenotypic effects) in carrion crows (Knief et al., 2019; see also Weissensteiner et al., 2019).

To detect extra-pair paternity events, we used all autosomal SNPs except those on chromosome 18 ($N = 752$ SNPs) to estimate pairwise identity-by-descent probabilities (k_{0-2}) and kinship coefficients (θ) between all nestlings. k_0 , k_2 and θ are well suited to distinguish full- from half-sibs (expectation full-sibs: $k_0 = 0.25$, $k_2 = 0.25$, $\theta = 0.25$; half-sibs: $k_0 = 0.5$, $k_2 = 0$, $\theta = 0.125$; Weir, Anderson, & Hepler, 2006). We made use of the PC-Relate approach (M. P. Conomos, Reiner, Weir, & Thornton, 2016), which provides accurate recent genetic relationship inference in samples with unknown or unspecified population structure. PC-Relate takes principal components calculated from genome-wide SNP data of related and unrelated individuals (PC-AiR algorithm) into account (M. P. Conomos, Miller, & Thornton, 2015; Conomos et al., 2016). PC-AiR in turn makes use of the KING-robust method (Manichaikul et al., 2010). The complete workflow is implemented in the R-packages GENESIS (v2.8.0; Conomos, Thornton, Gogarten, & Brown, 2017) and SNPRelate (v1.12.1; Zheng et al., 2012). In nests where we had an unequal number of full- and half-sibs, we defined the larger group as within-pair and the smaller group as extra-pair young.

We intended to test whether the colour difference between the parents of a brood predicted the occurrence of extra-pair paternity. However, we neither sampled parental genotypes nor phenotypes because exhaustive catching of both parents at the nest is impractical. Thus, we inferred the possible two-locus genotypes (*chr18+NDP*) of the parents from the nestling genotypes taking the genotype frequencies along the hybrid zone transect into account. Then, we used the estimated genotypic effects of *chr18* and *NDP* on plumage colour (Knief et al., 2019) to transform genotypes into colour phenotypes. There are 81 possible parental genotype combinations of two-locus, two-allele genotypes (3 genotypes of the first locus in the first parent \times 3 genotypes of the second locus in the first parent \times 3 genotypes of the first locus in the second parent \times 3 genotypes of the second locus in the second parent). For each brood, we first excluded all combinations that were not consistent with Mendelian inheritance. Next, we weighted each parental two-locus genotype by its frequency estimated from all broods at

the same position along the transect. If a genotype was not present at a brood's location, we excluded all combinations that were composed of this parental genotype. After scaling the remaining parental genotype frequencies to a sum of 1, we multiplied the frequency of each parental combination with the predicted plumage colour difference between the parents (based on the principal component values capturing the vast majority of variation in the colour phenotype; see Knief et al., 2019). The sum thereof reflects the weighted colour difference between the most likely parents of a brood. To illustrate the spatial distribution of the parental colour differences, we fitted a generalized additive model (GAM) using the position along the hybrid zone transect as the sole predictor (see Knief et al., (2019) for details on how this position was calculated). Since all individuals of a brood had the same values for the independent and dependent variable, we used only a single individual for fitting the model and used brood size as a weights argument.

We then tested whether the colour difference between parents had an effect on extra-pair paternity, expecting EPP to be more common in nests where the difference was large. Due to sample size dependency of brood-based estimates (Eccard & Wolf, 2009), we quantified both the proportion of broods with EPP and the proportion of extra-pair offspring. To estimate EPP per brood, we fitted a generalized linear model (GLM) with a binomial error structure using the occurrence of at least one extra-pair young within a brood as the dependent variable (1 = at least one extra-pair young present in brood, 0 = all within-pair young in brood). To estimate the proportion of extra-pair offspring, we used every individual as our dependent variable (1 = extra-pair young, 0 = within-pair young) and fitted a generalized linear mixed-effects model (GLMM) with a binomial error structure and nest ID as a random intercept. In both models, we fitted the most likely colour difference between the parents as the sole continuous predictor. All analyses were implemented in R (v3.4.3; R Core Team, 2017) and the packages mgcv (v1.8-22; Wood, 2011) and lme4 (v1.1-21; Bates, Mächler, Bolker, & Walker, 2015).

3 | RESULTS

We sampled 152 offspring from 55 nests with a median number of 3 (range = 1–5) nestlings across the European hybrid zone. We used the information from the *chr18* and *NDP* genotypes of the offspring to define the plumage colour difference between their parents. Independent from the ancestry on chromosome 18, we inferred relatedness between nestlings using the kinship coefficient. Then, we combined these two data sets to assess whether nests where the colour difference between parents was large also have higher EPP—conditional on having more than one offspring in the nest.

Most individuals from the hybrid zone were homozygous for the carrion or hooded crow diplotype on chromosome 18 ($N = 98$ individuals in 43 nests). The remainder were F1 hybrids ($N = 42$ individuals in 21 nests) and backcrosses ($N = 12$ individuals in 10 nests). There was no biased sex ratio in F1 hybrids ($p = .64$), lending no support

for Haldane's rule which states that if one sex suffers from hybridization, then it will be the heterogametic sex (females in birds). The inferred plumage colour differences between the parents of a nest were highest for those nests containing offspring that were heterozygous for the *chr18* diplotype and lowest for those containing offspring homozygous for the dominant carrion crow *chr18* diplotype. Broods with offspring homozygous for the recessive hooded crow *chr18* diplotype were intermediate (Figure 1a). Consistent with increasing genotypic diversification, the inferred plumage colour differences between the parents of a nest peaked towards the centre of the hybrid zone (Figure 1b).

Full- and half-sib pairs could reliably be separated based on their kinship coefficients (Figure 2). Only for one brood, relationships could not be unambiguously resolved. That nest contained three individuals, with two full-sib ($\theta = 0.23$ and $\theta = 0.26$) and one half-sib ($\theta = 0.12$) relationship. Interestingly, one nestling was a purebred carrion crow and two were F1 hybrids. We removed the entire brood from the EPP analyses.

Overall, the extra-pair paternity rate was low. From a total of 43 nests with more than one offspring ($N = 138$ nestlings), three nests contained at least one extra-pair young, which translated to an extra-pair nest rate of 6.98% ($\pm 1SE = 3.96\% - 12.01\%$). There were 4 extra-pair young (maximally 2 per brood), translating into an extra-pair young rate of 2.90% ($\pm 1SE = 1.77\% - 4.72\%$). The weighted plumage colour difference between possible parents (see methods) had an effect neither on the extra-pair nest

rate ($\chi^2_1 = 0.03$, $N = 43$, $p = .86$) nor on the extra-pair young rate ($\chi^2_1 = 0.006$, $N = 138$, $p = .94$).

4 | DISCUSSION

The observed extra-pair paternity rates of 6.98% per brood and 2.90% across all offspring are much lower than a previous estimate from a carrion crow population ($N = 59$ nests) in which most pairs breed cooperatively (EPP rate 26% per brood; Baglione et al., 2002). The only three broods of unassisted pairs all had no extra-pair paternity, which is consistent with the link between EPP rate and breeding system described across bird species (Brouwer & Griffith, 2019). Our EPP estimates rank in the lower third of all socially monogamous birds studied so far (Brouwer & Griffith, 2019) and are similarly low as in other corvid species. This may be expected because EPP rates show a strong phylogenetic signal on the level of the family and order (Brouwer & Griffith, 2019).

The EPP rate did not significantly covary with the colour difference between parents of a brood. EPP rates had been measured in three hybridizing bird species before (Reudink, Mech, & Curry, 2006; Vallender, Friesen, & Robertson, 2007; Veen et al., 2001). Similar to the crow system, in two of the studies, there were small or no costs associated with hybridization and hetero-specific social pairs did not show higher EPP rates (Reudink et al., 2006; Vallender et al., 2007). In the third hybridizing species pair,

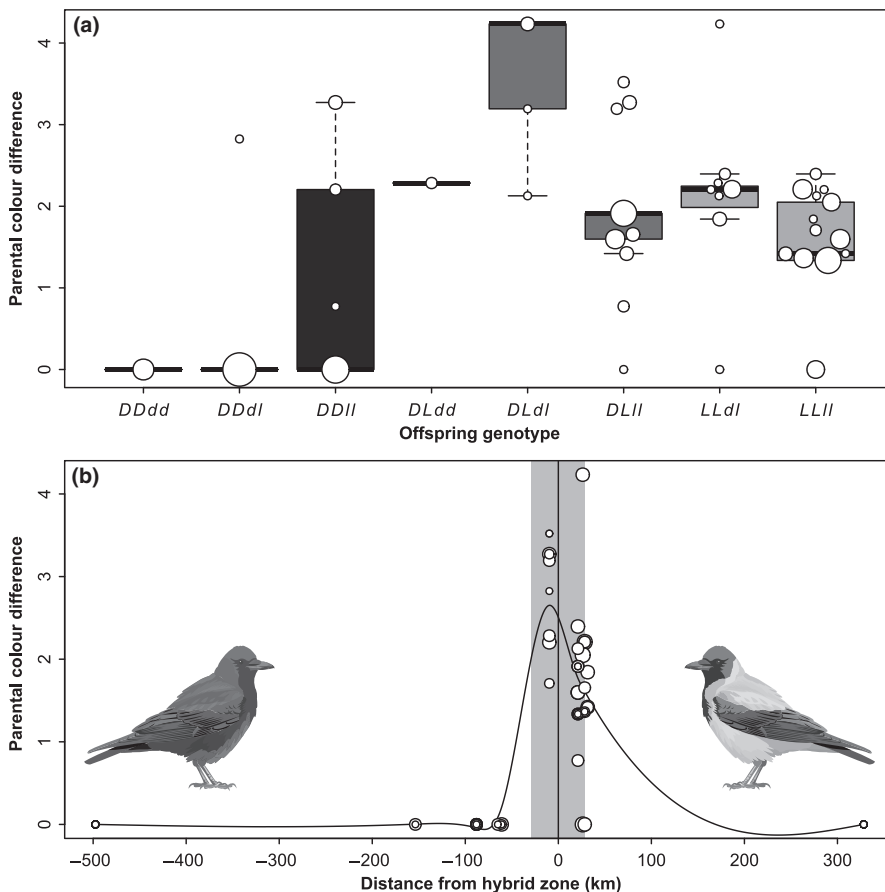


FIGURE 1 Inferred plumage colour differences between possible parents of offspring (a) with a given genotype on *chr18* and NDP weighted by genotype frequency of each sampling location (see methods) and (b) along the hybrid zone transect. In (a), the capital letters refer to the *chr18* genotype and the small letters to the NDP genotype (*D*, *d* = dark alleles, carrion crow; *L*, *l* = light alleles, hooded crow). Point size reflects sample size across all populations. In (b), the centre and width of the hybrid zone are depicted by the vertical line and the dark grey shading, respectively. Both had been estimated in Knief et al. (2019) using *chr18* allele frequencies. Point size reflects the brood size. Crow images courtesy of Dan Zetterström, modified by Joshua Peñalba

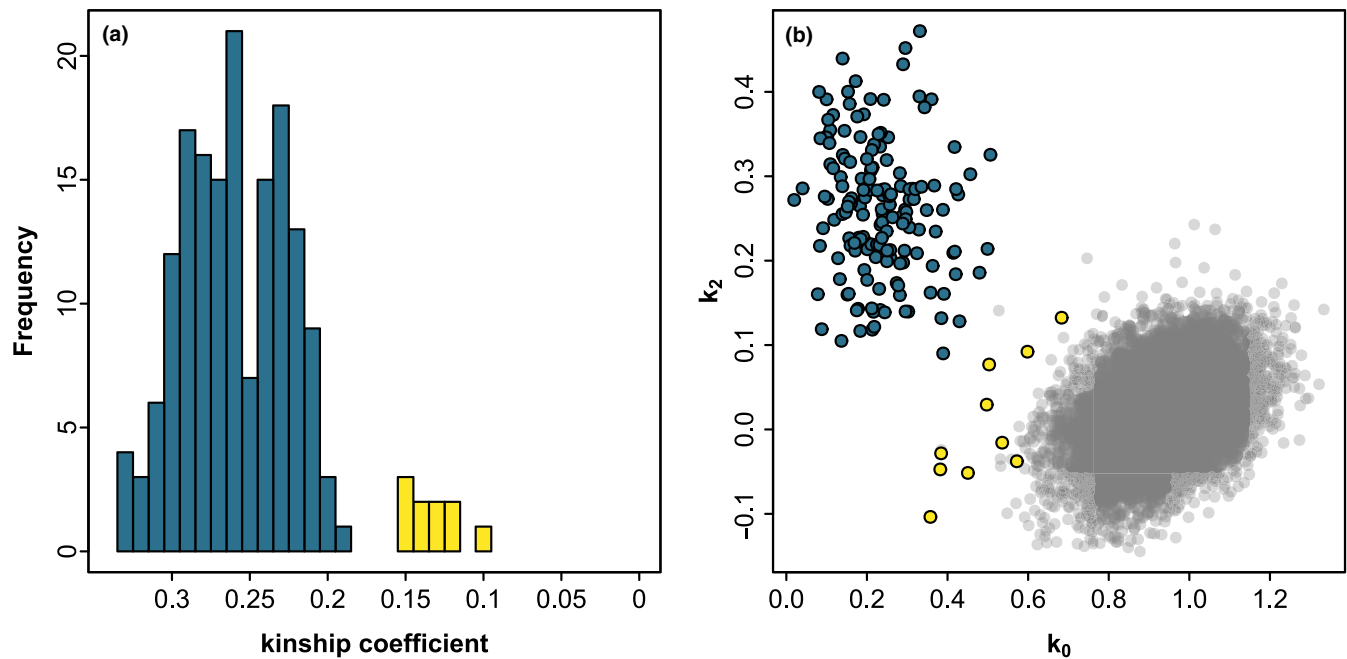


FIGURE 2 Relatedness between all samples measured as (a) the kinship coefficient (θ) and (b) IBD-sharing probabilities k_0 and k_2 . In blue full-sibs and in yellow half-sibs, identified using both nest and genetic information. In panel (b), in grey all pairwise relatedness estimates between nests

the costs of hybridization were much higher. Hybrid females were sterile and hybrid males suffered a fitness reduction through mate choice (Veen et al., 2001), which makes the occurrence of reinforcement more likely (Liou & Price, 1994). Indeed, all extra-pair young of females that had a heterospecific social partner were sired by conspecific males (extra-pair young rate of 14.5% versus 59% for conspecific and heterospecific pairings, respectively; Veen et al., 2001).

In summary, extra-pair copulations are part of the reproductive repertoire in the crow hybrid zone with the potential to reduce the costs associated with hybridization but—taking the colour difference between parents as a proxy—we found no higher extra-pair rates in hybrid broods. It has been shown through simulations that restricted dispersal with assortative mating (imprinting) and induced post-zygotic selection against minority phenotypes could maintain the crow hybrid zone (Brodin & Haas, 2009; Metzler et al., in press), such that no other selective forces need to be invoked. Yet, hybrids seem to suffer a small reduction in fitness (Saino, 1990; Saino & Bolzern, 1992; Saino & Villa, 1992), and an increased EPP rate in heterospecific pairs could have evolved through reinforcement. To ultimately test this idea, larger sample sizes, estimates of hybrid fitness and extra-pair paternity rates from pure and experimentally induced mixed pairs of allopatric carrion and hooded crow populations are needed (Howard, 1993).

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CONFLICT OF INTEREST

We declare we have no conflict of interests.

AUTHOR CONTRIBUTION

UK, CMB and JBWW conceived the study. CMB and JBWW conducted fieldwork. CMB generated the genetic data. UK analysed the data and wrote the manuscript with input from JBWW. All authors contributed to the revision of the final version of this manuscript, approved and agreed to be held accountable for the content therein.

ETHICAL APPROVAL

Permissions for sampling of wild crows were granted by Regierungspräsidium Freiburg (Aktenzeichen: 55-8852.15), Landratsamt Zwickau (364.622-N-Her-1/14), Landratsamt Mittelsachsen (55410704 Beringungserl-Voigt_14), Landratsamt Vogtlandkreis (364.622-2-2-88841/2014), Landratsamt Meißen (672/364.621-Kennzeichnung von Tieren-18935/2013), Landratsamt Bauzen (67.3-364.622:13-01-Krähen), Landesdirektion Sachsen (24-9168.00/2013-4), Landesamt für Verbraucherschutz,

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DATA AVAILABILITY STATEMENT

Data are available as the Appendix S1.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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