


Short Communication

The first case of a successful brood from a double hybrid mixed pair (*Dendrocopos syriacus* × *Dendrocopos major* (Picidae))

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Hybridization occurs between numerous bird species, with some of them capable of producing backcrosses. However, little information is available concerning mating between pairs of hybrids. In this case study, a mixed pairing is reported between hybrids of Syrian and Great Spotted Woodpeckers, *Dendrocopos syriacus* and *Dendrocopos major*, respectively. Mixed phenotypic and genotypic traits were found in the male, the female and their offspring. The possible consequences of hybridization and its effect on the Syrian Woodpecker, which is protected under the Bird Directive of the European Union, are discussed.

Keywords: backcrosses, Great Spotted Woodpecker, hybridization, Syrian Woodpecker.

INTRODUCTION

Hybridization is a relatively frequent phenomenon in birds (Aves; Randler 2002). It has been reported in numerous families between members of many species; however, the extent of knowledge on interspecific mating and breeding is still unsatisfactory (McCarthy 2006). Hybrids are often hard to detect and determine because of intermediate or mixed external characters that often overlap with intraspecific traits of the parental species. Studies on hybridization are even more challenging if hybrids are fertile, resulting in backcrossing and introgression (Randler 2002).

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Hybrids are known among numerous taxa of woodpeckers (McCarthy 2006). Among pied woodpeckers (genus *Dendrocopos*), hybrids have been reported or suspected for 10 out of 12 species. The majority of examples concern hybridization within a group of closely related woodpeckers: Great Spotted *Dendrocopos major*, Syrian *Dendrocopos syriacus*, White-winged *Dendrocopos leucopterus* and Sind *Dendrocopos assimilis* (McCarthy 2006). The best known is hybridization between Great Spotted and Syrian Woodpeckers, occurring mostly at the northern edge of the latter's range (Gorman 1997, Dudzik & Polakowski 2011). The range of the Syrian Woodpecker has expanded from the Middle East to southeast, central and eastern Europe over the past 120 years. This has rendered it sympatric with the common Great Spotted Woodpecker (Michalczyk 2014), although their ecological niches only partially overlap (Figarski & Kajtoch 2018a). Recent studies have shown that hybrids can constitute up to 7% (field data) or even 20% (genetic data) of individuals in wild urban populations (Michalczyk *et al.* 2014, Figarski & Kajtoch 2018b, Gurgul *et al.* 2019). Increasing cases of territory overlap and mixed pairs abundance are also being reported (Figarski & Kajtoch 2018b). However, none of the previous reports on mixed pairs referred to double hybrid mixed pairs (i.e. both parents being of mixed origin, or *D. syriacus* × *D. major* hybrids).

This note presents an interesting case of a successful brood from a double hybrid mixed pair *D. syriacus* × *D. major* determined based on plumage characteristics and genetic data.

MATERIALS AND METHODS

Field research conducted in 2021 in the Krakow agglomeration of southern Poland investigated breeding sites of woodpeckers (Syrian and Great Spotted) following methods described in Kajtoch and Figarski (2017) and Figarski and Kajtoch (2018a). Hybrids were determined according to the key of morphological (plumage) characters already described by Figarski and Kajtoch (2018b). Additionally, some birds were caught with the use of a net and ringed for monitoring purposes. Samples were collected through non-invasive methods (feathers were collected from cavities after birds had fledged, as they fell from adults when measuring the birds or from juvenile corpses – birds already found dead). We used two feathers per adult and corpse, and three fresh and well-preserved feathers of juveniles taken from the tree hole (therefore these juvenile feathers could not be assigned to particular individuals, and were numbered as samples 1, 2 and 3). These feathers were used for subsequent genetic assignment of individuals to the species according to a simplified protocol (Michalczyk *et al.* 2014). Three genetic markers that have previously proved to be effective in species and hybrid

determination were sequenced: mitochondrial Control Region (CR; mtDNA), Z-linked brahma protein intron 15 (BRM) and autosomal transforming growth factor intron 5 (TGF). These three DNA fragments were amplified using primers developed for studies on *Dendrocopos* woodpeckers (mtCR-F/mtCR-R, tgfn5-F/tgfn5-R, brmin5-F/brmin5-R; Michalczyk *et al.* 2014), and then Sanger sequenced using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3100 Automated Capillary DNA Sequencer. Heterozygous positions in nuclear introns were determined based on the presence of double peaks in chromatograms. Newly obtained sequences have been deposited in GenBank under accession numbers: ON060673–ON060678 (CR), ON060679–ON060684 (TGF) and ON101504–ON101509 (BRM). Obtained sequences were aligned and compared with available sequences of ‘pure’ birds from a previously published study (Michalczyk *et al.* 2014). These reference sequences were obtained from urban populations of Syrian and Great Spotted Woodpeckers (including the population from Kraków city, and thus the area, where the double hybrid mixed pair was found). Moreover, samples were sexed with the use of primers 1237L/1272H (Khan *et al.* 1998).

RESULTS

In May 2021, an unusual pair of woodpeckers was found in an area of old-growth deciduous park (50.0196°N, 19.9982°E). Two adults were noted with plumage suggesting their hybrid origin; they were captured for photographs and ringing. The nest was active with four chicks, two of which fledged prematurely and two fledged naturally, but no photographs of chicks were obtained and incomplete plumage data were obtained. One dead chick (corpse) was pulled from the cavity by the female.

The plumages of the female and male woodpeckers showed a mix of traits for both Great Spotted and Syrian Woodpeckers, known to be specific for hybrids (Table 1; Fig. 1). The juvenile birds also expressed some mixed or intermediate features; however, they could not be observed in detail (Table 1; Fig. S1). For comparison, plumage of ‘pure’ birds (males and females of Syrian and Great Spotted Woodpeckers) are presented in Figure S2. The male parent and one of the juveniles that left the nesting cavity expressed Syrian-like vocalizations, whereas the female parent had Great Spotted-like vocalizations. The juveniles in the cavity were loud, as is common with a Great Spotted Woodpecker brood.

Genetic data supported the hybrid origin of all samples, with all birds having mixed genotypes (Table 2; Fig. S3). Sexing of samples confirmed sex for the adult female and male, and showed that the dead juvenile and feather 3 were from females, whereas feathers 2 and 3 were from males (Table 2; Fig. S4). Both adult birds

Table 1. Phenotypes of examined woodpeckers determined for traits according to Figarski and Kajtoch (2018a).

Characteristic	<i>Dendrocopos syriacus</i> – reference	<i>Dendrocopos major</i> – reference	Adult female	Adult male	Juvenile in cavity, younger	Juvenile in cavity, older	Juvenile, volatile fugitive
Outer rectrices	Black with white bars	White with black bars	White with black bars	White with black bars	Not visible	Not visible	White with black bars
Undertail coverts	Pink	Red	Red	Red	Not visible	Not visible	Not visible
Flank feathers	Clearly streaked	Generally without stripes	Streaked	Without stripes	Not visible	Not visible	Streaked
Cheek	Fuzzy dark spot beyond the eye in juveniles	Clean (whitish)	Clean	Clean	Fuzzy dark spot	Fuzzy dark spot	Fuzzy dark spot
Post-auricular stripe	Lack of black line ('white neck')	Black, connected with hind-crown	Black line with small break under hind-crown	Very short black line	Intermediate black line	Intermediate black line	Intermediate black line
Red nape (in males)	Large	Short	–	Short	–	–	–
Lore feathers	Whitish	Black	Whitish	'Dirty' (greyish)	'Dirty' (greyish)	Whitish	Not recorded



Figure 1. Photographs of captured adult hybrids (female – a, c; and male – b, d). Diagnostic traits of plumage indicating hybrid status of these birds are marked with arrows (details regarding state of these traits differentiating Syrian and Great Spotted Woodpeckers, as well as their hybrids, are listed in Table 1). Photos: Łukasz Kajtoch.

could not be F_1 hybrids, as most of the diagnostic single nucleotide polymorphisms were not heterozygous (as should be expected for first-generation hybrids). Determined single nucleotide polymorphisms in samples from juvenile feathers showed that feather 3 must be from a different individual compared with feathers 1 and 2, though feathers 1 and 2 could be from the same individual. Feather 3 had the same genotype as the sample taken from the corpse, but it could not belong to the dead bird (which was already rotten). Therefore, we deduced that collected samples from juveniles were from at least three individuals (one dead and two alive).

DISCUSSION

Both phenotypic and genetic traits supported the hybrid origin of the observed pair of woodpeckers. Their mixed

or intermediate plumage features were consistent with current knowledge about the appearance of Syrian \times Great Spotted Woodpecker hybrids (Figarski & Kajtoch 2018b). The plumage of birds had more states specific for Great Spotted than for Syrian Woodpecker, with some exceptions such as short or intermediate post-auricular stripe in all birds, streaked flank feathers in female, and whitish lore feathers in female or greyish in juveniles. The limited number of loci used was inadequate for detailed determination of their origin. It is most probable that both adult birds were backcrosses of F_1 hybrids with Great Spotted Woodpecker, but the female was matrilineal Great Spotted Woodpecker, whereas the male was matrilineal Syrian Woodpecker. This finding differs from the previous study, which found that mixed pairs are mostly formed by Syrian Woodpecker females, thereby suggesting some

Table 2. Genotypes of examined woodpeckers determined for three markers. SW – Syrian Woodpecker alleles determined, GW – Great Spotted Woodpecker alleles determined. Additionally results of sexing are provided.

Genetic marker	Adult male	Adult female	Juvenile corpse	Juvenile feathers 1 and 2	Juvenile feather 3
Control Region (mtDNA)	SW-like	GW-like	GW-like	GW-like	GW-like
Brahma i.15 (Z-linked)	GW-like	SW-like	Mixed	GW-like	Mixed
Transforming growth factor i.5 (autosomal)	GW-like	Mixed	GW-like	Mixed	GW-like
Chromo-helicase-DNA binding region (Z- and W-linked)	Male	Female	Female	Male	Female

Abbreviations: GW, Great Spotted Woodpecker; mtDNA, mitochondrial DNA; SW, Syrian Woodpecker.

unbalanced selection of sexes (Figarski & Kajtoch 2018b). The observed juveniles in the present case possessed some intermediate genotypes, indicating that they were double cross hybrids, being the offspring of two hybrid birds. It seems that the whole family had more Great Spotted than Syrian characteristics. This is consistent with current trends in the examined population: Great Spotted Woodpeckers are increasing in number, whereas Syrian Woodpeckers are declining (<https://monitoringptakow.gios.gov.pl/>; Michalczyk & Michalczyk 2015). Hybrid individuals have a greater chance to mate with Great Spotted Woodpeckers than Syrian Woodpeckers, which could lead to genomic homogeneity, and possibly also to despeciation; however, this would only be for local sympatric populations.

Detection of a double hybrid mixed pair of Syrian × Great Spotted Woodpeckers and their successful breeding, with four out of five hatched chicks surviving until fledging, is an interesting phenomenon. In a previous review of hybrid birds (McCarthy 2006), there is no report of such a case. However, multiple examples of hybridizing birds in which virtually all individuals within the zone are later-generation hybrids or backcrosses strongly suggests that some double crosses are present within such populations. Apparently, detection of such (possibly rare) offspring of hybrid × hybrid pairings is challenging in the natural environment, meaning this could be an overlooked phenomenon.

Apart from its scientific contribution to understanding the mechanisms of hybridization in birds, the present case could have other serious implications. Hybridization is frequent between Syrian and Great Spotted Woodpeckers (Michalczyk *et al.* 2014, Figarski & Kajtoch 2018b, Gurgul *et al.* 2019). Knowing that hybrids and backcrosses are viable and fertile, this could present a serious threat to the duration of Syrian Woodpecker populations on the verge of the species range in central Europe. Winkler (1971) suggested a moving hybrid zone between these two species as Syrian Woodpeckers expanded into Central Europe, but he found the interbreeding tended to decline rapidly once this species became numerous and widespread. On the other

hand, recent studies show a strong decline on the verge of the Syrian Woodpecker's range (Michalczyk & Michalczyk 2015). It is presently unclear whether decline causes or is caused by intensive hybridization. This species is rare in Europe and protected under the European Union's Bird Directive, meaning that if a substantial part of its population is influenced by hybridization and probably gene introgression, the protection of Syrian Woodpecker could be challenging or even impossible in some areas. The risk is that some populations could vanish as a result of the expansion of hybrids, as in the case of other threatened birds, for example ducks (Greig 1980). The question remains as to how such hybrid individuals should best be treated from the conservation point of view.

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AUTHOR CONTRIBUTIONS

Łukasz Kajtoch: conceptualization (only); Investigation (equal); methodology (equal); manuscript writing (only). **Bartłomiej Kusal:** Investigation (equal); methodology (equal).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Data Availability Statement

All data obtained for this study are presented in the manuscript and supplementary files.

ETHICS STATEMENT

The study was executed within the standards and procedures laid down by Polish law.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Photographs of observed adult male (a) and female (b) hybrid woodpeckers, and some of their offspring (c, d). Photos: Łukasz Kajtoch.

Figure S2. Photographs of ‘pure’ adult Syrian Woodpeckers (a – male, b – female), and Great Spotted Woodpeckers (c – male, d – female). Photos: Paweł Głowacki (Warsaw, Poland).

Figure S3. Simplified alignments of three markers used for genotyping of woodpeckers.

Figure S4. Results of sample sexing based on primers 1237L/1272H (Khan *et al.* 1998).