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作品名稱　Whose feather is that? A cross-views between a naturalist and a molecular biologist

得獎獎項　二等獎

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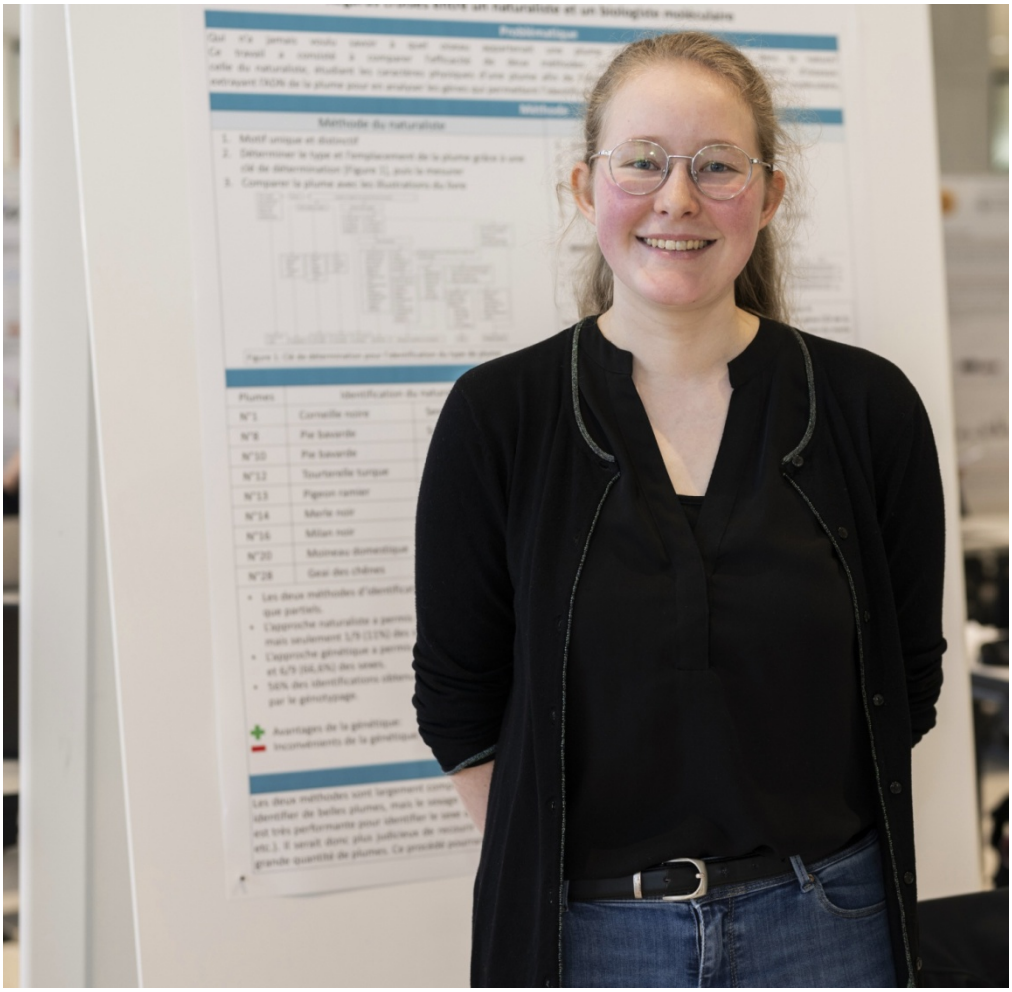

Abstract

Identifying the species or sex of a bird based on a feather found in nature is often challenging, even with the help of reference books. However, determining the presence of a rare species in a habitat using an indirect presence indicator, such as a feather, can help in implementing specific measures for preserving the species. The aim of this study is to investigate whether DNA genotyping is better than specialized books when identifying bird feathers.

To answer this question, I collected feathers in the wild and, with the help of two books, tried to identify their species and sex. Then, assisted by Dr Gwenaël Jacob (UNIFR), I isolated two genes in nine selected feathers. The investigated genes were the CHD gene for sexing and the COI gene for species identification. To do this, the DNA was first extracted from the feathers, purified, and amplified by PCR. Subsequently, an electrophoresis was performed to sex the samples and check that the PCR amplification had worked properly. Finally, the samples were sequenced by the Microsynth laboratory (St-Gall), and the obtained sequences were entered into the NCBI database.

A comparison of the results obtained with each of the two different methods shows that the identification with specialized books was fairly successful. 56% of the species identification made with the books were indeed confirmed by genotyping. DNA analysis provided a different result only for feather #16. However, 33% of genetic identification failed, either due to genetic material quality or laboratory errors. As it was possible to identify the sex of only one sample (feather #14) with the books, it was not possible to make a true comparison of the two approaches. However, as genetic sexing worked well (one failure, feather #28), it can be inferred that genetic sexing is more effective than using books.

This work demonstrated that DNA is not infallible and that sometimes books are equally effective in identifying bird species from a feather. However, in sexing bird, DNA remains more efficient. Thus, one can conclude that DNA genotyping is not superior but rather complementary to specialized books for identifying bird feathers.

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1 Introduction

In addition to my three hens, many other birds live in my garden, or at least pass through it, leaving behind them numerous feathers. Curious by nature, I have often tried to guess which species populate my garden by trying to identify the owner of a feather found on the ground. Was it a rook, a common magpie, or even a carrion crow? Now, this is a relatively difficult exercise, even with the help of a book. Of course, a seasoned ornithologist could easily identify a common magpie feather at first glance. But he would be in a lot more trouble if the feather in question was a down feather rather than a contour feather or if he had to determine the sex of the bird. Wouldn't it be easier to extract DNA from this feather and, through genotyping, to identify for certain the species (and possibly even the sex) to which it belongs? The purpose of this process is to correlate a genetic variation located at a precise point in the genome with a given individual or group of individuals. Thus, it would enough to identify one or more typical variations of a bird species to determine with certainty to which species a feather belongs thanks to the extracted DNA. Such information could, for example, make it possible to prove the presence of a rare species in a specific area and, therefore potentially, to better protect it. My research question arose from this reflexion namely: "Is DNA-based genotyping better than specialized books in the identification of bird feathers?" To attempt to answer these questions, I experimented with both approaches: that of the naturalist and that of the molecular biologist. I collected a number of feathers at the beginning of autumn 2021 and initially attempted to identify them with the help of specialized books [1] [3]. From the same feather, I then extracted DNA and performed genotyping at a laboratory of UniFR. In this paper I describe the two different methods I tested, from theory to practice, before comparing the various results. Finally, I put both approaches in perspective, discuss them and provide conclusions to answer my research question.

2 Material and Methods

2.1 Phenotyping: feather identification using specialized books

The first stage was to collect and identify the feathers. This is a demanding task. Fortunately, books are full of advice and offer a methodical approach to phenotyping i.e., the description of an observable characteristic of a living being [18].

The method used for the identification of the feathers can be summarized as follows:

Initially one should look quickly for a unique and known pattern that would enable the feather to be identified immediately, with a view to saving a considerable amount of time [Figure 1] [5].

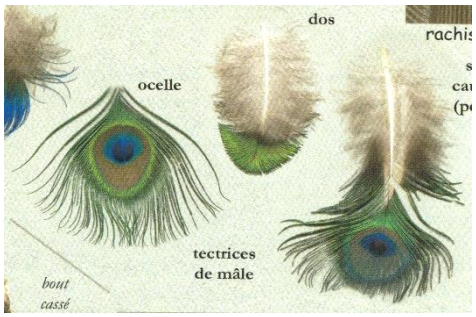


Figure 1: *Tectrices of male Indian peafowl. Black, blue bronze and green ocellus is a unique pattern which enables to identify immediately the sex and the species.*

If the feather has no distinctive pattern, the identification of the species will be a little more complicated. The first step is to distinguish between “large” and “small” feathers by testing their rigidity [7]. To do this, simply press the feather against your palm and observe its behaviour [Figure 2].

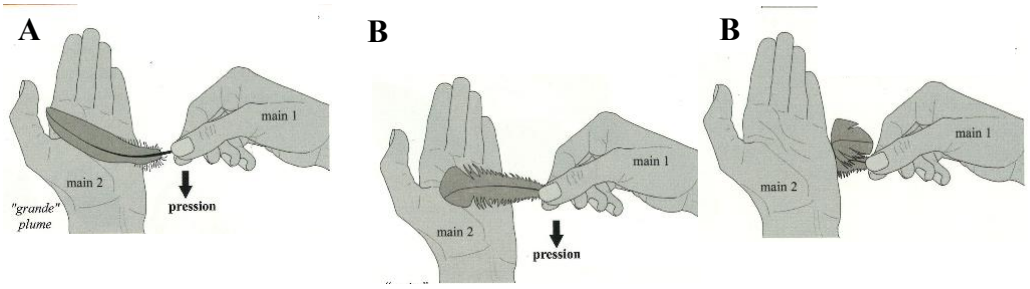


Figure 2: *Feather rigidity test. The feather bends only slightly and resists when the pressure of the hand increases, so it is said to be “large” (A). The feather resists only slightly and bends easily without breaking when the pressure of the hand increases, so it is said to be “small” (B).*

The rigidity of the feather is decisive in identifying the location of the feather on the bird (rectrices, remiges, tectrices, etc.), which later makes it easier to identify the species from which the feather comes [Figure 3].

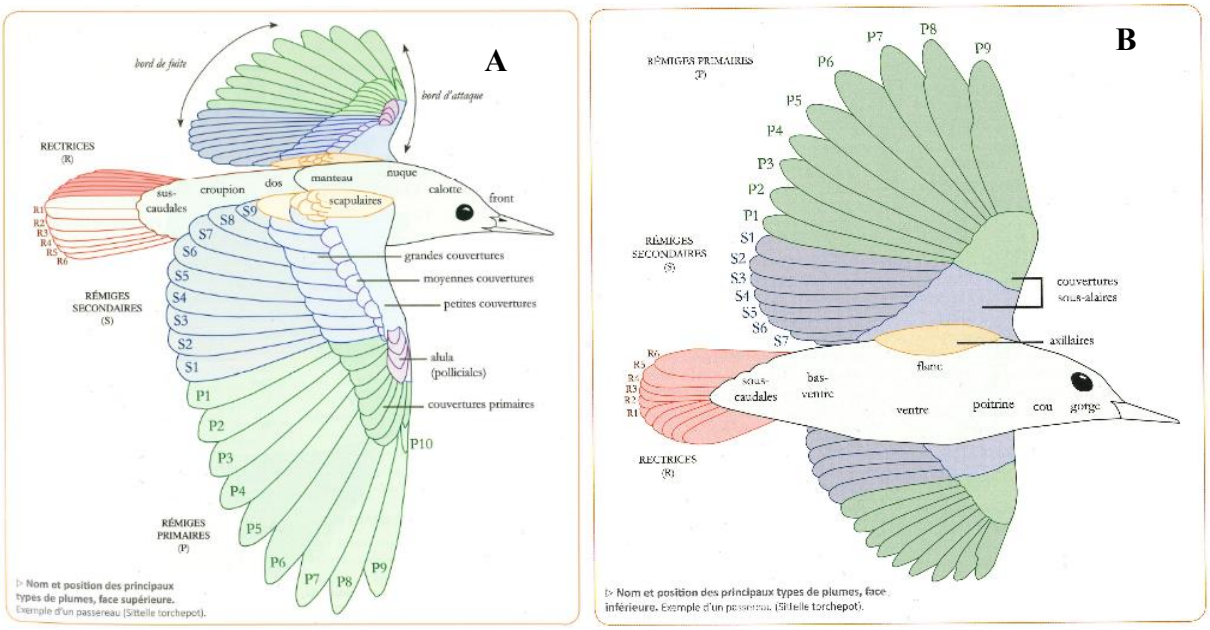
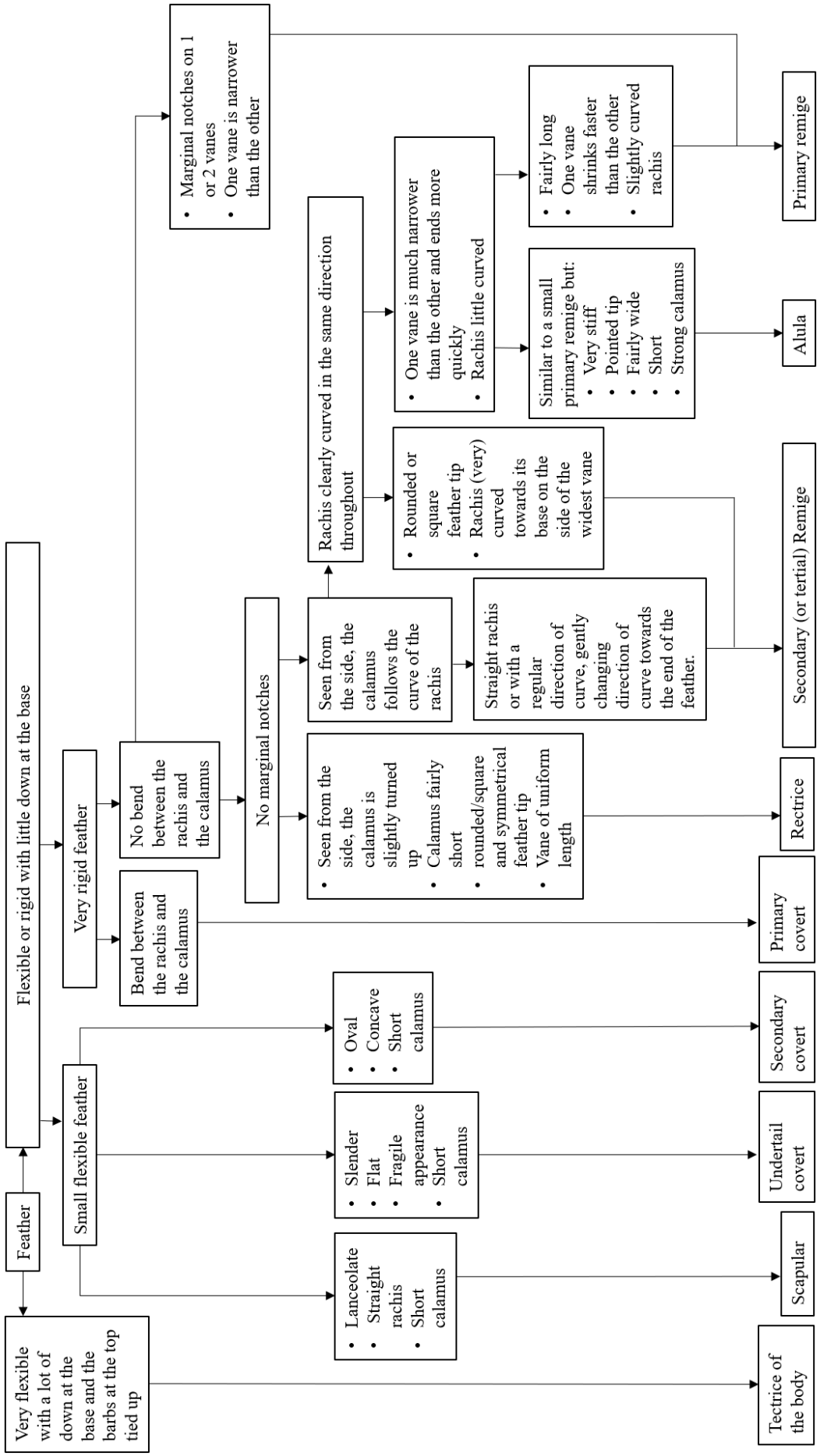


Figure 3: *Locations and names of the different feathers as seen from above (A) and below (B) the bird. The green feathers are the primary remiges (P) and their coverts. Birds generally have between 10 and 11 primary remiges. The blue feathers are the secondary remiges (S) and their coverts. Most birds have 9 secondary remiges (not including any tertials remiges). The red feathers are the rectrices (R), covered by the uppertail and undertail coverts. The purple feathers are the alulas. Birds have between 3 and 7 per wing [6]. In yellow, these are the shoulder feathers called scapulars (above) and axillaries (below) [4]. The other feathers are tectrices [6]. The rectrices, primary and secondary remiges are also called flight feather or pennae or contour feathers. These are numbered. The rectrices are numbered starting from the centre of the tail. The two feathers in the centre of the tail are R1, then the two feathers next to them are R2, and so on. The remiges are a little more complicated. Their numbering varies according to region, custom and author. In general, the primary and secondary remiges are numbered starting from the "wrist". P1 and S1 are the feathers closest to the "wrist" of the wing, followed by P2 and S2, and so on.*

The diagram below was generated to identify the precise location of the different feathers, according to their rigidity, coupled with other characteristics such as the presence of contour notches or the shape of the rachis [7].


Feather
Very flexible with a lot of down at the base and the barbs at the top tied up
Tectrice of the body
Flexible or rigid with little down at the base
Small flexible feather
Lanceolate
Straight rachis
Short calamus
Scapular
Slender
Flat
Fragile appearance
Short calamus
Undertail covert
Oval
Concave
Short calamus
Secondary covert
Very rigid feather
Bend between the rachis and the calamus
Primary covert
No bend between the rachis and the calamus
No marginal notches
Seen from the side, the calamus is slightly turned up
Calamus fairly short
rounded/square and symmetrical feather tip
Vane of uniform length
Rectrice
Seen from the side, the calamus follows the curve of the rachis
Straight rachis or with a regular direction of curve, gently changing direction of curve towards the end of the feather.
Secondary (or tertial) Remige
Rachis clearly curved in the same direction throughout
Rounded or square feather tip
Rachis (very) curved towards its base on the side of the widest vane
Marginal notches on 1 or 2 vanes
One vane is narrower than the other
One vane is much narrower than the other and ends more quickly
Rachis little curved
Similar to a small primary remige but:
Very stiff
Pointed tip
Fairly wide
Short
Strong calamus
Alula
Fairly long
One vane shrinks faster than the other
Slightly curved rachis
Primary remige

Once the location of the feather has been identified, it should be measured. This will help define the search. Its size can be used to classify it according to the size diagram below [2] [Figure 4]. This indicates the category which the feather belongs to, which allows finding targeted information in the book. Thus, it is possible to get an overall idea of which species it might belong to. Finally, the feather must be compared with the illustrations and descriptions in books.

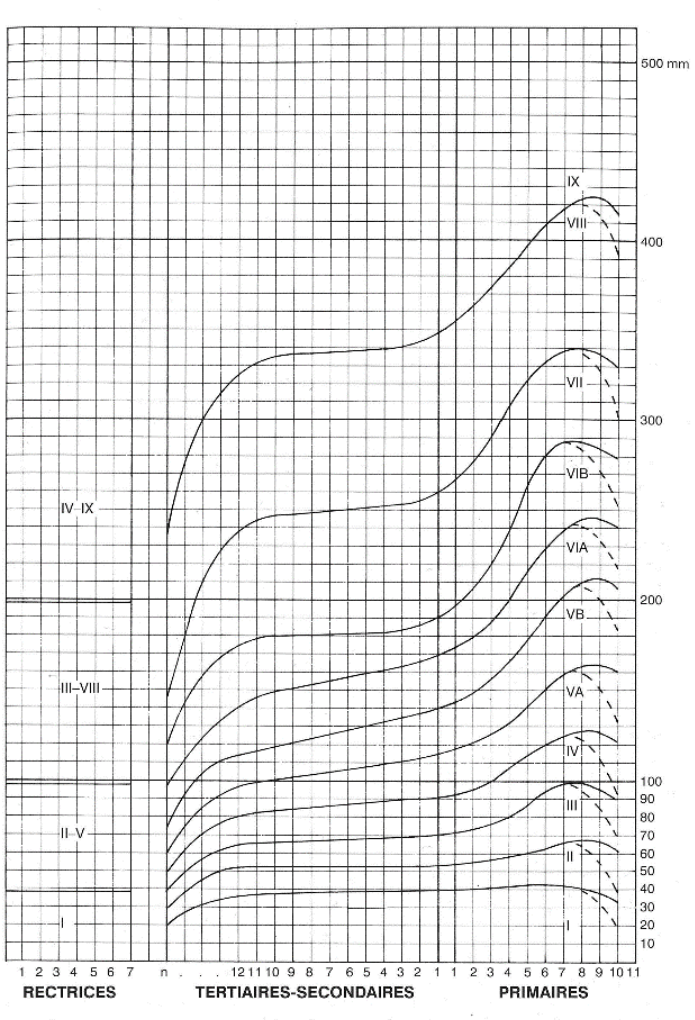


Figure 4: *Size diagram. The X coordinate shows the names of the three types of feathers (primary remiges, secondary/tertials remiges and rectrices). The Y coordinate shows the size of the feather in mm (from 0-500mm). In the centre, you can read which category (from I to IX) our feather falls into. Species are classified according to the size of the largest primary remiges. The curves delimit the categories according to the size and type of feathers. This diagram is valid for all the feathers listed in the book [4], but the list is not exhaustive, so it is possible that the species of a feather does not appear in the category where it should be.*

2.2 Genotyping: feather identification using DNA

Once the feathers have been identified in the classical naturalist way using books, the next step is to genotype (and sex) them using the DNA they contain, in order to validate the identification and compare methods. But how does it work?

2.2.1 Background information

What is genotyping?

Genotyping is a molecular biology technology [16] aimed at identifying a genetic variation located at a specific point in the genome and attributing it to a given individual or a group of individuals [14]. These sought-after genetic variations for differentiating individuals at the DNA level exist in two forms: microsatellites and single nucleotide polymorphisms [14]. "A microsatellite (or microsatellite sequence) is a DNA sequence formed by a continuous repetition of motifs composed of 1 to 4 nucleotides, most often". "Single nucleotide polymorphism (SNP) is the variation (polymorphism) of a single base pair in the genome between individuals of the same species" [Figure 5]. These two genetic variations, which are very commonly found in the genome, are hereditary and therefore passed from generation to generation [19] [21]. This implies that individuals of the same species share specific microsatellites and single nucleotide polymorphisms, enabling their identification as members of the species in question. Regardless of the application domain, the process is fundamentally the same. First, it is necessary to find, extract, and purify the DNA from the collected sample. Then, DNA must be amplified using polymerase chain reaction (PCR) technology, and the different fragments obtained must be separated by electrophoresis. Next, the DNA portion possessing the polymorphic sequence, allowing for individual identification, should be sequenced. Finally, the obtained DNA sequence can be compared with databases containing the desired genotypes [22].

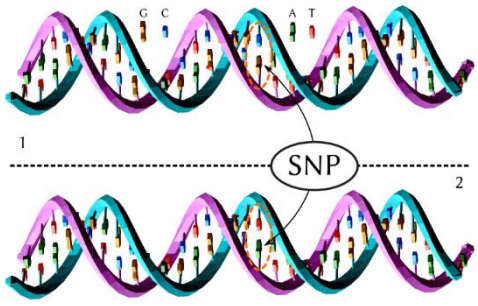


Figure 5:
Nucleotide polymorphism. DNA sequence no. 1 differs from DNA sequence no. 2 in only one place. A G base (Guanine) in DNA 1 is replaced by an A base (Adenine) in DNA 2.

PCR-based Sexing

Birds have a different sex determination system to humans. It doesn't operate on the XY system, where females have two X chromosomes, and males have one X and one Y chromosome. In birds, females are heterogametic, possessing one W and one Z chromosome. Males, on the other hand, have two Z chromosomes and are therefore homogametic [15]. A gene known as the CHD gene (Chromodomain-Helicase-DNA-binding gene) is found on both sex chromosomes. It is expressed in two alleles, CHD-W on the W chromosome and CHD-Z on the Z chromosome. The size of the introns (non-coding parts of the gene) is different from one allele to another,

resulting in different lengths and therefore different migration rates during electrophoresis [15] [Figure 6]. When the sample comes from a male, which has two identical alleles (CHD-Z) that migrate at the same rate, only one DNA band is visible in the gel. In a female, however, as there are two alleles (CHD-W and CHD-Z) of different sizes, which do not migrate at the same pace, two DNA bands are observed in the gel. Thus, it is possible to determine the sex of a DNA sample from a bird by amplifying the DCH gene and conducting a simple electrophoresis. In summary, if only one DNA band appears, it is a male. On the other hand, it two DNA bands are visible, it is a female [Figure 6].

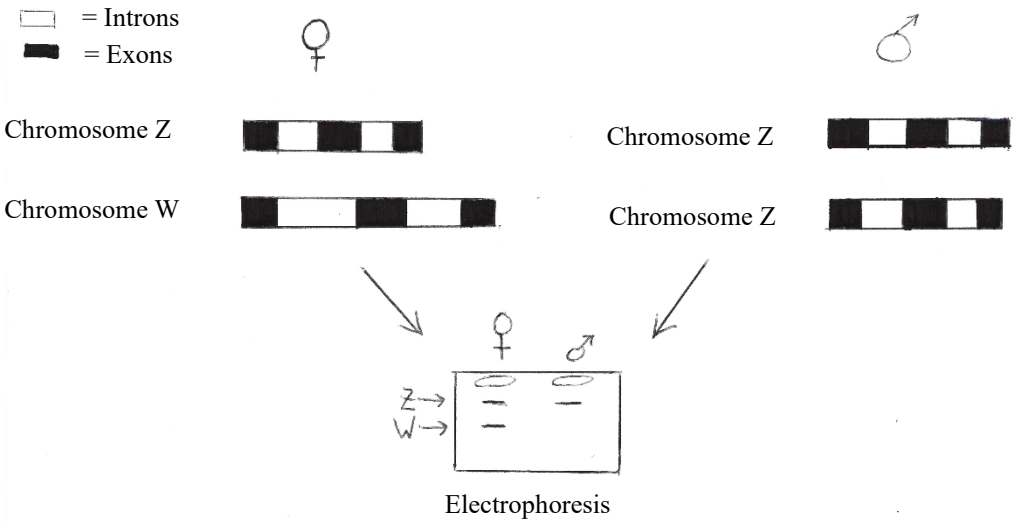


Figure 6: *Diagram explaining sexing in birds.*

DNA sequencing

To identify the species to which a feather belongs, it is necessary to amplify and sequence a gene, called COI (Cytochrome Oxidase I), where the sought-after polymorphisms are located. Unfortunately, the information conveyed by these polymorphisms is relatively limited because the gene is found in mitochondrial DNA. However, the polymorphisms are considered to be neutral, as most mutations do not induce changes in the amino acid sequence of proteins. Although these polymorphisms always make it possible to identify the genus of a bird, it is sometimes not possible to determine the species of the individual if it is genetically too close to another. For example, it is challenging to identify a particular bird species within the Corvidae family, due to low degree of polymorphism.

2.2.2 Laboratory protocols

Excision of the base from the feather

To extract DNA from a feather, a small blood clot is taken from the top of the calamus (upper umbilicus), which is the remnant of the axial artery [Figure 7]. The axial artery is the blood vessel that supplied the feather during its development [10]. At the end of growth, the axial artery resorbs, leaving only this small visible blood clot filled with nuclear and mitochondrial DNA in the region of the upper umbilicus [10]. For this reason, the entire calamus is cut with scissors into a tube to ensure the capture of the blood clot. Before 2005, only the tip of the calamus was collected because this small blood clot was not yet known. Researchers were still able to extract DNA, but in much smaller quantities and of lower quality. Amplification failures and genotyping errors were frequent [10].

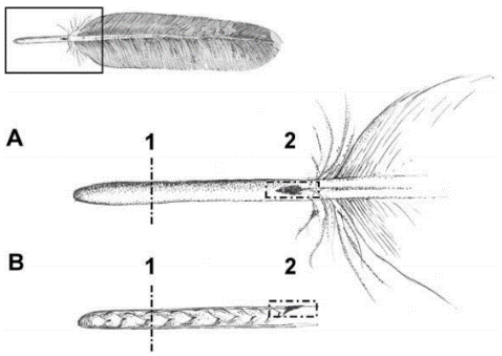


Figure 7: *Diagrams of the calamus of a feather. (A) Calamus of a feather. (B) Longitudinal cross-section of the calamus. (1) Area where the calamus was cut in the past. (2) Area where the blood clot is located.*

DNA extraction

After excising the small blood clot from the calamus, it is necessary to extract the DNA. To do this, we used the "DNeasy, Blood & Tissue Kit (50)". To begin with, all the necessary products for DNA extraction must be vortexed and centrifuged [Figure 8] to ensure proper mixing. Then, the proteins present in the samples need to be destroyed using proteinase K. Afterwards, the samples must be incubated for a minimum of 1 to 3 hours to break down the cell and the nuclear membranes which hold the genetic material. The next step is to make the DNA insoluble by adding 100% ethanol. Then comes the cleaning stage during which the samples are centrifuged three times, allowing the genetic material to be completely separated from the other components of the solution. At this point, all the other components dissolved in the liquid can pass through a filter, except the DNA, which has become insoluble. Finally, Buffer AE is added to the DNA to resolubilize it, allowing it to pass through the filter and be pipetted into a new tube.

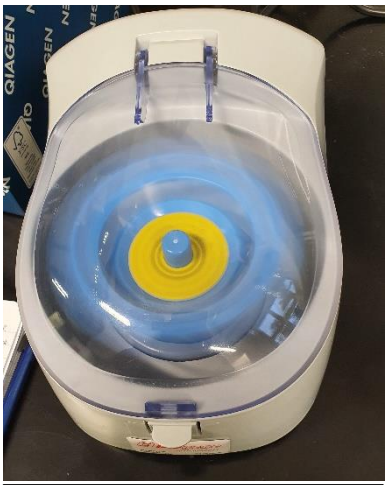


Figure 8:
Small centrifuge in rotation.

Sexing a DNA sample by polymerase chain reaction (PCR)

To sex a DNA sample, it is necessary to first amplify the genetic material. The chemical components used for this purpose, in addition to the various DNAs, are: the "Multiplex Kit" product and the primers P2 (TCTGCATCGCTAAATCCTTT) [9] and P8 (CTCCCAAGGATGAGRAAYTG, with R for A or G and with Y for C or T) [9]. To begin with, the various products must be vortexed and centrifuged to ensure they are properly mixed. Next, a solution with the correct proportions of each chemical component must be prepared in a tube, then this solution should be divided into different tubes and finally, the various DNAs should be added to them (in case there are multiple DNAs to amplify). Finally, the tubes should be placed in a PCR machine and the PCR program should be set according to the primers used [Figure 9].

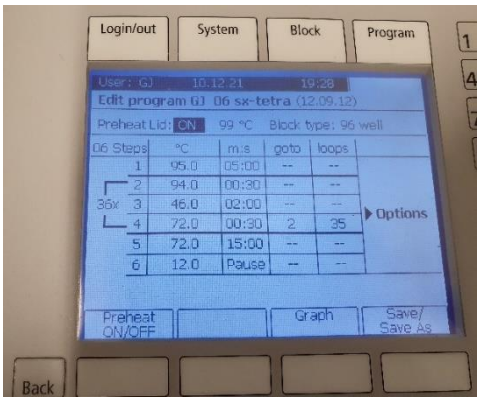


Figure 9: *PCR programme for sexing*

Species identification by polymerase chain reaction (PCR)

To sequence a DNA sample, it is first necessary to amplify it through PCR. The chemical components used for this purpose, in addition to the various DNAs, are: "Type-it" product and the COI-F and COI-R primers. To begin with, the different products must be vortexed [Figure 1'] and centrifuged separately to ensure they are properly mixed. Then, a solution with the correct proportions of each chemical element must be prepared in a larger tube before dividing it into different tubes and finally adding the various DNAs (in case there are multiple DNAs to amplify). Finally, the tubes should be placed in a PCR machine [Figure 40], and the PCR program should be set according to the primers used.

Figure 10: *Vortex the small tubes before PCR.*

Alternatively, primers COI-F and COI-R could be replaced by GAF-407 (GRGGRCAAATATCATTYTGAGG, where R could be A or G and Y could be C or T) [20] and (GAR-627GGRTTGTTTGAGCCYGATTCG, where R could be A or G and Y could be C or T) [11]. In this case, the PCR programme would be adapted to the primers used.

Electrophoresis

Electrophoresis is used to check whether a DNA sample has been correctly amplified before being sequenced and to determine the sex of a bird. To carry out an electrophoresis, the first step is to prepare the agarose gel for electrophoresis. To do this, 100ml of TBE must be mixed with 2g of 2% agarose, then the solution must be heated in the microwave and left to cool. The next step is to add "Cyber Sage 5%" intercalating agent, which will mark DNA fragments to make them fluorescent under UV light. Then, the solution is poured into an electrophoresis tray, where the gel solidifies [Figure 11]. While it's solidifying, Loading Day is added to the samples to make the DNA denser so that it stays at the bottom of the gel. Finally, the size marker "1 Kb Plus DNA Ladder" of 15'000 bp and the amplified DNA samples are loaded into the different wells of the gel before placing the gel into the electrophoresis machine and starting it.

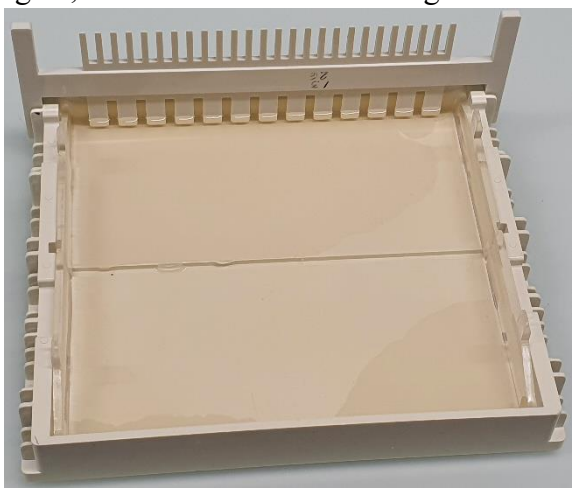


Figure 11: *Solidifying gel in an electrophoresis tray.*

Preparatory work before sequencing

Before the samples were sequenced, the DNA concentration had to be adjusted to between 2-6 ng/µl and the samples purified. Sequencing and purification of the fragments were carried out by the Microsynth laboratory (St. Gallen).

Preparatory work before results analysis

Before being able to submit the sequenced genetic material to the NCBI (National Center for Biotechnology Information) database, the sequences should still be processed using computer software (Sequencher 4.9). This includes cutting sequences of primers, generating a consensus between the 5'-3' and 3'-5' strands, and ultimately converting the files into FASTA format to meet the database requirements.

3 Results:

3.1 Phenotyping: feather identification using the naturalist method with specialized books

3.1.1 Feather no. 1

This feather is rigid and doesn't have down at the base. It doesn't show a bend between the calamus and the rachis and has two notches [Figure 12]. Moreover, one vane is much narrower than the other. It is therefore a primary remix. Its size is about 29 cm, which corresponds to the measurements of the carrion crow and the rook. The shape, size and black colour of the feathers could correspond to those of these two species. However, the tip of the feather looks more like a carrion crow than a rook [Figure 13]. Unfortunately, the place where the feather was found does not help with its identification, because it corresponds exactly to the habitat of both species. However, as the carrion crow population (80,000-120,000 pairs) [13] is much larger than that of the rook (5,800-7,300 pairs) [12], the chances of it being a carrion crow feather are greater. We can therefore presume, but with no certainty, that the feather belongs to the carrion crow species.

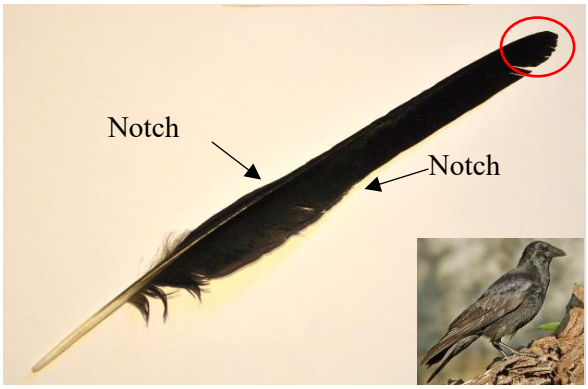


Figure 12: *Feather no. 1, from a carrion crow. It was found on 28 August 2021 under a roadside walnut tree in Grolley.*

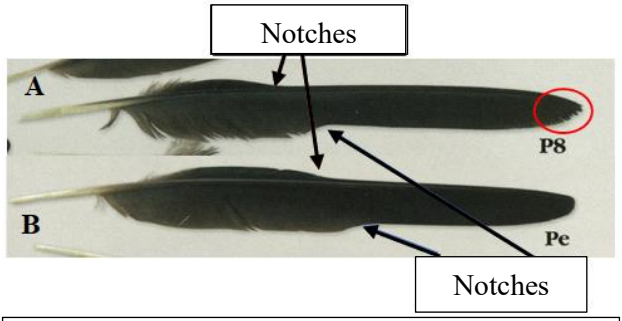


Figure 13: *Models of the primary remiges of carrion crows and rooks. (A) Model of the 8th primary remix of a carrion crow. (B) Model of an outer primary remix of a rook.*

The sex remains unknown.

3.1.2 Feather no. 8:

The feather is rigid and doesn't have down at the base. It doesn't show a bend between the calamus and the rachis and doesn't have contour notches [Figure 14]. Seen from the side, the calamus is slightly turned up. Is it therefore a rectrice. Its size is about 17,5 cm which corresponds to the

Figure 14: *Feather no. 8, from a common magpie. She was found on 3 September 2021 under a tree in the inner courtyard of the Collège St-Michel in Fribourg.*

measurements of the common magpie. The black colour and the blue, green and bronze highlights on the outer vane [Figure 14] are typical of this species. Finally, the feather is very similar to its illustration in the book [Figure 15]. We can therefore presume that the feather belongs to a common magpie.
The sex remains unknown.

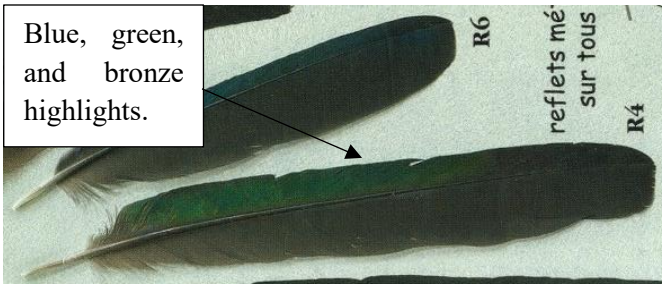


Figure 15: *Model of the 4th and 6th rectrix of a common magpie.*

There was an error during the laboratory phase. As a result, it was not possible to sequence or sex the feather's DNA. There will therefore be no genetic result for this feather.

3.1.3 Feather no. 10:

The feather is rigid and doesn't have down at the base. It doesn't show a bend between the calamus and the rachis. The feather has vanes with regular edges [Figure 16]. Seen from the side, the calamus follows the curve of the rachis, which is clearly curved in the same direction along the entire length of the feather. Finally, the tip of the feather is square and the rachis is strongly curved towards its base on the side of the internal vane [Figure 16]. It is, therefore, a secondary remix. Its size is about 13 cm, which corresponds to the measurements of the common magpie.
The black colour and the blue, green and bronze highlights on the outer vane are typical of this species [Figure 16]. Finally, the feather is very similar to its illustration in the book [Figure 17]. We can therefore presume that the feather belongs to a common magpie.
The sex remains unknown.

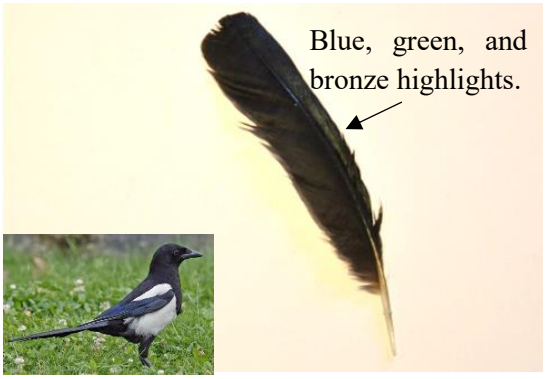
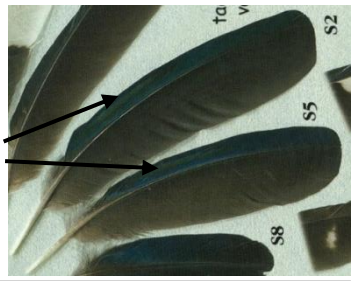


Figure 16: *Feather no. 10 from a common magpie. It was found in the courtyard of the Collège St-Michel in Fribourg.*



Blue, green, bronze highlights.

Figure 17: *Model of the 5th and 2nd secondary remix of the common magpie.*

An error was made during the laboratory phase. As a result, it was not possible to sequence or sex the feather's DNA. There will therefore be no genetic result for this feather.

3.1.4 Feather no. 12:

This feather is quite rigid and doesn't have down at the base. It shows a bend between the calamus and the rachis. It is therefore a primary covert. Its size is about 6,2 cm. The feather is light grey, tending to beige [Figure 18]. A light white border can be seen at the tip of the feather and on the outer vane [Figure 19]. The feather is like those of the Eurasian collared dove and of the common wood pigeon [Figure 20]. However, it is a little small for a common wood pigeon's primary covert. In addition, some Eurasian collared doves have already been observed in the area where the feather was found. Finally, the feather is like its illustration in the book [Figure 20]. We can therefore presume that the feather belonged to an Eurasian collared dove. However, its identification is rather uncertain. In fact, the only criteria for ruling out the common wood pigeon is that it is slightly too small and that Eurasian collared turtle doves have been seen in the area where the feather was collected.

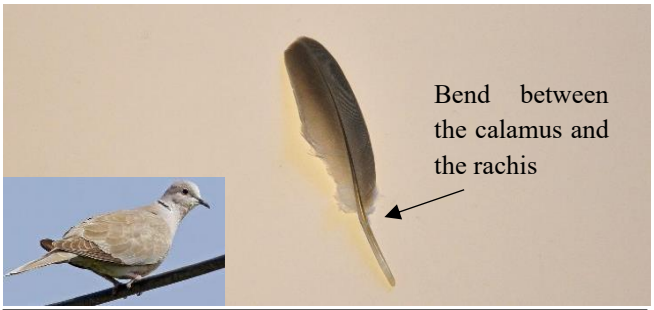


Figure 18: *Feather no. 12 from an Eurasian collared dove. It was found in a garden under a hazel tree in Grolley.*

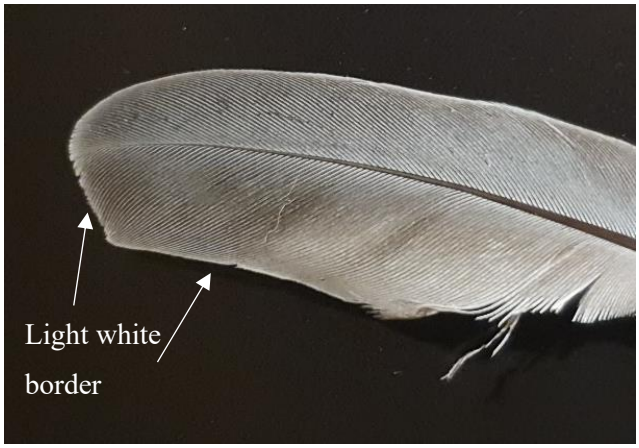


Figure 19: *Detail of the feather no. 12*

The sex remains unknown.

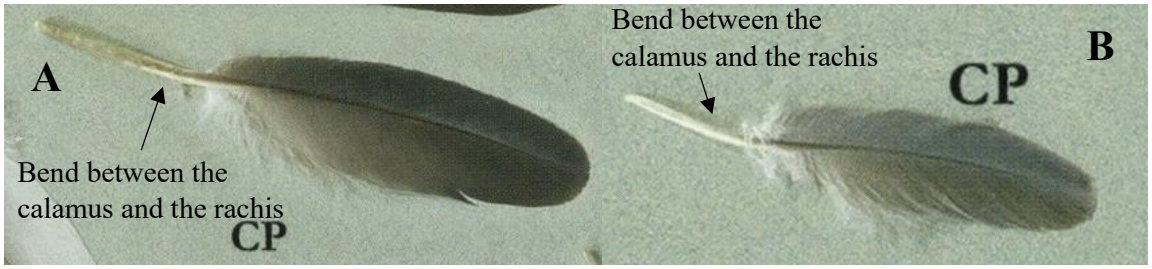


Figure 20: *Primary covert model of a common wood pigeon and Eurasian collared dove: (A) common wood pigeon, (B) Eurasian collared dove.*

3.1.5 Feather no. 13:

This feather is rigid and doesn't have down at the base. It doesn't show a bend between the rachis and the calamus, has a notch and one of its vanes is much narrower than the other [Figure 21]. It is therefore a primary remix. Its size is about 21 cm, which would correspond to the measurements of the common wood pigeon and would exclude the Eurasian collared dove whose feathers are smaller. The feather is dark grey with a clear white border on the outer vane [Figure 22]. Finally, it is also very similar to the illustration in the book [Figure 23]. we can therefore presume that the feather belongs to a common wood pigeon.

The sex remains unknown.

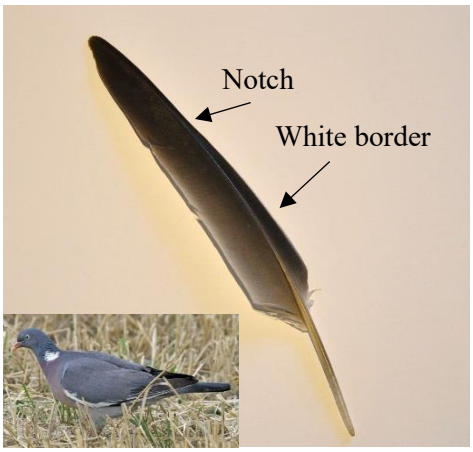


Figure 21: *Feather no. 13 from a common wood pigeon. It was found under a weeping willow beside a stream in a garden in Grolley.*

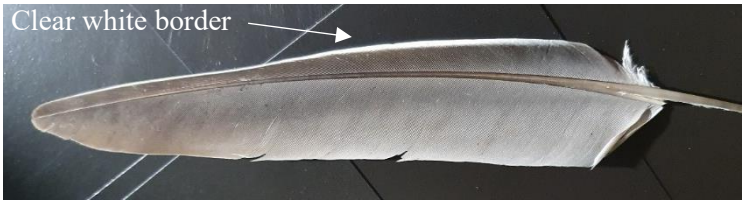


Figure 22: *Detail of feather no. 13.*

Figure 23: *Model of the 9th primary remige and an outer primary remige.*

3.1.6 Feather no. 14:

This feather is rigid and doesn't have down at the base. It doesn't show a bend between the calamus and the rachis [Figure 24]. It has a notch and a vane that is clearly narrower than the other [Figure 24]. It is therefore a primary remix. It is about 10,5 cm long, the same size as a common blackbird. A fin light brown border is visible on

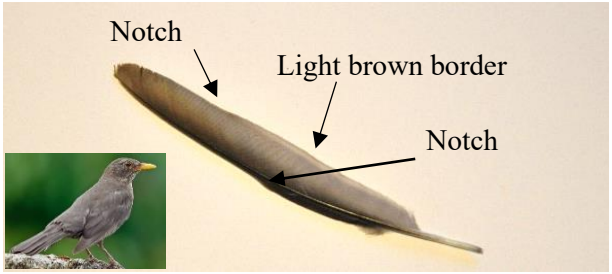


Figure 24: *Feather no. 14 from a common blackbird. It was found in a garden under an oak tree*

the outer vane. Finally, the feather is very similar to the illustration of the common blackbird in the book [Figure 25]. The feather also looks like a ring ouzel's feather, but it has a grey border on the outer vane which is not present on the collected specimen [Figure 25]. Furthermore, the geographical distribution of the ring ouzel seems to exclude it [17]. We can therefore presume that the feather comes from a common blackbird.

As the feather is more grey than black, we can presume that it could belong to a female, whose feather are generally lighter than these of males.

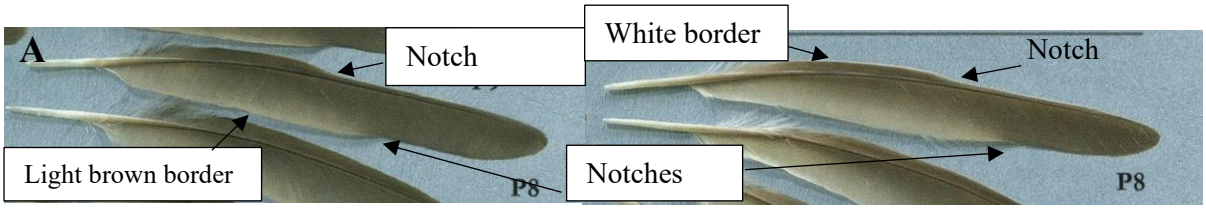


Figure 25: *Models of the 8th primary remix of common blackbird and ring ouzel. (A) blackbird, (B) ring ouzel.*

3.1.7 Feather no. 16:

This feather is rigid with little down at the base. It doesn't show a bend between the calamus and the rachis and has vanes with regular edges [Figure 26]. Seen from the side, the calamus follows the curve of the rachis, which is clearly curved in the same direction along the entire length of the feather. Finally, the tip of the feather is rounded and the rachis is strongly curved towards its base on the side of the internal vane. It is therefore a secondary remix. Its size is about 22,5 cm, which corresponds to the measurements of the black kite. The feather is brown with dark stripes and a little white down at the base [Figure 26]. There is no white between the dark stripes, which rules out the possibility that the feather came from a common buzzard. Finally, the feather is similar to the illustration of the black kite in the book [Figure 27]. One can therefore presume that the feather belongs to a black kite.

Figure 26: *Feather no. 16 from a black kite. It was found in gravel in front of Grolley primary school on 13 September 2021.*

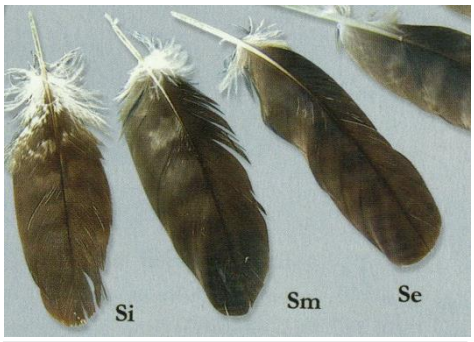


Figure 27: *Models of inner, middle and outer secondary remiges from a black kite.*

The sex remains unknown.

3.1.8 Feather no. 20:

The feather is quite rigid and doesn't have down at the base. It doesn't show a bend between the calamus and the rachis and has vanes with regular edges [Figures 28]. Seen from the side, the calamus follows the curve of the rachis, which is clearly curved in the same direction along the entire length of the feather. The top of the feather is square with a slit and the rachis is very curved towards its base on the side of the widest vane [Figure 28]. It is therefore a secondary remige. It is about 5 cm long, the same size as a house sparrow. A russet border can be seen on the outer vane and a large light grey border on the inner vane [Figure 29]. Finally, the feather is very similar to its illustration in the book [Figure 30].

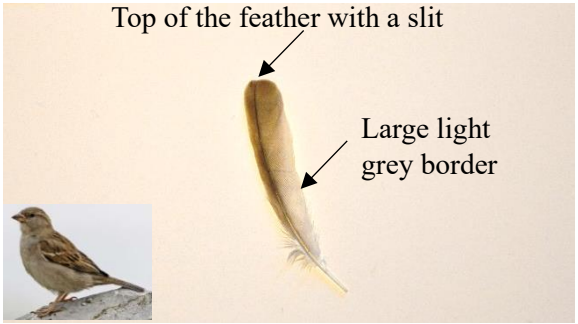


Figure 28: *Feather no. 20 from a house sparrow. It was found at the side of a road, under a plane tree in Grolley, on 19th September 2021.*

The sex remains unknown.

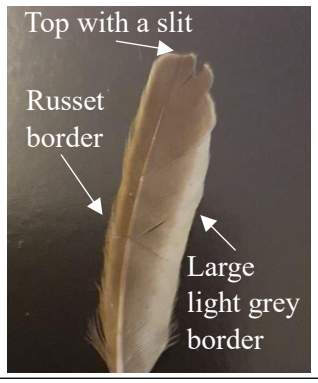


Figure 29: *Details on feather no. 20*

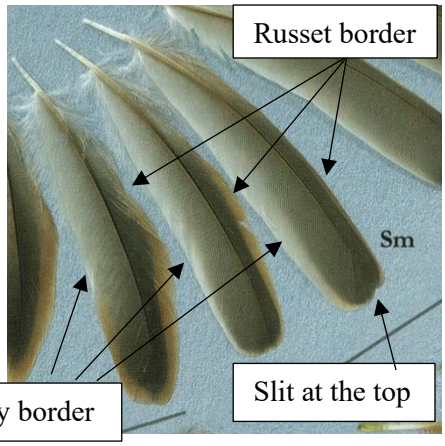


Figure 30: *Models of the 7th and the 6th secondary remiges and median secondary remiges of house sparrows.*

3.1.9 Feather no. 28:

It is not necessary to identify the location of the feather for this specimen. The feather has a very distinctive pattern on the outer vane that immediately identifies the species to which it belongs [Figure 31]. The blue stripes are the hallmark of the Eurasian jay, as shown in the illustration in the book [Figure 32]. Moreover,

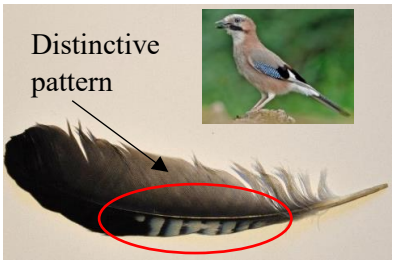


Figure 31: *Feather no. 28 from an Eurasian jay, It was found under an isolated pine above Cerniat (1256m) on 3 October 2021.*

Figure 32: *Models of the 7th and the 5th secondary remiges of Eurasian jay.*

the place where the feather was found is a good match for its habitat.
The sex remains unknown.

3.2 Genotyping: Feather's identification using DNA

3.2.1 Sexing results:

An electrophoresis was conducted on December 17, 2021 to verify if the PCR had actually worked before sending the samples to be sequenced and to determine the sex of the birds to which the feathers belonged. The electrophoresis shows that the PCR for species identification did not work very well [Figure 33]. Only wells #2 and #8 exhibit a DNA band with fragments measuring approximately 220 base pairs. These two DNA samples were successfully amplified during the PCR, but they are the only ones. For wells #3, 4, 5 and 6 there was a problem during the PCR, which remains unidentified.

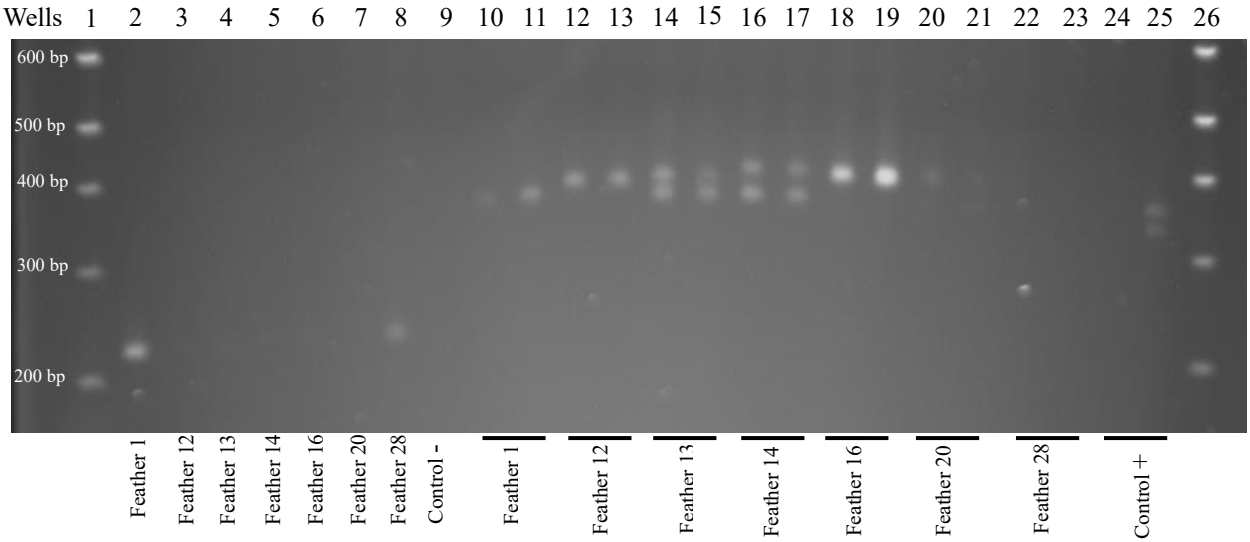


Figure 33: *Electrophoresis gel showing PCR fragments of approximately 220, 330, 380, 400, 410 and 420 base pairs, resulting from the amplification of the COI and CHD genes. Well #1 contains the 1 Kb Plus DNA Ladder size marker of 15,000 base pairs. Wells #2 to #9 contain DNA from feathers 1, 12, 13, 14, 16, 20, 28, and the negative control prepared for species identification. Wells #10 to #25 contain DNA from feathers 1, 12, 13, 14, 16, 20, 28 and the positive control prepared for sexing. Well #26 contains the 1 Kb Plus DNA Ladder size marker of 15,000 base pairs. Presence of DNA bands in wells #2, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 16, 20, 21 and 25.*

However, sex determination worked very well. All wells, except for wells #22 and #23, show one or two DNA bands with fragments measuring between 380 and 420 base pairs, making it possible to identify the sex of the individuals [Table 1].

Feathers	Number of bands	Fragments size [bp]	Sex
Feather 1	**1**	380	Male
Feather 12	**1**	400	Male
Feather 13	**2**	380 et 410	Female
Feather 14	**2**	380 et 420	Female
Feather 16	**1**	410	Male
Feather 20	**2**	360 et 400	Female
Feather 28	/	/	Unknown
Control +	**2**	330 et 360	Female

Table 1: *Sexing results*

3.2.2 Sequencing results:

After performing another PCR and another electrophoresis control, the DNA samples were sent for sequencing to the Microsynth laboratories in St. Gallen. Raw sequences and database comparison are provided in Appendix.

DNA from feather no. 1:

The result of the DNA sequencing of feather no.1 shows that this feather belonged to a carrion crow (Corvus corone). Unfortunately, it was not possible to compare the sample with the genetic material of the rook, as it is not present in the NCBI database. Therefore, it is not possible to conclusively rule out that it is not a rook. However, since the 177 base pairs of the DNA fragment exactly match those in the database, we can still conclude that the feather does indeed belong to a carrion crow, confirming the naturalist's initial identification.

DNA from feather no. 12:

The results of the DNA sequencing of feather no. 12 indicate that the feather belongs to an Eurasian collared dove (Streptolia decaocto). The 166 base pairs of the DNA fragment match the database model with only 90% similarity, but the only non-matching bases are either Y bases (= could C or T) or N bases (= any bases), which in itself is consistent with the model DNA

fragment. Furthermore, the genetic material of the feather does not have a close match with that of any other species of Columbidae. Therefore, the genetic results confirm the naturalist's initial identification.

DNA from feather no. 13:

The results of the DNA sequencing of feather no. 13 indicate that the feather belongs to a common wood pigeon (Columba palumbus). The 177 base pairs of the DNA fragment perfectly match those in the database, confirming the initial identification made using the naturalist's method.

DNA from feather no. 14:

The results of the DNA sequencing of feather no.14 indicate that the feather belongs to a common blackbird (Turdus merula). The 177 bases of the DNA fragment match exactly with the database, confirming the initial identification made using the naturalist's method.

DNA from feather no. 16:

The results of the DNA sequencing of feather no. 16 indicate that it belongs to a red kite (Milvus milvus) and also to a black kite (Milvus migrans). For both species, the 177 bases of the DNA fragment match exactly with the database. Sequencing is therefore unable to determine which species the feather belongs to, as the two species are too closely related genetically.

DNA from feather no. 20:

Unfortunately, the sequencing failed. The DNA was in too poor condition to obtain usable results. Therefore, the initial identification made using the naturalist's method, although very likely correct, cannot be confirmed through DNA analysis.

DNA from feather no. 28:

The results of the DNA sequencing of feather 28 indicate that the feather belongs to an Eurasian jay (Garrulus glandarius). The 177 bases of the DNA fragment match exactly with that in the database, confirming the initial identification made using the naturalist's method.

4 Discussion

Once the results of the sequencing of the feathers have been analysed, the final stage of this work consisted of comparing the two methods of identification, namely that of the naturalist using specialized books and that of the molecular biologist using DNA genotyping.

4.1 Sexing comparison:

As only one sample could be sexed using the naturalist's method (with specialized books), a true comparison of the two approaches could not be made. However, as genetic sexing worked very well (only one failure, feather No. 28), it can be deduced that DNA sexing is more effective than sexing based on phenotyping.

4.2 Species identification comparison:

A comparison of the results obtained using the two different techniques shows that the first identification was relatively successful [Table 2]. Unfortunately, it is not possible to compare the results obtained using the two methods for feathers 8 and 10, as an error was made during DNA extraction.

Feather	Naturalist identification (with specialized books)	Molecular biologist identification (with DNA)
Feather no. 1	Carrion Crow	Carrion crow
Feather no. 8	Common magpie	/
Feather no. 10	Common magpie	/
Feather no. 12	Eurasian collared dove	Eurasian collared dove
Feather no. 13	Common wood pigeon	Common wood pigeon
Feather no. 14	Common blackbird	Common blackbird
Feather no. 16	Black kite	Black kite and red kite
Feather no. 20	House sparrow	/
Feather no. 28	Eurasian jay	Eurasian jay

Table 2: *Comparison of results obtained using the two methods.*

Additionally, the two different approaches cannot be compared for feather 20, as its sequencing failed, most likely due to damaged DNA. Furthermore, one of the results is surprising: according to the sequencing data, feather no. 16 appears to belong to a black kite and a red kite. This result suggests that these two species are genetically very close, with identical DNA in this specific fragment of the COI gene, making it impossible to confirm the identification made with the naturalist's method. In conclusion, DNA-genotyping of the feathers confirms that 5 out of the 9 initial identifications made with specialized books. This means that 56% of the selected feathers were correctly identified using the naturalist's method [Figure 34]. Considering the loss of two samples and the unsuccessful sequencing, genotyping even confirmed 5 out of 6 identifications.

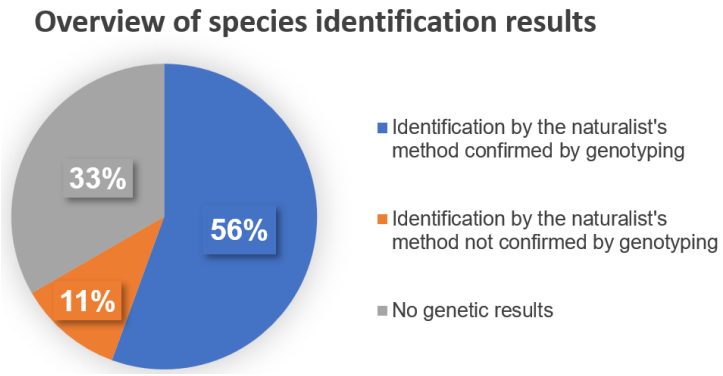


Figure 34: *Overview of species identification results*

5 Conclusion

In view of these results, it is possible to conclude that genotyping using DNA allows a much more reliable and refined identification compared to that of the naturalist. Indeed, with books alone [1][3], it would have been impossible for me to determine the sex of the birds from which the feathers came. Furthermore, only DNA allowed to confirm the uncertain identification of the species of feather no. 12. Without genotyping, it would have been impossible to determine with certainty that it was an Eurasian Collared Dove feather. However, this work also demonstrates that DNA has its limits. It can be too degraded to be sequenced, the laboratory protocols or equipment can be of poor quality, not to mention the potential errors made by the biologist during the process. Furthermore, the genome of the species to be identified must already be included in a database; otherwise, it is absolutely impossible to determine to which species a feather belongs by using DNA alone. Finally, as exemplified with the feather no. 16, two species can be genetically too close for a gene, such as the COI gene, to allow for precise species identification from the sample, as observed with the feather no. 16 sample. In this case, only an identification carried out using the naturalist's method allows for determining the species to which the feather belongs. Thus, it is legitimate to wonder whether the cost of conducting such DNA identification is truly worthwhile, especially if the feather is easily identifiable with the help of a book. Therefore, I would answer to the question "Is genotyping using DNA better than specialized books for identifying bird feathers?" by "no", genotyping using DNA is not better but complementary to the naturalist's method for identifying bird feathers. In fact, it would probably be wiser to use this method only to confirm a doubtful identification or to refine it, for example, for sexing an individual. Indeed, most of the time, only DNA allowed for a clear identification of a bird's sex. However, this does not always work, as observed with the feather no. 28. Finally, it would also be conceivable and certainly more interesting to use this genotyping procedure to assist in the identification of bird species via droppings, as these are often difficult to identify. Bird droppings are regularly used, in addition to feathers, when searching for the potential presence of a species in a given area.

6 Acknowledgements

I would like to express my sincere gratitude to the University of Fribourg and Mr. Gwenaël Jacob for granting me access to the university laboratories and for their guidance throughout the entire process.

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Finally, a heartfelt thank you to my parents, who supported me throughout my work and helped with the proofreading.

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Illustrations

Cover page: *Title page*, personal drawing in black felt pen produced in Grolley on 16th March 2022.

Fig. 1: *Tectrices of male Indian peafowl*, personal scan of page 337 of chapter [8], taken at EPAC in Bulle on 17th March 2022.

Fig. 2: *Feather rigidity test*, personal scan of page 27 of chapter [8] taken at EPAC in Bulle on 5th January 2022.

Fig. 3: *Locations and names of the different feathers as seen from above and below the bird*, personal scan of page 13 of chapter [6], taken at EPAC in Bulle on 5th January 2022.

Fig. 4: *Size diagram*, personal scan of [2] performed at EPAC in Bulle on 5th January 2022.

Fig. 5: ECCLES, David, *A Single Nucleotide Polymorphism*, 2021. Consulted on 29th November 2021 on <https://fr.wikipedia.org/wiki/Polymorphisme_nucl%C3%A9otidique >

Fig. 6: *Diagram explaining sexing in birds*, personal drawing made in Grolley on 16th March 2022.

Fig. 7: *Diagrams of the calamus of a feather*, personal screenshot of article [10], taken at Collège St-Michel in Fribourg on 12th October 2021.

Fig. 8: *Small centrifuge in rotation*, personal photo taken at UniFR in Fribourg on 10th December 2021.

Fig. 9: *PCR programme for sexing*, personal photo taken at UniFr in Fribourg on 10th December 2021.

8 Appendix

8.1 Sequencing results

These figures illustrate the different sequencing results. They show the alignment of the sequenced DNA, called Query, with a DNA sequence in the NCBI database, called Subject (Sbjct).

DNA from feather no. 1:

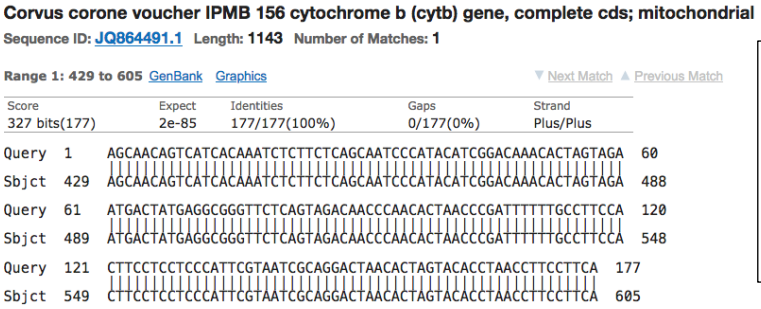

Figure 35: *Results of DNA sequencing from feather no.1. Concordant alignment of the sequenced DNA fragment from feather no. 1, called Query, with the template DNA in the NCBI database, called Sbjct. Base numbering of each fragment at the ends of the sequences. The sequenced fragment has no gaps.*

DNA from feather no. 12:

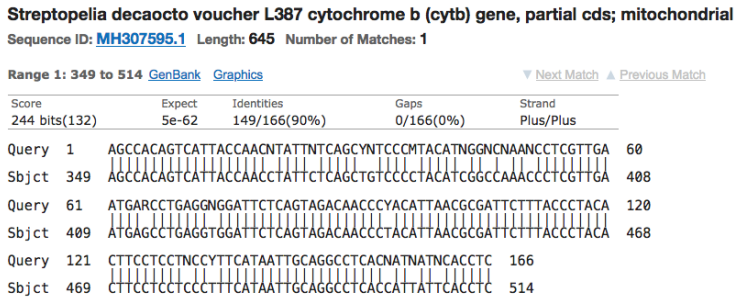

Figure 36: *Results of DNA sequencing from feather no. 12. Partial alignment of the sequenced DNA fragment from feather no. 12, called Query, with the template DNA in the NCBI database, called Sbjct. Base numbering of each fragment at the ends of the sequences. The sequenced fragment has no gaps.*

DNA from feather no. 13:

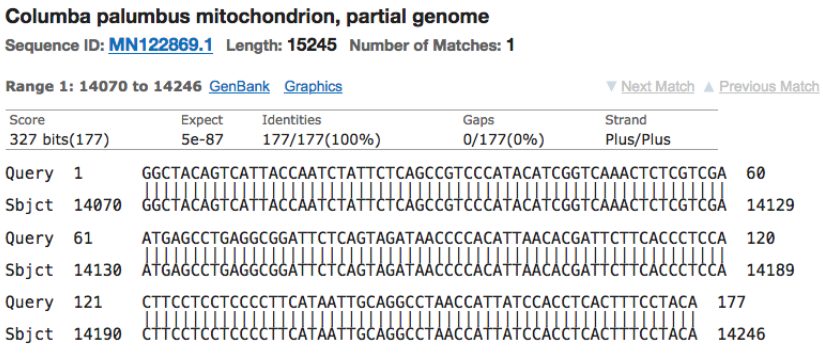

Figure 37: *Results of DNA sequencing from feather no. 13. Alignment of the sequenced DNA fragment from feather no. 13, called Query, with the template DNA in the NCBI database, called Sbjct. Base numbering of each fragment at the ends of the sequences. The sequenced fragment has no gaps.*

DNA from feather no. 14:

Turdus merula mitochondrion, complete genome

Sequence ID: MN122910.1 Length: 16748 Number of Matches: 1

Range 1: 14093 to 14269 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
327 bits(177)	5e-87	177/177(100%)	0/177(0%)	Plus/Plus

```
Query  1      GGCTACAGTAATCACCAACCTATTCTCAGCAATCCCCTACATCGGCCAAACACTAGTAGA  60
Sbjct  14093  GGCTACAGTAATCACCAACCTATTCTCAGCAATCCCCTACATCGGCCAAACACTAGTAGA  14152
Query  61     ATGAGCCTGAGGGGGATTCTCAGTAGACAACCCCACACTGACGCGATTCTTCGCCCTCCA  120
Sbjct  14153  ATGAGCCTGAGGGGGATTCTCAGTAGACAACCCCACACTGACGCGATTCTTCGCCCTCCA  14212
Query  121    CTTCCTCCTCCCATTCGTCATTGCAGGGCTCACACTAGTACATCTCACCTTCCTGCA  177
Sbjct  14213  CTTCCTCCTCCCATTCGTCATTGCAGGGCTCACACTAGTACATCTCACCTTCCTGCA  14269
```

Figure 38: *Results of DNA sequencing from feather no. 14. Alignment of the sequenced DNA fragment from feather no. 14, called Query, with the template DNA in the NCBI database, called Sbjct. Base numbering of each fragment at the ends of the sequences. The sequenced fragment has no gaps.*

DNA from feather no. 16:

Milvus milvus mitochondrion, partial genome

Sequence ID: MN122837.1 Length: 17883 Number of Matches: 1

Range 1: 14088 to 14264 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
327 bits(177)	5e-87	177/177(100%)	0/177(0%)	Plus/Plus

```
Query  1      GGCCACAGTCATCACAAACCTATTCTCGGCCATCCCATACATCGGACAAACCATCGTAGA  60
Sbjct  14088  GGCCACAGTCATCACAAACCTATTCTCGGCCATCCCATACATCGGACAAACCATCGTAGA  14147
Query  61     ATGGGCCTGAGGGGGATTCTCCGTAGACAACCCTACCCTTACCCGATTCTTCGCCCTACA  120
Sbjct  14148  ATGGGCCTGAGGGGGATTCTCCGTAGACAACCCTACCCTTACCCGATTCTTCGCCCTACA  14207
Query  121    CTTCCTACTCCCATTCCTAATCGCAGGCCTCACCTTAATCCATCTCACCTTCCTCCA  177
Sbjct  14208  CTTCCTACTCCCATTCCTAATCGCAGGCCTCACCTTAATCCATCTCACCTTCCTCCA  14264
```

Figure 39: *First results of DNA sequencing from feather no. 16. Concordant alignment of the sequenced DNA fragment from feather no. 16, called Query, with a first template DNA from the NCBI database, called Sbjct. Base numbering of each fragment at the ends of the sequences. The sequenced fragment has no gaps.*

Milvus migrans migrans voucher AMNH 531936 cytochrome b (cytB) gene, partial cds; mitochondrial

Sequence ID: AY994399.1 Length: 1066 Number of Matches: 1

See 1 more title(s) See all Identical Proteins(IPG)

Range 1: 325 to 501 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
327 bits(177)	7e-92	177/177(100%)	0/177(0%)	Plus/Plus

```
Query  1    GGCCACAGTCATCACAAACCTATTCTCGGCCATCCCATACATCGGACAAACCATCGTAGA  60
Sbjct  325  GGCCACAGTCATCACAAACCTATTCTCGGCCATCCCATACATCGGACAAACCATCGTAGA  384
Query  61   ATGGGCCTGAGGGGGATTCTCCGTAGACAACCCTACCCTTACCCGATTCTTCGCCCTACA  120
Sbjct  385  ATGGGCCTGAGGGGGATTCTCCGTAGACAACCCTACCCTTACCCGATTCTTCGCCCTACA  444
Query  121  CTTCCTACTCCCATTCCTAATCGCAGGCCTCACCTTAATCCATCTCACCTTCCTCCA  177
Sbjct  445  CTTCCTACTCCCATTCCTAATCGCAGGCCTCACCTTAATCCATCTCACCTTCCTCCA  501
```

Figure 40: *Second DNA sequencing result from feather no. 16. Concordant alignment of the sequenced DNA fragment from feather no. 16, called Query, with a second template DNA from the NCBI database, called Sbjct. Base numbering of each fragment at the ends of the sequences. The sequenced fragment has no gaps.*

DNA from feather no. 28:

Garrulus glandarius glandarius isolate Gglagla13 cytochrome b (cytb) gene, partial cds; mitochondrial

Sequence ID: EF602128.1 Length: 586 Number of Matches: 1

Range 1: 73 to 249 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
327 bits(177)	5e-87	177/177(100%)	0/177(0%)	Plus/Plus

```
Query  1    GGCTACAGTTATCACTAACCTTTTCTCAGCAATCCCATATATTGGACAAACACTAGTAGA  60
Sbjct  73   GGCTACAGTTATCACTAACCTTTTCTCAGCAATCCCATATATTGGACAAACACTAGTAGA  132
Query  61   ATGACTCTGAGGCGGATTCTCAGTAGACAACCCTACCCTAACTCGATTTTTCGCTTTCCA  120
Sbjct  133  ATGACTCTGAGGCGGATTCTCAGTAGACAACCCTACCCTAACTCGATTTTTCGCTTTCCA  192
Query  121  CTTCCTCCTCCCTTTTGTAATCGCAGGACTAACACTAGTCCATCTGACCTTCCTACA  177
Sbjct  193  CTTCCTCCTCCCTTTTGTAATCGCAGGACTAACACTAGTCCATCTGACCTTCCTACA  249
```

Figure 41: *Results of DNA sequencing from feather no. 28. Concordant alignment of the sequenced DNA fragment from feather no. 28, called Query, with the template DNA in the NCBI database, called Sbjct. Base numbering of each fragment at the ends of the sequences. The sequenced fragment has no gaps.*

【評語】050021

The aim of this research is to determine the species and sex of birds by means of comparing the book referencing and DNA genotyping. This research topic is interesting. The design is good and the report is clear. For the species determination, DNA and combined phenotyping are both required. And both techniques need skill training and close supervision to make sure the use of primers are correct. This will be very helpful for future field work.

This study uses very few birds for the analysis, and individual variations are not considered. It will be nice to include a phylogenetic analysis of all the species.