




Geslachtsbepaling jonge raven (*Corvus corax*) door PCR genotypering

- **Robert Jan Veldman**
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- Chantal Kradolfer
- Karlijn Zoutenbier, Tom Broekhuis, Esmée Arents, Livia Veenemans, Cherin Mohamed, Samar Sajadi, Tess van de Mheen, Kenan Akin, Ghislaine van Gilse (sep 2020 - jul 2022)



BIOLOGIE EN MEDISCH LABORATORIUMONDERZOEK (LIFE SCIENCES)

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Wat is de oorzaak van leukemie, allergie of dementie? Hoe ontdek je met moderne DNA- en eiwittechnieken afwijkingen die een ziekte kunnen verklaren? Hoe verbeter je een vaccin tegen griep, ebola of COVID om een epidemie te voorkomen? Hoe kunnen we medisch onderzoek zo uitvoeren dat er minder proefdieren nodig zijn? En hoe verwerk je de enorme hoeveelheid data die biologische experimenten kunnen opleveren? Maakt dit jou nieuwsgierig? Dan is de hbo-opleiding Biologie en Medisch Laboratoriumonderzoek (Life Sciences, BML) iets voor jou!

Vraagstelling



Foto: Rinus Jansen



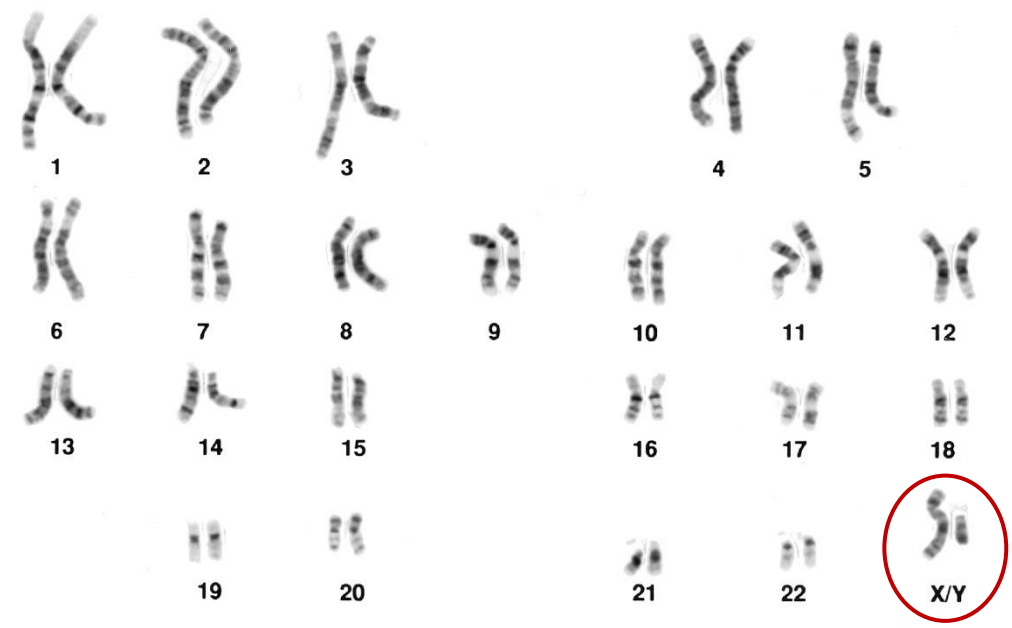
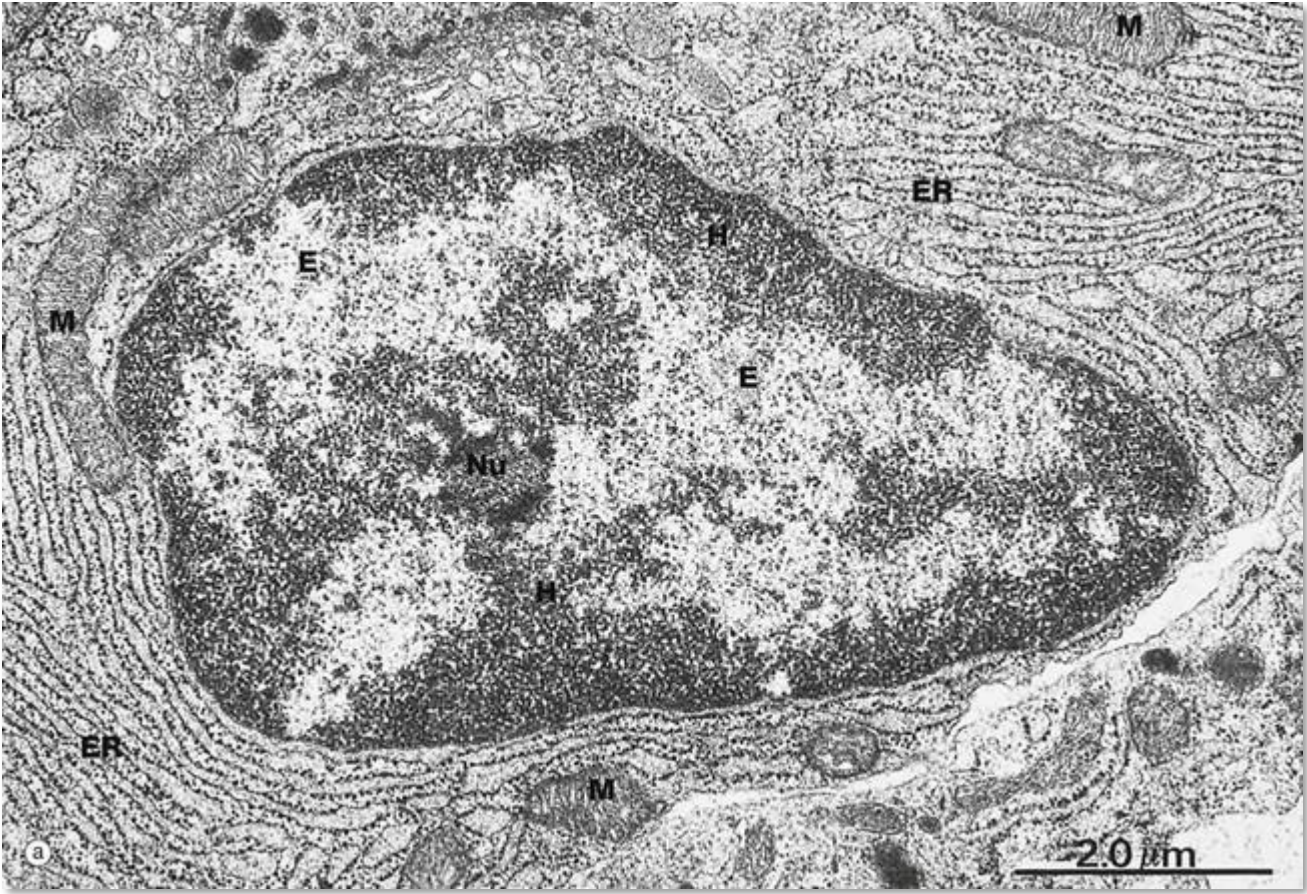
Foto: Wim Steenge

- Kan a.d.h.v. veermateriaal het geslacht worden bepaald?
- Bestaat er een correlatie tussen geslacht en bepaalde biometrische kenmerken?

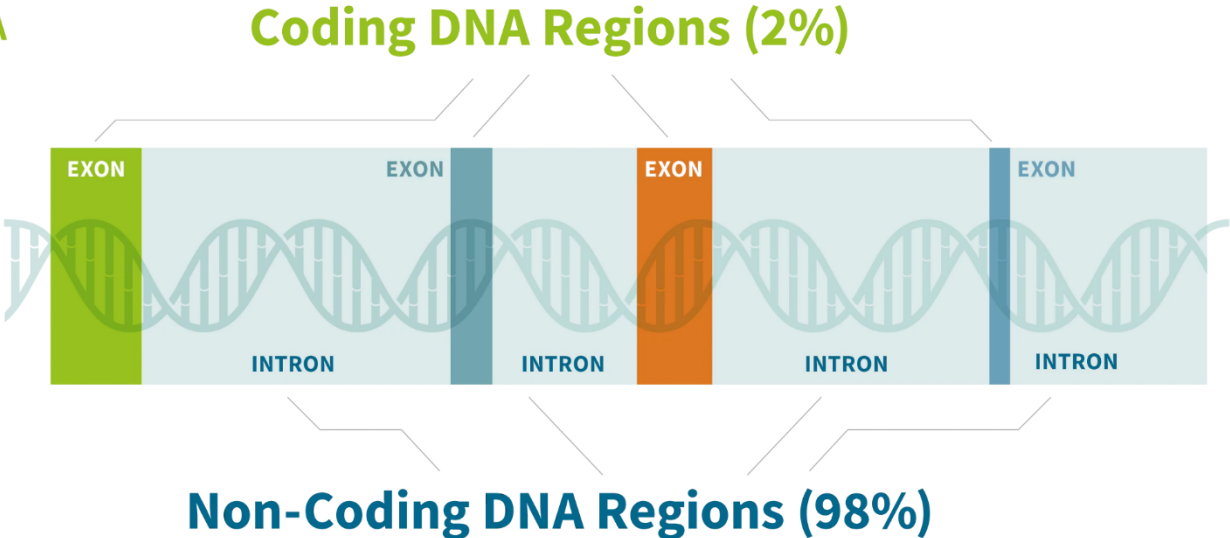
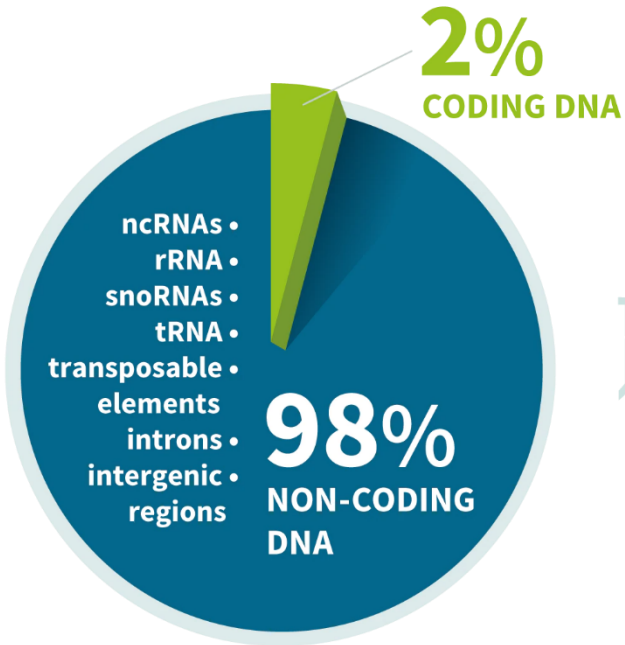
Genotype *versus* phenotype



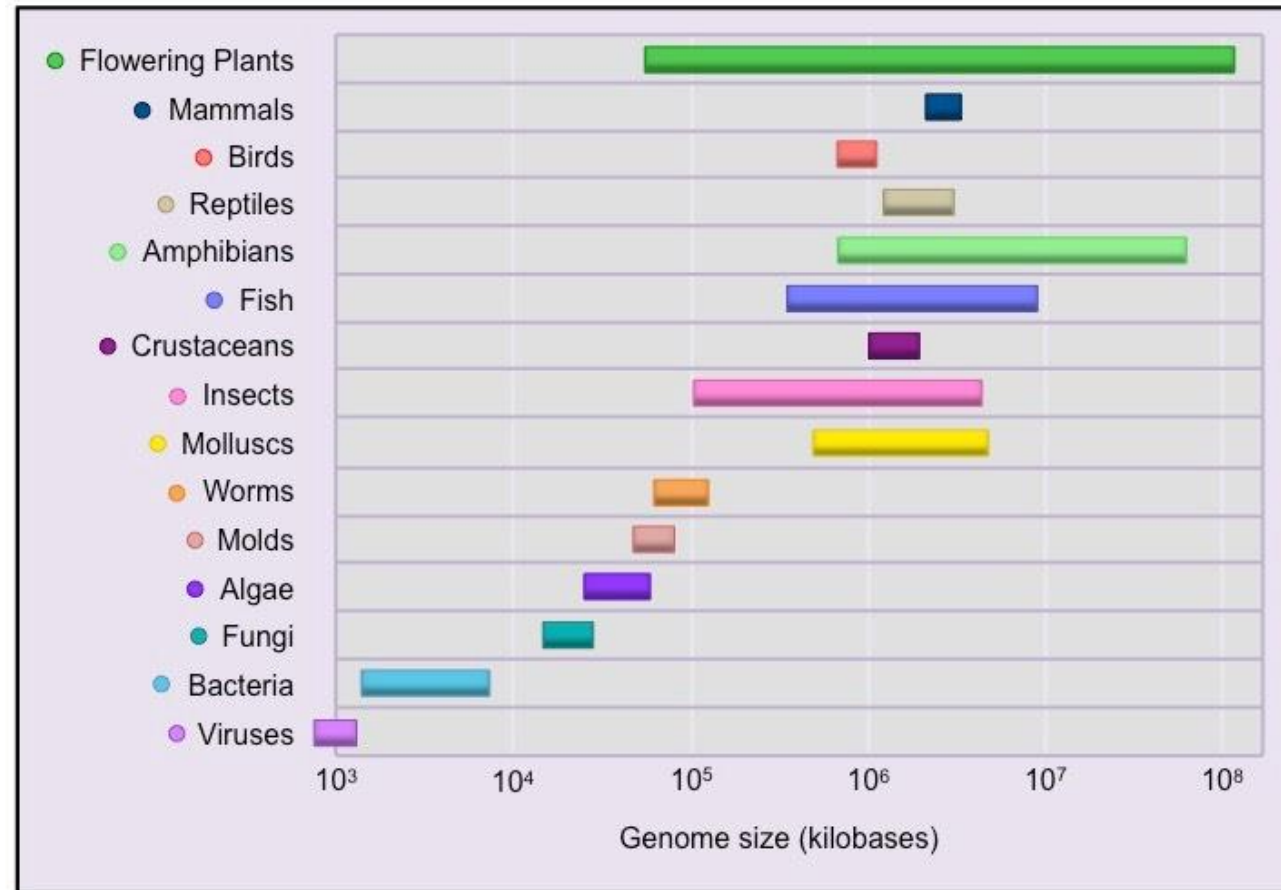
DNA en chromosomen



Genen en genomen



Vogels hebben relatief kleine genomen



“Compared with other animal groups, the genome of birds is generally small, ranging from 1 Gb to 2.1 Gb. It is hypothesized that smaller genomes are conducive to reducing metabolic costs, which is generally an adaptation to flight.”

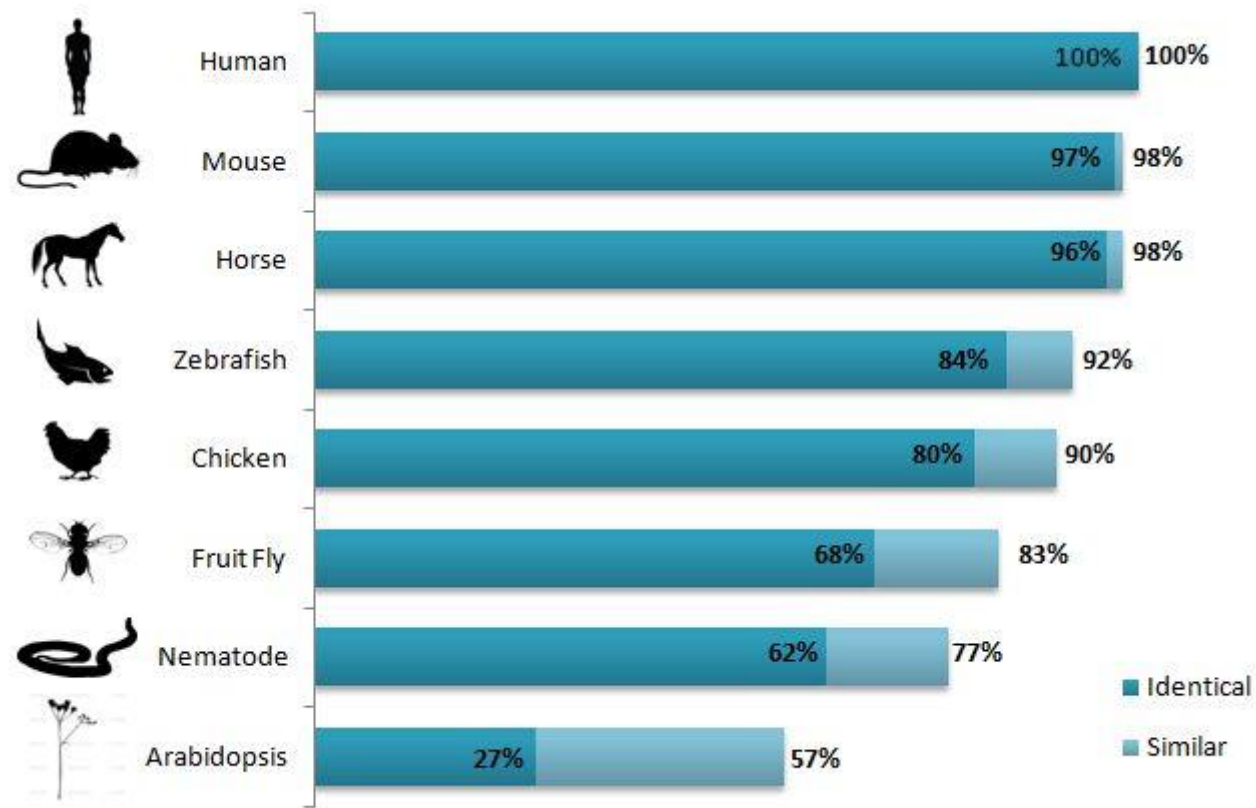
Comparative Genomics and Evolution of Avian Specialized Traits
Lei Wu, Xiaolu Jiao, Dezhi Zhang, Yalin Cheng, Gang Song, Yanhua Qu and Fumin Lei
Current Genomics (2021) 22(7): 496-511

Vogels hebben relatief weinig genen

	Organism	# of protein-coding genes
viruses	HIV 1	9
	<i>Influenza A virus</i>	10-11
	Bacteriophage λ	66
	Epstein Barr virus	80
prokaryotes	<i>Buchnera sp.</i>	610
	<i>T. maritima</i>	1,900
	<i>S. aureus</i>	2,700
	<i>V. cholerae</i>	3,900
	<i>B. subtilis</i>	4,400
	<i>E. coli</i>	4,300
eukaryotes	<i>S. cerevisiae</i>	6,600
	<i>C. elegans</i>	20,000
	<i>A. thaliana</i>	27,000
	<i>D. melanogaster</i>	14,000
	<i>F. rubripes</i>	19,000
	<i>Z. mays</i>	33,000
	<i>M. musculus</i>	20,000
	<i>H. sapiens</i>	21,000
	<i>G. gallus</i>	16,736



Overeenkomst genomen



CHD gen als universele marker voor geslacht vogels

RESEARCH ARTICLE

Sex Determination in 58 Bird Species and Evaluation of CHD Gene as a Universal Molecular Marker in Bird Sexing

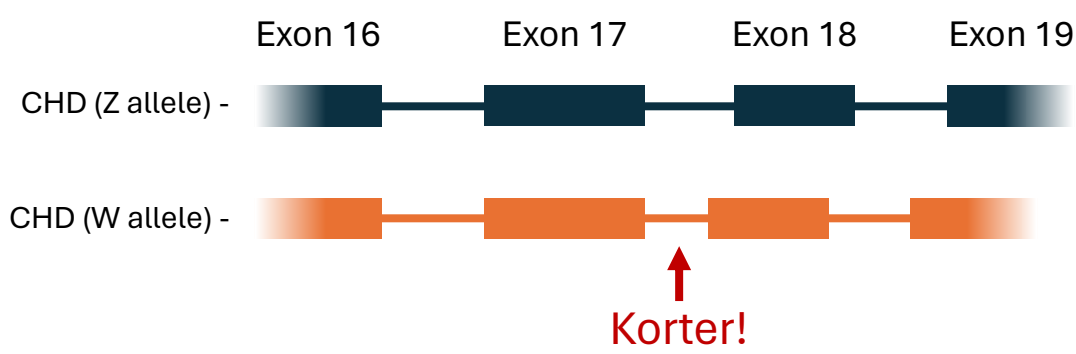
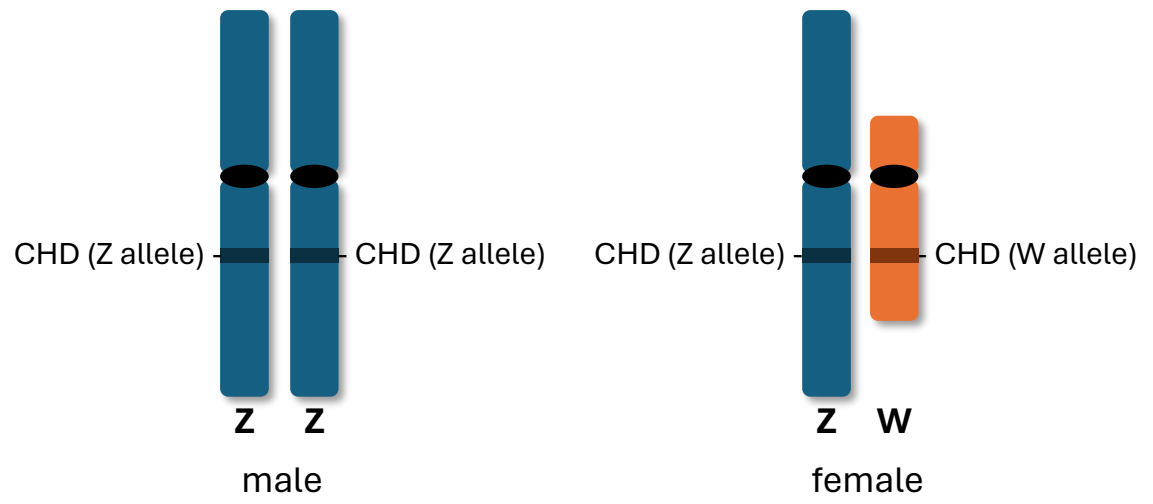
Milos Vucicevic,^{1*} Marija Stevanov-Pavlovic,¹ Jevrosima Stevanovic,¹ Jasna Bosnjak,¹ Bojan Gajic,² Nevenka Aleksic,¹ and Zoran Stanimirovic¹

¹Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Serbia

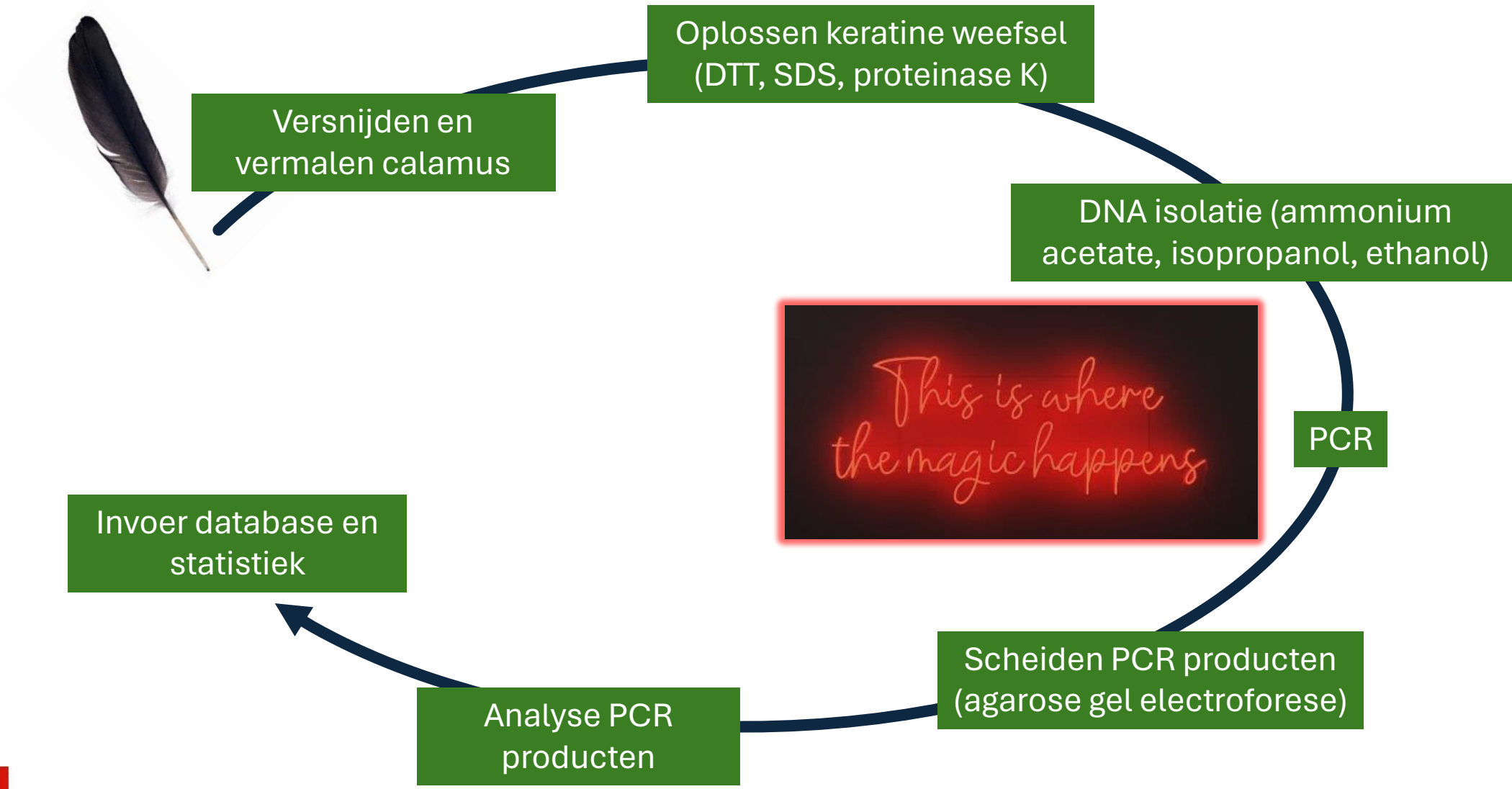
²Department of Parasitology, Faculty of Veterinary Medicine, University of Belgrade, Serbia

The aim of this research was to test the CHD gene (Chromo Helicase DNA-binding gene) as a universal molecular marker for sexing birds of relatively distant species. The CHD gene corresponds to the aim because of its high degree of conservation and different lengths in Z and W chromosomes due to different intron sizes. DNA was isolated from feathers and the amplification of the CHD gene was performed with the following sets of polymerase chain reaction (PCR) primers: 2550F/2718R and P2/P8. Sex determination was attempted in 284 samples of 58 bird species. It was successful in 50 bird species; in 16 of those (*Alopochen aegyptiaca*, *Ara severus*, *Aratinga acuticaudata*, *Bucorvus leadbeateri*, *Cereopsis novaehollandiae*, *Columba arquatrix*, *Corvus corax*, *C. frugilegus*, *Cyanoliseus patagonus*, *Guttera plumifera*, *Lamprotornis superbus*, *Milvus milvus*, *Neophron percnopterus*, *Ocyphaps lophotes*, *Podiceps cristatus*, and *Poicephalus senegalus*), it was carried out for the first time using molecular markers and PCR. It is reasonable to assume that extensive research is necessary to define the CHD gene as a universal molecular marker for successful sex determination in all bird species (with exception of ratites). The results of this study may largely contribute to the aim. *Zoo Biol.* 32:269–276, 2013. © 2012 Wiley Periodicals, Inc.

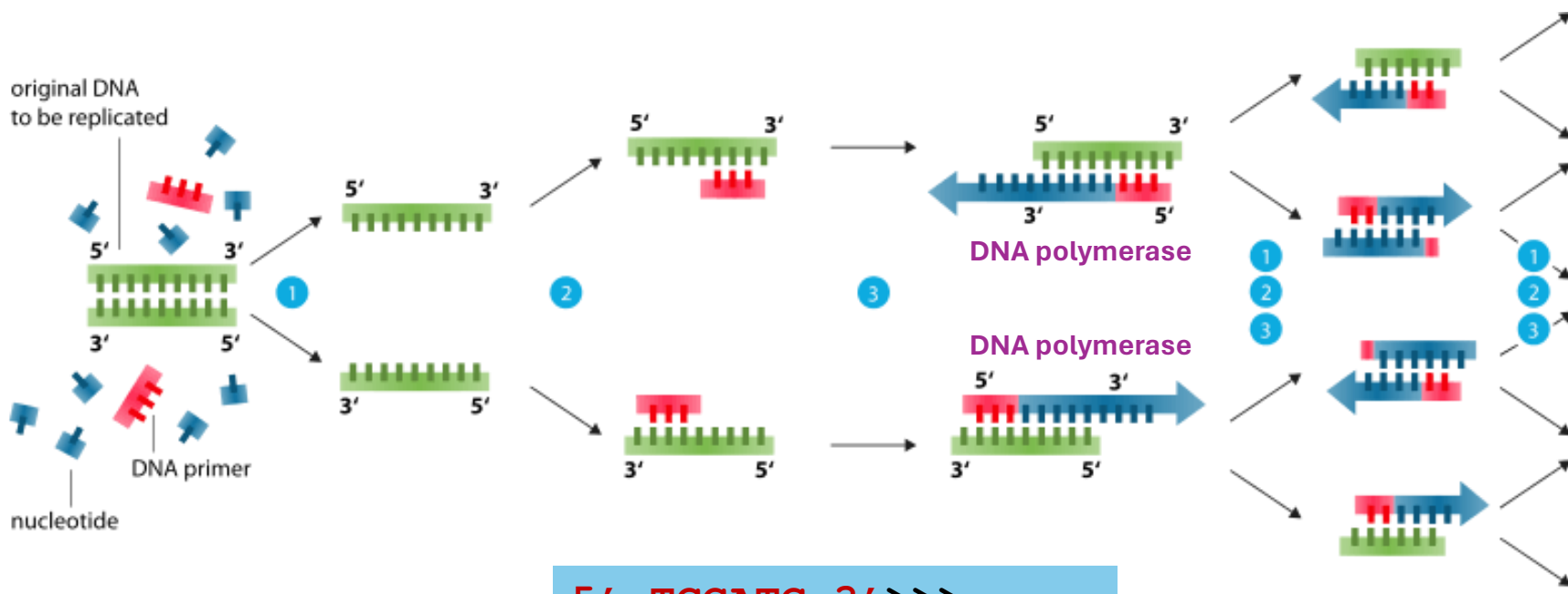
Variatie in intron lengte CHD1 gen



Aanpak



Polymerase chain reaction



- 1 Denaturation at 94-96°C
- 2 Annealing at ~68°C
- 3 Elongation at ca. 72 °C

5' -TCCATC-3' >>>
3' -AGGTAGCGCGTATA-5'

Primers (18 à 25 basen)

- Zéér specifiek
- Paar: bakenen gebied af

Cyclisch proces (25 à 35 verdubbelingen)

- Zéér gevoelig (weinig startmateriaal nodig)



Genome Data Viewer

Corvus cornix (NA) Assembly: ASM73873v6 (GCF_000738735.6) Chr Z (NC_046357.1)

Search assembly: CHD1

NC_046357.1: 71,525,359 - 71,592,151

Gene: CHD1 Transcript: XM_039566510.1

Genes, NCBI Corvus cornix cornix Annotation Release 104, 2021-02-16

Genes, Ensembl Rapid release 2023-10

RNA-seq exon coverage, aggregate (filtered), NCBI Corvus cornix cornix Annotation Release 104 - log base 2 scaled

RNA-seq intron-spanning reads, aggregate (filtered), NCBI Corvus cornix cornix Annotation Release 104 - log base 2 scaled

RNA-seq intron features, aggregate (filtered), NCBI Corvus cornix cornix Annotation Release 104

Unplaced/unlocalized scaffolds: 17

Tracks and User Data

BLAST

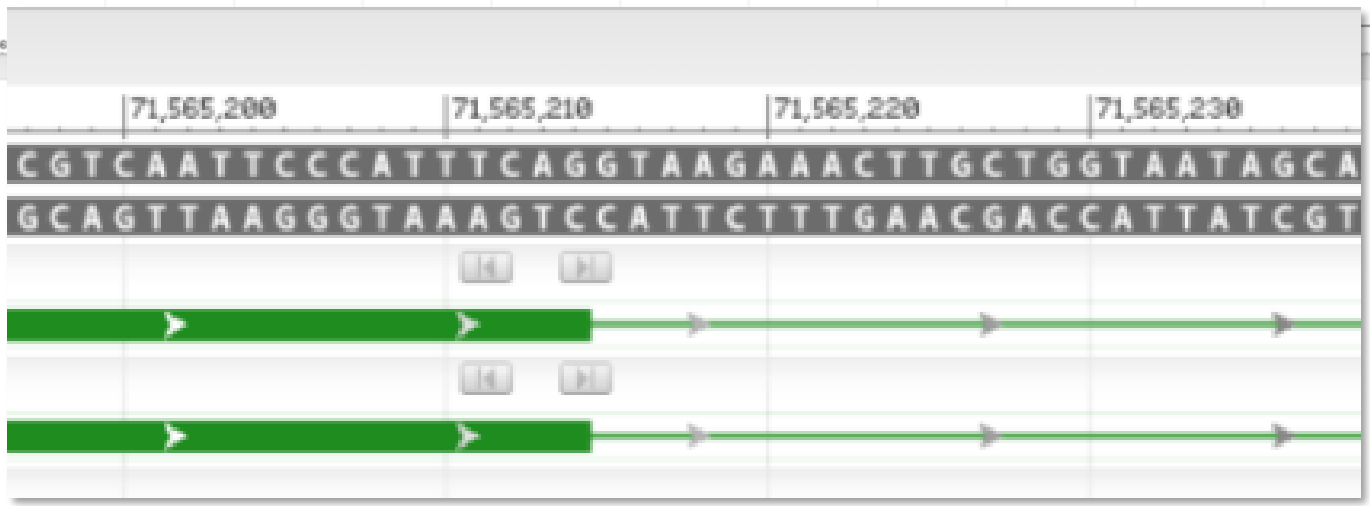
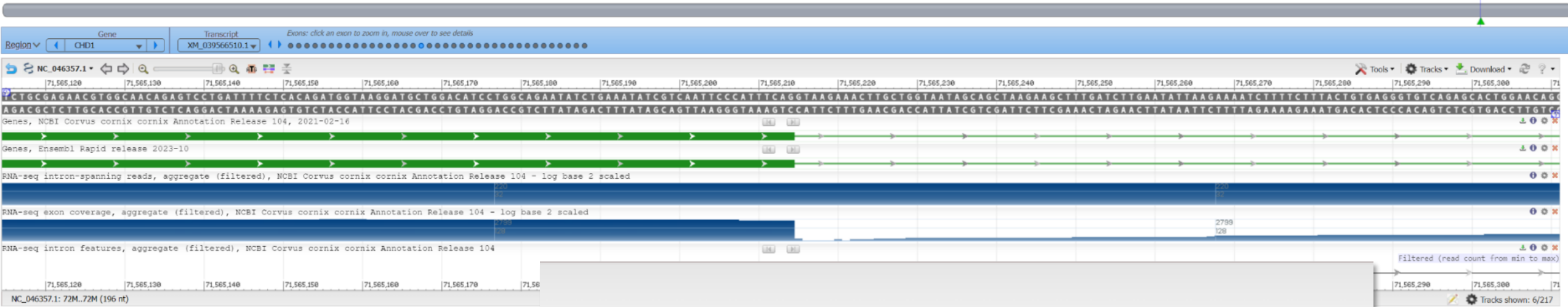
Tracks by Accession

History

- Europese raaf (*Corvus corax*) nog niet gesequenced
- Bonte kraai (*Corvus cornix*) wel



Genoom kraai



Problemen

- Genoom *Corvus corax* niet gesequenced
- Alleen sequence data Z-chromosoom beschikbaar, niet van W-chromosoom
- CHD1 gen ligt soms (o.a. bij kip) op complementaire streng
- Exon nummering loopt anders dan verwacht
- Aanpassing 2550/2718 primers (Vucicevic *et al.*) gedaan op basis van *cornix* genoom
- Intron 17 variabel genoeg of andere intronen nemen?
- Extra primer-paren ontwikkeld tegen exon 17 en 18.

Primer ontwerpen

Primers ontworpen tegen exon 17 en exon 18 CHD1 gen Corvus cornix

2550F original GTTACTGATTCGTCTACGAGA
 2550F adapted CTACTGATTCGTCTGCGAGA

2718R original ATTGAAATGATCCAGTGCTTG
 2718R adapted ATTAAAATGATCCAGTGCTTGT

Primers ontworpen tegen exon 17 en exon 18 CHD1 gen Corvus cornix

2550F original GTTACTGATTCGTCTACGAGA
 2550F adapted CTACTGATTCGTCTGCGAGA

2718R original ATTGAAATGATCCAGTGCTTG
 2718R adapted ATTAAAATGATCCAGTGCTTGT

Name	Sequence (5'>3')	Length	GC	Tm	Selfc	Self3c
2550F adapted	CTACTGATTCGTCTGCGAGA	20	50.0	56.9	4	4
2718R adapted	ATTAAAATGATCCAGTGCTTGT	22	31.8	54.3	4	0
Amplicon (Z)		660				

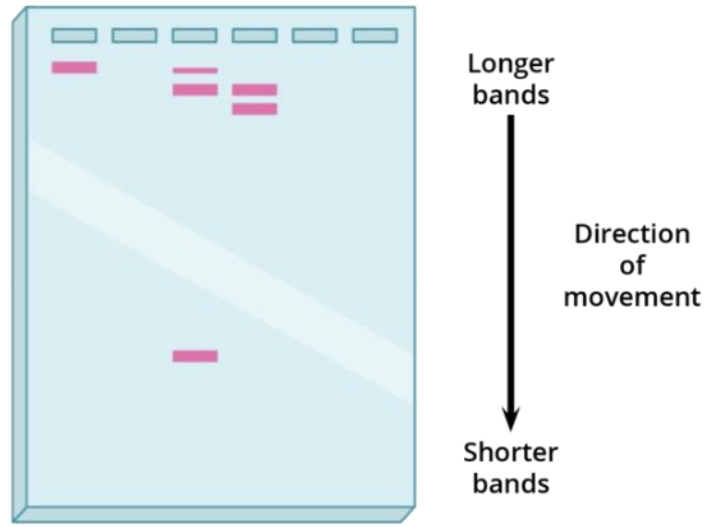
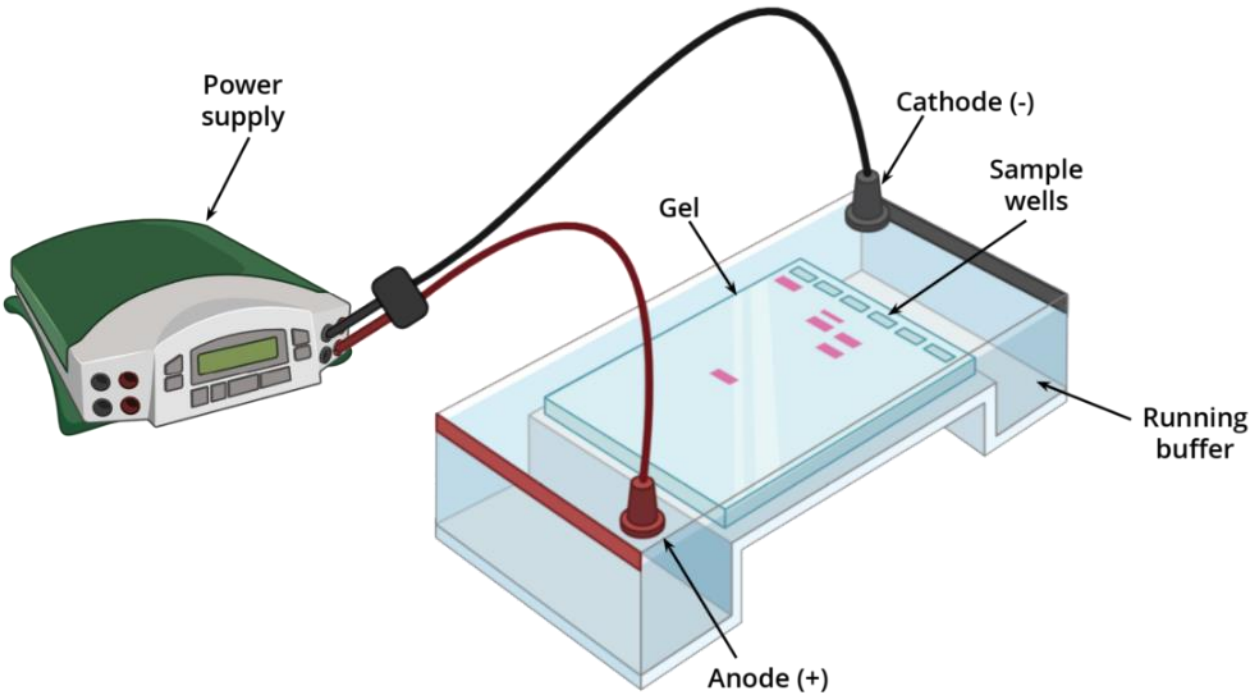
CATTTAATACGTAGCAGTGGGAACTAATCCTTCTTGACAAGCTACTGATTCGTCTGCGAGAACGTGGCAACAGAGTCCTGATTTTCTCACAGATGGTAAGGATGCTGGACATCCTGGCAGAATATCTGAAATATCGTCAATTCCCATTTCAGGTAAGAACTTGCTGGTAATAGCAGCTAAGAAGCTTTGATCCTGAATATTAAGAAAATCTTTCTTTACTGTGAGGGTGTGAGAGCACTGGAACAGGTTGTCCAGAGGTTATGGAATTTTGTCTCTTGTGGAGGTCCAAAAGCTACCTTGGCATGACCTTGGGCAGCATGCTTTAGTTGTCCCTGTCTGAGTAGGGGATTTAGACAAGTTGACCCCTCAGCAGTCCCTTTTCAGCTTTGTTTCGTGATTATGTGACCGTTACCCCTTCAATTAAGAAAAGTGGAAAGAAAATGCATTCTTTTTTCTAGAAGGGGACTGGCTCTATGATGAGTGTTATTTGGAAGTAAAACAGATGAACTAAAAATTATGTGAGCTGTTTATTTACTTACTTTTTTTTCCCTTACATAACAGTTTCAGCAGCTGACAATTGAAGTTGCTCTGATTTTGAATATAGTATAAAAATTATTTTTTAACTGTAGTTCTCAATCTCTTTAGAGACTTGATGGATCAATAAAAGGGGAATTGAGGAAACAAGCACTGGATCATTTTAATGCAGAAGGATCAGAG

Name	Sequence (5'>3')	Length	GC	Tm	Selfc	Self3c
RVe NCBI for	CGAGAACGTGGCAACAGAGT	20	60.6	55.0	4	1
RVe NCBI rev	GCTTGTTCCTCAATTCCTT	21	47.6	58.5	4	0
Amplicon (Z)		629				

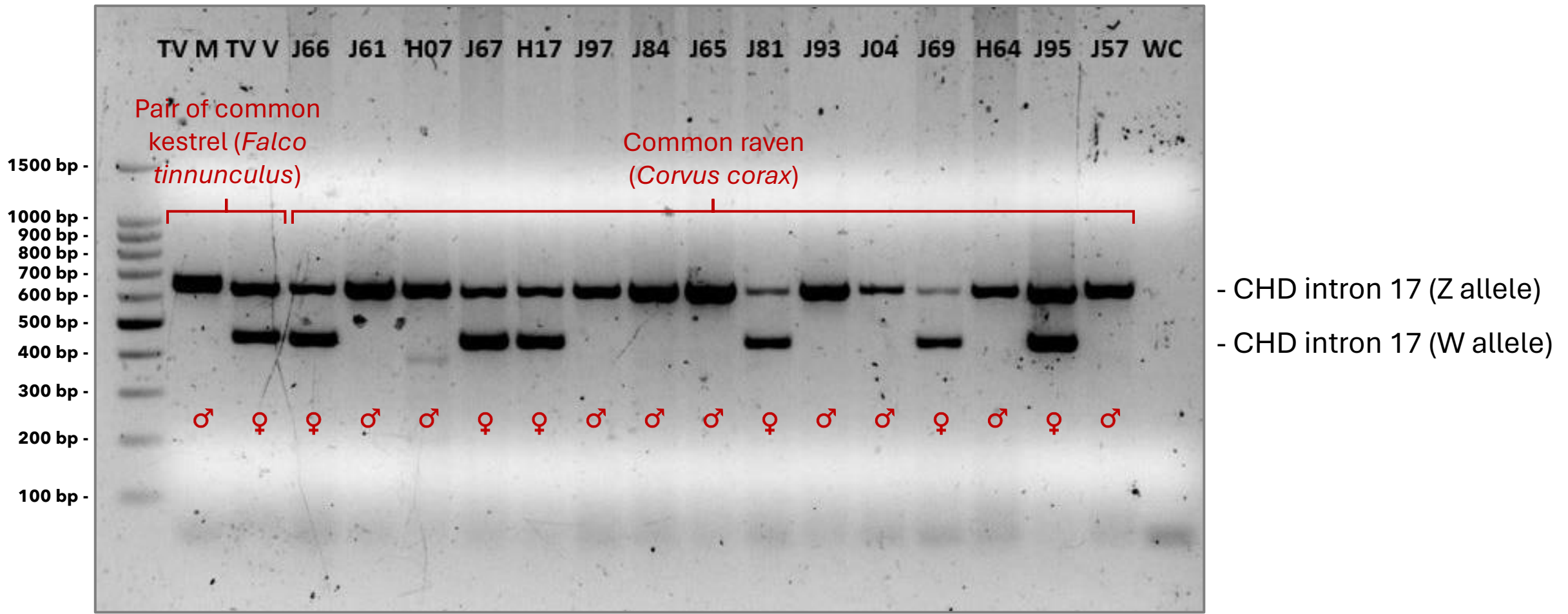
CATTTAATACGTAGCAGTGGGAACTAATCCTTCTTGACAAGCTACTGATTCGTCTGCGAGAACGTGGCAACAGAGTCCTGATTTTCTCACAGATGGTAAGGATGCTGGACATCCTGGCAGAATATCTGAAATATCGTCAATTCCCATTTCAGGTAAGAACTTGCTGGTAATAGCAGCTAAGAAGCTTTGATCCTGAATATTAAGAAAATCTTTCTTTACTGTGAGGGTGTGAGAGCACTGGAACAGGTTGTCCAGAGGTTATGGAATTTTGTCTCTTGTGGAGGTCCAAAAGCTACCTTGGCATGACCTTGGGCAGCATGCTTTAGTTGTCCCTGTCTGAGTAGGGGATTTAGACAAGTTGACCCCTCAGCAGTCCCTTTTCAGCTTTGTTTCGTGATTATGTGACCGTTACCCCTTCAATTAAGAAAAGTGGAAAGAAAATGCATTCTTTTTTCTAGAAGGGGACTGGCTCTATGATGAGTGTTATTTGGAAGTAAAACAGATGAACTAAAAATTATGTGAGCTGTTTATTTACTTACTTTTTTTTCCCTTACATAACAGTTTCAGCAGCTGACAATTGAAGTTGCTCTGATTTTGAATATAGTATAAAAATTATTTTTTAACTGTAGTTCTCAATCTCTTTAGAGACTTGATGGATCAATAAAAGGGGAATTGAGGAAACAAGCACTGGATCATTTTAATGCAGAAGGATCAGAG



Gel electroforesis



Voorbeeld gel met PCR producten



Resultaten

Phenotypic parameter	Males*	Females*	<i>p</i> -value
Wingspan (mm)	243.5 ± 50.9 (n=47)	243.8 ± 46.8 (n=54)	0.98
Age (days)**	32.2 ± 6.1 (n=47)	32.2 ± 5.4 (n=54)	1.00
Weight (gr)	997.3 ± 160.4 (n=47)	933.6 ± 102.2 (n=54)	0.02
Claw size (mm)	110.5 ± 6.2 (n=47)	105.4 ± 5.7 (n=54)	<0.01
Leg thickness (mm)	10.2 ± 0.6 (n=47)	10.0 ± 0.6 (n=54)	0.07

USING MORPHOMETRICS TO DETERMINE THE SEX OF COMMON RAVENS

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DEREK CRAIGHEAD

Craighead Beringia South, PO Box 147, Kelly, Wyoming 83011 USA

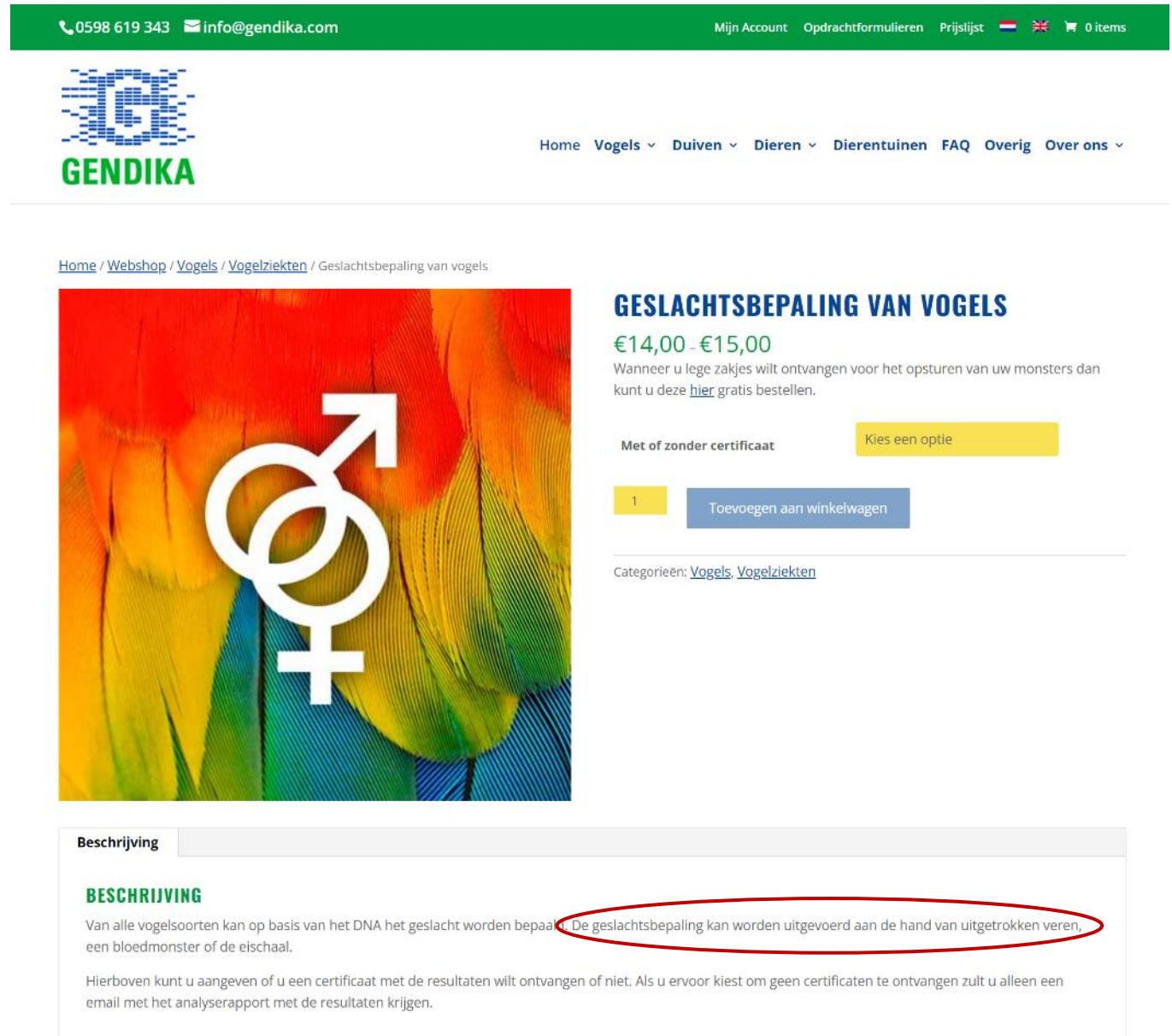
ABSTRACT—We tried using morphometrics to determine sex for a population of Common Ravens (*Corvus corax*) in northwestern Wyoming. We attempted to correlate 13 external measurements to sex using discriminant function analyses. Sex was verified with a DNA test that identified females with 2 PCR-amplified gene copies (1 each from the W and Z chromosomes) and males with 1 gene copy (only Z chromosome). We created a predictive model of sex of ravens for easy field use. We found that by using 2 separate discriminant functions with footpad length and body mass measurements simultaneously, we were able to correctly classify 97% of female samples, 91% of male samples, and had an unknown category that included 15% of samples.

Conclusies

- Genotypering ravengeslacht op veermateriaal is mogelijk (bevestiging literatuur)
- Lichaamsgewicht en klauwgrootte zijn (gecombineerd) gecorreleerd aan geslacht (praktische bruikbaarheid?)
- Genotypering blijft nodig

Hoe verder?

- Als studentproject afgerond (Ontwikkelwerk > uitvoerend)
- Dit project valt buiten programma instituut
- Afspraak met analist m.b.t. Kleine aantalen bijzondere samples (bijv. gezenderde raven)
- Commerciële labs?



The screenshot shows the Gendika website interface. At the top, there is a green navigation bar with contact information (0598 619 343, info@gendika.com) and links for 'Mijn Account', 'Opdrachtformulieren', 'Prijzlijst', and flags for the Netherlands and the United Kingdom. Below this is the Gendika logo and a main navigation menu with categories like 'Home', 'Vogels', 'Duiven', 'Dieren', 'Dierentuinen', 'FAQ', 'Overig', and 'Over ons'. The main content area features a product page for 'GESLACHTSBEPALING VAN VOGELS' with a price range of €14,00 - €15,00. A large image of a colorful parrot with a white male and female symbol overlaid is prominent. The product description includes a 'Beschrijving' section with the text: 'Van alle vogelsoorten kan op basis van het DNA het geslacht worden bepaald. De geslachtsbepaling kan worden uitgevoerd aan de hand van uitgetrokken veren, een bloedmonster of de eischaal.' The phrase 'aan de hand van uitgetrokken veren' is circled in red. There are also buttons for 'Kies een optie', 'Toevoegen aan winkelwagen', and a quantity selector set to '1'.

Einde

