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Factors affecting DNA quality in feathers used for non-invasive sampling

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Abstract

The development of genetic methods broadens the scope of non-invasive sample types. Large shed feathers are good material for genetic analyses, since they are easy to collect and a single feather can provide sufficient amount of DNA for PCR based methods. Previous studies demonstrated that feather quality and type affect the DNA quality extracted from the feather tips. Besides the tip, the superior umbilicus part of the shaft is also proposed as appropriate source of DNA. In our study we examined whether some feather parameters (physical condition, type and size) and storage time affect amplification success of DNA extracted from the superior umbilicus of shed Eastern Imperial Eagle (*Aquila heliaca*) feathers. We also tested the effects of sunlight, temperature and humidity on DNA extracted from Domestic Goose (*Anser anser domesticus*) feathers with amplification of fragments of various sizes, modelling the environmental conditions of the moulting season. While good quality feathers usually provided sufficient DNA, the usability of the DNA extracted from moderate quality feathers were affected by feather type. DNA quality was influenced in order of importance by humidity, direct sunlight and heat. Our findings support the usability of DNA samples derived from the superior umbilicus of shed feathers, and help to schedule field work with the careful consideration of our results about feather quality and environmental factors.

32

33 **Keywords**: *Aquila heliaca*, *Anser anser,* shed feathers, non-invasive sampling, DNA quality, DNA
34 fragmentation

35

36 **Introduction**

37

38 In the past years the use of DNA based methods became more frequent in ecological studies (Haig et
39 al. 2011), e. g. DNA can be used for genetic tagging of individuals for capture-recapture studies (e.g
40 Solberg et al. 2004, Lukacs and Burnham 2005, Mondol et al. 2009) or for the preparation and
41 monitoring of species protection activities (e.g. Negro and Torres 1999, Crompton et al. 2009). As the
42 application of non-invasive sampling techniques allows studying populations without the individuals
43 needed to be captured, these methods get more important in ecological genetics. It makes sampling
44 easier and less stressful for the studied animals (Waits and Patkeau 2005; Beja-Pereira et al. 2009),
45 and can be money- and timesaving. Furthermore, this way of sampling is the only possibility for
46 genetic analyses in the case of several endangered and rare species, or when capturing is extremely
47 difficult and could harm the individuals.
48 However, there are several serious limitations that coincide with the obvious advantages of non-
49 invasive sampling. Most animal tissues sampled this way provide DNA of low copy number and
50 quality (Taberlet et al. 1999). These are either dead tissues containing only few damaged cells, or
51 excrements that are often contaminated with foreign DNA, contain significant amount of PCR
52 inhibitors, and become digested quickly by endogenous nucleases (Monteiro et al. 1997; Murphy et
53 al. 2003a; Murphy et al. 2003b; Nsubuga et al. 2004; Hájková et al. 2006; Beja-Pereira et al. 2009).
54 Moreover, there are several uncertainties concerning the sampled specimen's species, sex or identity.
55 Besides this the amount of samples derived from one single individual can be a limiting factor when
56 assigning individual DNA-profiles (Gagneux et al. 1997; Reiners et al. 2011). However with
57 adequate laboratory practice and caution, DNA isolated from such samples can be sufficient for
58 individual identification (Stoneking 1995; Taberlet and Luikart 1999; Waits and Patkeau 2005;
59 Rudnick et al. 2005).
60 In contrast to hair, faeces and urine, shed feathers usually are easier to collect from individuals and
61 yield better DNA (Harvey et al. 2006; Rudnick et al. 2005 and 2007). Collection of moulted feathers
62 of larger sized birds such as pigeons (Seki 2006) or bigger is relatively easy compared to other non-

63 invasive sampling methods, and in most cases the target species can be identified based on the feather
64 (Rudnick et al. 2007). There are several methods published for DNA extractions from feathers
65 (Taberlet and Bouvet 1991; Horváth et al. 2005; Bayard De Volo et al. 2008) and most commonly the
66 feather tip is used (e.g. Hogan et al. 2008; Gebhardt et al. 2009). Another possible DNA source is the
67 superior umbilicus part of the feather shaft (Horváth et al. 2005). This suggests higher yield and
68 better DNA quality. This method is well cited, however it is applied only in a few studies, mostly for
69 large raptors since relatively big feathers with visible and isolable superior umbilicus are needed (e.
70 g. Seki 2006; Hailer et al. 2007; Martínez-Cruz et al. 2007; Banhos et al. 2008; Alcaide et al. 2010;
71 Väli et al. 2010; Miller et al. 2011).
72 Hogan et al. (2008) studied feather tip samples of the Powerful Owl (*Ninox strenua*) in order to
73 determine whether the type and condition of the feather affects the DNA yield. According to their
74 results, the initial condition of the feathers is essential regarding the outcome of the studies and feather
75 type has no influence on it.
76 The quality of non-invasively collected shed feathers highly depends on field conditions (Johansson
77 et al. 2012). High relative humidity, high temperature and sunlight can cause visible changes in
78 feathers, but they are also known to damage DNA. Humidity causes the most noticeable physical
79 damage, as the calamus is getting sponge-like, creating openings for various microorganisms.
80 Murphy et al (2003a) demonstrated that after 60 days in a humid area, DNA quality extracted from
81 bear faeces dropped considerably. The effects of heat and sunlight are more subtle. At higher
82 temperature, DNA was found more uncoiled and therefore more exposed to the effects of UV-B
83 radiation (Li and Paulsson 2002), which is the most effective component of sunlight reaching the
84 ground, although UV-A radiation may also be of importance (Ravanat et al. 2001).
85 Quality of the extracted DNA depends also on storage conditions of the samples, because the risk of
86 high endogenous nuclease activity. Storage of faeces samples was studied thoroughly and faeces is
87 recommended to be stored at -20°C in order to inhibit enzymatic activity (Wasser et al. 1997, Murphy
88 et al. 2003a, Roeder et al. 2004). However, the effect of storage time and conditions has to be
89 considered when using samples like hair or feathers as well. Despite that these sample types are less
90 exposed to enzymatic processes, several external factors (humidity, UV radiation and decomposers)
91 can possibly influence DNA yield.
92 The most common method to determine the quality of DNA is to measure the concentration of the
93 extracted DNA solution. This can be sometimes impractical, because the spectrophotometer measures

DNA, RNA, protein fragments and single nucleotides as well, and this can lead to the overestimation of the DNA concentration (Teare et al. 1997). Furthermore, it does not provide information about the degree of fragmentation, which can be well predicted by the visualisation of the extracted DNA solution on agarose gels by the extent of smearing. More accurate estimates can be made by the amplification success of different long fragments (Hogan et al. 2008, Zayats et al. 2009).

In our study we tested the findings of Hogan et al. (2008) on large shed feathers, namely that good quality feathers yield better quality DNA and the feather type is of less importance are valid if the sample source is the superior umbilicus part of the feather shaft. We examined the effect of physical condition, feather type, size and storage time on shed feathers of Eastern Imperial Eagles (*Aquila heliaca*). We also investigated the effects of sunlight, temperature and humidity on fresh Domestic Goose (*Anser anser domesticus)* feathers and determined the most threatening environmental factors that have to be taken into consideration when collecting and storing shed feathers for genetic analyses.

Methods

Sample collecting and handling

Shed feathers of Eastern Imperial Eagles were collected between June and August in all years from 1997 to 2006 under active nests in East-Hungary (46°20'–48°35'N, 19°30'–21°35'E). Collected feathers were individually tagged, categorized (Table 1) and measured (calamus diameter, calamus and vane length); if calamus diameter was less than 1.5 mm, the sample was excluded from the study. Medium-sized miscellaneous feathers, like scapulars, primary coverts and alula feathers were grouped together since there was no difference in their average size (calamus diameter: t = -0.5963, df = 93, p = 0.5524 and calamus length: t = 1.648, df = 93, p = 0.1024, Welch tests).

Based on their physical appearance feathers were ranked into three categories (Fig. 1): "poor" (n = 6) degraded, calamus in very poor condition, damaged superior umbilicus; "abraded" (n = 81) visible degradation of the calamus, slight degradation of the superior umbilicus and "good" (n = 494) calamus in good condition, no damage at the superior umbilicus. Feathers were stored in plastic bags under dry, dark and cool (room temperature or below) conditions until sample preparation.

125 Goose primaries were gathered in a goose farm that breeds parent stock for "Babat" Grey Landes
126 geese in Babatpuszta, Hungary. To ensure that all feathers are in the same condition at the start of the
127 experiments feathers were plucked at the early phase of moulting, when their bases were already
128 loose, so feathers could be easily removed and geese were not exposed to significantly more stress
129 than at a usual veterinary examination. The main difference between the usability of feather tips as
130 DNA source of plucked and moulted feathers is the condition of living tissue which sticks on the
131 outer basal surface of the feather shaft. As the sampled superior umbilicus is not inside the skin, it
132 was assumed that its quality is not different from freshly moulted feathers. Therefore, they can model
133 the initial condition of large shed feathers in order to examine the effects of different environmental
134 factors. Plucked feathers were stored in a freezer until the start of the treatments.
135
136 ***Treatments on goose feathers***
137
138 *Natural sunlight*
139 Feathers were attached to the bottom of a plastic box, covered with UV transparent foil (JK
140 International GmbH), to exclude external humidity. To keep the feather completely dry previously
141 desiccated silica gel granulates were placed in the box. After the experiment the silica gel was not
142 saturated, so the interior of the box was assumed to be absolutely dry. The box was placed in
143 September for 20 days on a southern facing window sill approximately 4m high. During the time of
144 the experiment the temperature varied between 12°C and 23.6°C.
145
146 *Artificial sunlight*
147 In order to create monitored circumstances we repeated the former experiment on the effects of
148 sunlight. We illuminated the feathers with a Repti Glo 5.0 (ExoTerra, 60cm, 20W) linear bulb which
149 produces a spectrum including UV-B, UV-A, visible and infrared light. In order to keep the treatment
150 comparable with the previous one, the length of the treatment was set to 100 hours, which is
151 approximately equal with the length of the average number of sunshine hours with the most intense
152 solar radiation in September in Hungary (5 hours/day for 20 days). In order to keep the feathers dry
153 we used the same method as described above. To ensure that the light bulb is the only light source
154 and to exclude exterior humidity the tray and the light source was covered with black nylon film. The
155 temperature was constantly below 30°C in the range 22-29°C.

156

157 *Dry heat*

158 Dry heat was produced in a laboratory oven (Memmert, ULE 500) set to a constant 30°C, this is 2-3
159 degrees higher than the average summer temperature in Hungary. The experiment lasted 100 hours.
160 To keep the feathers dry, desiccated silica gel was placed next to them into the plastic bags containing
161 the feathers.

162

163 *Heat and humidity*

164 Feathers were placed into a water bath on a polystyrene tray, to avoid direct contact with the water.
165 The temperature was set to a constant 30°C temperature. The treatment lasted 100 hours.

166

167 *Disinfection in treatments "dry heat" and "heat and humidity"*

168 In the pilot study, we found mould-like discolouration on the feathers at the end of the experiment. In
169 order to check the effects of the presumed microorganisms the surface of the feathers in these groups
170 was disinfected (Descosept, Dr. Schumacher GmbH). Additionally, in these treatments both the water
171 bath and the water used in it was thoroughly cleaned and exposed to UV-C light for 8 hours. Dry heat
172 was produced in the same laboratory oven as in the non-disinfected *dry heat* treatment group
173 (Memmert, ULE 500) but it was previously sterilized on 170°C for one hour.
174 As the feathers could be placed into the instruments only after sterilization, the circumstances cannot
175 be considered completely sterile. All other experimental settings were identical to the non-disinfected
176 groups.

177

178 *Control group*

179 No special treatment was applied, feathers were stored at -20°C until sample preparation.

180

181 The superior umbilicus part of the feather shaft was cut immediately after the treatments (goose
182 feathers) or within one year after collection (eagle feathers) and stored at -20°C in a microfuge tube
183 until DNA extraction. Sample preparation of both eagle and goose feathers was carried out as
184 described by Horváth et al (2005). DNA extraction was carried out according to the standard salting
185 out method (Gemmel and Akiyama 1996). In order to facilitate the digestion process 10 μl
186 dithiothreitol (DTT; 1M) was given to each sample (Weigmann 1968).

187

188 *DNA quality control*

189 To estimate fragmentation of the extracted DNA from both eagle and goose feathers, two methods
190 were used: (1) extracted DNA solution was visualized by agarose gel electrophoresis, and (2)
191 genomic regions of various lengths were amplified by PCR (Hogan et al. 2008; Zayats et al. 2009).
192 In eagle feathers sex-chromosome-linked CHD gene introns were amplified with the primers
193 (*2550F/2718R*) and procedures described by Fridolfsson and Ellegren (1999). To test the results of
194 the treatments on goose feathers three nuclear loci of small, medium and large sizes were amplified.
195 Two microsatellite loci *Bcaμ6* (approximately 150 bp, Buchholz et al. 1998) and *Ans24*, (approx. 300
196 bp, Weiß et al. 2008) were chosen and partial sequences of the α-actin gene with the primers *Act2* and
197 *Act4*, amplifying a 985 bp sequence (Rodríguez et al. 2002). All PCRs were carried out according to
198 the published procedures.
199 At the electrophoretic visualisation, we used a three-grade scale for ranking the quality of the
200 extracted DNA and all PCR products except for the CHD gene introns: "not visible (0)" means no
201 visible DNA or PCR product; "detectable (1) " indicates clearly visible, but smeared DNA or visible
202 but very light PCR product; and "clear (2)" represents well defined and clearly visible DNA or PCR
203 product. For the CHD products, the scale was dichotomous because initially this reaction was used
204 for testing the usability of eagle samples, meaning if this amplification failed the sample was omitted
205 from further analyses: "unsuccessful (X)" represents indefinite PCR product or no product at all, and
206 "successful (S)" represents amplifications with definite PCR product. DNA solutions were visualized
207 on 0.8%, PCR products on 2% agarose gels.
208

209 *Statistical analyses*

210

211 Conditional inference trees were built to analyze the effects of physical condition, feather type,
212 calamus diameter and storage time on eagle feathers, and to reveal the effects of sunlight, artificial
213 light, temperature and humidity on goose feathers. Conditional inference trees are unified framework
214 of recursive binary partitioning with piecewise constant fits statistically confirmed by permutation
215 tests developed by Strasser and Weber (1999). The covariates measured at different scales are
216 selected unbiased, and multiple test procedures are applied to determine whether the recursion needs
217 to stop. Splitting criterion of the tree was set to $p = 0.2$ (in eagles) and $p = 0.1$ (in geese), that is, only

218 the variables with p-values less than 0.2 and 0.1 are represented in the tree. The criterion was defined
219 so that the next node after the last significant (p = 0.05) difference was also represented. The
220 minimum number of observations in a terminal node was set to five (eagle feathers) and to 16 (goose
221 feathers, as this was the sample size in each group).Test type was set to “Bonferroni” in order to get
222 multiplicity adjusted p-values (Strasser and Weber 1999). Statistical analyses were carried out with
223 the R 2.12.1 statistical program (R Development Core Team 2011), and to estimate the conditional
224 inference trees the package “party” was used (Hothorn et al. 2011).
225
226
227 **Results**
228
229 *Eagle feathers – effects of physical condition, feather type, calamus diameter and storage time*
230
231 Amplified PCR product could be defined in two of six cases (33.3%) in the poorest quality group, in
232 66 cases (81.4%) in the abraded group and in 429 cases (86.8%) in the good quality group. Regarding
233 the feather type, success rate ranged between 82.1% (greater covert feathers) and 92.5% (tail
234 feathers).
235 Good and abraded quality feathers yielded less fragmented DNA than poor quality feathers (Fig. 2,
236 first and second node, p = 0.002, and 0.018, respectively). In addition, amplification success of
237 abraded quality feathers differed (although not significantly) according to feather type, with less
238 definable PCR product in greater coverts, secondaries and tertials (Fig. 2, fourth node, p = 0.121).
239 As storage time and calamus diameter had no significant effect on amplification success, these
240 variables are not represented on the conditional inference tree.
241
242 *Goose feathers – effects of light, heat and humidity*
243
244 Altogether 112 feathers were used in the experiments, with 16 feathers assigned to each group. To get
245 preliminary estimates on the DNA fragmentation, extracted DNA solutions were visualized on
246 agarose gels and graded according to their degree of smearing.
247 The amplification of both the 151 bp long *Bcaμ6* and the 300 bp long *Ans 24* fragments yielded at
248 least moderate quality PCR products in all groups. The 985 bp long part of the *α-actin* gene could

249 only be amplified in the control group and in groups treated with *dry heat* (with or without
250 disinfection) and artificial sunlight. These results correspond with the preliminary expectations made
251 after the electrophoretic visualisation of extracted DNA solutions.

252 The size of the amplified fragment was the first splitting factor on the conditional inference tree (Fig.
253 3). It divided the amplification success rates into two groups: large versus medium and small (node 1,
254 $p < 0.001$). On the left branch the control group was separated from the other groups (node 2) as it
255 yielded slightly better results, although this difference was only marginally significant ($p = 0.072$). On
256 the right branch, the group treated with *heat and humidity* had the poorest amplification success in
257 both small and medium sized fragments (node 5, $p < 0.001$). At the next node (node 7), samples
258 treated with *natural sunlight* had worse DNA quality ($p = 0.003$) than the samples stored in dark (i.e.
259 no light treatment was included) or treated with *artificial sunlight*. Similarly to the previous splitting
260 node there was no difference according to the fragment size. At node 8, the results were divided
261 according to the size of the amplified fragment ($p = 0.008$). In medium sized fragments, amplification
262 success did not differ significantly among the groups, but it did in small fragments, according to the
263 treatments. At the amplification of small fragments the groups *disinfected dry heat* and the *control*
264 yielded better PCR success with marginally significant value (node 9; $p = 0.069$) than the groups
265 *disinfected heat and humidity* and *dry heat*.

266

267 **Discussion**

268

269 *Eagle feathers – effects of physical condition, feather type, calamus diameter and storage time*

270

271 Our results confirm and broaden the findings of Hogan et al. (2008) about the importance of feather
272 quality with the use of the superior umbilicus as DNA source. Even though feather type did not affect
273 amplification success considerably, the groups *primaries*, *medium sized feathers* and *tail feathers*
274 yielded better results than *greater coverts*, *secondaries and tertials* as seen in Fig. 2. Although our
275 results are not entirely clear on that, we suggest using feathers from the first group when using
276 superior umbilicus as DNA source. As feathers with calamus diameter below 1.5 mm were excluded
277 from the analyses, it is likely that small feathers sampled at the superior umbilicus would yield less
278 DNA and worse amplification results, similarly as it was found previously (Hogan et al. 2008). The
279 fact that storage time had no significant effect on DNA quality can be explained by the storage

280 conditions, as feathers were stored in a dry and dark room, and the removed blood clots were placed in
281 a freezer (-20°C) until DNA extraction.
282
283 *Goose feathers – effects of fragment size, sunlight, heat and humidity*
284
285 Estimating the level of DNA fragmentation via electroforetic visualisation of the extracted DNA
286 solution was ambiguous. In the minor fragments (*Bcaμ6* and *Ans24*), amplification success was well
287 predicted by the results of the DNA grading, but in the 985 bp *α-actin* fragment, amplification success
288 was incidental. This result together with the basic stability data from full DNA electrophoresis implies
289 that this evaluation method gives reliable estimates on the usability of samples when using short and
290 medium length fragments.
291 Nevertheless, the amplification success was considerably affected by the size of the amplified
292 fragment as the amplification success of the large fragments differed significantly from the medium
293 and small ones (Fig. 3, node 1). In this large fragment length, any type of treatment had negative effect
294 on PCR success (although not significant), compared to the control group. Moreover, even in the
295 control group, only few samples gave evaluable PCR product. This suggests that shed feathers are
296 rarely suitable for the PCR-based analyses of such large fragments (985 bp), and even small impacts
297 can largely reduce their usability. This coincide with other studies, which also suggested to prefer
298 small or medium sized fragments for non-invasive genetic tagging of animals (e. g. Taberlet et al.
299 1999, Broquet et al. 2006, Beja-Pereira et al. 2009) since genotyping error rates correlate with the
300 fragment size (Hoffman and Amos, 2005).
301 Among the treatment factors, humidity reduced PCR performance to the greatest extent. Amplification
302 success of the smaller fragments (both medium and small) in the group treated with *heat and humidity*
303 was significantly lower than in the groups with any other treatments (*dry heat with and without*
304 *disinfection, heat and humidity with disinfection, artificial sunlight* and the *control*; Fig. 3, node 5).
305 This poor performance can be assigned to the activity of decomposing microorganisms. Even in our
306 experimental circumstances mould spores and hyphae could be detected (stained with Cotton Blue
307 dye) on humidity-treated feathers either in disinfected or non-disinfected cases, but not on *control* and
308 *dry heat* treated ones. This implies that surface disinfection is not sufficient to prevent DNA
309 degradation. Since humidity can both provide optimal circumstances for fungi and other keratin-
310 degrading microorganisms (Onifade et al. 1998), and change the texture of the rachis exposing the

311 matrix (Lingham-Soliar and Bonser, 2009), it can be assumed that the blood clot was damaged by
312 these microorganisms. Therefore, shed feathers exposed to wet (or warm and wet) environment for
313 longer periods are expected to fail in DNA-based work.
314 Within the remaining groups, *natural sunlight* was the most important splitting factor (Fig. 3, node 7).
315 Its negative effect on PCR success did not differ according to the fragment size, being present in both
316 150 and 300 bp range, suggesting that the sunlight can cause damage that prevents the amplification of
317 fragments below the 300 bp range as well. This means that feathers exposed to direct sunlight for long
318 time might be inefficient as DNA source even if they are seemingly well preserved.
319 The fact that feathers treated with *artificial sunlight* had one of the best amplification results among all
320 treatments was surprising, compared to the poor performance of the group treated with natural
321 sunlight. It is possible that the proportion of components in the natural and artificial sunlight were
322 different and this resulted in less damage on the DNA in the latter case, since photosensitization and
323 the size of DNA damage depends on the wavelength of light (Kielbassa et al. 1997, Ravanat et al.
324 2001).
325 Effect of fragment size was present also in the shorter PCR products. In the medium sized 300 bp
326 fragment PCR success was independent of any treatment, but in the smallest PCR product, *dry heat*
327 and *disinfected humid heat* resulted slightly worse amplification success compared to *control* and
328 *disinfected dry heat* treatment, suggesting that heat and humidity deteriorates DNA even in the shortest
329 fragments, and that disinfection can partly improve the results by hampering microorganisms.
330
331 Our study was carried out to find the threatening environmental factors that influence the quality of
332 DNA extracted from the superior umbilicus of large flight feathers. However in the case of smaller
333 feathers or other sampling methods than the superior umbilicus we suggest to follow the screening
334 protocol developed Hogan et al. (2008) in order to achieve good quality DNA.
335 In conclusion, to get best result when working with non-invasively sampled feathers, we suggest using
336 good quality feathers that are collected as soon as possible after moult, especially in localities where
337 they could be exposed to humidity and direct sunlight. In these samples, type and size of the feathers
338 are of less importance, and when stored properly (in freezer or in a dark, dry and cool place), storage
339 time can be prolonged. Using standard DNA extraction methods, the superior umbilicus proved to be a
340 practical source of genetic material in case of small and medium sized fragments. This is confirmed by
341 our finding that moderate quality feathers provided nearly the same quality DNA as good quality

342 feathers (81.4% and 86.8%); although in the former case the feather type can affect the results.
343 Nevertheless, even in ideal conditions, DNA extracted from these moulted feathers will be suboptimal
344 when amplifying large nuclear fragments.
345
346 **Acknowledgements**
347
348 We would like to thank the Hungarian Imperial Eagle Working Group and the Anser-Branch Ltd.
349 (Babatpuszta, Hungary) for providing the feathers and their cooperation. We are grateful to Dr. Iren
350 Siller, for her observations on the mould and to Dr. Andrea Harnos, for her help in the statistical
351 analyses. We also would like to thank Dr. János Kis and two anonymous reviewers for their valuable
352 comments on the manuscript.
353 All work described here comply with the current laws of Hungary.
354
355
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Tables and figures

Table 1 Type, quality and calamus diameter of Eastern Imperial Eagle (*Aquila heliaca*) feathers examined in the study

Feather type	Quality (n)				Calamus diameter (mm)
	good	abraded	poor	total	
Primaries	122	23	1	146	5.5 – 8.9
Secondaries and tertials	137	20	1	158	3.3 – 6.7
Tail feathers	66	13	1	80	5.5 – 7.2
Medium size feathers [a]	102	11	2	118	2.0 – 5.3
Greater coverts [b]	67	11	1	79	1.9 – 5.2
Total	496	82	6	581	

[a] scapular, primary covert, alula, > 3 mm calamus diameter
[b] 1.5-3 mm calamus diameter

Fig. 1 Typical physical appearance of feathers belonging into three quality categories: poor ("0"), abraded ("1") and good ("2") with enlarged view of the superior umbilicus below.

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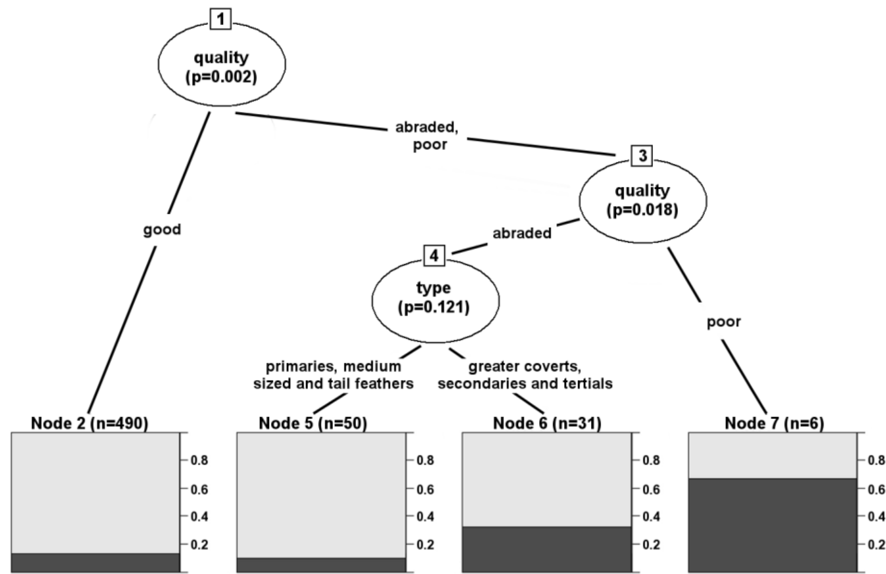

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572

573 **Fig. 2** Conditional inference tree for the effects of feather quality and feather type on the success of
574 amplification of the CHD1W/Z fragments from Eastern Imperial Eagle feathers. P - values are shown
575 at nodes 1, 3, 4; light grey areas represents the rate of successful amplifications of the CHD1 fragment,
576 dark grey areas represents the rate of failed amplifications; variable names are shown in the circles,
577 sample sizes are represented on the top of the boxes.

578

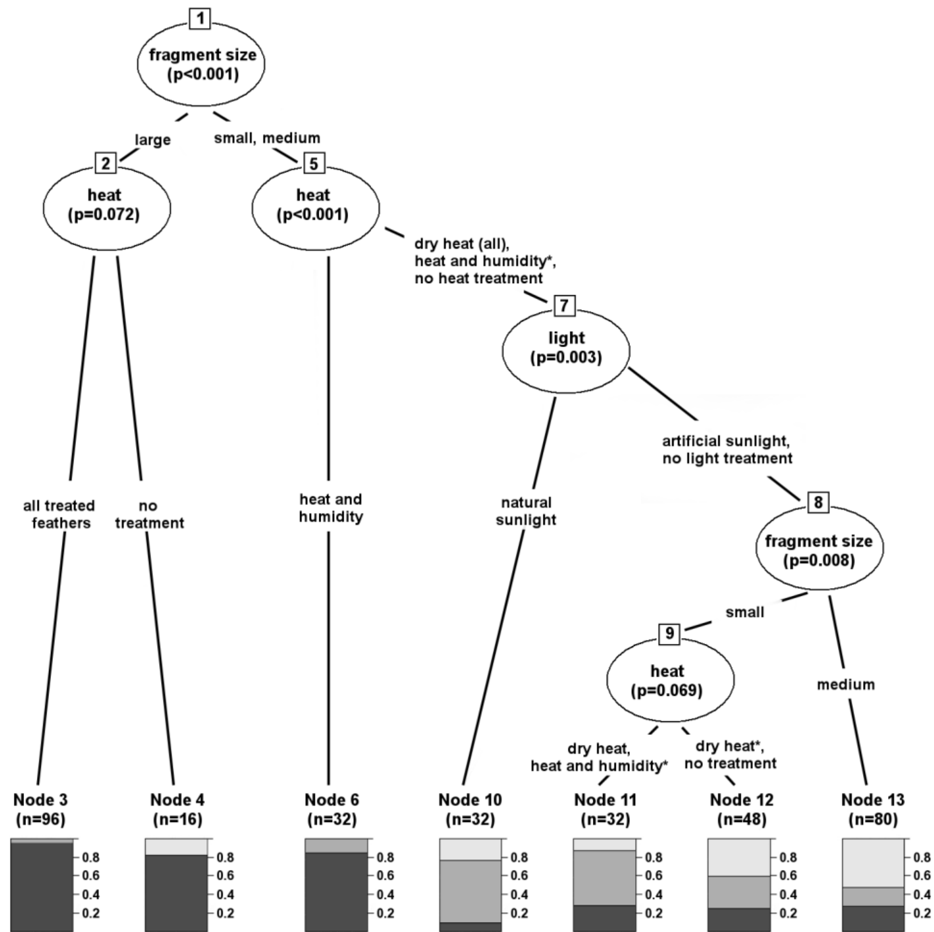

Fig. 3 Conditional inference tree for the effects of fragment size, heat, humidity and light on the amplification success of three nuclear loci (Act2/Act 4 - partial sequence of the α-actin gene, 985 bp-, Ans 24 – 300 bp, Bcaµ6 – 151 bp) in goose feathers; p – values are shown at nodes 1, 2, 5, 7, 8 and 9; light grey area represents the rate of PCR product with good quality, medium grey area represents the rate of poor quality PCR products and dark grey area represents the rate of failed amplifications; variable names are shown in the circles, sample sizes are represented on the top of the boxes.
* indicates disinfected feathers and experimental environment

All figures were edited with the Gimp 2.6 software.