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# Integration of wild and captive genetic management approaches to support conservation of the endangered Japanese golden eagle

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1     **Title:**           Integration of wild and captive genetic management  
2                            approaches to support conservation of the endangered Japanese  
3                            golden eagle  
4  
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26     DNA analysis, Population management, Population Viability Analysis

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**Abstract:**

The loss of biological diversity within species has the potential to significantly reduce resilience in the face of environmental change. Conservation of genetic variation needs to consider all available sources of diversity within a species, and approaches are required to integrate population management across traditionally separate wild and captive population domains. Here we report on a study that utilises different types of genetic analysis at different taxonomic scales and across an *in situ* – *ex situ* transition to support conservation planning for the Japanese golden eagle, a subspecies in serious regional decline. Mitochondrial DNA sequencing and nuclear DNA profiling are used to investigate subspecies differentiation and diversity in the natural population, revealing relatively high levels of variation in Japan. These results are compared with data from a newly established conservation breeding programme that indicates good representation of wild genetic variation in the captive founders. However, subsequent population viability analysis (PVA) to examine the demographic and genetic future of the captive population demonstrates the severe effects of existing reproductive skews, suggesting that this population is not sustainable without intensive genetic management. Lastly, the use of available molecular tools to validate and reconstruct pedigrees in Japanese golden eagle are evaluated and discussed in the context of captive and wild conservation management. The paper highlights the importance of producing and utilising comparative molecular genetic data across the population management spectrum and the benefits of PVA to support the implementation of integrated conservation plans.

50           1. **Introduction**

51       Despite the best efforts of conservationists, the decline of natural wildlife populations  
52       has led to many taxa being on the verge of disappearing from the wild. With notable  
53       exceptions, extinction of our most charismatic species has so far been avoided,  
54       however we are witnessing huge reductions in both numbers and diversity within  
55       species (World Wide Fund for Nature, 2016), with extirpation of populations  
56       effectively hollowing-out the genetic variation that enables adaptive responses to local  
57       and global environmental change. The issue of intra-specific diversity reduction is  
58       now recognized explicitly within the Convention on Biological Diversity (Aichi  
59       Target 13 of SCBD, 2010). As well as being of widespread concern to field  
60       conservationists, it is the focus of great attention from the *ex situ* conservation  
61       community who are responsible for genetic management of threatened species in  
62       captivity (Lacy, 1987; Lacy, 2013). Captive genetic management is primarily  
63       managed through individual-level pedigree analysis based on studbook records (Balou  
64       and Lacy 1995, Ivy and Lacy 2012). However, with incomplete pedigree data in many  
65       captive populations limited knowledge of kinship, and a move away from intensive  
66       management altogether in some species (Wildt et al. 2012), molecular genetic data are  
67       being viewed as the potential solution to validate, contextualise and sometimes simply  
68       correct theoretical genetic management estimates (Fienieg and Galbusera 2013). In  
69       this regard, the measurement and management of extant genetic diversity provides a  
70       common and potentially unifying theme to wildlife conservation, from extensive  
71       population management to intensive individual level husbandry.

72  
73       The synthesis of conservation strategies for a single species across different  
74       geographic, social and management scales, sometimes referred to as the One-Plan  
75       approach (Conde et al., 2013), is increasingly being seen as an important framework  
76       for effective species conservation (Redford et al., 2012). It is underpinned by a need  
77       to understand, utilize and maintain the total genetic diversity available within all  
78       living members of a species, along with the possibility of contributions from  
79       biobanked material. Conservation genetic data should be available to inform both  
80       policy makers and wildlife managers in relation to a range of questions arising within  
81       an integrated approach, from extensive to intensive management. The list of  
82       applications for genetic data and management advice to species conservation is long  
83       and well established (Frankham et al. 2009), and a number of studies have started to

84 bridge traditional conservation domains through measurement of captive and wild  
85 population genetic diversity (Stanton et al., 2015; Milián-García et al., 2015; Hvilson  
86 et al., 2013). However, comparative wild and captive molecular genetic data is still  
87 not generally applied across the spectrum of issues faced in conservation planning for  
88 a single species. In this paper we report on a study that integrates global evolutionary  
89 species history and subspecies diversity in the wild, with measures of founder  
90 diversity and forecasts of genetic variation within a captive conservation breeding  
91 programme.

92

### 93 *1.1 The evolutionary and conservation status of the golden eagle in Japan*

94 The golden eagle (*Aquila chrysaetos*) has six sub-species found across a circumpolar  
95 distribution in the temperate northern hemisphere. Due to its total population size  
96 (estimated at 300,000 individuals), the IUCN has classified this species as Least  
97 Concern (IUCN Red List 2013), however at the subspecies level and below, some  
98 populations of golden eagle are in severe decline.

99 The Japanese golden eagle (*A. c. japonica*) is one such sub-species, distributed only in  
100 Japan and possibly a part of the Korean Peninsula (Masuda et al., 1998). Unlike other  
101 sub-species, the Japanese golden eagle has adapted to heavily-forested mountainous  
102 areas, where its breeding success is considered to be related to the availability of older  
103 deciduous broad-leaved forests where large gaps in tree cover allow the eagles to hunt  
104 ground prey (Yui et al., 2005). Since 1981 the breeding success rate has decreased  
105 from 55.3 % to 24.6 % in Japan (The Society for Research of Golden Eagle, 2014)  
106 (Figure 1) and in 1995 the Japanese Ministry of the Environment classified this  
107 species as nationally endangered. The population size has been steadily decreasing  
108 since records began in 1981 (Figure A1) and there are now estimated to be only  
109 around 500 individuals in Japan (The Society for Research of Golden Eagle, 2014).  
110 Yui et al. (2005) suggested that reduced breeding may be associated with decreases of  
111 patchy forest cover and increases in overcrowded forest plantations. Inbreeding  
112 depression as a result of small population size has been also considered as a part of  
113 the reason for low breeding success, however there is no empirical evidence for this  
114 assertion and no studies to examine individual relatedness have been conducted. In an  
115 attempt to conserve the species, the Japanese Ministry of the Environment conducts  
116 ecological surveys, works to improve the environment around nest sites, and has  
117 commissioned conservation genetic analysis to inform management strategy. Despite

118 these efforts there is increasing concern over the long-term future of the species in  
119 Japan.

120

121 A global evolutionary genetic study of golden eagles by Nebel et al. (2015) using  
122 mtDNA control region sequencing revealed thirty haplotypes (maternal lineages)  
123 divided into two groups: Holarctic (21 types) and Mediterranean (9 types). The  
124 Japanese golden eagle belongs to the Holarctic group and, based on a small sample  
125 number, was found to be relatively diverse, displaying five haplotypes. Although  
126 three of these were found only in Japanese samples, the other two were shared across  
127 Eurasia and there was no clear phylogeographic relationship among them. The only  
128 previous study to focus on the Japanese golden eagle (Masuda et al. 1998) targeted the  
129 mtDNA pseudo-control region. This work also described five haplotypes however  
130 only two of these were confirmed to originate from known Japanese locations (the  
131 study included Korean, Chinese and unknown origin samples) and the two mtDNA  
132 data sets are not directly comparable, limiting their interpretation in relation to  
133 Japanese population diversity. However Masuda et al. also undertook a karyotypic  
134 study that suggests Japanese golden eagles display a different microchromosomal  
135 pattern to those previously observed at either the eastern or western ends of the  
136 Eurasian continent. This potentially supports a level of population distinctiveness for  
137 the Japanese golden eagle that would place constraints on future population recovery  
138 options.

139

#### 140 *1.2 Captive golden eagles in Japan*

141 Given the known status of wild Japanese golden eagles, the Japanese Association  
142 of Zoos and Aquaria (JAZA) has acted to establish and manage a captive breeding  
143 programme from 1997. From a total of 16 founder birds, forty Japanese golden eagle  
144 individuals are alive in eight zoos. Seven founders are still living, with all live  
145 offspring derived from five of these seven (2014 studbook data) (Figure 2). To  
146 confound matters further, one breeding founder male that has given rise to ten living  
147 O1 offspring and two living O2 offspring is considered by zoos to potentially be of a  
148 different subspecies based on its unconfirmed origin and observed body size, and this  
149 entire family of thirteen birds has therefore been excluded from the breeding  
150 programme. This leaves a total of 27 live birds from which to breed. Within this  
151 group zoo-keepers are trying to minimize inbreeding by strategic mating based on

152 studbook information, but no genetic data is available to support current management  
153 and not all birds have successfully bred. In attempting to develop the captive  
154 population into a sustainable, representative insurance population for the Japanese  
155 golden eagle, research is required to understand their relationships and comparative  
156 diversity to the wild population, and to forecast their demographic and genetic  
157 trajectories.

158

### 159 *1.3 Research objectives*

160 The aim of this study was to conduct an integrated genetic analysis of wild and  
161 captive Japanese golden eagles to inform our understanding of sub-species population  
162 biology and support the management of the captive population down to the level of  
163 the individual. To achieve this we attempted to answer a number of questions  
164 relevant to applied conservation genetic management in Japan: 1. What levels of  
165 genetic diversity and population structure exist in wild Japanese golden eagles?; 2. To  
166 what extent has founder effect impacted the conservation value of the captive  
167 population?; 3. Are we able to inform individual management of wild and captive  
168 birds using molecular estimates of relatedness?; and 4. How can we sustain long-term  
169 genetic diversity and demographic viability of the captive population? These  
170 questions were addressed through undertaking phylogenetic, population genetic and  
171 individual level analyses, in combination with simulations of future population  
172 genetic change over time.

173

## 174 **2. Materials and Methods:**

### 175 *2.1 Sampling*

176 Fifty-one samples were obtained from the wild population (Iwate prefecture  $n = 46$ ,  
177 Tochigi prefecture  $n = 3$ , Aomori prefecture  $n = 2$ ) (Figure 3). Feather ( $n = 41$ ), crop  
178 pellet ( $n = 2$ ), egg membrane ( $n = 2$ ), and faecal ( $n = 1$ ) samples were collected under  
179 18 nest sites between 1999 and 2014, with no repeated sampling of single nests across  
180 years. Muscle samples ( $n = 4$ ) were collected from incidental carcasses, and a talon  
181 sample ( $n = 1$ ) was collected from a private specimen. Twenty samples were obtained  
182 from the captive population including nine wild origin founder birds and eleven  
183 captive bred birds. Sixteen of these birds remain alive in captivity. Zoo keepers  
184 contributed to collect these samples (feather  $n = 15$ , blood  $n = 5$ ). All samples were  
185 preserved at  $-20\text{ }^{\circ}\text{C}$  until DNA extraction.

186

## 187 2.2 DNA analysis

188 Moulded feathers were processed to target the blood spot and basal tip for DNA  
189 extraction, as the described by Horvath et al. (2005). All DNA extractions were  
190 conducted using the Qiagen DNeasy Blood & Tissue Kit, or QIAamp DNA Stool  
191 Mini Kit (Qiagen, GmbH, Hilden, Germany) following the manufacturers' protocols.

192

193 To investigate the evolutionary history and diversity of the *A. c. japonica* sub-species,  
194 the Control Region (CR, D-loop) and pseudo Control Region ( $\psi$ CR) of mtDNA were  
195 sequenced. The CR primer pair amplify a 402 bp fragment, and primer sequences are  
196 as follows: modGOEA\_CR1L 5'-CCCCCGTATGTATTATTGTA-3' and  
197 GOEA\_CR595H 5'-GCAAGGTCGTAGGACTAACC-3' (Nebel et al., 2015). The  
198 mtDNA  $\psi$ CR, located in *A. c. japonica* between tRNA<sup>Glu</sup> and tRNA<sup>Phe</sup>, was isolated  
199 by Masuda et al. (1998), as the CR (Haring et al., 1999). The  $\psi$ CR primer pair  
200 amplify 444 bp fragment, and primer sequences are as follows: E-ACH 5'-  
201 CTCTCCAAAATCTACGACCTGAA-3' and IE-ACH  
202 5'CGTTGTAAACTTCAACTACAGAA-3' (Masuda et al., 1998). All PCR reactions  
203 were conducted under the same conditions: a final volume of 10  $\mu$ l, containing 1  $\mu$ l  
204 DNA, 5  $\mu$ l Multiplex PCR Master Mix (Qiagen), 0.2  $\mu$ M of forward and reverse  
205 primer, 0.1  $\mu$ g of T4 gene 32 Protein (Nippon Gene, Tokyo, Japan). The  
206 thermocycling conditions were as follows: initial step of 94°C for 15 min; 45 cycles  
207 of 94°C for 30s, 55°C for 45s, 72°C for 45s; and a final extension of 72°C for 30 min.  
208 The High Pure PCR Product Purification Kit (Roche, Basel, Switzerland) was used to  
209 purify PCR products. The Big Dye Terminator v3. 1 Cycle Sequencing Kit (Roche)  
210 and an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) were used to  
211 determine nucleotide sequences.

212 Microsatellite genotyping was conducted to investigate genetic diversity using 16  
213 loci derived from Japanese golden eagle (Sato et al., 2015). These microsatellite  
214 markers were synthesized with fluorescent labels and amplified in three separate PCR  
215 reactions. Multiplex 1 included AQJ03 (FAM), AQJ10 (FAM), AQJ30 (HEX),  
216 AQJ36 (HEX), AQJ59 (NED). Multiplex 2 included AQJ08 (NED), AQJ27 (FAM),  
217 AQJ49 (FAM), AQJ56 (HEX), AQJ66 (FAM). Multiplex 3 included AQJ19 (FAM),  
218 AQJ28 (HEX), AQJ34 (HEX), AQJ40 (FAM), AQJ52 (NED), AQJ53 (FAM). PCR  
219 reactions and conditions were the same as mtDNA analysis. The annealing



220 temperature of every multiplex set and cycle number was 55°C and 45 cycles.  
221 Amplicon size was measured using an ABI PRISM 3130xl Genetic Analyzer (Applied  
222 Biosystems), and genotypes were scored by eye with Peak Scanner Software 1.0  
223 (Applied Biosystems). These PCR to genotyping steps were repeated three times for  
224 each sample to control for allelic drop-out.

225

### 226 *2.3 Mitochondrial DNA analysis*

227 Mitochondrial CR,  $\psi$ CR, and concatenated CR +  $\psi$ CR sequences were determined  
228 and aligned using MEGA 6.0 software (Tamura et al., 2013). Median-joining  
229 networks for separate CR,  $\psi$ CR, and CR +  $\psi$ CR haplotype data were generated and  
230 visualised using PopArt (<http://popart.otago.ac.nz>). Haplotype sequence diversities ( $h$ )  
231 and haplotype richness ( $hr$ ) were calculated using Contrib 1.4 (Petit et al., 1998) to  
232 assess mtDNA genetic diversity within and among the sample localities. Where  
233 informative, result data were combined with those of previous studies to create larger  
234 datasets for re-analysis.

235

### 236 *2.4 Nuclear DNA analysis*

237 For the microsatellite data, the number of alleles ( $N_a$ ), observed ( $H_o$ ) and expected  
238 heterozygosities ( $H_e$ ), and inbreeding coefficients ( $F$ ) were calculated in GenAlEx  
239 6.501 (Peakall and Smouse, 2006). In addition, deviation from Hardy-Weinberg  
240 equilibrium (HWE) of wild samples was tested using GENEPOP on the web  
241 (Raymond & Rousset, 1995). Allelic richness was calculated in HP-Rare (Kalinowski,  
242 2005). Population genetic structure was investigated within the wild population and  
243 between the wild and captive birds using Principle Coordinates Analysis (PCoA,  
244 GenAlEx 6.501).. Evidence for a demographic bottleneck in the Iwate (wild)  
245 population was examined using the programme BOTTLENECK (Cornuet and Luikart  
246 1997) run under default parameters.

247

### 248 *2.5 Evaluation of molecular tools for kinship inference*

249 Given the completeness of the golden eagle captive pedigree, there was an  
250 opportunity to examine the power of the DNA profiling system to validate familial  
251 relationships and potentially infer pairwise kinship in captive or wild populations  
252 where breeding data is not available. Probabilities of parental exclusion were  
253 calculated to estimate the power to validate familial trios or parent-offspring pairwise

254 relationships (Jamieson & Taylor 1997, implemented in GenAlEx 6.501).  
255 Determining pairwise relatedness in the absence of pedigree data has been the subject  
256 of some debate in the *ex situ* conservation community, as traditional molecular  
257 relatedness coefficients that rely on accurate estimates of population allele frequencies  
258 are often confounded by non-random mating within captive populations (Ivy et al.  
259 2016; Cabellero & Toro, 2002; Montgomery et al. 1997). The use of molecular co-  
260 ancestry to infer kinship, based on allele sharing among individuals, has been  
261 proposed as a more robust alternative and was implemented here using the program  
262 MolKin v.2 (Gutiérrez et al 2005). This approach was used to compare molecular  
263 estimates of co-ancestry against studbook records for twenty-two parent-offspring  
264 pairs, nineteen sibling pairs, twelve grandparent-grandchild, six avuncular pairs and  
265 assumed unrelated individuals, in order to evaluate the power of the DNA profiling  
266 system for assisting in pedigree reconstruction of both captive and wild birds.

267

## 268 *2.6 Population Viability Analysis*

269 Simulations were conducted to forecast the change in captive population size and  
270 genetic diversity under different scenarios over 200 years using Vortex 10 (Lacy and  
271 Pollak, 2015). First, biological and technical parameters were estimated from  
272 studbook data or information collected directly from zoo keepers (Table A1). Based  
273 on these fixed parameters, a series of preliminary simulations were run to determine  
274 the management conditions required to maintain population numbers and diversity  
275 from a founding size of 27 birds (the current breeding population) with known  
276 molecular genetic diversity data from twenty individuals (nuclear and mitochondrial  
277 DNA markers included). Three key breeding parameters that could be potentially  
278 subject to management intervention/control were identified and varied to explore a  
279 range of conditions under which the starting population could be sustained  
280 demographically and genetically: i) ‘Maximum kinship within mate pair’ enables  
281 management control of inbreeding but indirectly limits population growth where  
282 sufficiently unrelated birds are not available to breed. Varying this parameter  
283 ( $x < k < 0.25$ ) revealed that a conservatively low relatedness threshold, while desirable,  
284 led to rapid population extinction, resulting in a kinship maximum value of 0.125  
285 being selected for future simulations. ii) ‘Mate monopolization’ refers to the  
286 proportion of adult males and females available to form breeding pairs which, in  
287 captivity, is largely under management control. The existing mate monopolization

288 rate was therefore adjusted based on an assumption that intensive management could  
289 increase the proportion of breeding adults and thereby enhance genetic diversity and  
290 reduce relatedness in subsequent generations. iii) The ability to supplement the  
291 captive population with wild sourced birds, or captive birds outside of the current  
292 breeding programme was also explored in order to maintain diversity and periodically  
293 add to the population size. For details of these simulated parameters see Table A1.  
294 The results of this preliminary simulation phase formed a range of management  
295 conditions under which a sustainable population was theoretically possible. Next, the  
296 actual management conditions and breeding restrictions currently in effect were used  
297 to forecast the future of the captive population and identify any issues relating to  
298 demographic or genetic sustainability (Table A1). Lastly, realistic modifications to  
299 management solutions were simulated in an attempt to mitigate declines in population  
300 size and diversity and develop a viable management strategy for the captive  
301 population of golden eagles in Japan. Supplementation from the wild of one male and  
302 one female at ten and two year intervals was simulated, reflecting a typical rate of  
303 wild bird rescue and a theoretical option for deliberate capture, respectively.  
304 Adjustments to mate monopolization were made to double the number of male and  
305 female birds breeding each year, from three pairs to six. While the focus of the PVA  
306 was on the sustainability of the captive breeding programme, simulated management  
307 solutions also included ‘harvest’ from the captive population to allow reinforcement  
308 of the wild population, thus establishing a preliminary model for reciprocal exchange  
309 of birds between wild and captive environments under a future one-plan approach.

310

### 311 **3. Results:**

#### 312 *3.1 Mitochondrial DNA analysis*

313 From fifty-one wild samples, twenty-seven samples returned both reliable CR and  
314  $\psi$ CR sequences, and four samples had reliable  $\psi$ CR sequences only. Wild samples  
315 displayed six CR haplotypes (H1, H2, H3, H4, H7, and H8) (Figure A2.1), with  
316 haplotype H1 observed in the Japanese subspecies for the first time, reinforcing the  
317 lack of Holarctic phylogeographic structure (Figure 4). At the pseudo-control region  
318 ( $\psi$ CR), wild Japanese samples displayed three haplotypes (a, b, and d) (Figure A2.2),  
319 generating total of seven combined (CR +  $\psi$ CR) mitochondrial sequence haplotypes  
320 (Figure A2.3). The distribution of these haplotypes in Japanese wild birds shows no

321 geographic pattern, with the most densely sampled region, Iwate, displaying nearly all  
322 observed mitochondrial lineages (Figure 3).

323 In the twenty captive samples, four samples failed to amplify both regions, but sixteen  
324 samples returned reliable sequences of both CR and  $\psi$ CR. Captive samples displayed  
325 five CR haplotypes (H2, H3, H4, H7, and H8) (Figure A2.1) and the same three  $\psi$ CR  
326 haplotypes as the wild birds (a, b, and d) (Figure A2.2), generating a total of six  
327 different combined (CR +  $\psi$ CR) mitochondrial sequence haplotypes (Figure A2.3).  
328 Overall haplotype diversity ( $h$ ) was marginally higher in the wild, which displayed  
329 three unique haplotypes compared to two in captivity, however this pattern reversed  
330 when rarefaction was applied to estimate haplotype richness ( $hr$ ), accounting for  
331 variation in sample size (Table 1).

332

### 333 *3.2 Nuclear DNA analysis*

334 Thirty-nine wild samples and twenty captive samples were genotyped for the sixteen  
335 microsatellites. Individual DNA profiling revealed that all wild samples were  
336 collected from different individuals. In the wild population, an average of 75.6 % of  
337 loci were genotyped per individual, with 75.6 % of samples genotyped per locus. As  
338 expected, due to better sample quality, genotyping in the captive population was more  
339 successful with an average of 91.6 % of loci genotyped per individual and 91.6 % of  
340 samples genotyped per locus. Two of the sixteen loci (AQJ27 and AQJ30) showed  
341 significant heterozygote deficiency ( $0.01 < P < 0.05$ ). Across all loci, allelic richness  
342 and private allelic richness were approximately equal between wild and captive  
343 populations, while observed heterozygosity in the wild was slightly lower than in  
344 captivity (Table 2).

345 The results of PCoA analysis revealed no population genetic structuring of wild  
346 samples among or within the three different sampling areas (Iwate, Aomori and  
347 Tochigi) (Figure A3). Analysis of wild versus captive golden eagles showed no  
348 differentiation of the two groups (Figure A4), however, when the captive birds are  
349 divided into wild-rescued founders and birds bred in captivity, the captive bred group  
350 show a much more limited distribution of genetic variance.

351 Bottleneck analysis showed weak evidence of a recent loss in effective  
352 population size with significant heterozygote excess observed under the infinite allele  
353 model for all three tests ( $0.01 < P < 0.05$ ), but not under the two-phase or stepwise  
354 mutation models of microsatellite mutation. There was no clear evidence of a mode-

355 shift from the expected L-shaped distribution of allele frequencies at mutation-drift  
356 equilibrium (Figure A5).

357

### 358 *3.3 Evaluation of molecular tools for kinship inference*

359 Studbook verification via DNA parentage exclusion analysis within the Japanese  
360 golden eagle population is achievable using the current 16-locus profiling system,  
361 with exclusion probabilities ranging from 0.99310 to 0.99999, depending on the  
362 particular scenario under consideration and the availability of parental samples. Co-  
363 ancestry estimates within the captive population, including selfing relationships,  
364 ranged from 0.18 to 0.91 (Table A3). While there is a clear trend of increasing  
365 molecular kinship with known pedigree relationship (Figure 5), a large degree of  
366 overlap was observed in molecular kinship among different levels of familial  
367 relatedness that would severely limit the utility of this DNA profiling system for  
368 pedigree reconstruction in captive or wild populations.

369

### 370 *3.4 Population Viability Analysis*

371 According to PVA results, the captive golden eagle population is unsustainable under  
372 current management conditions, with simulations leading to population extinction  
373 within 200 years (mean = 155 years) (Figure 6a). During this time continuous  
374 decreases in population size were accompanied by significant losses in nuclear and  
375 mitochondrial diversity.

376 Exploration of demographic and genetic forecasts with reduced mate-  
377 monopolization (> proportion of adults breeding) leading to a marked increase in  
378 population sustainability, with continuous population growth from  $n=27$  to a mean of  
379 over  $n=80$  individuals in the first 100 years, as a greater proportion of birds  
380 successfully bred (Figure 6b). However this was followed by a population collapse in  
381 the second 100 years due to an increase in inbreeding reducing the number of  
382 available mate pairs with mean kinship below the threshold of  $F=0.125$ , as indicated  
383 by continual marked reductions in nuclear and mitochondrial genetic diversity in all  
384 simulations (Figure 6b).

385 Individual supplementation from the wild every 10 years retarded loss of genetic  
386 diversity but as a standalone intervention did not result in population growth (Figure  
387 6c), as breeding rate could not achieve replacement levels. A combination of regular  
388 supplementation and reduced mate-monopolization was successful over two hundred

389 years, with a stable mean population size of 95 birds (carrying capacity,  $n < 100$ )  
390 retaining the majority of founder genetic diversity (Figure 6d).

391 Increasing individual supplementation from the wild to one pair every two years  
392 also resulted in a stable population, but at a slightly lower population number ( $n = 80$ )  
393 (Figure 6e); however such supplementation is considered unrealistic without  
394 reciprocal wild releases ('harvest'). Simulated harvest at the same rate (one pair every  
395 two years) resulted in a sustainable genetic captive population but a demographic  
396 population that failed to increase in size ( $n = 30$ ) (Figure 6f).

397  
398

#### 4. Discussion:

399 As pressures on natural populations increase, so does the importance of conservation  
400 genetic management of both wild and captive populations. Addressing questions  
401 relating to diversity and inbreeding, founder effects and likely future retention of  
402 genetic variation is key to informing current and future best practice conservation  
403 management. To date, very few studies have been conducted that integrate empirical  
404 data on molecular genetic variation across *in situ* and *ex situ* populations, and that  
405 combine current genetic data with long-term demographic and genetic forecasts in  
406 zoo-based conservation breeding programmes. In this study we have demonstrated  
407 how these issues can be practically addressed through application of traditional  
408 molecular genetic tools to support an integrated approach to species conservation.

409

##### 4.1. Genetic diversity and population structure in Japanese golden eagles

410 At a global level, as three of the six CR haplotypes observed in Japan have also been  
411 observed across Eurasia, there is no clear evidence that the Japanese golden eagle is  
412 evolutionarily distinct from other populations. This is perhaps not surprising given the  
413 possibility that golden eagles may be able to migrate from Japan to continental Asia  
414 (Masuda et al. 1998). However previous karyotype results indicating that the  
415 Japanese subspecies may display a different number of microchromosomes compared  
416 to its continental conspecifics (Masuda et al. 1998), does raise the possibility that  
417 nuclear differentiation has occurred. This should be further investigated before  
418 drawing any conclusions on the relative evolutionary and conservation significance of  
419 the Japanese golden eagle, or prior to considering reinforcement of the Japanese  
420 population from the continent.

421  
422

423 Within Japan, the mtDNA haplotype results show that Japanese golden eagles are  
424 composed of multiple lineages and are diverse in the context of the global population,  
425 displaying 35% of all known holarctic CR haplotypes; more than any other  
426 geographic region described by Nebel et al. (2015). Taken alongside the observation  
427 of additional pseudo-control region haplotypes, these findings suggest that the  
428 Japanese subspecies maintains higher diversity than previously thought (Masuda et al.  
429 1998), despite a declining population. Within Japan, the lack of phylogeographic  
430 structure, as evidenced by the same haplotypes being observed at either end of their  
431 distribution (Figure 3), is also likely to reflect the high dispersal ability of golden  
432 eagles and this has probably helped to retain species diversity and avoid lineage  
433 extirpation that affects less mobile species with more highly structured distributions.  
434 Based on the current data, there is no evidence of need for a regional management  
435 approach to golden eagle conservation within Japan. Further geographic sampling  
436 would help to validate this finding and enable the nuclear data from Iwate to be used  
437 within a Japan-wide evaluation of population genetic structure. A similar conclusion  
438 was drawn for golden eagles in mainland Scotland, UK (Ogden et al. 2015), where  
439 much larger sample sizes over a similar area revealed a lack of population structure  
440 using nuclear DNA microsatellites and a recommendation for management as a single  
441 population unit.

442

443 The pseudo control region has been previously detected only in Picidae, Cuculidae,  
444 Falconidae, Accipitridae, and the suboscines group of Passeriformes (Mindell et al.,  
445 1998; Haring et al., 1999). The use of both pseudo and true control regions provides  
446 additional resolution with which to assess lineage diversity of these species. Within  
447 our study, the combination of control region and pseudo control region sequence data  
448 also allowed the integration of different published datasets. In Iwate prefecture, nine  
449 mitochondrial (concatenated CR +  $\psi$ CR) haplotypes were recorded from samples  
450 collected at only thirteen nest sites, suggesting that a relatively high number of female  
451 lineages persist in the region. Such results are consistent with low female dispersal  
452 and indicate that the population has not yet entered a genetic bottleneck during which  
453 haplotype diversity would be expected to drop and nuclear diversity would show an  
454 elevated level of heterozygosity relative to allelic diversity, which was not observed in  
455 our microsatellite dataset.

456



457 4.2. Assessing founder effect in captive Japanese golden eagles

458 It is relatively unusual to have the opportunity to directly compare genetic diversity in  
459 a declining natural population with that present in a recently established captive  
460 breeding programme. Comparative analysis of the wild and captive (zoo) populations  
461 indicated roughly equivalent levels of diversity and no differentiation between the two  
462 groups, suggesting that the current breeding programme encompasses most of the  
463 extant genetic variation in the Japanese golden eagle observed to date. This is a  
464 positive finding in terms of the establishment of an insurance population for the  
465 Japanese subspecies, and is likely due to the fact that founder individuals were  
466 captured from all over Honshu island (Akita prefecture, Miyagi prefecture, Niigata  
467 Prefecture, and Fukui prefecture (Figure 2). While it could be argued that these  
468 results also give rise to concern about the likely loss of diversity in the wild over the  
469 past 50 years, there was no evidence of a strong nuclear genetic bottleneck having  
470 occurred, at least in the Iwate region. Nevertheless, despite small samples being  
471 collected from a further seven localities from northern to southern Honshu island, our  
472 samples were mainly collected from Iwate prefecture and temporal samples are not  
473 available for comparison, therefore it is not possible to be certain from the current  
474 dataset that diversity is not being lost over time in other areas of Japan.  
475 Importantly, our findings do suggest that founder effects, caused by creating a captive  
476 population using only a fraction of wild population diversity, will have been  
477 minimized. Founder effects are a key concern in *ex situ* conservation management  
478 but have until now been treated as an ‘elephant in the room’, with studbook breeding  
479 programmes defining 100% gene diversity as being the diversity contained within the  
480 founders, irrespective of whether those founders represent genetic diversity in the  
481 species as a whole. This can lead to somewhat spurious studbook measures of  
482 (theoretical) genetic diversity, where for example in zebras, captive populations with  
483 high studbook gene diversity have significantly lower evolutionary molecular genetic  
484 diversity than populations with much lower studbook diversity (Ito et al. 2017). The  
485 ability to quantitatively compare molecular genetic diversity in wild source and zoo  
486 founder populations provides conservation managers with much more detailed and  
487 accurate information with which to plan long-term species conservation measures.  
488 Previous studies of this nature have typically recorded reduced mitochondrial DNA  
489 diversity but similar levels of nuclear microsatellite DNA diversity (Stanton et al,  
490 2015; Muñoz-fuentes et al, 2008, Shen et al.2009; McGreevy et al. 2011) in captivity,



491 suggesting that while captive populations may largely be avoiding significant genetic  
492 loss in captivity, their founders do not represent wild lineage diversity. Our findings  
493 indicate that from a Japanese perspective, the founders are more representative of the  
494 extant wild population, although more comprehensive geographic sampling is  
495 required to confirm this.

496

#### 497 *4.3. Individual level molecular genetic management through pedigree reconstruction*

498 Verification of familial relatedness is a well-known issue in studbook-based breeding  
499 programmes and the application of molecular data to resolve pedigree gaps or  
500 reconstruct entire pedigrees has been employed to address this problem (Fienieg and  
501 Galbusera 2013). The approach used here demonstrates the power of DNA parentage  
502 approaches to verify studbook pedigree relationships in the Japanese golden eagle,  
503 however it has limited power to reconstruct multi-generational pedigrees *de novo*. In  
504 wild populations, use of pedigrees to investigate population genetic processes relevant  
505 to conservation, such as inbreeding depression, is considered superior to using indirect  
506 estimators of pairwise relatedness or co-ancestry based on molecular markers  
507 (Pemberton 2008). In the absence of a known wild pedigree one solution would be to  
508 perform a two-step process whereby molecular co-ancestry estimates are used  
509 alongside DNA parentage/sibship analysis to reconstruct a pedigree, which is  
510 subsequently used to directly calculate pairwise relatedness within the population.  
511 Achieving molecular pedigree reconstruction is likely to require significantly larger  
512 numbers of molecular markers (Ivy et al. 2016), but if achieved for the wild Japanese  
513 golden eagles, would offer significant insights into conservation relevant demographic  
514 and genetic processes in this declining population. To this end a study to generate  
515 thousands of genome-wide SNP markers in the Japanese golden eagle is now  
516 underway.

517

#### 518 *4.4. Sustaining captive genetic diversity and demographic viability*

519 Our results suggest that genetic diversity within the captive population represents a  
520 high proportion of extant wild genetic diversity. However, it has not been possible  
521 within this study to evaluate historic Japanese diversity and, while it may appear that  
522 the captive breeding programme has avoided a severe founder effect, it is clear from  
523 the pedigree data that the early generations of breeding have led to an extreme skew in  
524 founder representation that threatens to decimate the genetic diversity of the captive

525 population over the next few decades. This is further evidenced by the distribution of  
526 genetic variance in the captive population under PCoA, with the distribution of birds  
527 actually bred in captivity, as opposed to founder individuals, displaying a much-  
528 reduced proportion of total observed genetic variation. The current captive population  
529 is essentially comprised of one big family (including 1 founder bird) and 5 non-paired  
530 founders. The current exclusion from the breeding programme of descendants from a  
531 questioned male (Figure 2) is not supported by its mitochondrial or nuclear DNA data,  
532 nor are there any signs of outbreeding depression in its offspring, and a karyotypic  
533 analysis of this individual bird is urgently recommended to inform a decision  
534 regarding the inclusion of this potentially genetically valuable family.

535

536 Successive generations will inevitably see further rapid loss of genetic diversity and  
537 increases in inbreeding, with PVA indicating terminal decline under current  
538 management conditions. The key issue in our study was the imposition on the PVA  
539 of a maximum pairwise kinship value to form a breeding pair ( $F=0.125$ ); once this  
540 threshold was reached, breeding ceased. This may be considered an artificial  
541 restriction to population survival, however the alternative is to continue breeding  
542 increasingly related birds, a process that rapidly leads to loss of diversity and  
543 increased risks of inbreeding, significantly reducing the conservation value of the  
544 captive population. Indeed, the constraint on relatedness of breeding pairs did appear  
545 to successfully prevent an increase of lethal alleles in all VORTEX simulations, which  
546 would have been indicative of inbreeding depression, although such a strategy creates  
547 a trade-off between inbreeding and population growth.

548

549 Our simulations yielded a solution that would require increasing the proportion of  
550 individual birds breeding, as well as supplementation from outside the programme.  
551 At present only three males and three females are selected to breed annually and  
552 breeding success for many pairs is relatively low. Increasing the number of pairings  
553 would involve greater institutional exchange of birds (or gametes for AI); increasing  
554 breeding success might be achieved through modifications to husbandry practices.  
555 Both routes to increased reproduction could be attempted but would need concerted  
556 management effort. In terms of supplementation, from 1970 to 2014 fifteen wild  
557 rescue birds were included into the captive population suggesting that  
558 supplementation with a pair of birds every ten years may be achievable through an

559 opportunistic approach. Increasing the frequency of supplementation to the levels  
560 required for a sustainable captive population (one pair every two years), without  
561 manipulating mate monopolization, was shown to be possible, however this could  
562 only be achieved through the deliberate removal of Japanese birds from the wild, or  
563 importation of birds from outside Japan; both options would require a number of  
564 biological and legal challenges to be overcome.

565

566 In the context of managing genetic diversity, supplementation of the captive  
567 population represents a form of genetic rescue (short term) or genetic restoration (long  
568 term) (Weeks *et al.* 2011). Although such terms were devised to describe  
569 conservation interventions in natural populations, a vision of integrated *in situ* / *ex situ*  
570 conservation management should allow for either type of population to benefit from  
571 augmentation of genetic diversity. Genetic rescue has been previously demonstrated  
572 to be effective in small population conservation (Frankham 2015) and its inclusion in  
573 PVA simulations has previously been applied to other species (Harrison *et al.* 2016),  
574 suggesting there is certainly scope to explore these ideas further in the Japanese  
575 golden eagle. Wild capture, combined with wild release, as a form of ongoing genetic  
576 exchange under full implementation of the one-plan approach, perhaps offers the best  
577 solution for maintaining an integrated *in-situ* / *ex-situ* conservation population of  
578 golden eagles in Japan. However, the initial models examined here suggested that the  
579 captive population is very sensitive to harvest suggesting that management planning  
580 would benefit from additional comprehensive simulation work to deliver  
581 recommendations for sustainable rates of reciprocal harvest / supplementation. While  
582 the timescale of simulated declines is relatively long (>100 years), due principally to  
583 the life history of the species, it is clear that intervention should be considered and this  
584 will be most effective if implemented immediately.

585

586 The application of PVA approaches such as Vortex have typically been to wild  
587 population demographic simulation, with consideration given to supplementation  
588 from captivity; here we are effectively reversing this application. Few other examples  
589 exist, although a similar approach taken toward the European captive eastern black  
590 rhino also highlighted the effects of strong reproductive skews on the long-term  
591 demographic and genetic sustainability of small closed populations (Edwards *et al.*  
592 2015), suggesting that population viability analysis of captive breeding programmes

593 should be considered more widely. The suggestion that non-intervention may lead to  
594 population extinction in Japanese golden eagles is clearly important and may have  
595 broad implications for other captive breeding programmes. Similar findings were  
596 made by Suter et al. (2014) who examined the captive Asian elephant population in  
597 Laos and concluded that its long-term viability is compromised under current  
598 management conditions. The removal of animals from the wild to supplement captive  
599 breeding programmes is often contentious and requires careful justification. However  
600 if captive populations are really to form reservoirs or insurance populations to support  
601 the survival of species, then it is important that their genetic diversity is representative  
602 of extant variation and that this diversity is actively managed over the long term.

603

#### 604 *4.5 Management Recommendations*

605 Based on the combined findings of this study, a number of management  
606 recommendations for the Japanese golden eagle can be made:

- 607 • The gradual but continuous decline in wild Japanese golden eagle numbers  
608 gives importance to the captive population as a conservation resource,  
609 justifying ongoing intensive management.
- 610 • Despite a genetically diverse founder base, under current projections a large  
611 proportion of diversity will be lost in the near future threatening the  
612 sustainability of the captive population and necessitating changes to  
613 management practice.
- 614 • To maintain the diversity of the captive population it should be supplemented  
615 with additional, unrelated individuals. Options for supplementary birds may  
616 include captive birds currently excluded from the breeding programme, wild  
617 Japanese birds, or birds from outside Japan.
- 618 • Genetic data suggest that all three options would be compatible with the  
619 evolutionary history of the species in Japan, however further investigations of  
620 karyotype (chromosomal make-up) and morphological differentiation should  
621 be performed to increase confidence in these findings.
- 622 • To create an integrated population management solution for the Japanese  
623 golden eagle, a model of reciprocal exchange between the wild and captive  
624 populations should be considered, with the aim of managing the number and  
625 genetic diversity of birds in both groups.

- 626 • Initial simulations indicate that supplementation of the captive population  
627 with birds every ten years combined with improved reproductive success  
628 would achieve sustainability; however, further Population Viability Analysis  
629 for wild and captive birds is recommended to test and develop alternative  
630 practical solutions.

631

#### 632 4.6 Conclusions

633 Future approaches to biological conservation will need to maximise the use of all  
634 available sources of biological diversity, from pristine wilderness to cryo-preserved  
635 biobanks. Understanding how these natural resources relate to one another and  
636 integrating them within conservation programmes will require the development of  
637 continuous population management systems, for which genetic data will likely act as a  
638 common currency. For the Japanese golden eagle, such an approach has  
639 demonstrated how its conservation can be informed by simultaneous assessments of  
640 wild and captive genetic diversity.

641

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787

## 788 **7. Appendix**

789 An appendix containing supplementary tables (Tables A1-A3) and figures  
790 (Figures A1-A5) is available online.

791 **8. Tables:**

792

793 Table 1: Haplotype diversity ( $h$ ) and haplotype richness ( $Hr$ ) of CR,  $\psi$ CR, and  
 794 concatenated CR +  $\psi$ CR of mtDNA, showing broadly similar levels of  
 795 genetic variation in captive and wild golden eagles.

796

		$N$	$h$	$unique$	$se$	$hr$
CR	wild	27	0.746	1	0.062	3.36
	captive	16	0.667	0	0.113	3.09
$\psi$ CR	wild	31	0.239	0	0.096	1.42
	captive	16	0.342	0	0.140	2.00
CR + $\psi$ CR	wild	27	0.764	3	0.067	4.91
	captive	16	0.733	2	0.102	5.00

$N$ , Number of samples;  $h$ , Haplotype diversity;  $unique$ , unique haplotypes;  $se$ , Standard error of  $h$ ;  $hr$ , haplotype richness (rarefied)

797

798

799 Table 2: Genetic diversity indices for sixteen microsatellite markers showing  
 800 similarity between levels of molecular diversity between current wild  
 801 (Iwate) and captive populations.

802

	$N$	$Na$	$Np$	$Ar$	$PAr$	$Ho$	$He$	$F$
wild	39	4.4	16	3.37	0.65	0.519	0.560	0.08
captive	20	4.1	10	3.40	0.68	0.590	0.550	-0.07

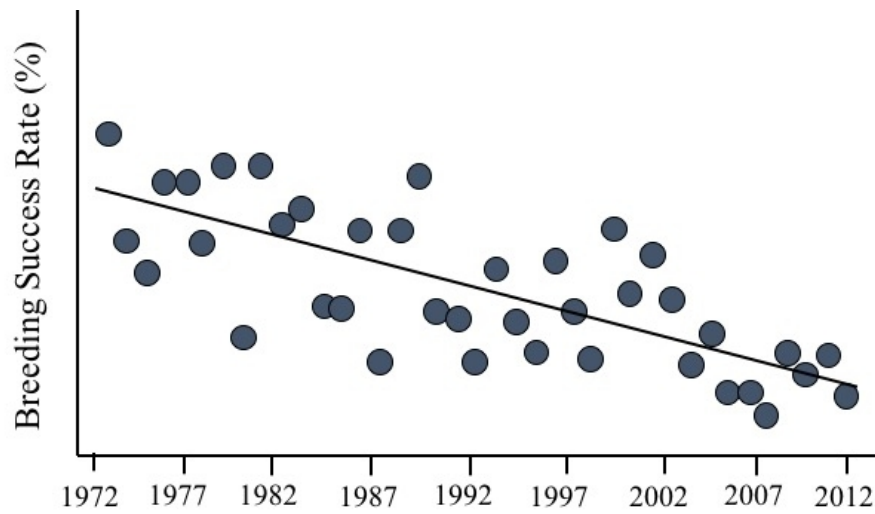
$N$ , number of samples;  $Na$ , number of Different Alleles;  $Np$ , private alleles;  $Ar$ , allelic richness;  $PAr$ , private allelic richness;  $Ho$ , observed heterozygosity;  $He$ , expected heterozygosity;  $F$ , inbreeding coefficient

803

804

9. Figures:

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Figure 1 The breeding success rate of Japanese golden eagle in Iwate-prefecture from 1972 to 2012. The success rate has gradually decreased from around 40% in the 1970's to around 10% in the 2000's.

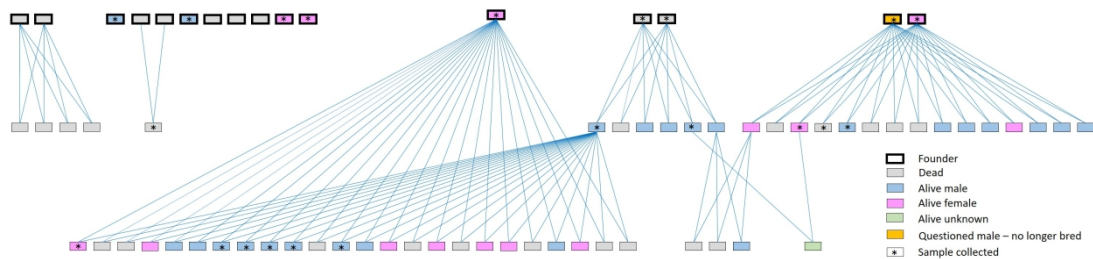
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Figure 2 The kinship tree of captive golden eagles in Japan, created from 2014 studbook data for a total of 40 living individuals, showing the current severe skew in breeding contribution. There were 16 founder birds, seven of which are still living. Grey = dead, blue = living males, pink = living females. One breeding founder male (orange) is considered as potentially a different subspecies (morphological variation), and all his descendants (12 offspring) are currently excluded from the breeding program.

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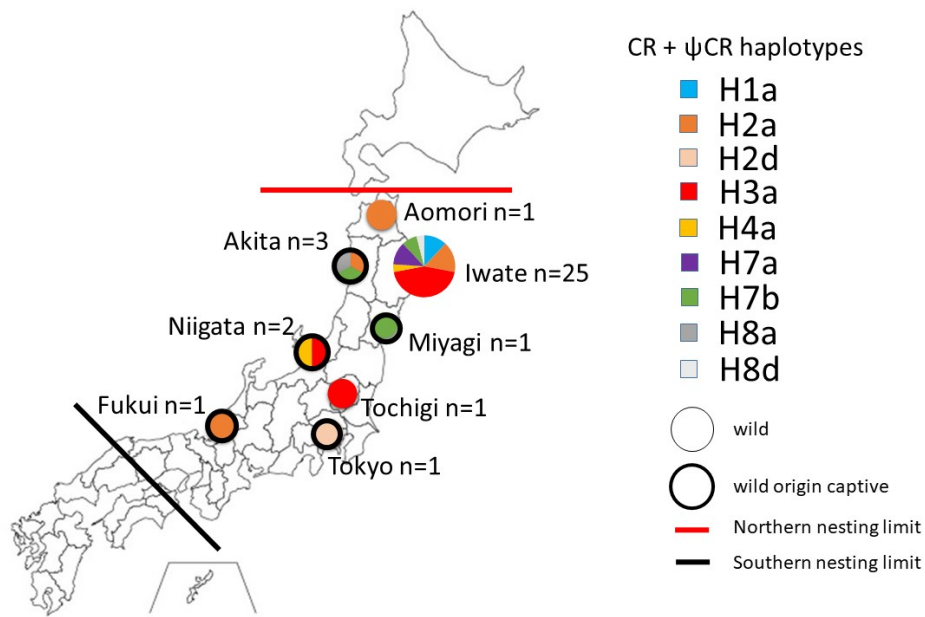
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Figure 3 The map of Japan with all mtDNA haplotypes of concatenated CR +  $\psi$ CR observed in wild origin individuals. There are nine haplotypes. Haplotypes H2d and H8a were only found only in wild-origin captive birds. Individuals from Iwate-prefecture (n = 25) display seven haplotypes.

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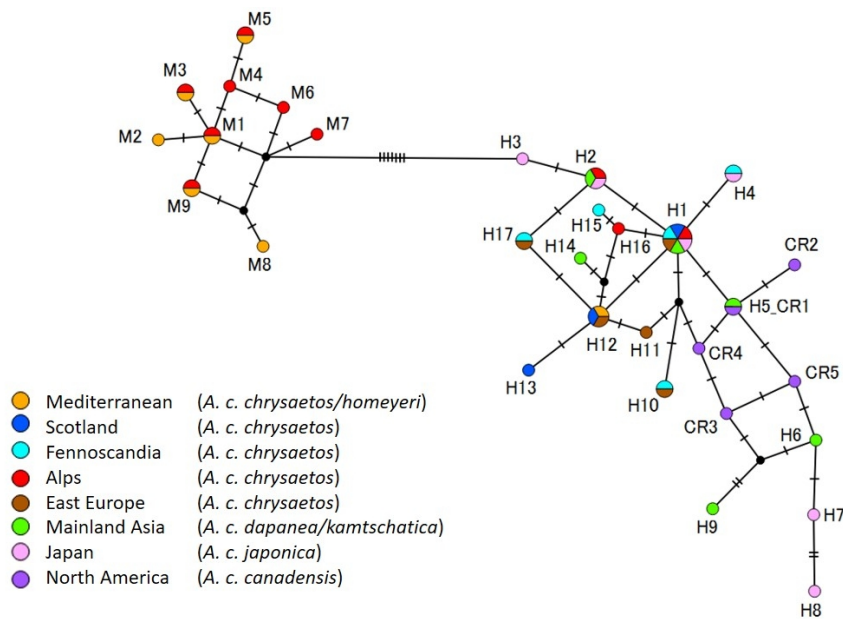
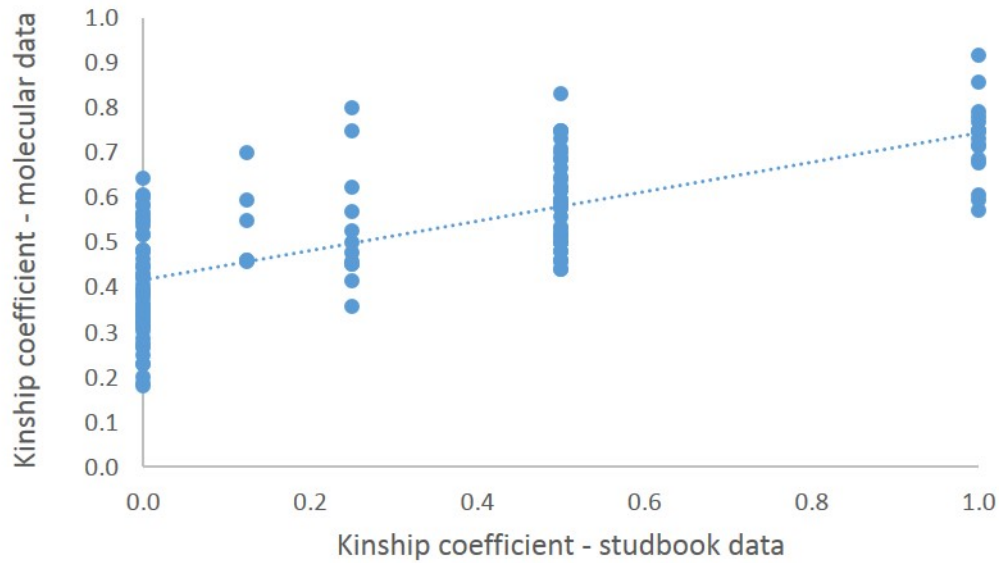


Figure 4 Median-joining network of the mtDNA control region haplotypes. 30 haplotypes are distributed globally in two lineages (haplotypes M, and haplotypes H/CR) (after Nebel et al., 2015). H1 is found in six areas including Japan. Six haplotypes (H1, H2, H3, H4, H7, and H8) are observed in Japan, three of which are currently unique to the country.

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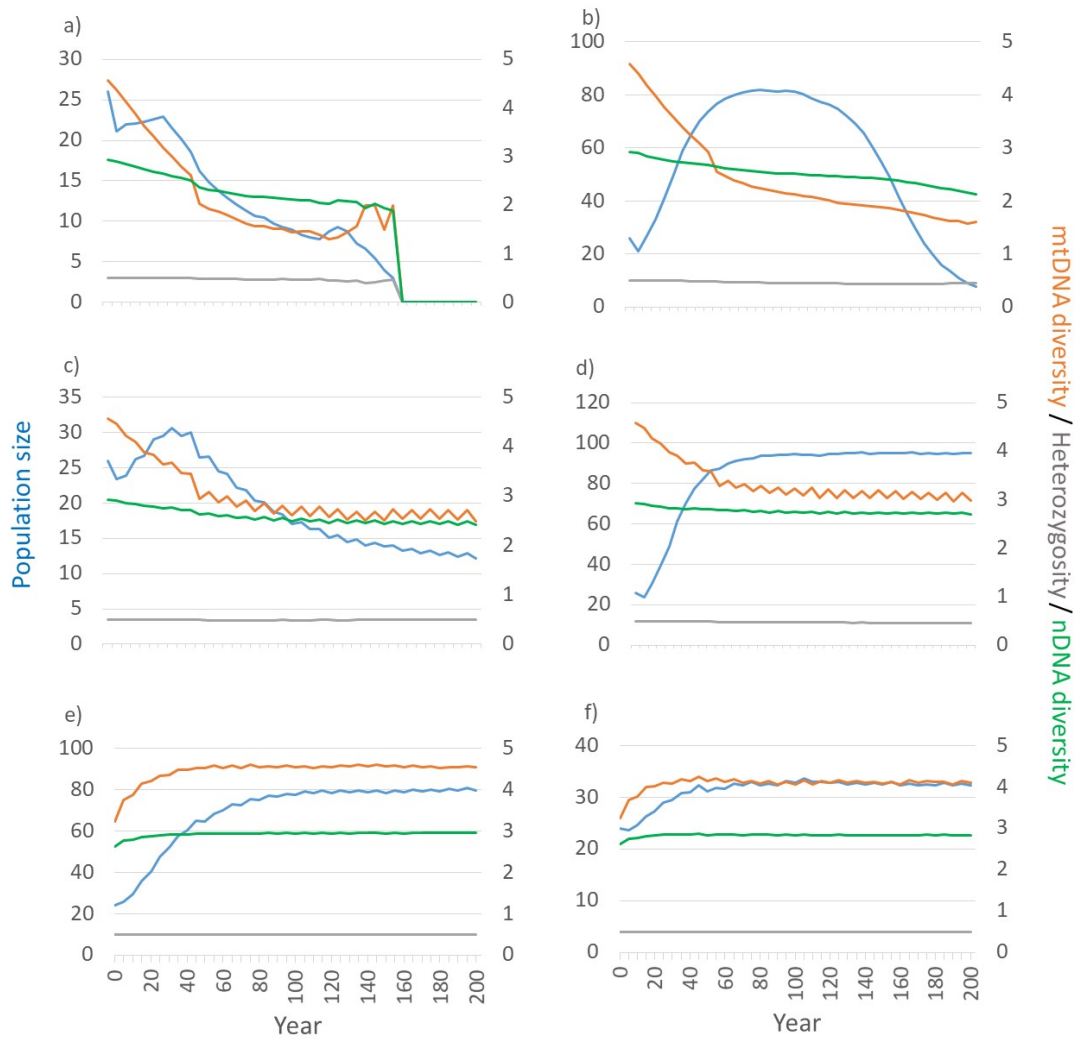
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Figure 5 Distribution of pairwise kinship coefficients in captive related individuals extracted from studbook (horizontal axis), and calculated from molecular profiling (vertical axis) by MolKin v.2. Coefficients of between self, parent-offspring or siblings, grandparent-grandchild, avuncular, and non-related pairs were defined as 1.0, 0.5, 0.25, 0.125, and 0 on the horizontal axis. The spread of molecular kinship coefficients for pairs within each relationship category resulted in only a weak correlation between the datasets, limiting the utility of this DNA marker system for predicting pedigree relationships.



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Figure 6

Vortex simulation results under six different scenarios: a) actual population parameters based on current management; b) increase in the proportion of breeders (18% to 36% in males, and 25% to 50% in females); c) supplementation with two unrelated individuals (1 male: 1 female) every 10 years; d) a combination of ‘b’ and ‘c’; e) supplementation with two unrelated individuals (1 male: 1 female) every 2 years; and f) supplementation (1 male: 1 female) and removal (to wild) of two individuals (1 male: 1 female) every 2 years.

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Population size (the blue line) is shown on the left hand axes; number of mtDNA haplotypes (orange), nuclear heterozygosity (grey), and number of nuclear DNA alleles (green) are on the right hand axes. Scenarios a) to c) show population collapse; scenarios d) to f) suggest sustainable populations over 200 years.

## 1 Appendix

### 2 Supplemental Tables

3 Table A1 Description of PVA parameters used in the different simulations

- 4
- 5 1. Simulations were run with a common set of Base Settings for the following
- 6 parameters: No. of iterations, Extinction, Inbreeding depression, EV
- 7 correlation, Catastrophe events and Carrying capacity (estimated as current
- 8 capacity in Japanese zoos).
- 9 2. Biological parameters were estimated based on known species biology with
- 10 modifications for the captive population based on consultation with zoo-
- 11 keepers. The reproductive system was 'Polygynous' (unlike the natural
- 12 system) because studbook managers can adjust breeding pairs annually. The
- 13 reproductive period of both male and female is 5 years old to 30 years old
- 14 (estimated from studbook), and maximum life span was forty-five years old
- 15 (as recorded in studbook). Maximum number of broods per year and progeny
- 16 per year are two, and the sex ratio at birth is 50%. The mortality rate from age
- 17 0 to 1 is 20%, 1 to 2, 2 to 3, 3 to 4, 4 to 5, annual mortality after age 5 are 5%
- 18 (estimated from studbook). There are no catastrophes and no harvests. In order
- 19 to prevent serious inbreeding,  $F < 0.125$  was selected based on a series of trial
- 20 simulations from  $F=0.03$  to  $F=0.25$  (not shown). The frequencies of 16
- 21 microsatellite loci and CR haplotypes were used to estimate heterozygosity,
- 22 the number of alleles per microsatellite and the number of mtDNA CR
- 23 haplotypes. The mean age in the initial population, taken directly from
- 24 studbook records, was 11.4 years old in males (17 individuals: one age 1, one
- 25 age 4, two age 5, two age 6, two age 7, three age 8, one age 12, one age 14,
- 26 one age 15, one age 16, one age 27, and one age 45) and 9.7 years old in
- 27 female (10 individuals: one age 1, one age 2, two age 3, one age 4, one age 8,
- 28 one age 11, one age 15, one age 20, and one age 30).
- 29 3. This set of fixed parameters (1. and 2. above) was then used as the basis for
- 30 conducting simulations of the actual population under current management
- 31 conditions with Actual Population Parameters (column 2) for: Maximum
- 32 kinship within a mate, annual supplementation, male mate monopolization (%)
- 33 and female mate monopolization (%). The results of these simulations are
- 34 shown at the foot of column 2 (survival and loss of genetic diversity)



35 measures). All population simulations went extinct within 200 years (Figure  
 36 6a).

37 4. Lastly these population parameters were adjusted within realistic limits in an  
 38 attempt to identify more sustainable management strategies for maintaining  
 39 genetic diversity and population numbers (column 3). Single parameter  
 40 adjustments improved simulation outputs (Figures 6b and 6c), however a  
 41 combination of parameter adjustments was required generate a long-term  
 42 sustainable solution (Figure 6d). Further simulations to increase  
 43 supplementation rate (2 birds every 2 years) and to assess the effects of  
 44 harvesting from the captive population for wild release were also simulated  
 45 (Figure 6e & 6f), but neither scenario is considered a current practical option.

<b>Vortex Parameter (Inputs to or outputs from the simulation)</b>		<b>Actual population parameters</b>	<b>Suggested management solution</b>	
<b>Base settings</b>	Iterations / time steps	1,000/200 years	1,000/200 years	
	Extinction	Only one sex remaining	Only one sex remaining	
	Inbreeding depression	6.29 (default)	6.29 (default)	
	EV correlation	0	0	
	Catastrophe events	0	0	
	Carrying capacity	100 individuals	100 individuals	
<b>Fixed (Biological or practical limitations)</b>	Reproductive system	Polygynous	Polygynous	
	Founder age / sex	Known-see table legend	Known-see table legend	
	Max. lifespan	45 years	45 years	
	Reproductive period	5 to 30 years old	5 to 30 years old	
	Max. broods per year	2	2	
	Max progeny per year	2	2	
	Distribution of broods per year (%)	0	79	79
		1	12.5	12.5
		2	8.5	8.5
	Mortality rate (%)	Age 0 to 1	20	20
		1 to 2	5	5
2 to 3		5	5	
3 to 4		5	5	
4 to 5		5	5	
After age 5	5	5		
<b>Controllable (potentially subject to management)</b>	Maximum kinship within mate pair	0.125	0.125	
	Annual suppl. from wild (10 years intervals)	0	1 m, 1 f	
	Male mate monopolization (%)	18	36	
	Female mate monopolization (%)	25	50	
<b>Output measures (Simulation results)</b>	No. birds surviving at 100 years	8.94	94.03	
	No. birds surviving at 200 years	0 (extinction)	94.95	
	% loss nuclear DNA diversity at 100 years	10.6	7.8	
	% loss nuclear DNA diversity at 200 years	100 (extinction)	9.2	
	% loss mtDNA diversity at 100 years	68.5	32.8	
	% loss mtDNA diversity at 200 years	100 (extinction)	34.9	

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Table A2 Pairwise kinship coefficients between captive individuals estimated from studbook (studbook kinship), and calculated from molecular profiling (Molecular kinship) by MolKin v.2.

Bird 1	Bird 2	Relationship	Studbook kinship	Molecular kinship
ZGE001	ZGE001	self	1.0	0.719
ZGE001	ZGE004	non-related	0.0	0.517
ZGE001	ZGE005	non-related	0.0	0.286
ZGE001	ZGE007	non-related	0.0	0.357
ZGE001	ZGE008	siblings	0.5	0.500
ZGE001	ZGE011	siblings	0.5	0.688
ZGE001	ZGE013	non-related	0.0	0.554
ZGE001	ZGE014	siblings	0.5	0.625
ZGE001	ZGE015	siblings	0.5	0.594
ZGE001	ZGE016	siblings	0.5	0.750
ZGE001	ZGE017	non-related	0.0	0.339
ZGE001	ZGE018	grandparent-grandchild	0.25	0.458
ZGE001	ZGE020	uncle-nephew	0.125	0.462
ZGE001	ZGE024	grandparent-grandchild	0.25	0.453
ZGE001	ZGE1921-f	parent-offspring	0.5	0.641
ZGE001	ZGE1921-m	parent-offspring	0.5	0.500
ZGE004	ZGE004	self	1.0	0.733
ZGE004	ZGE005	non-related	0.0	0.327
ZGE004	ZGE007	parent-offspring	0.5	0.558
ZGE004	ZGE008	non-related	0.0	0.483
ZGE004	ZGE011	non-related	0.0	0.545
ZGE004	ZGE013	parent-offspring	0.5	0.596
ZGE004	ZGE014	non-related	0.0	0.385
ZGE004	ZGE015	non-related	0.0	0.567
ZGE004	ZGE016	non-related	0.0	0.583
ZGE004	ZGE017	parent-offspring	0.5	0.462
ZGE004	ZGE018	non-related	0.0	0.313
ZGE004	ZGE020	non-related	0.0	0.538
ZGE004	ZGE024	non-related	0.0	0.600
ZGE004	ZGE1921-f	non-related	0.0	0.517
ZGE004	ZGE1921-m	non-related	0.0	0.483
ZGE005	ZGE005	self	1.0	0.679
ZGE005	ZGE007	parent-offspring	0.5	0.442
ZGE005	ZGE008	non-related	0.0	0.268
ZGE005	ZGE011	non-related	0.0	0.275
ZGE005	ZGE013	parent-offspring	0.5	0.458
ZGE005	ZGE014	non-related	0.0	0.229
ZGE005	ZGE015	non-related	0.0	0.357
ZGE005	ZGE016	non-related	0.0	0.313
ZGE005	ZGE017	parent-offspring	0.5	0.481
ZGE005	ZGE018	non-related	0.0	0.182
ZGE005	ZGE020	non-related	0.0	0.271
ZGE005	ZGE024	non-related	0.0	0.339
ZGE005	ZGE1921-f	non-related	0.0	0.321
ZGE005	ZGE1921-m	non-related	0.0	0.304
ZGE007	ZGE007	self	1.0	0.607
ZGE007	ZGE008	non-related	0.0	0.339

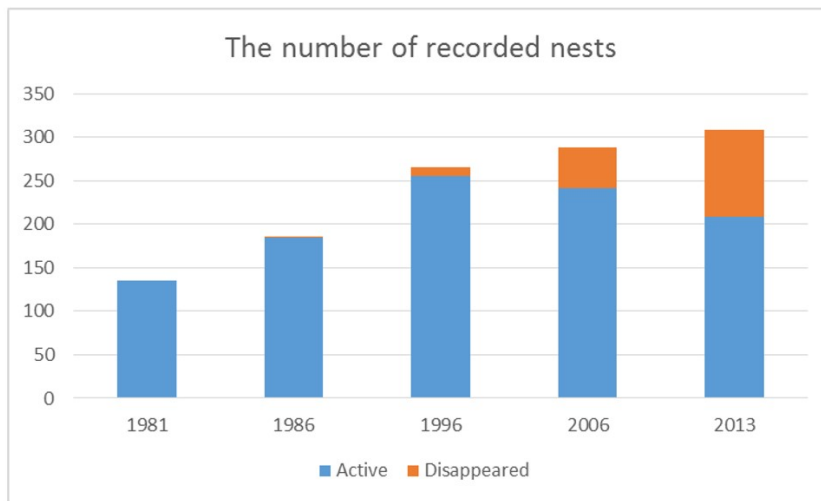
ZGE007	ZGE011	non-related	0.0	0.350
ZGE007	ZGE013	siblings	0.5	0.500
ZGE007	ZGE014	non-related	0.0	0.188
ZGE007	ZGE015	non-related	0.0	0.429
ZGE007	ZGE016	non-related	0.0	0.350
ZGE007	ZGE017	siblings	0.5	0.442
ZGE007	ZGE018	non-related	0.0	0.200
ZGE007	ZGE020	non-related	0.0	0.386
ZGE007	ZGE024	non-related	0.0	0.446
ZGE007	ZGE1921-f	non-related	0.0	0.321
ZGE007	ZGE1921-m	non-related	0.0	0.375
ZGE008	ZGE008	self	1.0	0.594
ZGE008	ZGE011	siblings	0.5	0.583
ZGE008	ZGE013	non-related	0.0	0.464
ZGE008	ZGE014	siblings	0.5	0.482
ZGE008	ZGE015	siblings	0.5	0.516
ZGE008	ZGE016	siblings	0.5	0.667
ZGE008	ZGE017	non-related	0.0	0.339
ZGE008	ZGE018	grandparent-grandchild	0.25	0.417
ZGE008	ZGE020	uncle-nephew	0.125	0.462
ZGE008	ZGE024	grandparent-grandchild	0.25	0.453
ZGE008	ZGE1921-f	parent-offspring	0.5	0.516
ZGE008	ZGE1921-m	parent-offspring	0.5	0.531
ZGE011	ZGE011	self	1.0	0.792
ZGE011	ZGE013	non-related	0.0	0.604
ZGE011	ZGE014	siblings	0.5	0.688
ZGE011	ZGE015	siblings	0.5	0.708
ZGE011	ZGE016	siblings	0.5	0.700
ZGE011	ZGE017	non-related	0.0	0.364
ZGE011	ZGE018	grandparent-grandchild	0.25	0.528
ZGE011	ZGE020	uncle-nephew	0.125	0.550
ZGE011	ZGE024	grandparent-grandchild	0.25	0.500
ZGE011	ZGE1921-f	parent-offspring	0.5	0.646
ZGE011	ZGE1921-m	parent-offspring	0.5	0.583
ZGE013	ZGE013	self	1.0	0.714
ZGE013	ZGE014	non-related	0.0	0.481
ZGE013	ZGE015	non-related	0.0	0.643
ZGE013	ZGE016	non-related	0.0	0.583
ZGE013	ZGE017	siblings	0.5	0.462
ZGE013	ZGE018	non-related	0.0	0.386
ZGE013	ZGE020	non-related	0.0	0.563
ZGE013	ZGE024	non-related	0.0	0.607
ZGE013	ZGE1921-f	non-related	0.0	0.482
ZGE013	ZGE1921-m	non-related	0.0	0.554
ZGE014	ZGE014	self	1.0	0.857
ZGE014	ZGE015	siblings	0.5	0.536
ZGE014	ZGE016	siblings	0.5	0.583
ZGE014	ZGE017	non-related	0.0	0.231
ZGE014	ZGE018	grandparent-grandchild	0.25	0.568
ZGE014	ZGE020	uncle-nephew	0.125	0.458
ZGE014	ZGE024	grandparent-grandchild	0.25	0.357
ZGE014	ZGE1921-f	parent-offspring	0.5	0.625
ZGE014	ZGE1921-m	parent-offspring	0.5	0.518
ZGE015	ZGE015	self	1.0	0.750
ZGE015	ZGE016	siblings	0.5	0.833

ZGE015	ZGE017	non-related	0.0	0.446
ZGE015	ZGE018	grandparent-grandchild	0.25	0.479
ZGE015	ZGE020	uncle-nephew	0.125	0.596
ZGE015	ZGE024	grandparent-grandchild	0.25	0.625
ZGE015	ZGE1921-f	parent-offspring	0.5	0.578
ZGE015	ZGE1921-m	parent-offspring	0.5	0.578
ZGE016	ZGE016	self	1.0	0.917
ZGE016	ZGE017	non-related	0.0	0.450
ZGE016	ZGE018	grandparent-grandchild	0.25	0.800
ZGE016	ZGE020	uncle-nephew	0.125	0.700
ZGE016	ZGE024	grandparent-grandchild	0.25	0.750
ZGE016	ZGE1921-f	parent-offspring	0.5	0.750
ZGE016	ZGE1921-m	parent-offspring	0.5	0.750
ZGE017	ZGE017	self	1.0	0.571
ZGE017	ZGE018	non-related	0.0	0.250
ZGE017	ZGE020	non-related	0.0	0.396
ZGE017	ZGE024	non-related	0.0	0.429
ZGE017	ZGE1921-f	non-related	0.0	0.321
ZGE017	ZGE1921-m	non-related	0.0	0.393
ZGE018	ZGE018	self	1.0	0.750
ZGE018	ZGE020	parent-offspring	0.5	0.500
ZGE018	ZGE024	non-related	0.0	0.396
ZGE018	ZGE1921-f	non-related	0.0	0.396
ZGE018	ZGE1921-m	parent-offspring	0.5	0.521
ZGE020	ZGE020	self	1.0	0.769
ZGE020	ZGE024	parent-offspring	0.5	0.731
ZGE020	ZGE1921-f	non-related	0.0	0.423
ZGE020	ZGE1921-m	siblings	0.5	0.615
ZGE024	ZGE024	self	1.0	0.781
ZGE024	ZGE1921-f	non-related	0.0	0.422
ZGE024	ZGE1921-m	parent-offspring	0.5	0.594
ZGE1921-f	ZGE1921-f	self	1.0	0.750
ZGE1921-f	ZGE1921-m	non-related	0.0	0.406
ZGE1921-m	ZGE1921-m	self	1.0	0.688

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54 **Supplemental Figures**



The Society for Research of Golden Eagle (2015)

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Figure A1 The number of recorded golden eagle pairs in Japan (modified from The Society for Research of Golden Eagle., 2015). The blue colour signifies active (observed) pairs, orange signifies pairs that have disappeared since the start of surveying. The total number of pairs increased from the 1970's as the survey expanded. An empty nest was founded in 1986 for the first time, and the number of missing pairs has steadily increased. In total, 99 pairs have disappeared from 1986 to 2013 and the total number of pairs is now dropping.

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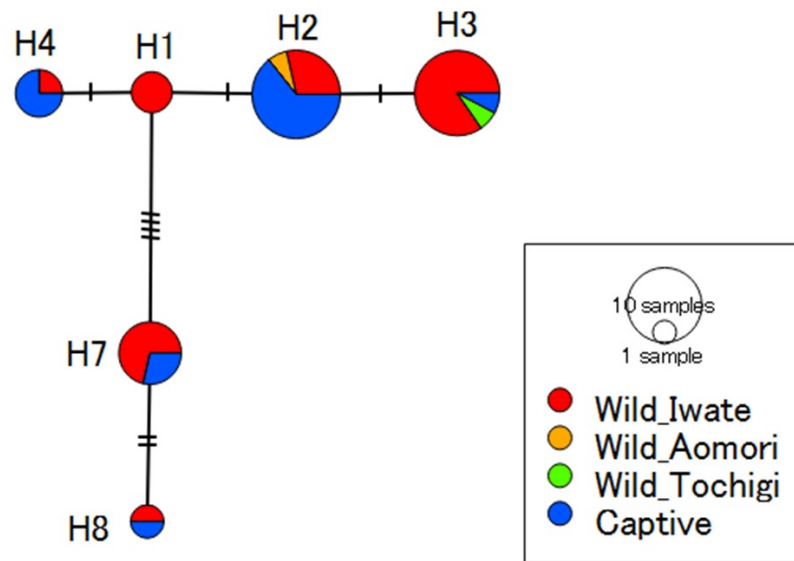
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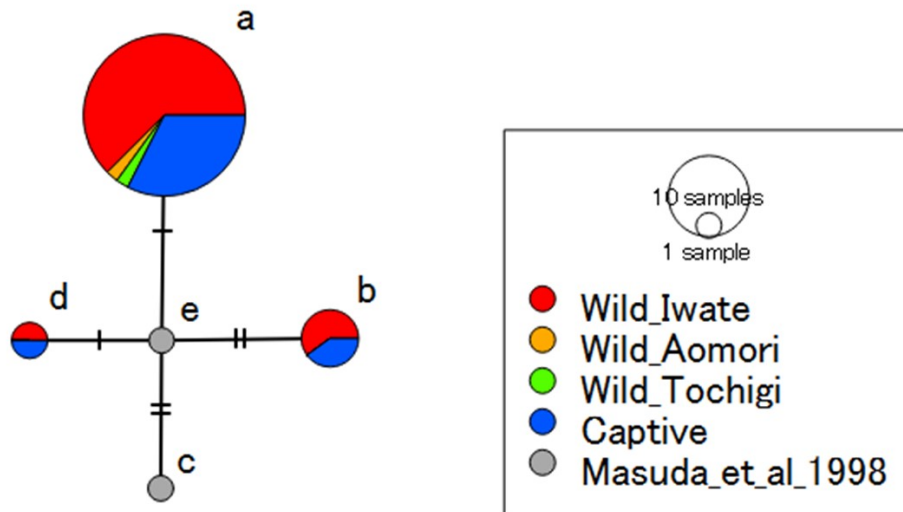


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68 Figure A2.1 Median-joining network of CR haplotypes founded in Japan. It  
 69 contains 6 haplotypes observed in 27 wild individuals and 16 captive  
 70 individuals. Red colour indicates haplotypes observed in the wild  
 71 Iwate-prefecture, orange in the wild Aomori-prefecture, green in the  
 72 wild Tochigi-prefecture, and blue found in captive population. The  
 73 circle size indicates the number of samples of each haplotype, and the  
 74 number of dashes between each haplotypes means the number of  
 75 nucleotide differences. All haplotypes are found in the wild (Iwate  
 76 population); H1 is absent from the captive population.

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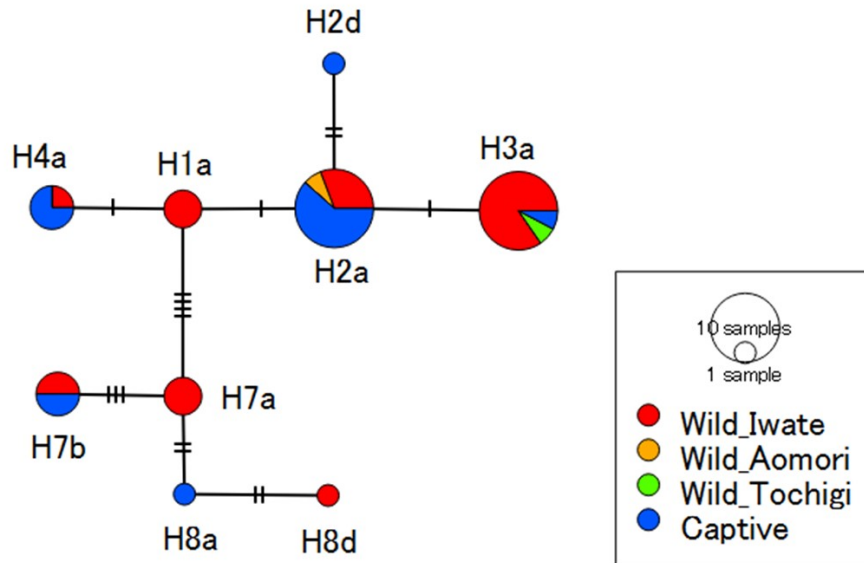


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79 Figure A2.2 Median-joining network of  $\psi$ CR haplotypes founded in Japan. It  
 80 contains five haplotypes, however haplotype e and c was not founded  
 81 from this study and referenced from Masuda et al. (1998). Three  
 82 haplotypes were founded from 31 wild individuals and 16 captive  
 83 individuals. Red colour indicates haplotypes observed in the wild  
 84 Iwate-prefecture, orange in the wild Aomori-prefecture, green in the  
 85 wild Tochigi-prefecture, and blue found in captive population. The  
 86 circle size indicates the number of samples of each haplotype, and the  
 87 number of dashes between each haplotypes means the number of  
 88 nucleotide differences.

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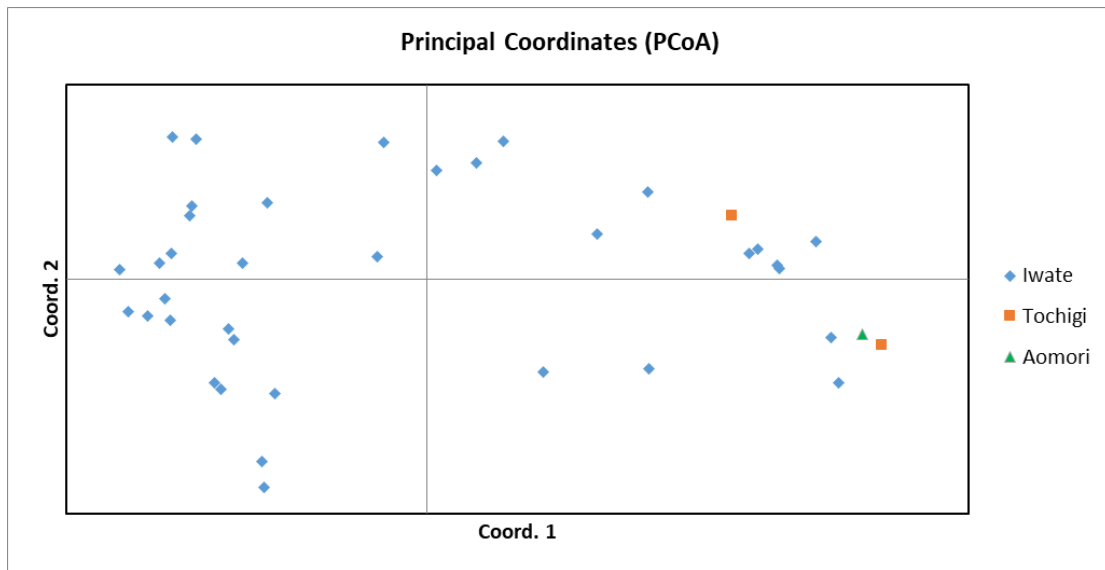
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Figure A2.3 Median-joining network of concatenated CR +  $\psi$ CR haplotypes found in Japan. It contains 9 haplotypes observed from 43 individuals. Circle size indicates the number of samples, and the number of dashes between haplotypes reflects the number of nucleotide differences. In addition to the sequence haplotypes described above, wild samples from one nest site showed additional novel haplotypes H18 and H19 (accession numbers: LC146690 and LC146691) in CR, and f (LC146689) in  $\psi$ CR; however, in all cases these haplotypes were observed at sequence bases showing clear heteroplasmy (H18/H1, H19/H1, and a/f-type heteroplasmy) and were therefore not included in diversity calculations.



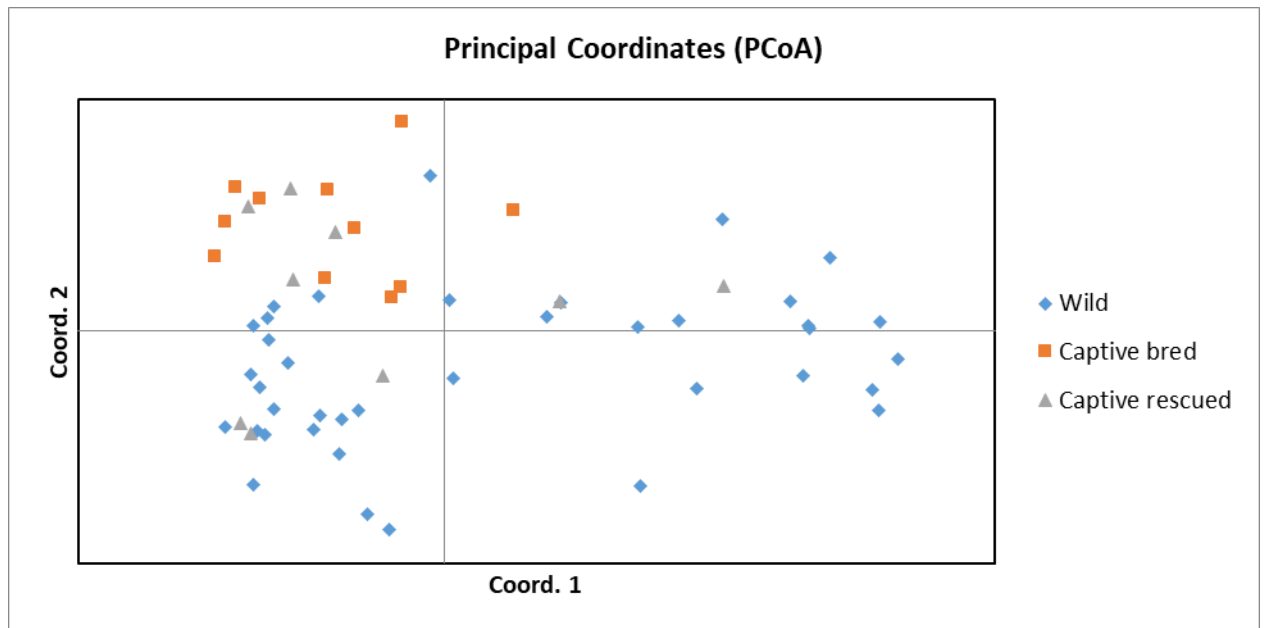


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Figure A3 Result of PCoA analysis calculated from sixteen microsatellite loci of wild Japanese golden eagles. Thirty six samples were collected from Iwate-prefecture (blue), two samples were collected from Tochigi-prefecture (orange), and one sample was collected from Aomiri-prefecture (green). Tochigi is more than 400 km away from Iwate, however data from the first two principle coordinates do not show any geographic discrimination within among samples.

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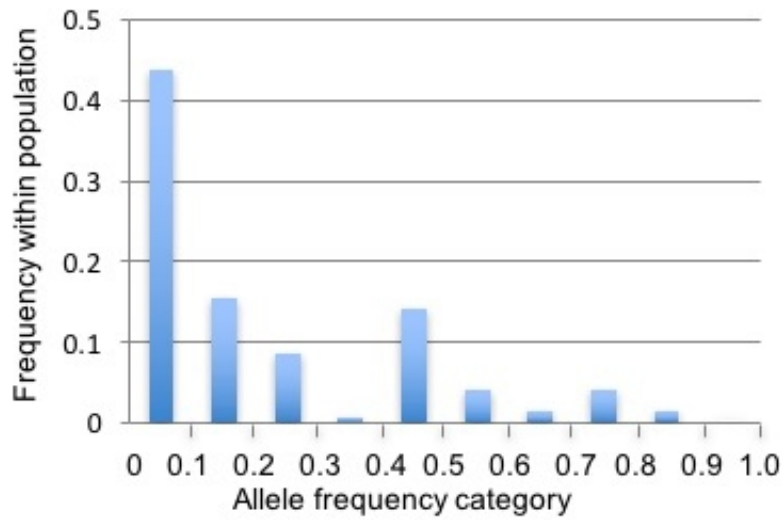
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Figure A4 Result of PCoA analysis calculated from sixteen microsatellite loci of wild and captive Japanese golden eagles. Thirty nine samples were collected from the wild (blue), and twenty samples from captive birds (orange and gray). Wild samples were collected from Iwate (n= 36), Tochigi (n = 2), and Aomori (n= 1). Nine captive samples were from rescued individuals (Akita, n = 4, Miyagi, n = 1, Niigata, n = 2, Tokyo, n = 1, Fukui, n = 1, gray), and remaining samples were captive bred (orange).



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Figure A5 Graph to investigate mode-shift in allele frequencies as an indication of a recent genetic bottleneck in the Japanese wild golden eagle population. The observed L-shaped distribution does not provide evidence for a genetic bottleneck, despite observed demographic decline.