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1 **Exploring genetic diversity in an Italian horse native breed to develop strategies** 2 **for preservation and management**

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9 **Abstract**

10 Genetic diversity is a key factor for both adaptation and response to selection. The loss of genetic
11 diversity causes a decrease in individual fitness, and it has a dramatic negative effect on the
12 population lifespan in the long term. This study aimed in exploring the genetic diversity at pedigree
13 level of the Bardigiano horse breed, which is a native breed from Italy shaped for living in rural
14 areas. In the 1977, the Bardigiano studbook was founded to preserve the breed and for improving
15 its use for riding and draft purposes. Pedigree data contained 9,469 horses, of which 3,416 were
16 alive. Demographic and genetic parameters were estimated on sub-populations to evaluate potential
17 genetic diversity differences among breeding and non-breeding animals, and animals showing
18 different breeding values (EBVs) for an index combining 18 conformation traits. Throughout the
19 studied period, inbreeding steadily increased, reaching in the last birth year cohort a mean value
20 equal to 0.10. The rate of inbreeding per generation, assuming a mean generation interval of 8.74
21 years, was equal to 1.64%. Moreover, significant differences on both average relatedness and
22 inbreeding among horses with high and low EBVs were shown. Our study unravelled the state of
23 genetic diversity in the Bardigiano breed, highlighting that breeding strategies for optimising the
24 contribution of breeding animals in the future generations are needed to ensure long term survival
25 of the Bardigiano horse breed.

26 **Keywords**

27 Genetic diversity, pedigree, Bardigiano horse breed, conservation, breeding strategies

28 **Introduction**

29 The Bardigiano horse breed is an Italian native population with excellent resilience, well adapted
30 to roughage diet, harsh climate and pasture conditions, and with an old breed history. Already in
31 the 19th Century the Bardigiano played an important role in human society, as it was used for
32 transportation in agriculture and for meat production (Libro Genealogico Cavallo Bardigiano,
33 2018). In the early 20th Century, the Bardigiano breed underwent introgression, mainly with two
34 native Italian breeds: Haflinger and Maremmano. After II World War, the number of Bardigiano
35 horses decreased dramatically, with only 5 stallions and 150 mares surviving the War (Di Stasio,
36 Perrotta, Blasi, & Lisa, 2008). To overcome the decrease in population size, the Bardigiano
37 studbook was founded in 1977. The main aim of the studbook is to preserve the Bardigiano
38 distinctive features while improving its use for riding and draft purposes. The current population
39 counts 3,416 Bardigiano horses; since introgression with other breeds is not allowed anymore, it is
40 classified as a closed population. Bardigiano horses have been bred in several Italian areas, mainly
41 in the North of Italy. Few breeders are also present outside Italy, such as in Germany, Switzerland
42 and Hungary. Up to date, the Bardigiano breed is classified as a small native population, no longer
43 at risk of extinction following FAO legislations (Sherf, 2000). However, due to the relatively small
44 size and the closed status of the current population, it is extremely important to implement breeding
45 strategies that avoid the risk of permanent loss of genetic material. Genetic diversity is a key factor
46 for both adaptation and response to selection. The loss of genetic diversity not only causes a
47 decrease in individual fitness, but it also has a dramatic negative effect on the population life span
48 in the long term. Inbreeding generally reduces performance, health and fertility at individual level
49 (Hill & Mackay, 2004; Sairanen, Nivola, Katila, Virtala, & Ojala, 2009), thus, it should be
50 monitored. Bottlenecks, limited introgression and limited number of individuals can radically
51 affect the rate of genetic diversity. To prevent the negative effects of the loss of genetic diversity,
52 the Food and Agricultural Organisation (FAO) set a maximum inbreeding rate of 1% per generation
53 (Sherf, 2000). Pedigree analysis can be considered as a useful tool to study genetic diversity in a
54 population and to detect changes that occur during breed formation, generation to generation
55 (Bokor et al., 2013). Inbreeding coefficients and average relationships over time have been used to
56 evaluate how genetic diversity evolved during breed formation (Hill and Mackay, 2004).
57 Demographic and genetic parameters, based on population structure by origin and based on
58 founders and ancestors contributions, have also been used to monitor the changes in a population

59 over a short period of time (Boichard, Maignel, & Verrier, 1997). These principles have been
60 applied to characterize the genetic diversity in several horse breeds, including both sport and
61 isolated breeds (Bussiman et al., 2018; Duru, 2017; Giontella, Pieramati, Silvestrelli, & Sarti, 2018;
62 Hamann & Distl, 2008; Hasler et al., 2011; Kjöllström, Gama, & Oom, 2015; Onogi, Shirai, &
63 Amano, 2017; Schurink, Arts, & Ducro, 2012; Vicente, Carolino, & Gama, 2012; Vostrá-Vydrová,
64 Vostrý, Hofmanová, Krupa, & Zavadilová, 2016; Vostry, Vostra-Vydrova, Hofmanova, Vesela, &
65 Majzlik, 2015). Those estimates can be in turn used to optimise breeding strategies to avoid loss of
66 genetic diversity and enhance breeding programs. Analyses at pedigree level are effective tools to
67 unravel the state of genetic diversity, especially when studying small and under-development
68 populations with often limited economic resources, causing the unavailability of more advanced
69 technologies as genomic data. Exploring genetic diversity at pedigree level does not need extra
70 economic resources, since from already-available data it is possible to identify indicators of genetic
71 variability. The aim of this study was to investigate the genetic diversity of the Bardigiano breed
72 at pedigree level to: 1) assess past and current trends in the genetic structure, 2) check the up-to-
73 date genetic variability, and 3) provide a starting point to optimise the breeding program.

74 **Material and Methods**

75 *Data available and quality control*

76 The data were provided by the breeding association of the Bardigiano horse breed (Libro
77 Genealogico Cavallo Bardigiano, Associazione Regionale Allevatori dell'Emilia Romagna, Parma,
78 Italy). The quality control (QC) of the data was performed on: inconsistency of dam and sire
79 registration number, birth date and sex identification. After QC of the data, the herd-book contained
80 9,469 horses (TP) which 3,416 were currently alive: 2,575 females (75.38%) and 841 males
81 (24.62%).

82 *Reference populations*

83 Horses currently alive were defined as reference population (RP), and their pedigrees were traced
84 back to the earliest recorded ancestors. The first ancestor recorded was traced back to 1939 and the
85 youngest horses included in the study were born in 2017. In addition to the RP, we distinguished
86 breeding mares and stallions from the reference population to analyse the current breeding
87 population (BP). To build the BP, two filters on age and number of offspring were applied: in the

88 case of mares, we included only female horses younger than 20 years old with at least 1 offspring;
89 in the case of stallions, we included only male horses younger than 20 years old with at least 5
90 offspring. The minimum number of offspring was designed considering the distribution of progeny
91 in the two genders separately. Since differences in the selection strategies are expected between
92 genetically superior and inferior animals, thus causing differences in their genetic variability, two
93 additional reference populations considering breeding values (EBVs) were defined. The EBVs are
94 indexes of 18 combined conformation traits, such as: height at wither, neck position, shoulder
95 length and leg correctness (PERCHÈ CE NE SONO SOLO 4, CONVIENE ELENCARLI TUTTI?).
96 Percentile classes of EBVs were calculated from the 3,416 currently alive horses and the 1st
97 percentile class and the 4th percentile class were considered as the two reference populations for
98 inferior (IP) and superior (SP) horses respectively (Table 1).

99 *Population structure & breeding strategy*

100 To characterize the Bardigiano population structure, several demographic parameters were
101 analysed: number of registered horses, number of sires and dams per birth year and the total number
102 of offspring per sire and dam. The geographical area of origin was known for 7,251 horses, thus
103 the population structure by province of origin was also considered. The term geographical area of
104 origin is referred to the province of origin, which is the Italian administrative division between
105 municipality and region. The F-statistics, F_{st} , F_{is} and F_{it} , were used to assess if sub-populations
106 genetic differences were present between and within areas (Caballero, 2000). The following
107 parameters were calculated within geographical area of breeding: number of individuals born,
108 percentage of individuals with sire born in the same area, percentage of individuals with sire born
109 in a different one. The geographical areas of breeding by municipalities were further classified
110 considering their altitude to assess whether the Bardigiano breeding has been more popular in
111 mountains, hills or low lands. The classification in altitude type was made following the guidelines
112 from the Italian National Statistical Institute (ISTAT, 2018); where mountains are defined as lands
113 higher than 700 meters from sea-level, hills: from 300 to 700 meters from sea-level and low lands:
114 lower than 300 meters from sea-level. To classify the altitude type within province of breeding, the
115 municipality of origin was used to assess the altitude classification. Thus, per each province, three
116 classes of altitude were calculated. The completeness of pedigree information was investigated by
117 using the equivalent complete generation (CGE), which is computed as the sum of $(1/2)^n$, where n
118 is the number of generations between individuals and each known ancestor (Maignel, Boichard, &

119 Verrier, 1996). The maximum number of fully traced generations was also considered for each
120 individual. Generation intervals (GI) were calculated for the following pathways: sire to son, sire
121 to daughter, dam to son and dam to daughter by using the average age of parents at the birth of
122 their offspring.

123 *Genetic variability*

124 Both the effective number of founders (f_e) and the effective number of ancestors (f_a) were
125 calculated as the minimum number of founders and ancestors explaining the observed genetic
126 diversity in the reference population, respectively (Lacy, 1989). To assess if the population
127 experienced bottlenecks, the ratio between f_e and f_a was calculated: when the ratio is close to unity,
128 the population is stable in terms of the numbers of effectively contributing animals. In contrast, if
129 the effective number of founders is larger than the effective number of ancestors this means that
130 some ancestors have played a major role in population formation (Boichard et al., 1997). The
131 founder genome equivalent (f_{ge}), which is defined as the effective number of founders with non-
132 random loss of founder alleles describing the observed genetic variability, was calculated in the
133 two population clusters concerning EBVs percentile class (SP and IP) and compared to the result
134 obtained from the RP.

135 The individual inbreeding coefficient (F), as the probability that an individual has two identical
136 alleles by descendant, was computed following Meuwissen and Luo, 1992. The rate of inbred
137 Bardigiano horses within generation was calculated: if a horse showed an inbreeding coefficient
138 higher than 0.05, then it was considered as inbred. The threshold of F ($F > 0.05$) was chosen
139 considering the distribution of the individual inbreeding coefficients in the RP. The average
140 relationship (AR) defines the mean relationship of each individual with the remaining of the
141 population and it can thus be interpreted as a representation of the animal contribution in the whole
142 pedigree. The trends of F and AR coefficients throughout the analysed generations were evaluated
143 with the GLM procedure in SAS (SAS Institute 9.4). The rate of the increase in inbreeding (ΔF)
144 was also calculated per generation, as well as the AR. Average F and AR were calculated per each
145 breeding province divided by altitude type (low lands, hills and mountains). The effective
146 population size (N_e) was estimated as follow: a) computing the regression coefficient of the
147 individual inbreeding coefficient in the TP over: i) the number of full generations traced; ii) the
148 maximum number of generations traced; and iii) the equivalent complete generations, and
149 considering the corresponding regression coefficient as the increase in inbreeding between two

150 generations, b) as individual increase in inbreeding in the population $N_e = \frac{1}{2\Delta F}$ for TP, RP and BP.
151 The ENDOG v4.8 software was used to carry out the above mentioned analyses (Gutierrez &
152 Goyache, 2005).
153 The degree of non-random mating practiced by breeders was assessed by comparing the F and the
154 AR among sires and dams belonging to the breeding population to the results obtained from the
155 reference population. Differences in the number of sires building the superior and inferior
156 populations (SP and IP), the inbreeding coefficient and the average relationship coefficients
157 between those two sub-populations were tested by two sample t-tests in SAS 9.4.

158

159 **Results**

160 *Population structure & breeding strategy*

161 From the '70s, a gradual increase of the number of registered horses was recorded, with the highest
162 peak in 2009 with 389 horses listed. Since 2010, a reverse bending in the annual number of
163 registered horses has been observed, dropping from 336 in 2010 to 165 in 2016 (complete year).
164 The average number of mares registered throughout the studied period was always significantly
165 higher than males ($p < 0.0001$), being on average more than 3 times more abundant. The number of
166 registered males over the 62 evaluated years ranged between 1 in 1939 to 178 in 2008. Up to 1976,
167 with the only exception of year of birth 1960, all the male horses registered to the studbook were
168 used for breeding. Since the foundation of the studbook in 1977, a steady decrease of the number
169 of males used for breeding was observed, reaching to the point where only 4.24 % of the males
170 born and registered in 2009 had offspring in the following years. The number of sires born in the
171 last birth cohort and used for breeding was equal to 50, which is the 5.9% of the males currently
172 present in the population (**Errore. L'origine riferimento non è stata trovata.**). The most
173 influenced stallion produced 222 offspring; half of the registered stallions produced more than 10
174 offspring and 13.8% of the sires produced one offspring only. The Number of offspring per
175 breeding mare ranged from 1 to 14, with an average of 2.60 offspring per mare. When considering
176 the 7,251 horses with information available on the geographical area of breeding, we found that
177 the Bardigiano has been bred in 14 areas in Italy, and in three countries outside Italy. Nevertheless,
178 93.5% of the horses were from four geographical areas only: Parma, Piacenza, Genova and La
179 Spezia, with 55.7% (4,041 horses) belonging to Parma only. Breeding strategies differed between

180 geographical areas of breeding: in the case of Parma, 64% of the stallions used for breeding were
181 local stallions, whereas in Piacenza, Genova and La Spezia, the majority were foreign stallions.
182 The least number of breeding stallions born and used in the area of origin was shown in La Spezia,
183 where only 4% of the horses registered within this area were born from native stallions (**Errore.**
184 **L'origine riferimento non è stata trovata.**). The F-statistics were calculated to evaluate the
185 within-population genetic differences between Parma, Piacenza, Genova and La Spezia, the values
186 were as follow: $F_{st}= 0.0038$, $F_{is}=0.0941$ and $F_{it}= 0.0122$. From the classification in classes of land-
187 type in respect to altitude (mountains, hills and low lands), it resulted that the majority of
188 Bardigiano horses have been bred in mountain and hilly areas: 3,117 (46%) and 3,234 (48%) horses
189 respectively. The equivalent complete generation (CGE) investigates the completeness of the
190 pedigree information, which resulted equal to 4.0 generations in the TP and 5.2 when considering
191 only alive horses (RP). Percentage of ancestors known per ancestral generation differed between
192 birth year cohorts. The maximum number of known ancestral generations ranged from 15 in the
193 last birth year cohort (2011-2017) to 9 in the first birth year cohort after the foundation of the
194 Bardigiano breeding association (1975-1983). Average equivalent complete generations differed
195 between cohorts of horses born from different birth year cohort, e.g.: 2011-2017 was 6.1, from
196 birth year cohort 1984-1992 was 2.9 and from birth year cohort 1966-1974 was 0.43. As expected,
197 pedigree of horses born in the most recent years was deeper and had a higher quality (**Errore.**
198 **L'origine riferimento non è stata trovata.**). The average generation interval (GI) calculated from
199 all the pathways was equal to 8.74 years; the GI in the maternal lineages was higher than in the
200 paternal lineages: mother-daughter = 8.67 years, and mother-son = 9.18 years, whereas father-
201 daughter = 8.45 and father-son = 8.65 years.

202 ***Genetic variability***

203 The effective number of founders (f_e) was equal to 20 and the effective number of ancestors (f_a)
204 was equal to 15 in the TP. When considering only alive animals, both f_e and f_a decreased, resulting
205 equal to 18 and 14, respectively. The ratio between f_e and f_a was equal to 1.30 and to 1.20 in the
206 TP and RP, respectively. The number of ancestors explaining the 50% of the observed genetic
207 diversity in the RP was equal to 8 animals. Conversely, when considering only animals belonging
208 to the SP cluster, the number of ancestors explaining the 50% of the observed genetic diversity
209 decreased to 4. Differences were found on the numbers of founder genome equivalents (f_{ge})
210 between the two sub-populations defined on percentile class of EBVs: in the case of SP, the number

211 of f_{ge} was 4.6, whereas in the case of IP, f_{ge} was equal to 11.0. The average inbreeding coefficient
212 (F) was equal to 0.06 (S.D. = 0.06) in the whole population (TP), when considering alive animals
213 (RP) F increased to 0.08 (S.D. = 0.06), ranging from 0 to 0.35 within population. Significant
214 differences ($p < 0.05$) in the F between the groups of superior and inferior animals were found:
215 0.10 ± 0.002 in the SP and 0.06 ± 0.002 in the IP group. Both the F and the rate of inbred animals
216 (RIA) calculated per birth year cohort increased steadily in the RP, reaching in the last cohort an F
217 of 0.10 and a RIA equal to 86.5%. Horses, showing a level of consanguinity equal or higher than
218 0.25, were found in all the birth year cohorts (Table 2). The rate of inbreeding per generation (ΔF),
219 assuming a mean generation interval of 8.74, was equal to 1.64% in the RP. In contrast, when
220 considering only breeding animals, ΔF increased to 1.93%. Average relatedness (AR) within
221 breeding animals did not significantly differ from the AR in the reference population and it was
222 equal in both populations to 11.0% ($p > 0.05$). In contrast, AR of stallions (13.0%) was higher, if
223 compared to AR of mares (11.0%) ($p < 0.05$). A similar pattern was shown when comparing AR
224 within superior and inferior animals in respect to their EBV ($AR_{SP} = 14.0\%$ and $AR_{IP} = 9.0\%$) ($p <$
225 0.05). Significant mean differences ($p < 0.05$) were shown for the comparison of F and AR between
226 provinces in all cases except between La Spezia and Genova. Average F and AR, calculated for
227 provinces of breeding nested with altitude type (low lands, hills and mountains), showed higher
228 average inbreeding and relationship among individuals belonging to mountain and hill areas
229 compared to low land locations in all provinces except for the case of La Spezia, however no
230 significant differences were found between altitude types within province. Animals belonging to
231 mountain areas in the province of Piacenza showed the highest average F and AR, being equal to
232 0.10 and 0.13 respectively (Table 3). The effective population size (N_e) in the TP resulted equal to
233 60.81, 26.41 and 18.26 when using the increase in breeding by maximum generation, equivalent
234 generation and complete generation, respectively. The N_e calculated in respect to the ΔF was equal
235 to 30.67 in the RP and to 26.32 in the BP. In **Errore. L'origine riferimento non è stata trovata.**,
236 the breeding values (EBVs) of the 66 sires building the BP were plotted against their average
237 relationship in the population. Sires with an AR lower than 13% and with an EBV higher than 50
238 were showed as rhombus representing the optimal in terms of potential breeding contribution. This
239 result supports the hypothesis that difference in the selection strategies between genetically
240 superior and inferior animals is expected, thus affecting their genetic variability. The 66.0% of the

241 sires showing an EBV higher than average (50) displayed also an AR higher than the average value
242 in the sampled sire population ($AR > 0.13$).

243 **Discussion**

244 The goal of the present study was to investigate the genetic diversity of the Bardigiano breed based
245 on pedigree information to propose breeding strategies for breed management and conservation.

246 *Population structure*

247 The number of Bardigiano horses registered to the studbook showed two separate trends: from the
248 beginning of breed formation to 2009 a steady increase was shown. On the other hand, from 2010
249 to current days a reverse bending was observed. Similarly, the number of stallions used for breeding
250 gradually decreased. The observed trend might be related to the decrease in the number of
251 Bardigiano horses used for meat production. This reduction might be the consequence of three
252 events. Firstly, the opening up of international markets of equine meat has increased the availability
253 of meat from foreign countries, thus reducing local production. Secondly, due to the Eurozone
254 crisis in the late 2009, a reduction of meat consumption has been generally observed in several
255 European countries. This crisis might have affected horse meat consumption in Italy, thus dropping
256 local horse meat production even further (Na, Minjun, & Wen, 2013). Finally, since the beginning
257 of the 21st century in the equine market the demand of horses for leisure activities has increased
258 deeply. Consequently, the breeding goal of many Bardigiano breeders has changed from meat
259 production to horses shaped for pleasure activities, which have a higher economic value, a longer
260 life span and thus an adverse influence in the number of horses registered per year. In the last 13
261 years, the number of living animals has been maintained fairly stable; from a previous study it
262 resulted equal to 3,556 animals, which is 140 animals higher than the current population (Sabbioni
263 et al., 2005). Although the Bardigiano is no longer classified as at risk of extinction, its population
264 size is considerably smaller than other Italian native breeds. Currently, the Bardigiano has a
265 registered population of 3,416 individuals which is lower than the Italian Haflinger, Murgese and
266 the Italian Heavy Draft populations: all of them have a population size larger than 5,500 animals
267 (Associazione Italiana Allevatori, 2018). A recent study on the Maremmano horse population
268 showed a registered alive population of 5,705 horses and a breeding population of 1,532, both of
269 them are nearly two times larger than in the Bardigiano breed (Giontella et al., 2018). Similarly,

270 the Bardigiano population size is smaller than international breeds, as an example, the number of
271 Bardigiano horses is roughly half of the current Lusitano population (Vicente et al., 2012). The
272 number of breeding animals in the Bardigiano breed and in the endangered Old Kladruber horse
273 population is very close: 742 and 612, respectively (Vostrá-Vydrová et al., 2016).

274 ***Breeding strategy***

275 Selection intensity differed between mares and stallions: 26.3% of the females have been used for
276 breeding and this figure becomes 7.8% for males. The proportion of breeding stallions and mares
277 resulted equal to 1 stallion every 10 mares. In European horse breeds for sport performances this
278 proportion is considerable smaller if compared to what we found in the Bardigiano breed: in the
279 Hanoverian population is equal to 20 mares each stallion and 1 stallion every 60 mares in the Dutch
280 harness population (Hamann & Distl, 2008; Schurink et al., 2012). Artificial insemination (AI)
281 improves stallion reproduction efficiency, increasing intensely the number of mated mares
282 obtainable per stallion. Nowadays, AI is broadly used in the majority of European horse breeds for
283 sport performances, whereas in the Bardigiano AI has not been implemented yet. Thus, the absence
284 of AI in the Bardigiano might be the reason why we observed both a lower mares/stallion ratio and
285 a limited average number of offspring per stallion. The number of sires born in the last birth cohort
286 and used for breeding was equal to 50, which is the 5.9% of the current total of male Bardigiano
287 horses. Once a year the pedigree database is updated regarding male horses that undergo surgery
288 for castration via owner declaration. Thus, this portion might be slightly higher as some of the male
289 horses considered as potential breeding animals in reality have been gelded. Breeding strategies
290 differed among areas where Bardigiano horses are commonly bred. Similarly to what previously
291 stated, as the use of AI has not been implemented yet in this breed, the exchange of genetic material
292 becomes uneven and challenging between more distant areas. The higher inbreeding and higher
293 relationship among individuals belonging to the cluster mountain area support this hypothesis. In
294 addition, the F-statistics analyses revealed higher F_{st} and F_{is} than what found in the Maremmano
295 breed, showing more genetic separation between areas of breeding and higher average inbreeding
296 within area of breeding (Giontella et al., 2018). To allow for a more uniform genetic flow between
297 areas, and to reduce the risk of genetic drift and inbreeding within local area, we suggest the
298 application of AI in this breed. However, if AI is introduced, breeding strategies for balancing
299 stallion's contributions are needed to reduce the risk of critical application of only the most popular
300 stallions. In recent years, strategies to optimise the contribution of breeding animals have been

301 implemented. Optimal contribution selection (OCS) was applied in the Norwegian and the North-
302 Swedish cold-blooded trotter, and the importance of annual mating quota per stallion was
303 highlighted to monitor stallion's contribution in the population (Olsen, Meuwissen, & Klemetsdal,
304 2013). Hence, especially if AI is introduced in the Bardigiano breed, OCS might be an effective
305 tool to balance stallion's contributions. Mating strategies to control future inbreeding were
306 considered in the indigenous Swiss horse breed, where they combined the best linear unbiased
307 prediction (BLUP) methodology with OCS theory (Hasler et al., 2011). Thus, OCS together with
308 BLUP might be used to monitor the Bardigiano genetic diversity while allowing for response to
309 selection for economically relevant traits. Even though in breeds potentially under endangered
310 status the most urgent matter is to conserve their genetic diversity, their probability to survive is
311 also highly dependent on their economic value. Thus, by improving economically relevant traits,
312 such as conformation, gaits and attitude traits via the breeding program, the profitability of
313 Bardigiano horses increases, hereafter reducing the risk of extinction. Therefore, the simultaneous
314 implementation of a selection program, which optimises the selection of breeding candidates both
315 in term of conservation and breeding values, is suggested to enhance the demand of Bardigiano
316 horses in the equine sector. The generation interval in the Bardigiano horse population was equal
317 to 8.74 years. In draft horse breeds the generation interval varies between 7.0 to 7.9 years for French
318 Comtois draught horse population and for the Austrian Noriker draught horse, respectively (Druml,
319 Baumung, & Sölkner, 2009; Verrier et al., 2010). Horse breeds for sport purposes showed generally
320 a longer generation interval: 10.3 for the Lusitano breed, 10.1 for the Andalusian horse population
321 and 10 years for the Hanoverian horses (Hamann & Distl, 2008; Valera, Molina, Gutiérrez, Gómez,
322 & Goyache, 2005; Vicente et al., 2012). The Dutch harness horse population presented a generation
323 interval akin to what we found in the Bardigiano, being equal to 8.6 years (Schurink et al., 2012).
324 Differences in generation interval among breeds might be related to the final destination of the
325 breed. Draught horse breeds mainly have a shorter generation interval, as this horse type is
326 commonly used for meat production and it is rarely used in competitions. In contrast, since horses
327 belonging to sport breeds attend competitions postponing the reproduction activity, they tend to
328 show longer generation intervals. The generation interval in the Bardigiano population fits in
329 between sport and draft breed-types.

330 ***Genetic variability***

331 Pedigree quality and depth appeared sufficient to calculate genetic diversity parameters. It is known
332 that the absolute level of inbreeding is highly dependent on the quality of the data available
333 (Schurink et al., 2012; Sørensen, Sørensen, Baumung, Borchersen, & Berg, 2008). Hence,
334 inbreeding rate appears to be a more appropriate parameter to assess genetic variability across time
335 within a breed. The average inbreeding in the reference population (RP) was 33.3% higher than in
336 the whole population, thus showing a possible increase in the inbreeding over time. This
337 assumption is confirmed by the rate of inbreeding per generation (ΔF), which resulted equal to
338 1.64% in RP. The Food and Agriculture Organization (FAO) stated that the value of ΔF should not
339 exceed 1% to avoid substantial loss of genetic material over time (Sherf, 2000); unfortunately, this
340 limit is not respected in the Bardigiano breed. In endangered horse populations we found similar
341 ΔF s: in the French Boulonnais draught horse population ΔF was equal to 1.38% and in the Old
342 Kladruber horse population was equal to 1% (Verrier et al., 2010; Vostrá-Vydrová et al., 2016).
343 Although the Bardigiano is not included in the list of endangered breeds, it currently shows a
344 critical increase in the inbreeding in the last generations. From the comparison between clusters of
345 EBVs, differences in the average relatedness were found between superior and inferior animals: on
346 average, horses with high breeding values were also more related to each other. Future inbreeding
347 might be kept under control by assuring that parents of future breeding animals are not closely
348 related. Thus, including the average relationship as an extra parameter of selection in the breeding
349 program might be an effective solution to avoid high relatedness among matings, thus reducing the
350 inbreeding in the coming generations.

351 **Conclusion**

352 Our study unravelled the state of genetic diversity in the Bardigiano breed, highlighting that further
353 research is needed to optimise the contribution of breeding animals in the upcoming generations.
354 To ensure long term genetic diversity within the Bardigiano breeding population, strategies to
355 optimise mating schemes are needed. One possible solution to monitor the future inbreeding and,
356 in turn, the genetic diversity in the population, is to implement efficient selection strategies.
357 Optimal Contribution Selection might be an effective tool to increase genetic gain while controlling
358 the rate of inbreeding, thus, enabling the maintenance of genetic diversity in the Bardigiano
359 population. Since using animals with lowest AR for breeding can minimise the inbreeding in the

360 coming generations, by balancing the contribution of the founders in the population, we suggest
361 accounting for the average relationship in the breeding program.

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366 autochthonous breeds.

367 **Declaration of interest**

368 The authors have no conflict of interest to declare.

369

370 **References**

- 371 Associazione Italiana Allevatori, A. equidi. (2018). Anagrafe Equidi. Retrieved May 30, 2018, from
372 <http://www.anagrafeequidi.it/menu.php>
- 373 Boichard, D., Maignel, L., & Verrier, É. (1997). The value of using probabilities of gene origin to measure
374 genetic variability in a population. *Genetics Selection Evolution*, 29(1), 5–23.
375 <https://doi.org/10.1186/1297-9686-29-1-5>
- 376 Bokor, Á., Jónás, D., Ducro, B., Nagy, I., Bokor, J., & Szabari, M. (2013). Pedigree analysis of the
377 Hungarian Thoroughbred population. *Livestock Science*, 151(1), 1–10.
378 <https://doi.org/10.1016/j.livsci.2012.10.010>
- 379 Bussiman, F. O., Perez, B. C., Ventura, R. V., Peixoto, M. G. C. D., Curi, R. A., & Balieiro, J. C. C. (2018).
380 Pedigree analysis and inbreeding effects over morphological traits in Campolina horse population.
381 *Animal*, 1–10. <https://doi.org/10.1017/S175173111800023X>
- 382 Caballero, A. (2000). Interrelations between effective population size and other pedigree tools for the
383 management of conserved populations Interrelations between effective population size and other
384 pedigree tools for the management of conserved populations, (July), 26–27.
385 <https://doi.org/10.1017/S0016672399004449>
- 386 Di Stasio, L., Perrotta, G., Blasi, M., & Lisa, C. (2008). Genetic characterization of the Bardigiano horse
387 using microsatellite markers. *Italian Journal of Animal Science*, 7(2), 243–250.
388 <https://doi.org/10.4081/ijas.2008.243>
- 389 Druml, T., Baumung, R., & Sölkner, J. (2009). Pedigree analysis in the Austrian Noriker draught horse:
390 genetic diversity and the impact of breeding for coat colour on population structure. *Journal of Animal*
391 *Breeding and Genetics*, 126(5), 348–356. <https://doi.org/10.1111/j.1439-0388.2008.00790.x>

- 392 Duru, S. (2017). Pedigree analysis of the Turkish Arab horse population: Structure, inbreeding and genetic
393 variability. *Animal*, 11(9), 1449–1456. <https://doi.org/10.1017/S175173111700009X>
- 394 Giontella, A., Pieramati, C., Silvestrelli, M., & Sarti, F. M. (2018). Analysis of founders and performance
395 test effects on an autochthonous horse population through pedigree analysis: structure, genetic
396 variability and inbreeding. *Animal*, 1–10. <https://doi.org/10.1017/S1751731118001180>
- 397 Gutierrez, J. P., & Goyache, F. (2005). A note on ENDOG: a computer program for monitoring genetic
398 variability of populations using pedigree information. *Journal of Animal Science*, 122(1992), 172–
399 179.
- 400 Hamann, H., & Distl, O. (2008). Genetic variability in Hanoverian warmblood horses using pedigree
401 analysis. *Journal of Animal Science*, 86(7), 1503–1513. <https://doi.org/10.2527/jas.2007-0382>
- 402 Hasler, H., Flury, C., Menet, S., Haase, B., Leeb, T., Simianer, H., ... Rieder, S. (2011). Genetic diversity
403 in an indigenous horse breed - implications for mating strategies and the control of future inbreeding.
404 *Journal of Animal Breeding and Genetics*, 128(5), 394–406. <https://doi.org/10.1111/j.1439-0388.2011.00932.x>
- 406 Hill, W. G., & Mackay, T. F. C. (2004). D. S. Falconer and introduction to quantitative genetics. *Genetics*,
407 167(4), 1529–1536. <https://doi.org/167/4/1529> [pii]
- 408 ISTAT. (2018). PRINCIPALI STATISTICHE GEOGRAFICHE SUI COMUNI. Retrieved June 1, 2018,
409 from <https://www.istat.it/it/archivio/156224>
- 410 Kjöllström, H. J., Gama, L. T., & Oom, M. M. (2015). Impact of inbreeding on fitness-related traits in the
411 highly threatened Sorraia horse breed. *Livestock Science*, 180, 84–89.
412 <https://doi.org/10.1016/j.livsci.2015.08.001>
- 413 Lacy, R. C. (1989). Analysis of founder representation in pedigrees: Founder equivalents and founder
414 genome equivalents. *Zoo Biology*, 8(2), 111–123. <https://doi.org/10.1002/zoo.1430080203>
- 415 Libro Genealogico Cavallo Bardigiano, A. R. A. dell'Emilia R. (Ed.). (2018). *La diversità genetica del*
416 *cavallo Bardigiano ad oggi* (First Edit). Parma. Retrieved from www.cavallobardigiano.it
- 417 Maignel, L., Boichard, D., & Verrier, E. (1996). Genetic variability of French dairy breeds estimated from
418 pedigree information. *Interbull Bulletin*, 14, 49–53.
- 419 Meuwissen, T., & Luo, Z. (1992). Computing inbreeding coefficients in large populations. *Genetics*
420 *Selection Evolution*, 24(4), 305. <https://doi.org/10.1186/1297-9686-24-4-305>
- 421 Na, L., Minjun, S., & Wen, H. (2013). Impacts of the Euro sovereign debt crisis on global trade and
422 economic growth: A General Equilibrium Analysis based on GTAP model. In *16th Annual*
423 *Conference on Global Economic Analysis, Shanghai, China*.
- 424 Olsen, H. F., Meuwissen, T., & Klemetsdal, G. (2013). Optimal contribution selection applied to the
425 Norwegian and the North-Swedish cold-blooded trotter - a feasibility study. *Journal of Animal*
426 *Breeding and Genetics*, 130(3), 170–177. <https://doi.org/10.1111/j.1439-0388.2012.01005.x>
- 427 Onogi, A., Shirai, K., & Amano, T. (2017). Investigation of genetic diversity and inbreeding in a Japanese
428 native horse breed for suggestions on its conservation. *Animal Science Journal*, 88(12), 1902–1910.
429 <https://doi.org/10.1111/asj.12867>
- 430 Sabbioni, A., Beretti, V., Zanon, A., Pagani, G. P., Superchi, P., Bonomi, A., ... Catalano, A. (2005).

- 431 Caratterizzazione demografica e variabilità genetica nel cavallo Bardigiano attraverso l'analisi di dati
432 genealogici. In *59th SISVet Congress* (pp. 451–452).
- 433 Sairanen, J., Nivola, K., Katila, T., Virtala, A.-M., & Ojala, M. (2009). Effects of inbreeding and other
434 genetic components on equine fertility. *Animal*, 3(12), 1662–1672.
435 <https://doi.org/10.1017/S1751731109990553>
- 436 Schurink, A., Arts, D. J. G., & Ducro, B. J. (2012). Genetic diversity in the Dutch harness horse population
437 using pedigree analysis. *Livestock Science*, 143(2–3), 270–277.
438 <https://doi.org/10.1016/j.livsci.2011.10.005>
- 439 Sherf, B. (2000). *World Watch List for Domestic Animal Diversity*, 3rd ed. FAO, Rome.
- 440 Sørensen, M. K., Sørensen, A. C., Baumung, R., Borchersen, S., & Berg, P. (2008). Optimal genetic
441 contribution selection in Danish Holstein depends on pedigree quality. *Livestock Science*, 118(3), 212–
442 222. <https://doi.org/10.1016/j.livsci.2008.01.027>
- 443 Valera, M., Molina, A., Gutiérrez, J. P., Gómez, J., & Goyache, F. (2005). Pedigree analysis in the
444 Andalusian horse: Population structure, genetic variability and influence of the Carthusian strain.
445 *Livestock Production Science*, 95(1–2), 57–66. <https://doi.org/10.1016/j.livprodsci.2004.12.004>
- 446 Verrier, E., Leroy, G., Blouin, C., Mériaux, J. C., Rognon, X., & Hospital, F. (2010). Estimating the effective
447 size of farm animals populations from Pedigree or molecular data: a case study on two French draught
448 horse breeds. *9th World Congress of Genetics Applied to Livestock Production, Leipzig (Germany)*,
449 (May).
- 450 Vicente, A. A., Carolino, N., & Gama, L. T. (2012). Genetic diversity in the Lusitano horse breed assessed
451 by pedigree analysis. *Livestock Science*, 148(1–2), 16–25. <https://doi.org/10.1016/j.livsci.2012.05.002>
- 452 Vostrá-Vydrová, H., Vostrý, L., Hofmanová, B., Krupa, E., & Zavadilová, L. (2016). Pedigree analysis of
453 the endangered Old Kladruber horse population. *Livestock Science*, 185, 17–23.
454 <https://doi.org/10.1016/J.LIVSCI.2016.01.001>
- 455 Vostry, L., Vostra-Vydrova, H., Hofmanova, B., Vesela, Z., & Majzlik, I. (2015). Genetic diversity in Czech
456 Haflinger horses. *Poljoprivreda/Agriculture*, 21(1 sup), 163–165.
457 <https://doi.org/10.18047/poljo.21.1.sup.38>
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460 **Tables**

461 **Table 1** Description of the data available in the entire pedigree database (TP), in the reference
 462 population (RP), in the breeding population (BP), in the superior population (SP) and in the inferior
 463 population (IP).

Parameters	TP	RP	BP	SP	IP
Number of horses	9,469	3,416	742	505	495
Number of males	2,113	841	66	119	35
Number of females	7,356	2,575	677	386	460
Number of horses with no progeny	5,928	2,252	0	236	215
Number of founder horses	1,698	648	35	14	61
Number of matings between full sibs	13	6	0	0	0
Number of matings between half sibs	259	103	16	8	16
Number of matings parent-offspring	162	51	10	2	8

TP = Total population; RP = Reference population; BP= Breeding population; SP = Superior population; IP = Inferior population

464 **Table 2** Average inbreeding coefficient, rate of inbred animals (RIA) and highest inbreeding
 465 coefficient calculated for each generation from 1975-1983 to 2011-2017 in the RP.

Birth year cohort	Inbreeding †	RIA % ‡	Highest F §
1975-1983	0.03	20.0	0.25
1984-1992	0.04	29.2	0.25
1993-2001	0.06	56.3	0.32
2002-2010	0.08	77.3	0.34
2011-2017	0.10	86.5	0.35

466 † Inbreeding: mean inbreeding coefficient calculated per generation. ‡ RIA: rate of inbred Bardigiano
 467 horses within generation in percentage, if a horse showed an inbreeding coefficient higher than 0.05, it was
 468 considered as inbred. § Highest F: highest individual inbreeding coefficient found within generation.

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479 **Table 3** Population structure, inbreeding and relationship among classes of horses born in the most
 480 popular areas for the Bardigiano breeding: Genova, Piacenza, Parma and La Spezia, considering
 481 the type of land (low land, hill and mountain areas).

<u>Location</u>	<u>N. †</u>	<u>Own Father % †</u>	<u>Foreign Father % †</u>	<u>Mean F ‡</u>	<u>Mean AR ‡</u>	<u>Highest F §</u>	<u>Highest AR §</u>
GE:							
Low Land	103	4	96	0.03	0.06	0.25	0.15
GE:							
Hill	204	6	94	0.05	0.09	0.25	0.18
GE:							
Mountain	548	9	91	0.05	0.10	0.25	0.17
PC:							
Low Land	116	1	99	0.07	0.10	0.27	0.15
PC:							
Hill	956	24	76	0.09	0.13	0.35	0.19
PC:							
Mountain	598	12	88	0.10	0.13	0.21	0.18
PR:							
Low Land	201	11	89	0.08	0.11	0.27	0.17
PR:							
Hill	1899	24	76	0.08	0.10	0.31	0.18
PR:							
Mountain	1941	49	51	0.09	0.12	0.35	0.19
SP:							
Low Land	15	0	100	0.01	0.04	0.08	0.13
SP:							
Hill	175	5	95	0.06	0.09	0.22	0.15
SP:							
Mountain	30	0	100	0.01	0.03	0.01	0.07

482 † Population structure parameters: N. = number of foals born within location. Own Father = percentage of
 483 foals born from stallions belonging to the same area. Foreign Father = percentage of foals born from stallions
 484 belonging to different area. ‡ Mean inbreeding (F) and average relationship (AR). § Highest inbreeding (F)
 485 and average relationship (AR) within location. GE: Genova, PC: Piacenza, PR: Parma and SP: La Spezia.

486

487 **Figure legends**

488 **Figure 1** Animals registered per year of birth divided by sex: female and male and male with
489 offspring.

490
491 **Figure 2** Bardigiano demographic distribution and population structure within areas of Italy.
492 Regions of Italy where the 96.5% of the Bardigiano horses have been bred are highlighted. Pie
493 charts represent the percentage of stallion origin used in the breeding program within area. The
494 percentage of breeding stallion born in the same area is represented with a striped pattern, whereas
495 the percentage of foreign breeding stallions is shown as full-fill pattern. GE: Genova, PC: Piacenza,
496 PR: Parma and SP: La Spezia.

497
498 **Figure 3** Percentage of known ancestors for horses born from 1957 to 2017 for four birth year
499 cohorts where major differences were shown: 1957-1965. 1975-1983. 1984-1992. and 2011-2017.
500 Ancestral generation 1 corresponds to parents, ancestral generation 2 to grandparents, et cetera.

501
502 **Figure 4** Breeding values plotted against the average relationship of sires to horses in the reference
503 population. The mean breeding value in the sampled sires was equal to 50 and the average
504 relationship to 0.13. The rhombuses represent the 20% best sires in term of optimal contribution.

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