1	Inbreeding may affect phenotypic traits in an Italian population of
2	Basset Hound dogs
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Inbreeding may affect phenotypic traits in an Italian population of Basset Hound dogs

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26 Abstract The influence of inbreeding depression on phenotypic traits of a Basset Hound dog 27 Italian population was evaluated. Moreover this paper reports the first results of a survey on 28 morphological traits and analyse the genetic variability of the population using genealogical 29 information. The following traits were studied: height at withers, height of chest, depth of chest, 30 body length, length at rump, ischiatic width of rump, circumference of chest, circumference of 31 cannon, length of ear, and length of nose. The inbreeding coefficient (F) of each dog was 32 computed by genealogical data. Traits were taken from 75 adult (mean age 2.17±1.545 years) 33 dogs (36 males and 39 females) belonging to 19 different Italian farms. A linear regression of 34 each trait on inbreeding coefficient was used to estimate inbreeding depression. ANOVA was 35 used to test the differences in morphological traits between the sexes, among breeders and among 36 animals with different F level. Despite the good level of genetic variability and the low 37 coefficients of inbreeding observed in the population, a significant weakly effect of inbreeding 38 on the depth of chest was observed. In addition, the present study revealed no significant 39 differences in morphological traits between sexes and among farms.

40 Keywords Basset Hound dog · Pedigree analysis · Morphological traits · Inbreeding depression

41

42 **1 Introduction**

43 The Basset Hound is a hunting dog, belongs to Group 6 (Segugi e cani per pista di sangue) of the 44 ENCI (Ente Nazionale Cinofilia Italiana) and over 400 puppies are registered every year. 45 Currently in Italy there are 32 official breeders, who breed dogs of American and British 46 bloodlines. Recently Swedish and Dutch lines were also introduced. The breed standard shows 47 rather accurate morphological and behavioural characteristics, although in this breed there are 48 many variables for the height, for the colour of the coat and for the deportment. Basset Hound 49 dog is also used as a model in studies on human hereditary diseases and their gene therapy 50 (Switonski, 2012); in Italy is frequently used for pet therapy (Tomezzoli, 2015).

51 In the selection the inbreeding is used as a mating method because it allows to fix the 52 characteristics and traits of the best representatives of a breed. However this mating method can 53 lead to high rates of inbreeding with disadvantageous phenomena: in fact, inbreeding can increase 54 genetic diseases (Ubbink et al. 1992), mortality of puppies (Van der Beek et al. 1999) and can 55 cause inbreeding depression (Ubbink et al. 1998, Ólafsdóttir and Kristjánsson 2008). This 56 phenomenon is often associated with fitness reduction (Keller and Waller 2002; Brzeski et al. 57 2014, Cecchi et al. 2016a) and can also lead to a decrease in selection response for economic traits 58 in the livestock species (Khan et al. 2007, Gonzalez-Recio et al. 2007, Hossein-Zadeh 2012, 59 Malhado et al. 2013, Niknafs et al. 2013, Silió et al. 2012, Mokhtari et al. 2014). The knowledge 60 of the influence of inbreeding on reproductive traits and on the manifestation of specific diseases 61 is crucial for breeders and veterinaries: over 1000 inherited conditions have been reported in dogs 62 (Leroy, 2011) and veterinarians can contribute to the solution of the problem of hereditary 63 disorders in dogs by diagnosing these diseases and helping breeders in the mating management. 64 A better management of the existing genetic variability should constitute an important concern 65 for clubs and breeders (Nicholas et al. 2011). Moreover, it is well known that dog breeders choose 66 animals on the basis of standard characteristics and the beauty of a breed is often equivalent to 67 the functionality and also to the health of an animal. For example the primary function of a Basset 68 Hound is hunting and dogs must follow traces in ravines, brambles and scrubs. So his skin should 69 not be a hindrance to movement and should not be excessive to be entangled and injured. The 70 ears must be long in order to capture the smells of the ground but not too long, thus risking 71 becoming an obstacle to the movement. Considering the health aspect of the animal dogs with 72 excessive skin can more easily meet dermatitis or too much loose skin that falls on the forehead, 73 inevitably leads to the formation of entropion and a laxity of the lower palpebral fissure makes 74 the eye too exposed to atmospheric agents and predisposed to ulcers and conjunctivitis (Società 75 Basset Hound Italiana, 2011). For all these reasons the purposes of the present study were to 76 explore the effects of inbreeding on morphological traits in order to estimate the magnitude of 77 changes associated with an increase in inbreeding and to analyse the genetic variability and 78 morphological traits of a Basset Hound population.

80

81 2 Materials and methods

82 The research was carried out on 19 different Basset Hounds dog Italian farms (most of 83 these had 1-3 dogs and/or one sex). Genealogical and morphological data of 75 adult (mean age 84 2.17±1.545 years) dogs (36 males and 39 females) were monitored. For each male and female 85 body measurements were carried out using a Lydtin stick and tape measure; dogs were put on a 86 flat floor and held by the respective owners (Cecchi et al. 2015). The ten body measurements 87 obtained were: height at withers (WH), height of chest (ChH), depth of chest (ChD), body length 88 (BL), length at rump (RL), ischiatic width of rump (RIsW), circumference of chest (ChC), 89 circumference of cannon (CaC), length of ear (EL), and length of nose (NL).

90 Concerning genealogical data the number of inbred dogs and the inbreeding coefficient and 91 the average numerator relationships of each dog were obtained using CFC software 92 (Sargolzaei et al. 2006). A linear regression was used do predict each morphological trait based 93 on inbreeding coefficient to estimate inbreeding depression. Values were considered statistically 94 significant for P<0.05 (Confidence Interval 95%). Solutions were given per 1% increase of 95 inbreeding.

96 For the analysis of the genetic variability of the population all measured dogs constitutes the 97 Reference Population (RP), while the complete genealogy of the RP constitutes the whole 98 population (WP), containing all founders, ancestors and their offspring; the base of population 99 (BP) was defined as individuals with one or both unknown parents. The distribution of inbreeding 100 in the whole population was analysed. Different levels of inbreeding were identified and dogs 101 were divided into nine classes according to its own F level: class 1 including non-inbred animals 102 (F = 0); all the other eight classes include inbred animals (class 2: dogs with an F value between 103 0 and 0.05; class 3: dogs with an F value between 0.05 and 0.10; class 4: dogs with an F value 104 between 0.10 and 0.15; class 5: dogs with an F value between 0.15 and 0.20; class 6: dogs with 105 an F value between 0.20 and 0.25; class 7: dogs with an F value between 0.25 and 0.30; class 8:

106 dogs with an F value between 0.30 and 0.35. A multivariate analysis of variance was used to test 107 the effect of sex, inbreeding level and farm on each morphological trait, including dog age at the 108 time of the study as covariate. Three variables were entered as fixed factors: sex in the first model, 109 different inbreeding class levels and sex in the second model and farm and in the third model. In 110 this last one model we considered only the farms (n=5) who breed more than 5 dogs (27 animals 111 in total, 11 males and 16 females); it was no possible consider sex factor because some of these 112 farms have only females. Morphological data were analysed by SAS-JMP software version 5.0 113 (2002).

114

115 **3 Results and discussion**

116 Table 1 reports morphological data in males and females. For all traits, between males and 117 females, no statistical significantly differences were observed. These results were also confirmed 118 by the measures of sexual dimorphism (m/f) that ranged from 0.99 for ChC, RIsW and NL to 1.02 119 for CaC. In the breed standard (ENCI), the height at withers is the only parameter for which range 120 is reported: from 33 to 38 cm (with no differences between males and females). In this research 121 the average withers height was 35.8 ± 1.57 cm in females and 36.0 ± 1.76 cm in males. These 122 values fall exactly in the expected ranges defined by the current breed standard. Only one male 123 in the sample exceeded the maximum value (39 cm) while a female had a lower value (32 cm). 124 The age at measurement did not show significant effects as well as no significant differences in 125 the traits were observed among dogs belonging to different breeders. A few number of study have 126 been performed on the morphological parameters of dog breeds; these studies have been 127 conducted with breed dogs with a very different morphology, such as Spanish water dogs (Barba 128 Capote et al. 1996), Bolognese dogs (Beretti et al. 2008), Lagotto dogs (Vaccari Simonini et al. 129 2007) and Bracco Italiano dogs (Cecchi et al. 2015). Also Jordana et al. (1999) had analysed 130 several morphological and behavioural characters of a series of breeds, including Basset Hound, 131 in order to improve the knowledge of relationships among these breeds. However, scores were assigned to each state of different characteristics in an arbitrary manner and no analytical 132

measurements were conducted. In our study 35 dogs out 75 (46.7%) of the Reference Population were inbred and all had an inbreeding coefficient less than 0.15 (Table 2). Therefore, considering the complete database, 47 dogs were inbred. Of these dogs, 22 dogs (68.75% of the inbred) showed an inbreeding value lower than 5% while only 3 dogs (9.37% of the inbred) showed an inbreeding value higher than 20%.

138 A significant regression equation was found for the depth of chest (ChD=32.6-0,659F) with a 139 R²_{ADJ} of 13.98%. Dogs' depth of chest decreased 0.659 (p<0.05) for each 1% increase of 140 inbreeding. No statistically significant effects were detected for all other traits. Table 3 reports 141 the morphological differences among animals with various F levels. The depth of chest was 142 significantly lower in animals with F values included between 5% and 10% (P<0.05) in 143 comparison to animals with F < 5%; this effect increased in animals with F values included 144 between 10% and 15% (P<0.01). A weakly reduction of ChC due to inbreeding was also 145 observed; this effect was even highlighted by Gandini et al. (1992) in Haflingers horse breed. The 146 effects of inbreeding on morphological traits have been studied in other horse breeds: Gomez et 147 al. (2009) found significant inbreeding depression for several biometrical measurements in 148 Spanish Purebred horses. On the other hand, Curik et al. (2003) in Lipizzan horse and 149 Sierszchulski et al. (2005) in Arabian horse found no effect of inbreeding on morphological traits. 150 Wolc and Balińska (2010) reported that inbreeding was associated with a decrease in whither 151 height in Sieraków horse, but not in Dobrzyniewo and in Kobylniki horse. To our knowledge, 152 there is only the paper by Cecchi et al. (2015) on the effect of inbreeding on traits in Bracco 153 Italiano dog showing that no inbreeding depression, computed using the same model, was 154 observed on the conformation traits. It is well known that different breeds and populations, as 155 well as different traits, vary in their response to inbreeding: some populations may show a very 156 pronounced effect of increased inbreeding for a trait, whereas other populations may not 157 demonstrate much of an effect. For example, Jansson and Laikre (2014) did not find any 158 correlation between inbreeding and health in 26 dog breeds in Sweden. As reported by Barczak 159 et al. (2009), in a given population, "bad" and "good" inbreeding effects are mixed. Furthermore,

160 the degree of inbreeding depression in a population depends on the extent of inbreeding, the 161 original frequency of deleterious recessives, the environment, and inbreeding depression may be 162 greater under more stressful conditions (Marr et al. 2006).

163 Table 4 summarizes the main genealogical parameters computed by CFC. Analysis of the 164 pedigrees of the 75 studied dogs (RP) showed that the complete database resulted in 333 dogs 165 (WP), distributed in ten traced generations. Sires and dams in total were 125 and 147, 166 respectively, with a ratio dams-sires of 1.18. This value was smaller than reported on Bracco 167 Italiano dog breed (1.63) (Cecchi et al. 2013) and on Bracco Francese dog breed (1.31) (Cecchi 168 et al. 2016b). Number of animals in the base population (one or both unknown parents: absolute 169 founders = ft) was 131. Average value of inbreeding (F) in the whole population (WP) was 1.1%170 while the average value of F in the reference population (RP) was 3.2% (7.7% in the inbred). Of 171 the 19 considered farms, 12 had inbred animals with an average F ranging from 0.1 to 10.7% 172 (data not shown). Many studies have explored inbreeding in dog breeds, showing different 173 average value of the pedigree coefficient inbreeding both within and amongst breeds, which also 174 varies across countries (Różańska-Zawieja et al. 2013). The results presented in this paper suggest 175 that this population of Basset Hound had a good level of genetic variability. In fact, our results 176 were generally smaller than those reported in many other dog breeds. In particular the average 177 coefficient of inbreeding observed in this Basset Hound population for the RP (3.2%) was smaller 178 than those reported by Cole et al. (2004) in a population of Labrador Retriever (F=22%) and 179 German Shepherd (26.2%) guide dogs and by Glażewska (2008) for a small population of Polish Hounds born in the period 1970-2004 (F=26.5%-37.0%). Also Voges and Distl (2009) reported 180 181 value of inbreeding coefficients ranging from about 4.5% for Bavarian Mountain Hounds to about 182 9.5% for Tyrolean Hounds. The average inbreeding coefficients estimated by Martinez et al 183 (2011) for Cimarrón Uruguayo dog breed, reached about 4-6% while in a study on breeds raised 184 in France (Leroy et al. 2006) the average coefficient of inbreeding ranged from 3.3% in 185 Bouledougue Francais to 12.4% in Barbet breed dog. In an Italian population of Pit Bull Terrier 186 dogs the average coefficient of inbreeding was 3.73% (Ciampolini et al. 2013), while the average

187 coefficient of inbreeding in an Italian population of the dog Braque Français type Pyrénées was
4.35% (Cecchi et al. 2016b). In some Italian breeds F value ranged from 2.27% in Lagotto
189 Romagnolo (Sabbioni et al. 2008), 6.29% in Bracco Italiano dog breed (Cecchi et al. 2013) to
10.81% in Bolognese dog breed (Sabbioni et al. 2007). On the other hand, values of inbreeding
191 smaller than 1% were reported by Cecchi et al. (2009) in an Italian colony of guide dogs (0.45%
192 in German Shepherd dogs, 0.38% in Labrador dogs and 0.49% for Golden Retriever dogs) and
193 by Różańska -Zawieja et al. (2013) in the Hovawart dogs (0.26-0.31%).

194

195 4 Conclusion

196 Despite the good level of genetic variability and the low coefficients of inbreeding of 197 this Basset Hound population, our data suggest that inbreeding has effect mainly on the depth of 198 chest starting from the F values between 10% and 15%. It should be noted that according to the 199 breed standard (ENCI), the chest should be neither narrow nor excessively descended. An 200 inbreeding-related reduction in the depth of chest could thus be advantageous for those animals 201 in which an excessive depth of chest is undesirable to the farmers. In fact, farmers considered this 202 as an unpleased feature because Basset Hound was created to follow traces in ravines, brambles, 203 scrubs and other place impossible to penetrate for other hounds; an excessive chest depth that 204 rubs into the ground, is not the most suitable for hunting and would can cause difficulty in 205 movements.

We do not know the effects that F values greater than 15% might have on these traits but it would be interesting to extend the sample size considering high inbred animals in order to verify the interesting results. Furthermore, it would be interesting to calculate inbreeding depression on other very important physiological aspects such as the neonatal survival, stress resistance, fertility, reproductive success, longevity, and birth weight.

211

212 **Disclosure statement**

213	The Authors report that they have no conflicts of interest. The Authors alone are responsible for
214	the content and writing of this article.
215	
216	ACKNOWLEDGMENTS
217	This work is supported by grant of University of Pisa (Ateneo – Prof Francesca Cecchi)
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- 336 Table 1 Means, standard deviation (SD) and Sexual Dimorphism for each morphological trait in
- both males and females
- 338 **Table 2** Distribution of inbreeding coefficients in the WP and in RP
- 339 Table 3 Mean and standard error (SE) for each morphological trait in dogs subdivided
- 340 according to different inbreeding class
- 341 **Table 4** Main genealogical parameters computed for dog pedigree

		Male	Female	Farm	Sexual
		Statistics	Statistics		Dimorphism
					(m/f)
		$Mean \pm SD$	$Mean \pm SD$		
Animals	N°	36	39		
Traits					
WH	cm	36.0±1.76	35.8±1.57	ns	1.00
ChC	"	72.4±4.85	73.28±3.87	ns	0.99
CaC	"	5.6±1.00	5.5±1.12	ns	1.02
ChD		32.6±0.93	32.6±0.90	ns	1.01
ChH	"	17.5±0.38	17.3±0.40	ns	1.01
BL	"	58.5±1.08	58.01±4.90	ns	1.00
RL	"	22.8±0.50	22.8±0.57	ns	1.00
RIsW	"	31.5±1.23	31.7±0.70	ns	0.99
EL	"	23.2±0.63	23.1±0.94	ns	1.00
NL	"	10.9±0.52	11.00±0.55	ns	0.99

344 WH: height at withers; ChC: circumference of chest; ChH: height of chest; ChD: depth of Chest;

345 CaC: circumference of cannon; BL: body length; RL: length at rump; RIsW: ischiatic width of

rump; EL: length of ear; NL: Nose length. ns: not significant.

347 Significant values for $P \le 0.05$.

348 m/f = ratio between mean values of males and females

351 Table 2

Range		WP*	RP**
		n°	n°
0.00 < F < 0.05	N°	22	14
0.05 < F < 0.10	"	6	6
0.10 < F < 0.15	"	16	15
0.15 < F < 0.20	"	0	0
0.20 < F < 0.25	"	2	0
0.25 < F < 0.30	"	0	0
0.30 < F < 0.35	"	1	0

****** RP: reference population

		F=0	0 <f≤0.05< td=""><td>0.05<f≤0.10< td=""><td>0.10<f≤0.15< td=""></f≤0.15<></td></f≤0.10<></td></f≤0.05<>	0.05 <f≤0.10< td=""><td>0.10<f≤0.15< td=""></f≤0.15<></td></f≤0.10<>	0.10 <f≤0.15< td=""></f≤0.15<>
		$Mean \pm SE$	$Mean \pm SE$	$Mean \pm SE$	$Mean \pm SE$
Animals	N°	40	14	6	15
Traits					
WH	cm	35.90±0.254	36.22±0.487	35.72±0.843	35.71±0.435
ChC	"	72.85ª±0.649	71.18 ^{ab} ±1.243	71.00 ^{ab} ±1.152	70.65 ^b ±1.111
ChH	"	17.37±0.061	17.47±0.116	17.57±0.202	17.23±0.104
ChD		32.75 ^{Aa} ±0.133	32.45 ^{Aa} ±0.255	31.52 ^{Ab} ±0.441	$30.69^{B} \pm 0.227$
CaC	"	5.51±0.162	5.52±0.311	5.92±0.539	5.48±0.278
BL	"	57.99±0.551	58.48±1.055	58.25±1.828	58.85±0.943
RL	"	22.77±0.082	22.90±0.157	22.72±0.272	22.72±0.141
RIsW	"	31.42±0.131	31.54±0.249	31.97±0.432	31.90±0.223
EL	"	23.14±0.121	23.27±0.232	23.87±0.402	23.19±0.208
NL	"	10.83±0.080	10.98±0.153	11.27±0.266	11.27±0.137

WH: height at withers; ChC: circumference of chest; ChH: height of chest; ChD: depth of Chest;
CaC: circumference of cannon; BL: body length; RL: length at rump; RIsW: ischiatic width of
rump; EL: length of ear; NL: Nose length.

358 Different letters on the same row show significant differences: ^{A, B}P \leq 0.01; ^{a, b}P \leq 0.05.

359

354 Table 3

360 Table 4

	No.
Whole population (WP)	333
	125
- Sires in total	147
- Dams in total	47
Inbreed in the whole population	47
Average F in the whole population	0.011
Average F in the inbred of the whole population	0.078
Reference population (RP)	75
Inbreed in the reference population	35
Average F in the reference population	0.032
Average F in the inbred of reference population	0.077
Base population (one or two unknown parents = f_t)(BP)	131