



Conservation priorities for the different lines of Dutch Red and White Friesian cattle change when relationships with other breeds are taken into account

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1 **Conservation priorities for the different lines of Dutch Red and White**
2 **Friesian cattle change when relationships with other breeds are taken into**
3 **account.**

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15

16

17 **Summary**

18 From a genetic point of view the selection of breeds and animals within breeds for
19 conservation in a national genepool can be based on a maximum diversity strategy. This
20 implies that priority is given to conservation of breeds and animals that diverge most and
21 overlap of conserved diversity is minimised. This study investigated the genetic diversity in
22 the Dutch Red and White Friesian (DFR) cattle breed and its contribution to the total genetic
23 diversity in the pool of the Dutch dairy breeds. All Dutch cattle breeds are clearly distinct,
24 except for Dutch Friesian breed (DF) and DFR, and have their own specific genetic identity.
25 DFR has a small but unique contribution to the total genetic diversity of Dutch cattle breeds
26 and is closely related to the Dutch Friesian breed. Seven different lines are distinguished
27 within the DFR breed and all contribute to the diversity of the DFR breed. Two lines show the
28 largest contributions to the genetic diversity in DFR. One of these lines comprises unique
29 diversity both within the breed and across all cattle breeds. The other line comprises unique
30 diversity for the DFR but overlaps with the Holstein Friesian breed. There seems to be no
31 necessity to conserve the other 5 lines separately, because their level of differentiation is very
32 low.

33 This study illustrates that, when taking conservation decisions for a breed, it is worthwhile to
34 take into account the population structure of the breed itself and the relationships with other
35 breeds.

36

37 **Keywords:** conservation, genetic diversity, population structure, relationships with other
38 breeds

39

40

41

42 **Introduction**

43 Farm animal breeds are recognized for different values, with economic, social, historic and
44 cultural aspects (Gandini and Oldenbroek 2007). Genetic diversity is the basis for the
45 development and survival of animal breeds. However, many traditional, local, farm animal
46 breeds have small (effective) population sizes, leading to a loss of their genetic diversity. It is,
47 therefore, especially important to maintain genetic diversity in these small populations of
48 farm animals (Fernandez, Meuwissen et al. 2011). Small populations of local breeds often
49 comprise genetic variation with cultural, historical, sociological and environmental values
50 (Hiemstra, De Haas et al. 2010) generally not present in the global highly productive breeds
51 that dominate modern intensive livestock production systems. Genetic management of local
52 breeds, is crucial for their own survival, and for maintaining diversity in the entire species,
53 because the genetic diversity between breeds is a substantial part of the genetic diversity
54 within the species (Wooliams and Toro 2007).

55 Maintaining high levels of within breed genetic diversity is the second important aim in
56 conservation genetic diversity within the species. Traditionally, animal breeders quantify genetic
57 diversity by analysing pedigrees, and estimating average kinships and inbreeding levels
58 (Gutiérrez, Altarriba et al. 2003; Wooliams and Toro 2007). Pedigree analysis may not be
59 adequate, since pedigrees are often not available in depth, so that a reliable quantification of
60 within breed variation may not be possible. Moreover, pedigrees are generally only known since
61 breed formation making analysis of between breed diversity by pedigree analysis impossible.

62 Methods based on pedigree analysis can now be complemented with molecular genetic
63 information facilitating analysis of diversity both within and across breeds (Boettcher, Tixier-
64 Boichard et al. 2010).

65 Besides small effective population size, local breeds may be threatened by indiscriminate
66 crossing with other breeds. Crossing may lead to increased genetic diversity in a population,

67 however at the expense of losing part or eventually all of the original genetic diversity in the
68 population (FAO 2007). Thus both within and across breed variation need to be considered in
69 order to preserve genetic diversity within species (Bennewitz, Eding et al. 2007; Wooliams
70 and Toro 2007; Boettcher, Tixier-Boichard et al. 2010; Roberts and Lamberson 2015).

71 Eding *et al* (2002) provided a framework to quantify relative amounts of both within- and
72 across population genetic diversity by using marker estimated kinships. In this method
73 kinships are estimated with the help of markers and the genetic diversity within a breed is
74 estimated as one minus the average kinship in that breed. The average kinship is also
75 estimated across breeds, so that the genetic diversity of a set of breeds can be determined.
76 Moreover, for each breed its contribution to the diversity of the total set can be quantified,
77 thereby quantifying both its unique diversity and the overlap with other breeds.

78 After the study of Eding *et al.* (2002) progress in genotyping techniques has increased the
79 number of available markers. The availability of dense molecular marker maps can provide a
80 more precise picture of the genetic background of breeds (e.g. distances, uniqueness), which
81 increase the capabilities for making decisions aimed at maintaining genetic diversity.

82 In this study the maximum diversity strategy was used to quantify the genetic diversity
83 (Bennewitz, Eding et al. 2007). This strategy selects breeds that contribute in a significant
84 way to the overall genetic diversity considering both within and across breeds diversity.

85 For local breeds, next to setting conservation priorities at breed level, a more detailed division
86 into lines can be helpful to determine conservation priorities within the breed.

87 The objective of this study was to quantify the genetic diversity in a numerically small breed
88 and its contribution to the total genetic diversity in other breeds of the same species in the
89 same country. For these objectives, we used the Dutch Red and White Friesian cattle (DFR)
90 and quantified the relationship with other Dutch dairy breeds. We assessed:

91 (a) The relationship of DFR with other Dutch dairy breeds and the contribution of the DFR to
92 the total genetic diversity in Dutch dairy cattle breeds.

93 (b) The genetic differences between lines within the DFR

94 (c) The contribution of the within line genetic diversity to the total genetic diversity in the
95 DFR, and to the gene pool of the Dutch dairy cattle breeds.

96

97 **Materials and methods**

98 *Animals and Genotypes*

99 A total of 68 Dutch Red and White Friesian cattle (DFR) animals (26 bulls and 42 cows) were
100 sampled. The DFR is a local breed in the North of the Netherlands. Anecdotally and
101 according to herdbook information it is closely related to the Dutch Friesian (DF) breed which
102 is one of the founding breeds of the Holstein Friesian which is now the dominant dairy cattle
103 breed in the world (Felius, Koolmees et al. 2011). Of the 68 sampled DFR animals, 48
104 animals were assigned to different lines, based on their ancestry from (founding) sires, within
105 the breed by the Dutch herdbook “Stichting Roodbont Fries Vee” (Table 1). Two other
106 groups consist of animals not (yet) registered in the herd book: one group from two farms
107 with some Holstein Friesian (HF) blood and another group of isolated animals originating
108 from the Dutch island Terschelling, from here on referred to as line 6 and 7 respectively.

109

110 Table 1. Number of samples per line of Dutch Red and White Friesian animals

Line	Name	#Bulls	#Cows	total
1	Jet	5	4	9
2	Marco-Kei	3	5	8
3	Koos	5	5	10
4	Reitsma	4	7	11
5	DF-line	8	2	10
6	Elsinga line		11	11
7	Terschelling	1	8	9
total		26	42	68

111

112 To obtain DNA we collected hair samples from the cows. From the bulls semen straws were
113 provided by the Centre for Genetic Resources, the Netherlands (CGN). Samples were chosen,
114 based on pedigree information of the herd book, so that they represent a wide variation in
115 origin within a line. Samples were genotyped using the BovineSNP50 BeadChip (Illumina
116 Inc., San Diego, CA, USA). All samples had a genotype call rate $> 85\%$. During the quality
117 check SNPs with a GenCall score ≤ 0.20 and call rate $\leq 85\%$ were deleted from the analyses
118 ($n=2,635$). Missing genotypes were imputed using Beagle with 20 iterations (Browning and
119 Browning 2009). The imputation was carried out for each chromosome independently. The
120 mean r^2 value for the accuracy of imputation provided by Beagle was 0.98. After these editing
121 steps 51,974 of the initial 54,609 SNPs remained.

122 Data from the DFR cattle were supplemented with data originating from studies with four
123 other Dutch breeds (Maurice-van Eijndhoven 2014; Pryce, Johnston et al. 2014; Maurice-Van
124 Eijndhoven, Bovenhuis et al. 2015). These data included 1,287 purebred cows; 989 were
125 Holstein Friesian (HF), 97 Groningen White headed (GWH), 137 Meuse-Rhine-Yssel
126 (MRY), and 64 Dutch Friesian (DF). Previously performed editing steps to remove
127 uninformative SNP are described by Hulsegge *et al.* (2013). In short, Holstein Friesian
128 animals were genotyped with a BovineSNP50 BeadChip and imputed to the BovineHD
129 BeadChip using Beagle (Browning and Browning 2009). The mean Beagle r^2 was 0.96 across
130 the imputed loci. Animals from the three other breeds (GWH, MRV and DF) were genotyped
131 with the BovineHD BeadChip. The editing steps comprised deleting SNP with call rate $<$
132 95% , GenCall score ≤ 0.20 and GenTrain score ≤ 0.55 . No MAF (minor allele frequency)
133 thresholds were applied in the editing procedure. To investigate whether differences in results
134 could arise with edits based on MAF, as is commonly done in other studies or applications,
135 the impact of MAF threshold 0.02 was evaluated. The preliminary analyses indicated that our
136 results and conclusions were hardly affected when not applying such editing step (results not

137 shown). After the editing steps 750,457 of the 777,962 SNP remained. These 750,457 SNP
138 contained 36,625 SNP that were also included in the DFR data after editing. For all animals,
139 genotypes on those 36,625 SNP were used in further analyses.

140

141 *Breed identity of DFR*

142 To investigate whether DFR is a breed with its own genetic identity, and to visualize the
143 relationship between DFR and the four other Dutch cattle breeds, principal component
144 analysis (PCA) was performed on the SNP genotypes (Price, Patterson et al. 2006) (Patterson,
145 Price et al. 2006) using the R-package Hierfstat (Goudet 2005). Genetic divergence between
146 each breed pair was quantified by calculating pairwise F_{ST} (Weir and Cockerham 1984) using
147 the R-package Hierfstat (Goudet 2005).

148

149 *Contribution of DFR to total genetic diversity in Dutch dairy cattle.*

150 To quantify the importance of DFR relative to the other breeds the marker estimated kinships
151 and the core set method of Eding *et al.* (2002) were used. In this method kinships are
152 estimated with the help of markers and the genetic diversity within a breed is estimated as one
153 minus the average kinship in that breed. The average kinship is also estimated across breeds,
154 so that the genetic diversity of the whole set can be determined. The total genetic diversity of
155 a set depends on the contribution of each breed to the total set. If all breeds contribute equally,
156 the total genetic diversity is equal to one minus the average within and across breed kinships.
157 Otherwise breed kinships have to be weighted by their contribution e.g.

158

$$g_{div} = 1 - \mathbf{c}'\mathbf{M}\mathbf{c},$$

159 with \mathbf{c} being the vector with n (number of breeds) contributions of each breed (summing up to
160 1) and \mathbf{M} being the $n \times n$ matrix with within and across breed kinships. So, if a relatively

161 uniform breed contributes more to the total set the genetic diversity of the total set will be
162 lower compared to when a relatively diverse breed contributes more.

163 In the core set method of Eding *et al.* (2002) the contribution of each of the breeds that
164 maximise the genetic diversity is estimated as

$$165 \quad \mathbf{c}_{\max} = \frac{\mathbf{M}^{-1}\mathbf{1}_n}{\mathbf{1}_n'\mathbf{M}^{-1}\mathbf{1}_n}$$

166 Where \mathbf{c}_{\max} is a vector with the contributions that maximises the diversity in the total set, and
167 $\mathbf{1}_n$ is a vector of n ones. The total diversity in the set is then estimated by:

$$168 \quad Div_{set} = 1 - \mathbf{c}_{\max}'\mathbf{M}\mathbf{c}_{\max} = \frac{1}{\mathbf{1}_n'\mathbf{M}^{-1}\mathbf{1}_n}$$

169 The contribution of each breed to this core set thus depends both on the between- and within-
170 breed components of genetic diversity. However, not only the contribution determines the
171 relative importance of a breed for the total genetic diversity. A breed may contribute a small
172 amount to the core set (e.g. when their within breed kinship is high) but nevertheless increase
173 the total genetic diversity considerably (e.g. when its across breed kinships are low).

174 Therefore, the average kinship of the core set when the breed is included is compared to the
175 average kinship of the core set when the breed is excluded (Eding, Crooijmans *et al.* 2002).

176 The required kinships were obtained by first computing a genomic relationship matrix (G)
177 according to Yang *et al* (2010) using the software Calc_grm (Calus 2013). Using those
178 genomic relationships, average within and between breed kinships were computed across all
179 pairwise relationships within and between breeds, including self-kinships.

180

181 *Contribution of lines to genetic diversity within DFR*

182 To visualize the separation of the different lines based on molecular genetic data, PCA was
183 used. The core set method was used to determine the relative contribution of each line to the

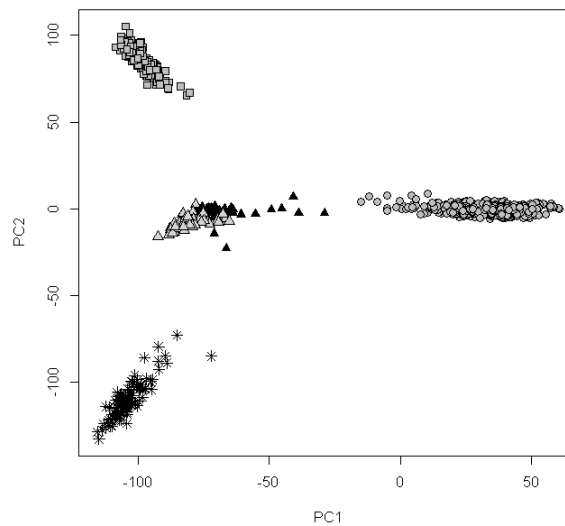
184 total genetic diversity in the DFR. The core set method was also performed with both the
185 DFR lines and the other breeds simultaneously, to determine the overlap of the contribution of
186 the individual DFR lines to the total genetic diversity with the contribution of other breeds.

187

188 **Results**

189 *Relationship of DFR cattle breed with other Dutch dairy breeds*

190 The combination of the first and second principal components (PC1 and PC2) separated
191 individual animals according to their breed (Fig. 1). PC1 distinguished the four local breeds
192 from the commercial breed HF. PC2 separated the local breeds MRY on the one hand and
193 GWH on the other hand from the Friesian breeds (DF, DFR and HF). Based on the first two
194 principal components overlap existed between the DF and DFR.



195

196 Figure 1 Principal component analysis (PCA) of five Dutch dairy cattle breeds based on 36
197 625 single-nucleotide polymorphisms (SNP's) [circle grey = Holstein Friesian (HF); star =
198 Groningen White headed (GWH); triangle grey = Dutch Friesian (DF); square grey = Meuse-
199 Rhine-Yssel (MRY); triangle black = Dutch Red and White Friesian (DFR)].

200

201 Genetic differentiation (pairwise F_{ST}) among breeds, confirmed that DFR is genetically
 202 closest to DF ($F_{ST}=0.056$) (Table 2). Pairwise F_{ST} values ranged from 0.056 (between DFR
 203 and DF) to 0.156 (between GWH and DF). The kinship values also indicated that DFR and
 204 DF were more related to each other than to the other breeds. DFR and DF had the highest
 205 average between-breed kinship (0.033) (Table 2). Average between-breed kinship ranged
 206 from -0.078 to 0.033.

207
 208 Table 2. Estimated pairwise F_{ST} as a measure of genetic differentiation (below diagonal) and
 209 average genomic kinship (above diagonal) between five Dutch dairy cattle breeds.

	GWH	DF	MRY	HF	DFR
G	-	-0.078	-0.057	-0.053	-0.068
DF	0.156	-	-0.067	-0.056	0.033
MRY	0.155	0.135	-	-0.031	-0.050
HF	0.132	0.111	0.110	-	-0.036
DFR	0.136	0.056	0.111	0.088	-

210
 211
 212 DFR showed the lowest average within-breed kinship (0.106) and GWH the highest (0.248)
 213 (Table 3). The total diversity of the Dutch cattle breeds was 0.926. All five breeds contributed
 214 almost equal to the overall genetic diversity (varying from 19.55% to 20.64%). The highest
 215 unique genetic diversity was observed for GWH (0.015) and the lowest for DFR (0.006).
 216 Nevertheless, the DFR contains some unique genetic diversity not present in the other Dutch
 217 breeds, although it is less than the unique diversity of the other breeds (Table 3).

218

219 Table 3. Average genomic kinship (f) within breeds and contribution of breeds to a core set in
 220 which the diversity is maximised (= average f minimised). Unique diversity is measured as
 221 the increase in f when the core set is formed without a contribution of that breed.

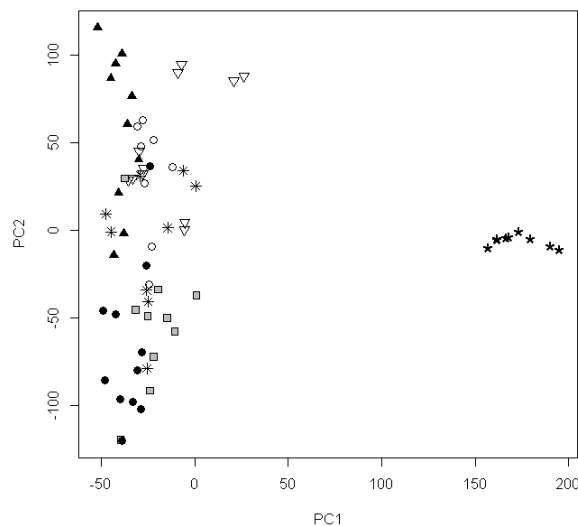
	f	contribution	Unique diversity
DFR (all lines)	0.106	19.84%	0.006
GWH	0.248	19.93%	0.015
DF	0.155	19.55%	0.007
MRY	0.199	20.04%	0.012
HF	0.174	20.64%	0.010
Core set	0.074		-

222

223

224 *Genetic differences between DFR lines*

225 PCA distinguished DFR line 7 from the other lines by the first principal component (Fig. 2).



226

227 Figure 2 Principal component analysis (PCA) of seven Dutch Friesian Red cattle lines on 36
 228 625 single-nucleotide polymorphisms (SNPs) [star = DFR line 1; circle white = DFR line 2;
 229 triangle point up black = DFR line 3; circle black = DFR line 4; square grey = DFR line 5;
 230 triangle point down = DFR line 6; asterisk = DFR line 7].

231

232 There was some differentiation among the other lines along the second principal component,
 233 but with a large overlap between the different lines. Genetic differentiation between the

234 different DFR lines was also confirmed by the pairwise F_{ST} , which varied between 0.012 and
 235 0.190 (Table 4). Consistent with the PCA results, the F_{ST} values indicated that line 7 clearly
 236 diverged from the other lines. Pairwise F_{ST} between DFR line 7 and the other six lines ranged
 237 from 0.149 to 0.190, while the maximum pairwise F_{ST} between the lines 1 to 6 was 0.078
 238 (between DFR lines 3 and 4). The F_{ST} values between DFR lines 1 to 6 were lower than the
 239 F_{ST} values between breeds (Table 2), meaning that the DFR lines 1 to 6 were more related to
 240 each other than the breeds were. The F_{ST} values between DFR line 7 and the other lines were
 241 somewhat higher than the values found between the breeds as presented in Table 2.

242 The average kinships between-line and within-line of the DFR breed are presented in Table 4
 243 and 5. Within-line kinship were higher (Table 5; varying between 0.131 and 0.478) compared
 244 with the between-line kinship (Table 4; varying between 0.041 and 0.157). The lines 1 to 5
 245 were more related to each other than to the lines 6 and 7. DFR line 7 showed the highest
 246 within-line kinship (0.478) and the lowest between-lines kinship (ranging from 0.041 to
 247 0.053). DFR line 6 had the lowest level of within-line and the second lowest level of between-
 248 line kinship.

249

250 Table 4. Estimated pairwise F_{ST} as a measure of genetic differentiation (below diagonal) and
 251 average genomic kinship (above diagonal) between 7 DFR lines.

	DFR line 1	DFR line 2	DFR line 3	DFR line 4	DFR line 5	DFR line 6	DFR line 7
DFR line 1	-	0.119	0.123	0.135	0.095	0.078	0.052
DFR line 2	0.042	-	0.140	0.114	0.091	0.080	0.053
DFR line 3	0.061	0.058	-	0.112	0.110	0.108	0.045
DFR line 4	0.036	0.056	0.078	-	0.157	0.069	0.043
DFR line 5	0.040	0.046	0.059	0.012	-	0.063	0.042
DFR line 6	0.048	0.049	0.057	0.063	0.046	-	0.041
DFR line 7	0.158	0.166	0.190	0.171	0.149	0.149	-

252

253

254 The contribution of each line (in %) to the DFR breed are shown in Table 5. All lines
 255 contributed to the diversity of the DFR breed. The highest contribution to the total diversity of
 256 the DFR breed was observed for line 6 (26.02 %), while line 3 showed the smallest
 257 contribution (6.81%). The total diversity of the DFR was 0.874. The largest part of diversity
 258 of most lines is represented in the other lines as well. The highest impact on the diversity was
 259 observed when line 6 or line 7 was removed, leading to a decrease in overall diversity of the
 260 DFR breed by about 2.3% and 1.6 %, respectively. Removing one of the lines 1 to 5 had only
 261 a small impact on the diversity. Apparently, the diversity contained in these lines is almost
 262 completely present in the other lines as well.

263

264 Table 5. Average genomic kinship (f) within lines and contribution of lines to a core set in
 265 which the diversity is maximised (= average f minimised). Unique diversity is measured as
 266 the increase in f when the core set is formed without a contribution of that breed/ line.

	DFR lines			All breeds/lines	
	f	contribution	Unique diversity	contribution	Unique diversity
DFR line 1	0.176	12.63%	0.005	13.26%	0.0002
DFR line 2	0.192	11.70%	0.004	11.90%	0.0002
DFR line 3	0.265	6.81%	0.001	15.37%	0.0003
DFR line 4	0.205	10.01%	0.002	16.35%	0.0002
DFR line 5	0.140	19.02%	0.008	14.97%	0.0002
DFR line 6	0.131	26.02%	0.020	14.82%	0.0002
DFR line 7	0.478	13.81%	0.014	13.21%	0.0004
Core set	0.126		-		

267

268

269 *Contribution of the DFR lines to the total genetic diversity.*

270 The average kinship between DFR lines and the Dutch cattle breeds are presented in Table 6.

271 This kinship varied from -0.079 to 0.085. The highest values were estimated between DFR

272 lines and DF, while the lowest values were observed between DFR lines and GWH. Line 6
 273 was the line most closely related to HF, and line 7 the line least related to DF.

274

275 Table 6. Average genomic kinship between Dutch cattle breeds and DFR lines.

	GWH	DF	MRY	HF
DFR line 1	-0.065	0.027	-0.046	-0.040
DFR line 2	-0.065	0.030	-0.048	-0.040
DFR line 3	-0.073	0.031	-0.054	-0.045
DFR line 4	-0.074	0.050	-0.058	-0.051
DFR line 5	-0.079	0.085	-0.065	-0.056
DFR line 6	-0.058	0.010	-0.046	-0.003
DFR line 7	-0.060	-0.007	-0.030	-0.017

276

277

278 Results of assessing the impact of removing one line from the DFR breed and calculating the
 279 contribution of each line (in %) to the pool of Dutch dairy cattle breeds with maximal genetic
 280 diversity are shown in Table 5. When considering all Dutch dairy cattle breeds, removing one
 281 of the DFR lines has a small impact on the diversity (loss of 0.0002 to 0.0004; Table 5).

282 When considering all breeds the contribution of DFR line 6 was considerably smaller
 283 (14.82%) compared to DFR lines analysed in separation (26.02%). This was due to the
 284 inclusion of the HF breed, removing the HF breed increased the contribution of line 6 with
 285 4,7% (results not shown). The contribution of DFR line 5 to the diversity across all breeds is
 286 also smaller (14.97%) compared to DFR lines only (19.02%). For DFR line 3 the contribution
 287 to the diversity across all breeds is larger (15.37%) compared to DFR lines only (6.81%).

288 Removing DF increased the contribution of DFR, especially by the contribution of line 5.

289 Thus analysing DFR in isolation of the other breeds suggests for some lines a larger
 290 proportion of unique diversity, while part of this diversity apparently is due to influences of
 291 the other breeds, in particular DF and HF, as revealed by the analysis including other breeds.

292 **Discussion**

293 *Relationship of DFR cattle breed with other Dutch dairy breeds*

294 Genetically, Dutch cattle breeds are clearly distinct from each other as shown by the PCA
295 results, except for DF and DFR. As expected from breed history, the DFR breed is closely
296 related to the DF breed (FAO 2007). These breeds were recorded as separate breeds for
297 slightly more than 100 years. Red offspring of the DF breed, born out of the combination of
298 two red-factor-carriers, could be incorporated in the DFR-breed. From 1970 DF and DFR
299 became rare. (Porter 2002). Genetic differentiation between the breeds (pairwise F_{ST}) and the
300 between breed kinship also indicated that DFR and DF were more related to each other than
301 to the other Dutch breeds. In European cattle breeds, pairwise F_{ST} values have been reported
302 i.e. ranging from 0.035 to 0.132 (Gautier, Faraut et al. 2007) and from 0.059 to 0.142
303 (Neuditschko 2011). The F_{ST} between DFR and DF of 0.056 is at the lower end of these
304 ranges. DFR showed a reasonable contribution (19.84%) to the total genetic diversity of
305 Dutch cattle breeds and contains a small amount of genetic diversity not present in the other
306 Dutch breeds. This contribution is comparable to the contribution of each of the other breeds.
307 Thus, although DFR and DF are closely related, the results of this study showed that DFR
308 has its own genetic identity, containing some genetic diversity not present in other breeds.

309

310 *Genetic management of lines within breeds*

311 Management of breeds subdivided in lines implies a compromise of different factors: first, the
312 maintenance of the highest possible levels of genetic diversity for the whole breed; second,
313 the preservation of the genetic differentiation between lines; and third, the restriction of
314 within-line diversity to acceptable levels, so inbreeding would not increase beyond these
315 acceptable levels (Fernández, Toro et al. 2008). The results of our study revealed a high level
316 of admixture between line 1 to 5. This reflects the similar origin of these lines. Consequently,

317 there seems to be no necessity to conserve these 5 lines separately, because their level of
318 differentiation is very low. The line with the highest overall contribution to diversity in DFR
319 is line 6. However, part of this diversity is due to some HF blood and therefore of lower
320 conservation value.

321 The pairwise F_{ST} values indicated that DFR line 7 had a high level of genetic differentiation
322 from other lines. This line has been bred for a considerable time in isolation from the other
323 lines, and apparently conserved genetic diversity not present anymore in the rest of the
324 population. However, this line showed high levels of inbreeding, and a low level of diversity.

325

326 *Contribution of lines within breeds to the total genetic diversity across breeds.*

327 A way to measure the influence of one line over the others in the DFR breed is to ascertain its
328 genetic contribution to diversity by removing this line from the whole DFR breed and
329 determining the remaining genetic diversity (Caballero and Toro 2002; Eding, Crooijmans et
330 al. 2002). However, the results are different when relationships of other Dutch cattle breeds
331 are taken into account. Some DFR lines contains a portion of genetic diversity which is also
332 represented in the other Dutch cattle breeds. Maximizing genetic diversity within a breed is
333 therefore not always the best strategy. Thus, our results demonstrate that when establishing
334 conservation programs, it is necessary to take relationships with other breeds into account as
335 well. Lenstra (2006) also indicated that for decisions on conservation priorities, the diversity
336 of all local breeds related to the endangered population should be taken into account in order
337 to assess their unique contribution to diversity.

338

339 *Assessing contributions of lines without pedigree relationship to herdbook animals.*

340 Previously, pedigree information was the most important information used for registration of
341 animals in a herdbook.. Use of genome-wide SNP information, now provides a way to assess
342 the relationship of animals without pedigree to animals registered in a herdbook. The Dutch

343 DFR herdbook “Stichting Roodbont Fries Vee” had assigned 48 sampled animals in this study
344 to five different lines. Two additional DFR lines were defined consisting of animals that were
345 not registered (DFR line 6 and 7). The lines might be considered as sub-populations, but there
346 are no formal restrictions on pairing animals from different lines with each other, whereas,
347 crosses between animals of different breeds are considered crossbreeds and not registered as
348 belonging to either breed. Consequently, in the context of diversity relationships between
349 lines are generally much higher than relationships between breeds.

350 For the lines without an official pedigree the results of this study showed similarities and
351 differences to the five lines (DFR line 1 to 5) with an official pedigree. This study indicated
352 that line 6, a group with some HF blood, indeed represents part of the HF genetic diversity.
353 Currently there seems to be no necessity to conserve DFR line 6. However, conserving line 6
354 *in situ* may be useful in practice for several reasons: first, this line consists of approximately
355 100 animals, while the total population size of DFR is 500; second, to increase the milk
356 production of the DFR breed; and third, to increase the genetic diversity of DFR and
357 consequently to decrease the chance of inbreeding. However, conserving line 6 should not be
358 at the expense of other lines.

359 This study distinguished DFR line 7 from the other DFR lines. However, considering all
360 Dutch cattle breeds line 7 is closely related to DFR and FH. This isolated group of animals
361 will maximize the level of genetic diversity for the whole DFR breed and will increase
362 genetic differentiation between lines, despite its high levels of inbreeding. Therefore, line 7
363 makes a unique contribution to the DFR cattle, and it is worthwhile to include this line
364 without an official pedigree in the herdbook. The DFR herdbook and breeders are now
365 considering the inclusion of line 6 and line 7 in the herdbook. It is often not possible, and
366 may also not be desirable, to conserve all breeds/lines, mostly due to financial limitations
367 (Bennewitz, Eding et al. 2007). As shown in this study, taking relationships with other breeds

368 into account can change conservation priorities within a breed, and thus may affect
369 conservation decisions made for this breed. This is not only applicable to the lines within a
370 breed in this study, but also for breeds within a species or in a gene pool of national breed as
371 in this study.

372 Conservation decisions also should take into account the degree of endangerment and costs of
373 conservations and economic, cultural and historic values of different characteristics of a breed
374 (Simianer, Marti et al. 2003) (Bennewitz, Eding et al. 2007). Endangerment of most DFR
375 lines is similar, however line 7 is clearly more endangered, since the owner has stopped active
376 farming. DFR line 6 had the highest overall contribution to diversity in DFR, however when
377 considering HF the contribution of DFR line 6 was considerably smaller, indicating that the
378 endangerment of line 6 is not really a threat for the DFR breed as a whole. Consequently,
379 conservation priorities based on genetic diversity coincides with priority based on degree of
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381

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