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Variance components using genomic information for two functional traits in Italian Simmental cattle: calving interval and lactation persistency. *by Cesarani et al.* Modern breeding programs aim to improve functional traits, such as fertility and/or lactation persistency. Calving interval is an easily accessible indicator of female fertility, but is lowly heritable and has moderate, unfavorable correlations with yield traits. Lactation persistency is also lowly heritable. We investigated the genetic background of calving interval and lactation persistency and estimated the genetic parameters that are needed to include both traits and milk production for the Italian Simmental breeding program. There is a potential to implement a single step-genomic prediction model by using both genomic and pedigree information.

VARIANCE COMPONENTS OF FUNCTIONAL TRAITS

Variance components using genomic information for two functional traits in Italian Simmental cattle: calving interval and lactation persistency

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ABSTRACT

Functional traits, such as fertility and lactation persistency, are becoming relevant breeding goals for dairy cattle. Fertility is a key element for herd profitability and animal welfare: in particular, calving interval (CI) is an indicator of female fertility that can be easily recorded. Lactation persistency (LPE), i.e. the ability of a cow to maintain a high milk yield after the lactation peak, is economically important and it is related to several other traits, such as feed efficiency, health, and reproduction. The selection of these functional traits is constrained by their low heritability. In this study, variance components for CI and LPE in the Italian Simmental cattle breed were estimated using genomic and pedigree information under the single-step genomic framework. A data set of 594,257 calving interval records (from 275,399 cows), 285,213 lactation persistency records (from 1563,389 cows) was considered. Phenotypes were limited up to the third parity. The pedigree contained about 2 million animals, and 7,246 genotypes were available. LPE was estimated using principal component analysis on test day records, with higher PC2 values associated to lower LPE and lower PC2 values to higher LPE, respectively. Heritability of CI and LPE were estimated using single-trait repeatability models. A multiple trait analysis using CI and production traits (milk, fat, and protein yields) was performed to estimate genetic correlations among these traits. Heritability for CI in the single-trait model was low (0.06 ± 0.002). Unfavorable genetic correlations were found between CI and production traits. A measure of LPE was derived using principal component analysis on test-day records. The heritability and repeatability of LPE were 0.11 ± 0.004 and 0.20 ± 0.02 , respectively. Genetic correlation between CI and LPE was weak but had a favorable direction. Despite the low heritability estimates, results of the present work suggest the possibility of including these traits in the Italian Simmental breeding program. The use of single-step approach can potentially provide better results for young genotyped animals without own phenotypes.

Keywords: fertility, persistency, genomic selection

INTRODUCTION

In the last century, the combined action of genetic, management, and feeding advancements has led to a substantial improvement of dairy cow performances. Such results have been, however, accompanied by a general deterioration in fitness traits (Sun et al., 2019). The progressive reduction of cow fertility is one of the most relevant examples of the unfavorable consequences of selection for production traits (Ma et al., 2019). Relationships between fertility and genetic improvement for milk production have been investigated (Castillo-Juarez et al., 2000; Lucy, 2001; Hayes et al., 2009; Walsh et al., 2011). Reproduction traits have been, therefore, included in breeding programs (Philipsson and Lindhé, 2003; López-Gatius, 2003; Miglior et al., 2005) even though a clear definition of phenotypes is still an issue. For example, fertility traits can be defined in many ways: time lengths (i.e. days open), or frequencies (i.e. number of inseminations needed to conceive). Generally, these traits are difficult to record routinely and have low heritability. Among fertility traits, calving interval (CI) is one of the easiest to record (Dal Zotto et al., 2007); however, it can be biased for not being available for cows that do not re-calve because of poor fertility. Other traits like conception or pregnancy rates could be better fertility traits, but they need additional resources to be collected. A longer CI is usually a consequence of more inseminations needed for the cow to conceive, with increased costs for the herd, and therefore, is undesirable. Moreover, cows with longer CI will have fewer calvings in their life. Selection for shorter CI would increase herd profitability: more calvings per cow, more offspring that can be sold or used as replacement, decreased feed costs, and less reproductive problems (Esslemont et al., 2001; Groenendaal et al., 2004; Atashi et al., 2013).

Reproductive performance is also strongly related to lactation persistency (LPE), i.e. the ability of the cow to maintain high levels of production after the lactation peak (Dekkers et al., 1998). LPE is a production trait connected to health, reproduction, and feed costs (Koloi et al., 2018). The relationship between CI and LPE in cattle has been investigated (Atashi et al., 2013; Němečková et al., 2015). Muir et al. (2004) reported a moderate, positive genetic correlation between CI and LPE

79 suggesting an unfavorable and antagonistic relationship between these two traits. This result was
80 confirmed by Atashi et al. (2013), who found that cows with shorter CI were less persistent in milk
81 production. Although there is a general consensus on the basic concept of LPE, generally defined as
82 the ratio between the milk measured in a certain period and the total milk yield, several measurements
83 have been proposed for this trait (Gengler, 1996; Sölkner and Fuchs, 1987; Cole and VanRaden,
84 2006; Strabel and Jamrozik, 2006; Togashi and Lin, 2006). Macciotta et al. (2006) have defined the
85 LPE in Italian Simmental using a principal component (PC) approach. The advantage of this approach
86 is that the measure of LPE is independent of milk production.

87 The Italian Simmental is a dual-purpose cattle breed farmed mostly in Northeastern Italy. Its
88 breeding program aims to improve both dairy and beef traits, and an economic selection index has
89 been developed for this purpose. Recently, the introduction of new functional traits such as CI and
90 LPE has been suggested; however, variance components for these traits were not available for this
91 breed in Italy. Thus, to investigate the genetic background of these two traits, the present study
92 focused on the estimation of genetic parameters for CI and its relationship with production traits and
93 LPE in Italian Simmental cattle using a genomic approach.

94

95 **MATERIALS AND METHODS**

96 *Calving interval*

97 CI phenotypes recorded from 275,399 cows in the period 1983 to 2017 were used. The number
98 of parities per cow ranged from 1 to 3, and the first record was mandatory in order to include a cow
99 in the analysis. A total of 594,257 CI records that were greater than 300 days and lower than 700 days
100 were retained for analysis.

101 Heritability and repeatability for CI were estimated using repeatability single-trait model:

102
$$y = \mu + \text{par} + a + pe + e \quad [1]$$

103 where **y** was the CI record; **μ** was the fixed effect of herd-year combination (103,467 levels); **par**
104 was the fixed effect of parity (three levels: 1-2, 2-3, 3-4); **a** was the random additive genetic effect

105 (465,633 animals in the relationship matrix); **pe** was the random effect of permanent environment
106 (465,633 levels); **e** was the random residual.

107 Genetic correlations between fertility and production traits were estimated using a multiple
108 trait animal model with the same structure of [1] considering CI, milk (MY), fat (FY) and protein
109 (PY) 305-d yield (kg). All available records for these 4 traits were included in the analysis (713,376
110 records from 274,759 cows). Average values of 5,687±1,676 kg, 221±81 kg, and 194±70 kg were
111 observed for MY, FY, and PY, respectively.

112

113 *Lactation persistency*

114 Genetic parameters for LPE were investigated using a data set with 285,213 lactation records
115 of 156,389 cows (parities ranging from 1 to 3) farmed in 5,344 herds. Each lactation (from 5 to 305
116 days in milk) was divided into seven intervals and one record per interval was kept. When more than
117 one test day per interval was available, the average value was used. Seven intervals were chosen
118 because the majority of cows have this number of controls available in the routine evaluation system
119 of Italian Simmental. The availability of at least one record before the 45th day of lactation and after
120 the 245th day of lactation was mandatory to include a cow in the analysis. Since milk, fat and protein
121 daily yields showed a similar decreasing trend along lactation, we decided to consider only milk yield
122 to define LPE. Thus, data were arranged in a multivariate framework and a principal component
123 analysis using SAS PROC PRINCOMP procedure was performed to extract eigenvalues and
124 eigenvectors of the phenotypic correlation matrix of test-day records. The second extracted principal
125 component (PC2) was used as an indicator of persistency (Macciotta et al., 2006). The PC2 scores
126 were analyzed with the following single-trait repeatability animal model:

$$127 \quad y = hy + par + a + pe + e \quad [2]$$

128 where **y** was the value of PC2 scores (i.e. LPE); **hy** was the combination between herd and year
129 (49,638 levels); **par** was the effect of parity (three levels: 1, 2 and 3); **a** was the random additive

130 genetic effect (333,003 levels); **pe** was the random effect of permanent environment (333,003 levels);
131 **e** was the random residual.

132 Finally, a two-trait model was used to estimate genetic correlations between CI and LPE of the
133 corresponding lactation: CI records between two consecutive parities (i.e. 1-2, 2-3 and 3-4) and the
134 LPE estimated for the corresponding lactations (i.e. first, second, and third lactations) were used (total
135 of 340,573 records).

136

137 ***Genetic parameter estimation***

138 Variance components and heritability (h^2) estimation for all the described models was
139 performed using a single-step genomic REML (ssGREML) approach with a combined relationship
140 matrix (**H**) built as described in Aguilar *et al.* (2010). Analyses were also done with pedigree
141 relationship matrix (**A**) with very similar results (not shown). Before the analyses, pedigree was traced
142 back for 5 generations. The full pedigree contained 1,981,728 animals of which 7,246 were genotyped
143 for 40,200 markers. Among all the genotyped animals, 3,358 were females with phenotypes and 2,045
144 were sires. The females with both phenotypes and genotypes came from 250 different herds and could
145 be dams of other phenotyped females. The other genotyped animals without phenotypes were half-
146 sib or relatives of phenotyped females. Table 1 shows the distribution of genotyped animals by year
147 of birth. Variance components were estimated using the average information REML (AIREML)
148 algorithm implemented in the blupf90 family programs (Miszta *et al.*, 2014).

149

150 **RESULTS AND DISCUSSION**

151 ***Calving interval in a single-trait, repeatability model***

152 The phenotypic average of CI for Italian Simmental (397.50 ± 68.32) is consistent with
153 previous reports on other cattle breeds. Values between 387 and 398 were reported for UK Holstein
154 (Wall *et al.*, 2003) and Australian Holstein (Haile-Mariam *et al.*, 2003), respectively. CI of 400 or
155 more days were found in Spanish dairy cattle (González-Recio and Alenda, 2005) and in Mexican

156 Holstein (Montaldo et al., 2010). Large values of CI were also reported for US Holstein (Tiezzi et al.,
157 2017).

158 Heritability (0.06 ± 0.002) and repeatability (0.11 ± 0.002) for CI in the present study, using a
159 single-trait model, were quite low. However, they were slightly higher than previous literature
160 reports. Values of 0.02 were estimated in Mexican Holstein (Montaldo et al., 2010) and in Xinjiang
161 Brown (Fu et al., 2017). Estimates ranging from 0.03 to 0.05 were reported for Australian dairy
162 (Haile-Mariam et al., 2003; 2008), UK Holstein (Wall et al., 2003), Irish Holstein (Olori et al., 2002),
163 Spanish dairy (González-Recio and Alenda, 2005), and Italian Brown Swiss (Dal Zotto et al., 2007).
164 It should be pointed out that heritability for fertility traits is generally low, as reported in a recent
165 review (Ma et al., 2019).

166

167 *Lactation persistency*

168 About 90% of the total phenotypic variance of test-day records was explained by the first two
169 principal components (PC). PC1 was related to the average level of milk yield whereas PC2 was
170 associated with the shape of the lactation curve. They explained about 78% and 12% of test-day
171 phenotypic variance, respectively. PC1 scores ranged from -7.60 to 14.54, with an average value of
172 0.14 ± 2.37 , and it showed a correlation of 0.97 with 305-d MY. PC2 scores ranged from -4.53 to 4.71.
173 Animals were grouped according to their PC2 scores into four classes: Class1 = from -4.53 to -1.04;
174 Class2 = from -1.03 to -0.57, Class3 = from -0.56 to -0.15; and Class4 = from -0.14 to 4.71. Average
175 milk yield for all the seven test day records were calculated separately for each PC2 class. These
176 average lactation curves for different PC2 class are shown in Figure 1. It can be clearly seen that the
177 average lactation curve for PC2 class4 exhibited the steepest negative slope. LPE tends to increase
178 for higher PC2 classes reaching the maximum in Class1. These results confirm the meaning of PC2
179 score as an indicator of the shape of the lactation curve.

180 PC2 (i.e. LPE) and MY showed a phenotypic correlation of -0.03, which was expected
181 because PC2 was defined to be independent of milk production in the calculations. However, the

182 genetic correlation between PC2 and MY was unfavorable (0.35 ± 0.03) because higher values of PC2,
183 i.e., lower LPE, are associated to higher MY. In fact, animals belonging to the Class1 (those with the
184 highest LPE) showed a slightly lower 305-d MY compared to the animals of Class4 (those with the
185 lowest LPE). LPE in this study was not defined by combining production levels at different lactation
186 stages, but using the PC2 values that just capture the shape of lactation curve without considering
187 production levels (PC are orthogonal). Thus, the genetic correlation observed between LPE and MY
188 could probably be a spurious result mediated by other variables. In the literature, estimates of genetic
189 correlation between LPE and MY show different magnitudes and signs according to the LPE
190 definition (Haile Mariam et al., 2003; Muir et al., 2004). Cole and Null (2009) found that genetic
191 correlation between LPE and MY changed magnitudes and signs according to the considered breed.
192 Some studies reported unfavorable genetic correlation between LPE and MY (Cobuci and Costa,
193 2012; Khorshidie et al., 2012; Pereira et al., 2012), whereas other studies reported favorable genetic
194 correlation between these two traits (Muir et al., 2004; Yamazaki et al., 2013).

195 Heritability (0.11 ± 0.004) and repeatability (0.20 ± 0.003) of LPE in a single-trait model were
196 rather low. Macciotta et al. (2006) reported lower heritability and repeatability for LPE using a
197 smaller, older dataset that comprised only phenotypic and pedigree information from the same Italian
198 Simmental population. In fact, heritability estimates for LPE in the literature exhibit a considerable
199 variation, with values ranging from 0.01 (Otwinska-Mindur and Ptak, 2015) to 0.50 (Koloi et al.,
200 2018). Such relevant differences can be ascribed to statistical model, breed, and trait definition. There
201 is no consensus in literature for measuring LPE. Grayaa et al. (2019) defined LPE as the difference
202 between milk production at 280 days in milk and at the lactation peak, and estimated heritability using
203 different multi-trait models: the estimates ranged from 0.05 when milk fat percentage was considered
204 to 0.21 when milk yield was included among the response variables. Strabel and Jamrozik (2006)
205 reported heritability estimates for LPE that ranged from 0.07 to 0.18 using the eigenvectors of the
206 (co)variance matrix of RRM coefficients. Higher heritability (0.18 ± 0.02) was reported for LPE for
207 first-lactation Canadian Holstein (Muir et al., 2004). Additionally, Cole and Null (2009), observed a

208 large heterogeneity in the h^2 of LPE in several dairy cattle breeds: from 0.09 to 0.26 and 0.18 to 0.28
209 for Milking Shorthorn and Guernsey, respectively.

210

211 *Calving interval, production traits, and lactation persistency*

212 Heritability for CI obtained with the multiple trait model was slightly higher compared to the
213 single-trait analysis (Table 2). Heritabilities for the production traits were close to the current
214 estimates for the Italian Simmental breed. Unfavorable, moderate genetic correlations were found
215 between CI and production traits (Table 2), as generally reported in literature. The magnitude of the
216 estimates obtained in the present study is not far from previous reports of about 0.5-0.6 (Pryce et al.,
217 2000; Dal Zotto et al., 2007; Fu et al., 2017). Deb et al. (2008) reported a genetic correlation of 0.4
218 between CI and MY in a native breed from Bangladesh. Antagonistic genetic correlation between
219 MY and CI was also observed by Haile-Mariam et al. (2003) and by Strapáková et al. (2016). The
220 latest reported a genetic correlation of 0.51 ± 0.11 , with higher MY observed for cows with longer
221 calving interval. Other similar reproductive traits, such as days open and days from calving to the
222 first service, show undesirable genetic correlations with milk production traits (Abe et al., 2009).

223 Heritability estimates for CI and LPE using the univariate or bivariate models were similar. A
224 weak, positive genetic correlation was observed between these two traits, whereas a near zero
225 phenotypic correlation was observed (Table 3). The positive genetic correlation reflected a favorable
226 association between CI and LPE because high values of CI are associated with high values of PC2,
227 which means lower LPE. On the contrary, lower values of CI (desirable) are related to lower values
228 of PC2 and, therefore, to higher LPE (desirable). However, reports about the genetic association
229 between CI and LPE are not always consistent. An undesirable association between CI and LPE was
230 reported by Atashi et al. (2013), who found that cows with short CI had a lower LPE. Unfavorable
231 genetic correlation between CI and LPE was reported also by Muir et al. (2004). Němečková et al.
232 (2015) reported no significant association between these two traits and Andersen et al. (2011) found
233 no significant differences on peak yield and peak day (i.e. traits associated with LPE) in different

234 calving interval groups. Haile-Mariam et al. (2003) concluded that the genetic correlation between
235 LPE and CI was almost zero. Apart from sampling effect, it should be pointed out that the different
236 definition of LPE used in the various studies may strongly affect the results.

237 An antagonistic relationship between productive and reproductive performances in cattle has
238 been observed: animals need energy to produce milk and to conceive at the same time and, therefore,
239 the energy balance during lactation is of great interest. However, fertility and production traits are
240 associated in a complex causal pattern because this relationship strongly depends on the considered
241 period of the lactation. The attention can be mainly focused on the energy balance during the lactation:
242 milk yield affects the energy balance mostly in the first part of the lactation when cows are
243 inseminated; in this period, body reserve mobilization and negative energy balance frequently occur
244 and the cow has not enough energy to conceive (Andersen et al., 2011; Beran et al., 2012; Duchacek
245 et al., 2014). Thus, a high level of milk production in early lactation (before or around the peak) could
246 reduce fertility with a subsequent delay of pregnancy. In the late part of the lactation, a switch in the
247 causal link between productive and reproductive performance occurs: in this period, energy
248 requirements for the fetal development are higher compared to the first part of lactation and, therefore,
249 pregnancy prioritizes the energy needs with an effect on milk production (that in the advanced stages
250 of lactation can be identified as LPE). More persistent cows with lower peak yield usually suffer less
251 strong energy imbalance during the lactation (Haile-Mariam et al., 2003). Results of the present study
252 highlighted a quite strong unfavorable genetic correlation between CI and MY, confirming the
253 negative relationship between reproductive and productive performance. Slightly favorable genetic
254 correlation between LPE and CI was also observed. However, such relationship between LPE and CI
255 could be influenced by MY. This is because selection for CI is unlikely to cause decline in MY, but
256 the selection for MY ignoring CI would have more negative impact on fertility due to the high
257 unfavorable genetic correlation between these two traits. Nevertheless, LPE has some positive
258 consequences for dairy cows because persistent cows could have less health and reproduction
259 problems, they are easier to manage, and there is a lower feeding cost (Sölkner and Fuchs, 1987;

Atashi et al., 2013). Thus, findings of the present work suggest the possibility to limit the fertility deterioration caused by the selection for MY by including CI and LPE as breeding goals.

In spite of the low h^2 for CI and LPE that has been confirmed also in the present study, it should be pointed out that genomic selection offers interesting perspectives for improving these functional traits, giving more phenotypes and genotypes are collected. García-Ruiz et al. (2016) showed that the genetic gain per year achieved in US Holsteins has been markedly larger for low heritability traits because of the considerable amount of data (e.g. somatic cell score, productive life, and daughter pregnancy rate). For estimating variance components, the main benefit of using genomic information in a single-step approach is the availability of more data that is reflected in the smaller standard errors (Forni et al., 2011; Veerkamp et al., 2011). The use of combined pedigree and genomic information using the single-step approach could have potential benefits for young candidates that have genotypes but no phenotypic records. However, in our case using A instead of H gave very close estimates (data not shown) because of the small number of genotyped animals. The similar results found using BLUP or ssGBLUP, even if with a small number of genotyped animals, showed the robustness of the latter methodology and the possibility to have better results by increasing the amount of genomic information.

CONCLUSIONS

Although of small magnitude, the heritabilities for CI and LPE show that these traits can be improved by genomic selection. The use of multi-trait models allowed to better understand the genetic connection between CI and LPE, showing that both traits should be included as breeding objective in order to prevent a deterioration of fertility.

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287

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419

420 **Table 1.** Distribution of genotyped animals by birth year.

Year of birth	Animals (n)
1963 - 1980	38
1981-1990	245
1991-2000	816
2001	118
2002	126
2003	148
2004	196
2005	179
2006	169
2007	196
2008	238
2009	269
2010	318
2011	336
2012	427
2013	859
2014	1048
2015	790
2016	389
2017	265
2018	76

421

422

423 **Table 2.** Heritability (diagonal), phenotypic (above diagonal) and genetic (below diagonal)
 424 correlation for calving interval and production traits using a 4-trait model.

	Calving interval	Milk yield	Fat yield	Protein yield
Calving interval	0.09±0.02	0.17	0.15	0.16
Milk yield	0.64±0.02	0.26±0.02	0.88	0.96
Fat yield	0.63±0.02	0.86±0.01	0.25±0.02	0.89
Protein yield	0.56±0.02	0.95±0.01	0.90±0.01	0.22±0.02

425

426

427 **Table 3.** Heritability, repeatability, and correlations (phenotypic and genetic) for calving interval and
428 lactation persistency using the two-trait model.

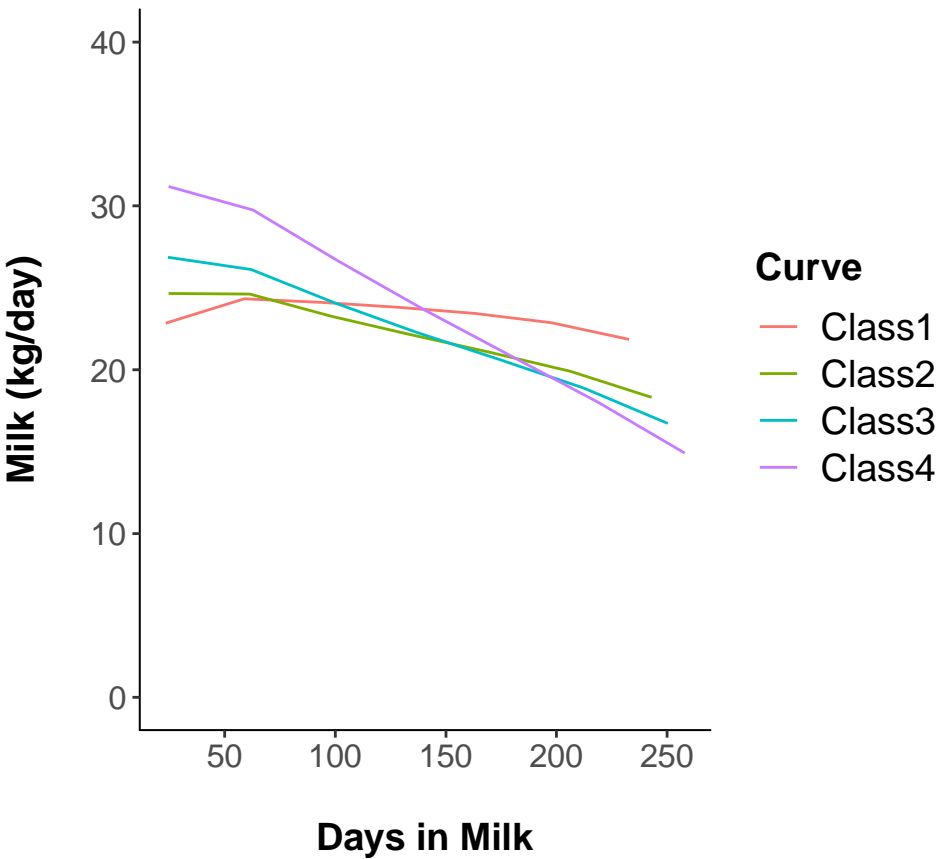
	Heritability	Repeatability	Correlation	
			Phenotypic	Genetic
Calving interval	0.05±0.01	0.11±0.01	-0.05	0.25±0.03
Lactation persistency	0.11±0.01	0.20±0.01		

429

FIGURE CAPTION

Figure 1. Average shape for the lactation curve using quartiles of the second principal component (PC2) that is associated with lactation persistency. Each curve is made using the average value of milk production (kg/day) of all animals belonging to the first (Class1), second (Class2), third (Class3), and fourth (Class4) quartile of PC2.

436 Cesarani. Figure 1.



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438