# The influence of genetic relatedness on dairy cows' social behaviour in Automatic Milking Systems

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## Abstract

Understanding social interactions and social preference among dairy cows is crucial for ensuring herd welfare and productivity. Our study aims to assess how genetic kinship and age within a herd of cows influences their social bonds through the application of Social Network Analysis (SNA). We investigated the social behaviour of 329 dairy cows during milking sessions in a free cow traffic barn equipped with an Automatic Milking System, and we compared their social behaviour during their first lactation and during subsequent lactations. Consistent social bonds were identified by measuring the time gap between consecutive entries of two cows into the same milking robot. If this time gap was less than 10 minutes, we considered it an association between the two cows, as it is highly likely that they were queuing together. We computed association indices between each pair of cows to estimate the proportion of time any two individuals associated and we built the association networks. Genetic kinship among cows was evaluated considering pedigree data of three generations of ancestors and integrated with SNA results. Our findings revealed the dynamic nature of cows' social behaviour, revealing that their preferences evolve over time. Specifically, younger cows demonstrated a tendency to form stronger social associations with specific individuals, influenced by genetic relatedness. In contrast, the higher number of connections observed in older cows suggests that they tended to be more socially connected with their herd mates. Additionally, they exhibited a preference for interacting with peers of similar age rather than with relatives.

Keywords: social interaction, social network analysis, genetic kinship, dairy cow, automatic milking system

# Introduction

Cattle are social animals that form structured groups characterized by hierarchies based on dominance and submission, alongside preferences in relationships. It is well known that cows have a non-random complex social organisation (Boyland et al., 2016; Fielding et al., 2021) influenced by multiple factors such as familiarity (Foris et al., 2022), a predisposition towards increased activity levels (Hansson et al., 2023) and genetic relatedness (Ozella et al., 2023). Modern commercial farming practices frequently neglect the importance of social relationships among animals, despite an increasing body of evidence highlighting the significant positive effects of maintaining these bonds. For instance, while regrouping is a common practice in dairy cow management, it is widely acknowledged to have adverse effects on both behaviour and production, including reductions in feed consumption, rumination, and resting times (Foris et al., 2022). Additionally, introducing new individuals into a group can lead to social tension and increased agonistic behaviours towards the newcomers (Scheurwater et al., 2021). In managing these dynamics, some dairy farms choose to introduce several animals simultaneously, while others prefer individual introductions. Previous research suggests that introducing cows in pairs (O'Connell et al., 2008) or in small groups (Foris et al., 2021) to a group can help mitigate the adverse impacts of regrouping on the newly introduced animals.

Given these considerations, it is crucial for farmers to carefully select the animals to move into new groups to avoid welfare and production-related issues that could potentially lead to economic problems. Therefore, it is necessary to understand the social preferences within a group of dairy cows. Social Network Analysis (SNA) has emerged as a valuable tool for investigating and evaluating the complexity of social relationships, providing a more precise understanding of the social structure of animals both at the group-level and at a dyadic-level (Croft et al., 2008). It has been extensively employed in research to explore the social behaviour and structure of cattle herds (e.g. Fielding et al., 2021; Foris et al., 2022).

In this study, we aimed to investigate how genetic relatedness within a herd of cows influences their social bonds. We studied the social associations of 329 dairy cows at an Automated Milking System (AMS) during milking events in a free cow traffic barn, both during their first lactation (primiparous phase) and in subsequent lactations (multiparous phase). We hypothesized that cows with a genetic relation would exhibit a higher propensity for social interaction compared to cows without genetic ties. Furthermore, we investigated whether individuals of similar age would be more familiar with each other, thus increasing the likelihood of interaction. These factors may prove to be a crucial variable for farmers to take into account when implementing regrouping strategies.

# **Material and Methods**

#### Data collection

The study was carried out on a commercial dairy farm in the northern part of Italy (Marene, Cuneo) equipped with Lely A4 Astronaut AMSs (Lely, Maassluis, the Netherlands). The barn was composed of two enclosed rectangular areas, each area hosted approximately 120 Holstein-Friesian lactating cows and contained two AMSs; the first area was designated for primiparous cows, while the second area housed multiparous cows. The cows were kept indoors throughout their entire production cycle with no access to pasture. All lactating cows had 24-hour access to the Automated Milking System (AMS) and were milked voluntarily, with cows being milked on average 2-3 times per day. Individual measurements were recorded using an electronic identification collar (Owes-H system, Lely, Maassluis, The Netherlands) fitted to each cow. This collar captured information such as the cow's unique identification number, entrance and exit time from the milking robot, milk yield, milk temperature, and composition of protein, fat, and lactose. The collected data was stored in a management software (Horizon Lely, Maassluis, The Netherlands). Data on individual milking traffic were collected from 329 lactating dairy cows over a two-year period. Milking records of the same 329 cows were collected during both their first lactation (primiparous) and their successive lactation (multiparous).

To identify consistent social associations between cows during milking at the AMS, we calculated the time gap between the entrance time of one cow and the entrance time of another cow in the same milking robot, on the same day, and in the same area following the method proposed by Marumo et al. (2022). If the time gap was less than 10 minutes, we considered it an association between the two cows. This specific time frame was chosen because if a cow entered the milking machine within 10 minutes of the previous cow entering, it was highly probable that they were queuing together, according to Marumo et al. (2022) and Ozella et al. (2023). Conversely, if the time gap was longer than 10 minutes, we excluded the event as it was unlikely that the two cows were queuing together. The average length of a milking event was 7 minutes per visit.

#### Symmetry of associations

For each pair of cows, we computed the number of times with which one cow followed the other at the AMS. It is possible that one cow may exhibit a higher number of times of following the other compared to the reverse scenario. To address this, we employed a Spearman rank correlation analysis for every pair.

#### Association index

To determine the strength of associations between two cows, denoted as a and b, during milking sessions, in relation to the number of opportunities they had to queue together in the same area and time period, we calculated an association index for each pair of individuals (Whitehead, 2008). We determined the total number of milkings where cows a and b were observed together in the same area  $(x_{ab})$ , as well as the number of milkings where comb where both cows were in the same area but only cow a  $(x_a)$  or only cow b  $(x_b)$  was identified by the milking robot. To calculate pairwise social associations, we employed the following formula:

$$AI = \frac{x_{ab}}{x_{ab} + x_a + x_b}$$

The resulting index ranges from 0, indicating that the two individuals were never associated during milking, to 1, indicating that they were always associated during milking. A higher index value reflects a stronger level of association between the pair of individuals.

#### Association networks

We constructed the social networks of the 329 cows when they were in the primiparous area and in the multiparous area. In these networks, each node represents a dairy cow, and an edge between two nodes (a and b) corresponds to the association index (AI<sub>ab</sub>) between them, calculated over the entire study period. We computed different network metrics including the node degree and the density of the network. The degree of a node in a network represents the number of unique individuals that the node has come into contact with. To address variations in the duration of time cows spent in different areas, we calculated the normalized degree of a node by dividing the total degree for each area by the total number of days each cow spent in that specific area. Additionally, we computed the network density, which reflects the proportion of existing connections in the network relative to the maximum possible connections. The network density can range from 0 to

1, with 0 indicating a network with no connections and 1 indicating a network with all possible connections. A density value closer to 1 suggests a denser network, indicating greater cohesion among the nodes.

## Genetic kinship

The pedigree information for the 329 cows involved in the study was gathered from both on-farm and public databases, merging and checking the correspondence between the two data sources. Where available, pedigree data up to the third generation of ancestors (i.e., great-grandparents) were collected. Individuals with no known parents in the pedigree were defined as founders and were assumed to be unrelated and in absence of inbreeding. The kinship coefficient between two subjects is defined as the probability that a randomly selected allele from a locus will be identical by descent (IBD) between them. This coefficient ranges from 0 (i.e., two unrelated individuals) to 0.5 (i.e., an individual with itself). The kinship matrix was then included in the social association analysis.

#### Pattern of social assortment

We investigated how genetic relatedness and similarity in age influence the strength of association among both primiparous and multiparous dairy cows. To do so, we employed the Mantel test, which compares distance matrices between association indices and both kinship coefficients and differences in ages between pairs of cows, calculated in months (where a value of 0 indicates cows born in the same year and month). This allowed us to assess the correlation between these variables and the association indices, providing insight into the underlying factors influencing social interactions within the cow population. We employed the Mantel test, utilizing 10,000 permutations, to compare both the kinship and age matrices with association matrices for both primiparous and multiparous cows.

#### Data analysis

We calculated the association indices and conducted statistical tests including the Spearman rank correlation test, the Two-sample permutation test and the Mantel test using the Python packages 'SciPy' v1.2.1 and 'p&lt' (Python Software Foundation). The network analysis was performed by using Python packages 'SciPy' v1.2.1 and 'NetworkX' v2.6.3 (Python Software Foundation). The genetic kinship matrix was built using the kinship2 R package (Sinnwell and Therneau, 2022).

# **Results and Discussion**

#### Symmetry of associations

We identified a total of 675,820 associations between one cow and the cow following it across the entire dataset, with 354,622 associations when the cows were primiparous and 321,198 when the cows were multiparous. We observed positive and significant associations between primiparous cows (Spearman rank correlation, rho = 0.70, p-value < 0.001) as well as multiparous cows (Spearman rank correlation, rho = 0.65, p-value < 0.001). These findings underscore the absence of a consistent order in entering the milking robot. This agreed with prior studies (Marumo et al., 2022; Ozella et al., 2023)

and indicates that the social interactions within the herd do not conform to a rigid hierarchy in relation to milking conduct. These findings enabled us to build symmetrical association networks, characterized by undirected network edges and symmetric weights assigned to the edges.

# Association indices

Table 1 presents the descriptive statistic of association index values. Primiparous cows showed the highest mean and median values compared to the multiparous ones. This suggests that, on average, primiparous cows exhibited slightly higher association indices compared to multiparous cows, indicating a stronger level of association during milking. Upon comparing the indices for primiparous and multiparous cows, our analysis unveiled a statistically significant difference between the association indices of these two groups (Two-sample permutation test: Z = 26.92; p-value < 0.001). Specifically, the youngest cows exhibited higher association indices. These observations might suggest a different social dynamic between the two groups, with younger cows appearing to be more inclined to form close social bonds or interact more actively within the group compared to multiparous cows. This pattern has been previously observed in similar settings (Ozella et al., 2023), indicating consistency in the social behaviour of cows based on their reproductive experience.

	mean±SD	median	range
Primiparous	0.013±0.13	0.010	0.0004-1
Multiparous	0.009±0.11	0.008	0.0002-0.57

# Networks structure of the herd

We constructed symmetrical association networks spanning the entire study period, each composed of 329 nodes. For primiparous cows, these networks contained 15,887 edges, while for multiparous cows, there were 29,947 edges. The network density, which reflects the proportion of actual connections to possible connections, was higher for multiparous cows (0.55) compared to primiparous cows (0.29). The higher number of edges and the greater network density observed in older cows suggest that they are more socially connected. This finding confirms a trend previously observed in a prior study (Ozella et al., 2023), conducted in a different barn but with a similar setting. The higher network density and increased number of connections observed in older cows imply that they tended to be more socially connected with their herd mates.

To evaluate the distribution of normalized degrees, we employed Kernel Density Estimation (KDE) and visualized the smoothed density estimation of the normalized degrees (Figure 1). The observed distribution of normalized degrees for both groups reveals a peak around 0.20. However, in younger cows, this peak appears slightly lower, indicating a comparatively smaller number of individuals with connections at this level. Additionally, there is a secondary peak around 0.60 for primiparous cows, which disappears as cows age. This bimodal distribution may signify the existence of two distinct subgroups among primiparous cows, some with lower and others with higher

levels of social interaction. On the other hand, multiparous cows exhibit a relatively uniform level of connectivity within the network across all individuals.

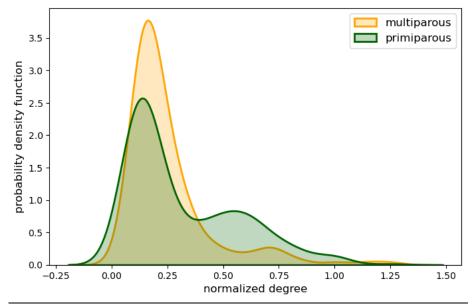


Figure 1. Kernel Density Estimation of the normalized degrees of the primiparous (green line) and multiparous (orange line) dairy cows.

#### Pattern of social assortment

Results from the Mantel test revealed positive and statistically significant correlations between the association indices of cows in both primiparous and multiparous stages and their relatedness coefficients (primiparous: r = 0.19, p-value < 0.001; multiparous: r = 0.04, p-value = 0.031). However, the correlation coefficient is higher in primiparous cows compared to multiparous cows, indicating that kinship primarily drives social associations at the milking robot when cows are in their first lactation compared to subsequent lactations. This finding aligns with previous research conducted in similar settings (Ozella et al., 2023), reinforcing the hypothesis that relatedness plays a more prominent role in shaping social associations among primiparous cows compared to multiparous.

On the other hand, we found that age similarity predominantly influences the relationships among older cows in their second lactation and beyond. We observed negative and significant correlations between the association indices of cows in both primiparous and multiparous stages and their age differences (primiparous: r = -0.03, p-value = 0.033; multiparous: r = -0.16, p-value < 0.001). This outcome could be expected in primiparous cows since the age difference in months can be quite low.

#### Conclusions

Our study demonstrates that the social behaviour of dairy cows is dynamic, with social preferences changing over time. Specifically, kinship primarily drives social associations among primiparous cows, while age similarity predominantly influences relationships among older cows in subsequent lactations. This suggests distinct mechanisms guiding

social interactions at different stages of lactation, with kinship playing a more prominent role in early lactation and age similarity becoming more influential as cows mature. Overall, our study provides valuable insights into the complex social dynamics of dairy cow herds, emphasizing the interplay between factors such as kinship, age, and social behavior. These findings contribute to a better understanding of herd management practices and can serve as a step forward in regrouping strategies, taking into account the social preferences of dairy cows, to enhance their welfare. This consistency across studies underscores the robustness of the observed trend and its potential applicability across different contexts, further supporting the generalizability of these findings in understanding the social dynamics of dairy cow herds.

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