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Effects of the introduction of single heifers or pairs of heifers into dairy-cow herds on the temporal and spatial associations of heifers and cows

Gesa Neisen [a,b,*], Beat Wechsler [a], Lorenz Gygax [a]

[a] Centre for Proper Housing of Ruminants and Pigs, Federal Veterinary Office, Agroscope Reckenholz-Tänikon Research Station ART, Tänikon, 8356 Ettenhausen, Switzerland
[b] Department of Behavioural Biology, University of Münster, Badestraße 13, 48149 Münster, Germany

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ABSTRACT

Investigations into the process of introducing heifers into dairy-cow herds may alleviate animal-welfare problems arising during social integration. The present study monitored the introduction of a single heifer and a pair of heifers known to each other on each of six dairy farms, and investigated the effects on the temporal and spatial associations of the heifers and cows. We automatically collected data on animal positions in three different functional areas (activity, lying and feeding areas) of cubicle housing systems. The positions of each animal were recorded once/min over six continuous days both before and after introduction of the heifers. A measure of synchronicity and distance within all possible dyads was averaged per cow or heifer and functional area to reflect the animals' temporal and spatial associations to other herd members. In addition, agonistic behaviour towards the heifers was recorded over the first 3 days after introduction by means of direct observations. For statistical evaluation, linear mixed-effects models were used.

Heifers introduced on their own faced almost double the rate of agonistic interactions experienced by heifers introduced in pairs ($7.19\,h^{-1}$ vs. $3.79\,h^{-1}$). They were more synchronous and at smaller distances with the cows in the activity and feeding areas than the heifers in the pairs. This pattern was inversed in the lying area, however. The same patterns also became visible from the perspective of the cows. Heifers introduced in pairs were more synchronous and at shorter distances with one another than with the cows. Cows were slightly but not significantly more affected in their dyadic synchronicity and distances after the introduction of pairs of heifers than after the introduction of single heifers.

High synchronicity and small distances of the single heifers with cows coincided with more frequent agonistic interactions, whereas heifers in the pairs were more synchronous and at shorter distances with each other and may have provided some mutual social support. We would thus recommend that heifers be introduced in pairs rather than singly into dairy herds composed of up to 50 dehorned cows kept in cubicle housing systems.

1. Introduction

Introduction of cows into established herds is a frequent and common dairy-management practice (Knierim, 1999; Nakanishi et al., 1993; Von Keyserlingk et al., 2008). During the cow-replacement process, heifers are usually introduced at about 2 years of age before first

* Corresponding author at: Department of Behavioural Biology, University of Münster, Badestraße 13, D-48149 Münster, Germany. Tel.: +49 251 83 21013; fax: +49 251 83 23896.
E-mail address: gesa_neisen@hotmail.com (G. Neisen).

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calving, so that they can habituate to the cows, the new housing system and management procedures such as entering the milking parlour. On their introduction, both heifers and cows must build new social relationships, which may affect the social organisation of the herd. Consequently, it is of interest to investigate not only the process leading to the establishment of social relationships between heifers and cows, but also the effects of the introduction of heifers on the social relationships between the cows.

Introducing unfamiliar heifers or cows into an established dairy herd may be associated with negative effects on animal welfare and performance. Several studies found increased rates of aggressive behaviour towards and displacements of newly introduced cows in the first few days after introduction (Knierim, 1999; Menke et al., 2000; Sato et al., 1990). Von Keyserlingk et al. (2008) also reported a decline in allogrooming of the new cows during the first 3 days after introduction compared with the day before introduction. Moreover, a reduction in feed intake, lying time, lying-bout duration, body weight and milk production was observed for the newly introduced cows (Nakanishi et al., 1993; Von Keyserlingk et al., 2008). In addition, Sowerby and Polan (1978) reported a decrease in milk yield of the herds to which the cows were introduced during the first month following their introduction.

Several studies have investigated strategies aimed at easing the introduction of unfamiliar individuals into established dairy-cow groups. Nakanishi et al. (1993) reported on introductions at night after dark (2000–2100 h), a time when he expected social activity, including agonistic interactions, to be low. Knierim (1999) and Menke et al. (2000) varied the number of heifers introduced at a given time. Knierim (1999) found fewer aggressive interactions and less affiliative behaviour between cows and heifers on the first day after introduction when three heifers were introduced simultaneously rather than just one.

The effect of the introduction of unfamiliar individuals into established cow herds on the affiliative relationships between the cows has hardly been investigated (but see Von Keyserlingk et al., 2008), even though the importance of these relationships in structuring a herd has previously been shown (Reinhardt and Reinhardt, 1981; Simonsen, 1979; Wasilewski, 2003). Affiliative relationships can be identified by measuring positive social interactions such as allo-licking (Sato et al., 1991; Wood, 1977), but because these behaviours do not occur frequently, they are not suitable for describing the social relationships of a large number of dyads in cow herds. Alternatively, spatial associations of herd members can be used to quantify affiliative relationships in cattle (Reinhardt and Reinhardt, 1981; Wasilewski, 2003), and such data can be collected automatically (Gygax et al., 2007).

In the present study, heifers were introduced into dairy herds singly and in pairs to study their socio-spatial integration and changes in spatial relationships among the cows. The relationships were characterised by dyadic synchronicity and distances, measured by an automatic position measurement system and presumably reflecting affiliative relationships. This spatial data was complemented by rates of agonistic interactions towards newly introduced heifers.

2. Methods

2.1. Farms and animals

Single heifers and pairs of heifers were introduced into dairy herds on six Swiss working farms with herd sizes of between 22 and 44 dehorned cows before introduction of the heifers (Table 1). On four farms (B, C, D, E), herd composition regarding cows was stable from at least 3 days before the first introduction of a heifer onwards. On farm A, one cow was removed 5 days into the experiment owing to health problems unrelated to the experiment. Consequently, this animal's data were excluded from all analyses. On farm F, herd composition remained unchanged from 1 day before the start of the experiment onwards. At this time, two cows were removed form the herd (start of dry period) and were replaced by three others which were due to calve shortly. These three cows were, however, familiar with the rest of the herd and had been away from the herd for their dry period (max. 42 days). On farm F, two more cows had to be removed in the final week of the experiment owing to health problems unrelated to the experiment. Consequently, their data was not taken into account for the second half of the experiment. Table 1 provides more detailed information on herd size, breeds, parity and lactation stage (days in milk) of the cows observed on the different farms.

On all six farms, the cows were kept in a loose-housing system (area: 7.0–11.0 m^2/cow) with lying cubicles (animal/cubicle ratio: 0.73–1.03) arranged in three to five rows. Some of the farms had an exercise yard, or let the cows onto pasture during part of the day (Table 1). When allowed on to pasture, cows did no longer have access to the barn. All farms had a single feed rack, except for farm F, where the feed rack was divided into three separate sections (animal/feeding-place ratio: 0.79–1.03). Cows on all farms were milked twice daily and had ad libitum access to water. Feed was provided once or twice daily (see Table 1).

2.2. Acquisition of animal-position data—tracking system

Animal-position data were collected automatically with a local position-measurement system (LPM®; www.lpm-world.com; ABATEC electronics systems, Regau, Austria), which recorded the positions of the animal transponders in the barns in two dimensions with an accuracy of up to 20 cm (Gygax et al., 2007). The system consisted of 8–11 antennas (depending on the size of the barn), fixed on the walls and directed towards the centre of the barn. Every cow and all of the newly introduced heifers wore an animal transponder, which transmitted a signal to the antennas at regular intervals. The antennas relayed the exact time of reception of the transponder signal to a central computing unit which calculated the 2D-position.

In principle, the system is capable of recording data at a frequency of 300 position estimates/s. Data was only sampled once a minute for each animal for the following

Table 1
Characteristics of the investigated herds, and farms (A to F), as well as information on data availability. cS: control week before single heifer was introduced; iS: after introduction of single heifer; cP: control week before pair of heifers were introduced; iP: after introduction of pair of heifers.

	A	B	C	D	E	F
Herd size and composition						
Introduction sequence[a]	P/S	S/P	P/S	S/P	S/P	P/S
cS/iS	24/25	24/25	26/27	29/30	30/31	44/43
cP/iP	22/24	25/27	24/26	30/32	31/33	43/45
Breeds[b]	BS	BS	Mixed	Mixed	BS	BS
Parity[c]	4.0 ± 0.5	2.6 ± 0.4	4.2 ± 0.5	3.7 ± 0.4	9.9 ± 6.4	3.3 ± 0.3
Days in milk[c]	179 ± 61	144 ± 62	174 ± 57	175 ± 54	236 ± 51	144 ± 47
Barn and management						
Feeding regime[d]	2×	2×	2×	1 × TMR	2×	1 × silage
Exercise yard	None	None	Temporary	Ad lib.	None	Ad lib.
Use of pasture	Until 17 h	Until 17 h	None	Mornings	Mornings	None
Percentage of available data corrected for system failures and time on pasture[e]						
cS	94	57	89	85	94	64
	67–99	36–67	67–97	67–100	83–100	0–74
iS	92	76	88	73	90	68
	56–100	0–92	59–97	0–100	0–100	0–77
cP	89	76	87	75	91	63
	31–96	49–85	72–94	44–100	39–100	41–74
iP	93	84	92	73	86	74
	0–100	62–100	0–96	44–100	72–94	0–89

[a] S = single heifer, P = pair of heifers.
[b] BS = mainly Brown Swiss; mixed = mainly Red and Black Holstein on farm C, Brown Swiss and Red Holstein on farm D.
[c] Mean ± StdErr.
[d] Cows were fed maize silage twice daily.
[e] Median and range of the percentage of available values per cow and week relative to the total number of measurement intervals in the barn.

three reasons: (1) to reduce the amount of stored data, (2) to increase the accuracy of the position estimates by averaging them over a short transmission interval, and (3) to prolong battery life. Consequently, the animal transponder switched between a 10-s transmission interval and a 50-s dormant period, and an average position estimate was stored at 1-s intervals for each animal in real time during the transmission interval. Because the system required about 2 s at the beginning of each transmission interval to calculate the coordinates with maximum precision, data transmitted in the first 3 s of the transmission interval were discarded. With the data of the remaining up to 7 s, a mean position was calculated. We thus ended up with a once-a-minute position estimate of each animal.

Because transponders were locally activated by switching on their batteries, it was not possible to synchronise the 10-s transmission interval among the animals. Given the average speed at which cows in a barn move and the length of the observation period, this small temporal variance among the position estimates of different animals was not considered relevant. Batteries in the transponders usually lasted 6 days, and were replaced during the morning feeding at the feed rack if necessary.

For data analysis, barns were virtually divided into three functional areas to which the animals' positions were assigned: the feeding area (transponder within 1 m of the feed rack), the lying area (transponder inside the lying cubicles) and the activity area (remaining barn area where animals could move about freely). The outdoor areas (exercise yard and pasture) were not covered by the automatic tracking system. Bearing in mind the animals' temporary absence from these areas, recordings within the barn for 57–94% of the observation time were available per animal (median per farm; Table 1). Except for a few transponders which broke down, most interruptions were short, with a median length of between 1 and 3 min in all animals. Thus, data in the barn was collected at 1-min intervals on an almost continuous basis.

2.3. Experimental design on farms

Setting up the automatic tracking system before the start of an experiment took about 3 days on each farm, including checks on the accuracy of the measurements.

On each farm, animal-position data was collected as described above for 4 weeks, 6 continuous days per week, 24 h a day. The cows were observed during the first week to set a baseline measurement. On the first day of week 2, one heifer was introduced into the herd on half of the farms. On the other half of the farms, a pair of heifers familiar to each other but not to the cows, were introduced (Table 1). At the end of the second week, data collection was suspended for a week, so that the introduced heifer(s) could become better acquainted with the cows. On farm F, one of the two heifers introduced in week 2 had to be removed on day 2 of the third week, owing to downer-cow syndrome.

In the fourth week, the collection of animal-position data was resumed, with the formerly introduced heifer(s) now being considered cows. In week 5, one heifer was introduced per herd if two had been introduced in week 2, and vice versa. In both week 2 and week 5, the heifers were introduced when the cows were either on pasture or fixed at the feed rack. They therefore had at least 15 min to explore the barn before the cows began interacting with them. The heifers were always introduced either in the morning after feeding, or in the afternoon after the pasture period or after feeding.

The heifers introduced had either been born and reared on the farm in question but separated from the cows since shortly after birth, or had been reared on a different farm. They were in the median 24 months old (range 18–36) and on day 159 into their pregnancy (range 50–258). Each heifer introduced to the herd took part in the experiment just once.

2.4. Calculation of agonistic and socio-spatial variables used as response variables

In addition to animal-position data, agonistic behavioural patterns (retreat by heifer; head-butts and fights involving the heifers) were recorded by direct observation during the first 3 days of the two introductory weeks. On farms where cows were fed twice daily, these observations took place directly after introduction of the heifers, as well as during and after feeding times. On the other farms with just one feeding time a day (Table 1), an additional observation period was created in which feed was pushed to the feed rack by the farmer. The observations were stopped whenever the newly introduced heifers lay down, went to the exercise yard or to the pasture, or when the cows were milked (average duration of observations, 284 min/farm and week; range, 170–475 min). For each heifer, the rate of all agonistic behaviour experienced was computed, taking into account total observation time for a given farm and week.

From the animal-position data of each individual (cows and heifers), two socio-spatial variables reflecting temporal and spatial proximity were calculated for all pairs of animals (dyads) and specific to the three defined functional areas (activity, lying and feeding areas), using a self-authored software (written in R; www.r-project.org, R Development Core Team, 2007). The two socio-spatial variables were defined as (1) *dyadic synchronicity*: percentage of observations in which the two animals of a dyad were in the same given functional area, divided by the total number of observations in which both animals were to be found; and (2) *dyadic distance*: the median distance observed within a dyad (of all observed distances < 10 m, m, because longer distances were assumed to be socially irrelevant) while they were in the same functional area. These values were saved in symmetric social matrices per herd.

In order to describe the degree of integration into a herd at the level of the individual, a value for *synchronicity* and *distance* was calculated for each animal as the median value for its dyadic synchronicity and dyadic distance values over all the possible partners of a focal animal, respectively.

2.5. Operationalisation of the research questions and statistics

For most of our evaluations, we created linear mixed-effects models and followed the approaches suggested by Pinheiro and Bates (2000) using R 2.6.1 (R Development Core Team, 2007). In these models, we used synchronicity and distance calculated for the three different functional areas (activity, feeding and lying areas) as response variables, and thus ended up with six models, one for each possible combination, for most of our questions. In order to reflect the experimental design and account for dependency in the data of the heifers of the pairs, the identity of the introductory week nested within farms was included as random effects. In the models that included an interaction, a step-wise backwards approach was used, i.e. the interaction was dropped from the model if it did not reach significance, but all main effects were retained. Thus, even if the main effects did not reach significance, we had an estimate of the differences based on our main effects available for reporting. Model assumptions were checked using a graphical analysis of the residuals and random effects.

2.5.1. Rate of agonistic behaviour experienced

We tested whether the single heifers or the heifers of the pairs were subject to more agonistic interactions from the cows. The rate of agonistic behaviour experienced was used as a response variable in a linear mixed-effects model. The sole fixed effect indicated the number of heifers introduced (factor with two levels: single heifer, heifers of the pair).

2.5.2. Synchronicity and distance of the introduced heifers with the cows

We tested whether heifers introduced on their own or in pairs achieved higher synchronicity and shorter distances with the cows. Synchronicity and distance of the introduced heifers with the cows was used as a response variable in linear mixed-effect models (six models). The number of heifers introduced was included as a fixed effect (factor with two levels: single heifer, heifers of the pair). The response variables for all models were log-transformed in order to account for the assumptions of normality and homoscedasticity in the errors.

2.5.3. Synchronicity and distance of cows with the heifers and with other cows

We wanted to know whether cows differed in dyadic synchronicity and dyadic distance with heifers introduced singly and in pairs. We further investigated whether these values wore modified depending on how synchronous and at what distance these cows were found with other cows before the introductions. Synchronicity and distance of each individual cow (with all other cows) in the week before the heifers were introduced served as a response variable in linear mixed-effect models (six models). The dyadic synchronicity and dyadic distance with the introduced heifers (continuous variable: dyadic synchronicity and dyadic distance with single heifer and the higher dyadic synchronicity and lower dyadic distance of the two values each with the heifers of the pair), the number of introduced heifers (factor with two levels: single heifer, pair of heifers) and their interaction were included as fixed effects.

2.5.4. Synchronicity and distance among the heifers of the pair and towards the cows

In addition to synchronicity and distance measured by the median, we were interested in the highest dyadic synchronicity and shortest dyadic distance formed by heifers of the pair with any of the cows. We wanted to know whether the dyadic synchronicity and dyadic distance among the heifers of the pair differed from their synchronicity and distance with the cows. Median and highest synchronicity as well as median and shortest distance among the heifers of the pair and between these heifers and the cows were used as response variables in linear mixed-effect models (12 models). The fixed effect described whether the value in the response variable was from among the heifers of the pair, or between each of the heifers of the pair and the cows (factor with two levels: within pair; with cows).

2.5.5. Effect of the introduced heifers on dyadic synchronicity and distance among the cows

We examined whether heifers introduced singly or in pairs had a stronger impact on dyadic synchronicity and dyadic distance between cows. The matrices of dyadic synchronicity and dyadic distance were correlated from before to after introduction specifically with the three different functional areas. We used a specialised correlation coefficient for social matrices resulting in a τ_{Kr} correlation coefficient (Hemelrijk, 1990). These correlation coefficients were tested for systematic differences resulting from the introduction of heifers singly and in pairs using the Wilcoxon test (two socio-spatial variables, three functional areas: six tests).

3. Results

3.1. Rate of agonistic behaviour experienced

The heifers introduced singly were subject to a higher level of agonistic interactions from the cows than those introduced in pairs (7.19 per hour and single heifer vs. 3.79 per hour and heifer of the pair; $F_{1,11} = 84.26$, $p = 0.033$).

3.2. Synchronicity and distance of the introduced heifers with the cows

Synchronicity and distance with the cows in the three functional areas did not differ significantly for the single heifers and the heifers of the pairs ($F_{1,5} < 2.90$, $p > 0.15$, Fig. 1). In the activity and feeding area, single

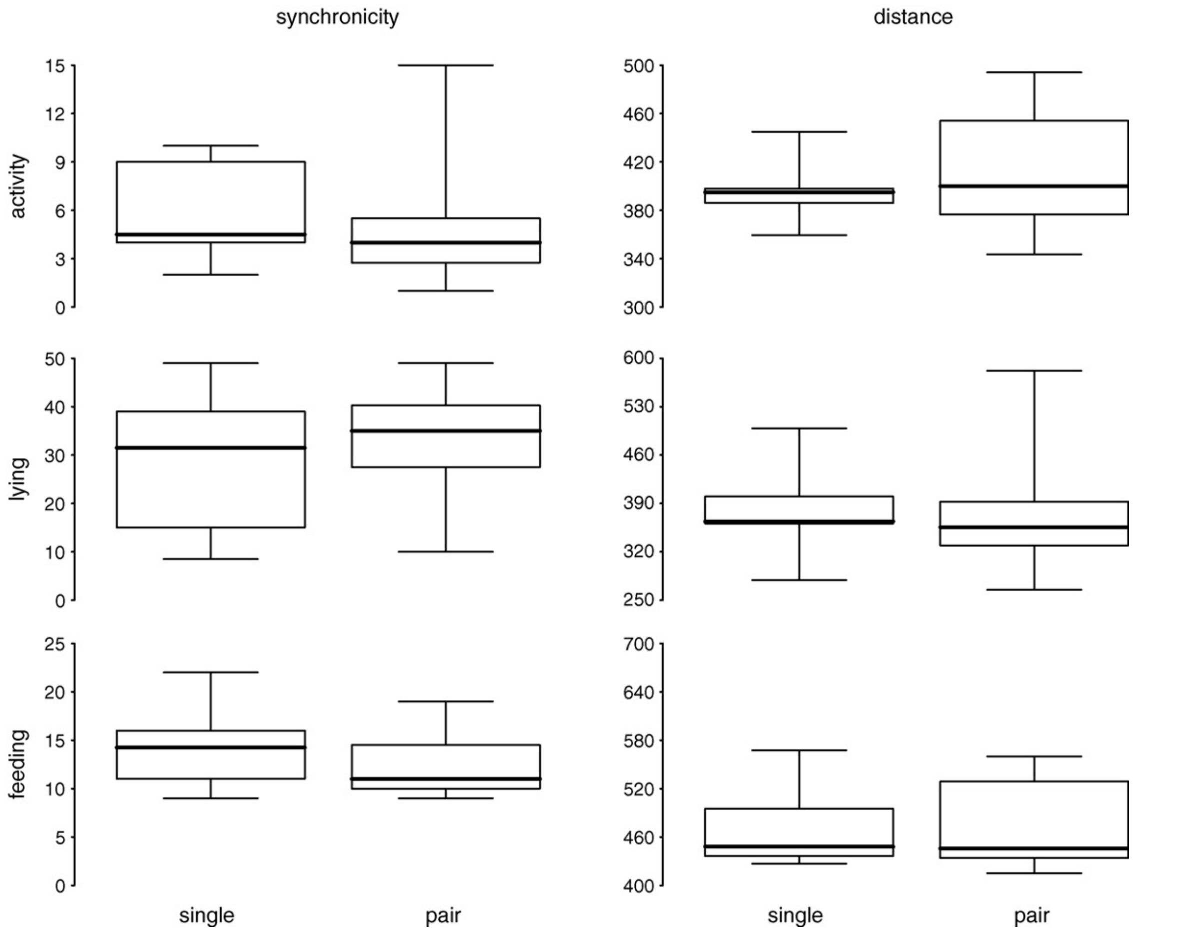

Fig. 1. Synchronicity (%) and distance (cm) between either a heifer introduced singly or heifers introduced in a pair and the cows in the activity, lying and feeding area.

heifers were on average slightly more synchronous (model based means 4.9% vs. 4.0% and 13.8% vs. 12.2%) and at shorter distances (4.0 m vs. 4.1 m and 4.68 m vs. 4.71 m) with the cows, whereas the reverse was true for the lying area (synchronicity: 24.9% vs. 29.5%, median distance: 3.72 m vs. 3.67 m).

3.3. Synchronicity and distance of cows with the heifers and with other cows

In general, there was a positive correlation between the synchronicity and distance of the cows before introduction of the heifers, and the dyadic synchronicity and dyadic distance of the cows with the introduced heifers, respectively, i.e., cows more synchronous and at shorter distances with one another before the introduction of the heifers were more synchronous and kept shorter distances to the introduced heifers. This positive correlation could be observed in all six combinations of synchronicity or distance and the three functional areas ($F_{1,332–345} > 20$, $p < 0.001$, reaching only a trend for the distance in the feeding area: $F_{1,333} = 3.59$, $p = 0.06$). In these analyses, a total of two outliers were detected among the 347 observations per analysis and these were excluded: one observation (0.3%) for synchronicity in both the activity and feeding areas.

In the activity and feeding area, cows were more synchronous and at shorter distances with single heifers than with the heifers of the pair (Fig. 2., top and bottom row), but this only achieved significance for synchronicity in the feeding area (all other $F_{1,5} < 3.24$, $p > 0.13$). In the feeding area, this difference was smaller for small values in synchronicity (interaction: $F_{1,332} = 10.27$, $p = 0.002$; Fig. 2, bottom row, left). In the lying area, the pattern for synchronicity was reversed, with the cows being more synchronous with the heifers of the pair than with the single heifer ($F_{1,5} = 6.88$, $p = 0.047$, Fig. 2, middle row, left), whilst the pattern corresponded to the other areas for the distance in the lying area but did not reach significance ($F_{1,5} = 0.56$, $p = 0.49$).

3.4. Synchronicity and distance among the heifers of the pair and towards the cows

Heifers of the pair were more synchronous with each other than with the cows in the activity area (model means: 7.5% vs. 5.2%, $F_{1,5} = 11.33$, $p = 0.020$). This difference in synchronicity did not attain significance in the lying area (35.5% vs. 32.6%, $F_{1,5} = 2.21$, $p = 0.20$) nor the feeding area (11.0% vs. 12.5%, $F_{1,5} = 2.34$, $p = 0.19$), although the difference was in the same direction in the lying area.

Also, the distance was shorter between the heifers of the pair than with the cows in all areas (activity area: 2.9 m vs. 4.1 m, $F_{1,5} = 29.83$, $p = 0.003$; lying area: 2.4 m vs. 3.8 m, $F_{1,5} = 60.26$, $p = 0.001$; feeding area: 3.6 m vs. 4.7 m, $F_{1,5} = 6.76$, $p = 0.048$; Fig. 3, left-hand column).

Heifers introduced in pairs were less synchronous among each other than towards the cow with which they reached the highest synchronicity (activity area: 7.5% vs. 10.1%, $F_{1,5} = 6.25$, $p = 0.055$; lying area: 35.5% vs. 40.8%, $F_{1,5} = 6.44$, $p = 0.052$; feeding area: 11.0% vs. 15.9%, $F_{1,5} = 23.14$, $p = 0.005$; Fig. 3, right-hand column). There were no significant differences in the distance among the heifers and the smallest distance reached with any of the cows, but the distance among the pair was longer on average than the smallest distance reached with cows (activity area: 2.6 m vs. 2.9 m, $F_{1,5} = 1.27$, $p = 0.31$; lying area: 1.8 m vs. 2.4 m, $F_{1,5} = 5.67$, $p = 0.06$; feeding area: 2.7 m vs. 3.6 m, $F_{1,5} = 4.02$, $p = 0.10$).

In summary, heifers of the pair were found at smaller distances to one another than their median distance with the cows and tended to be more synchronous with each other than with the cows. In contrast, their highest synchronicity value with any of the cows was larger and their shortest distance value tended to be smaller than those values observed among the pair.

3.5. Effect of the introduced heifers on dyadic synchronicity and distance among the cows

Correlation coefficients between the matrices of dyadic synchronicity as well as dyadic distance were calculated for the cows between the situation before and after introduction of the heifer(s). None of the Wilcoxon tests comparing these correlation coefficients between the introduction of a single heifer and a pair of heifers reached significance ($p > 0.16$). The median difference between the correlation coefficient for the introduction of a pair minus the coefficient for the introduction of a single heifer was negative in five out of six possible combinations of the synchronicity or distance and three functional areas, however (range: -0.003 to -0.049), indicating weaker correlations if a pair rather than a single heifer was introduced. In other words, matrices of dyadic synchronicity values and distances were consistently less well correlated from before to after an introduction if a pair of heifers was introduced rather than a single heifer.

4. Discussion

4.1. Patterns due to introduction of single or pair of heifers

In this study, we examined the influence of the introduction of single heifers and pairs of heifers on synchronicity and distance between heifers and cows and among cows within the dairy herd.

From the point of view of both the introduced heifers (Section 3.2) as well as the cows (Section 3.3), singly introduced heifers were more synchronous and at shorter distances towards the cows in the activity and feeding areas than the heifers of the pair; the converse, however, applied for the lying area. These differences between the functional areas may ask for a differentiated interpretation.

Close associations (high synchronicity and short distances) are usually seen as part of the affiliative aspects of a relationship. However, animals showing high rates of agonistic interactions might also be recorded as using a given barn area synchronously and staying in close proximity. For example, the newly introduced heifers may have found it difficult to avoid aggressive cows in time and space due to the restricted amount of space in a barn.

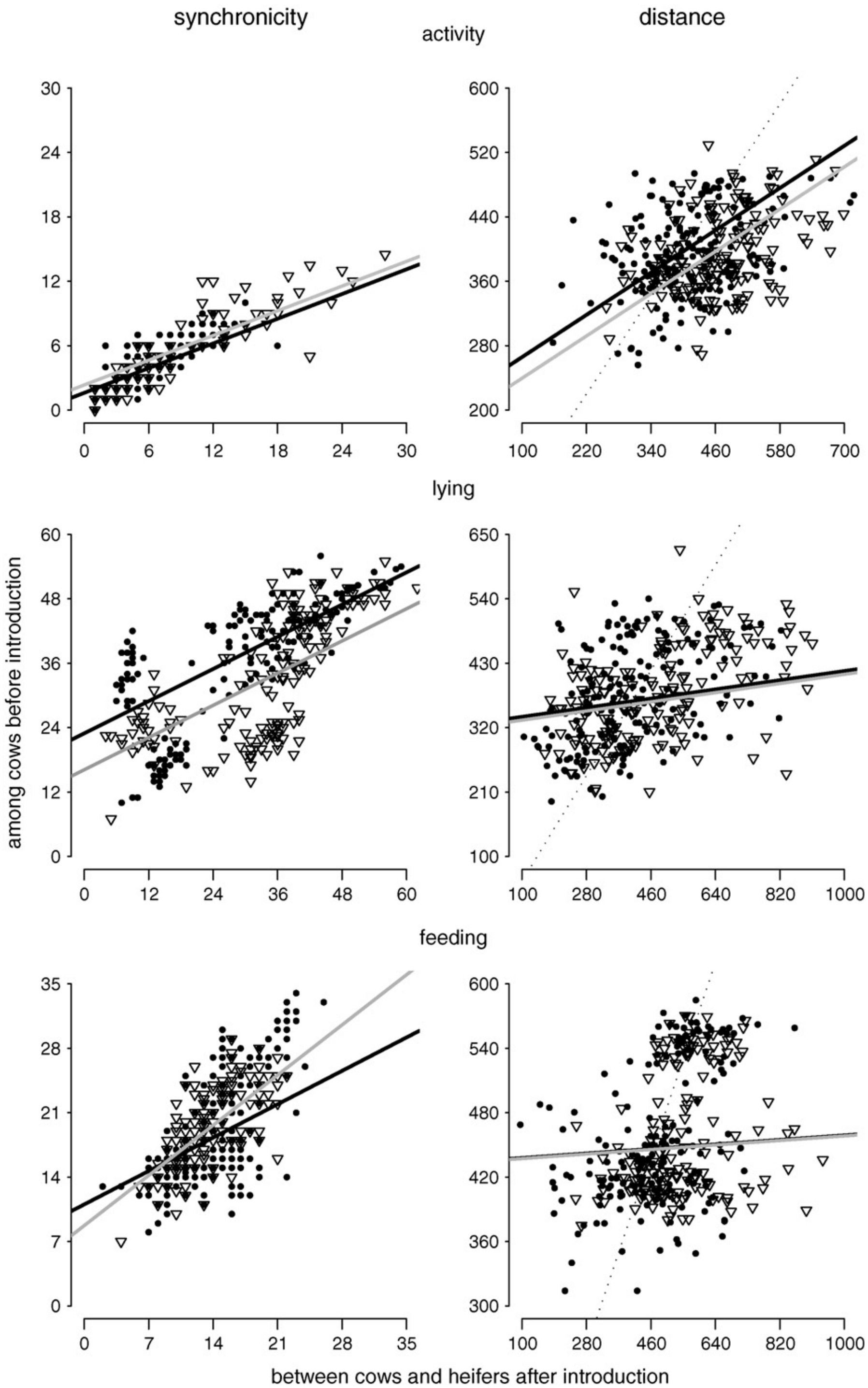

Fig. 2. Synchronicity (%) and distance (cm) among the cows before the introduction of the heifers vs. synchronicity and distance between the cows and the single heifer (●, model estimate: black line) and cows and the heifers of the pair (▽, model estimate: grey line) in the activity, lying and feeding areas. For synchronicity, scales are adjusted on both axes so as to span the same range to illustrate a 1:1 ratio more easily. A 1:1 slope is indicated with a dotted line in the graphs of the distance.

Thus, the closer association observed in the activity and feeding areas of cows and singly introduced heifers compared to cows and heifers in pairs may just reflect the doubled rate of agonistic interactions experienced by the single heifers. This notion is supported by the fact that cows with high synchronicity and at short distances to other cows that are likely to play a central role in the social structure of the herd had especially close associations with the introduced heifers (Section 3.3). However, direct agonistic interactions are short and the newly introduced

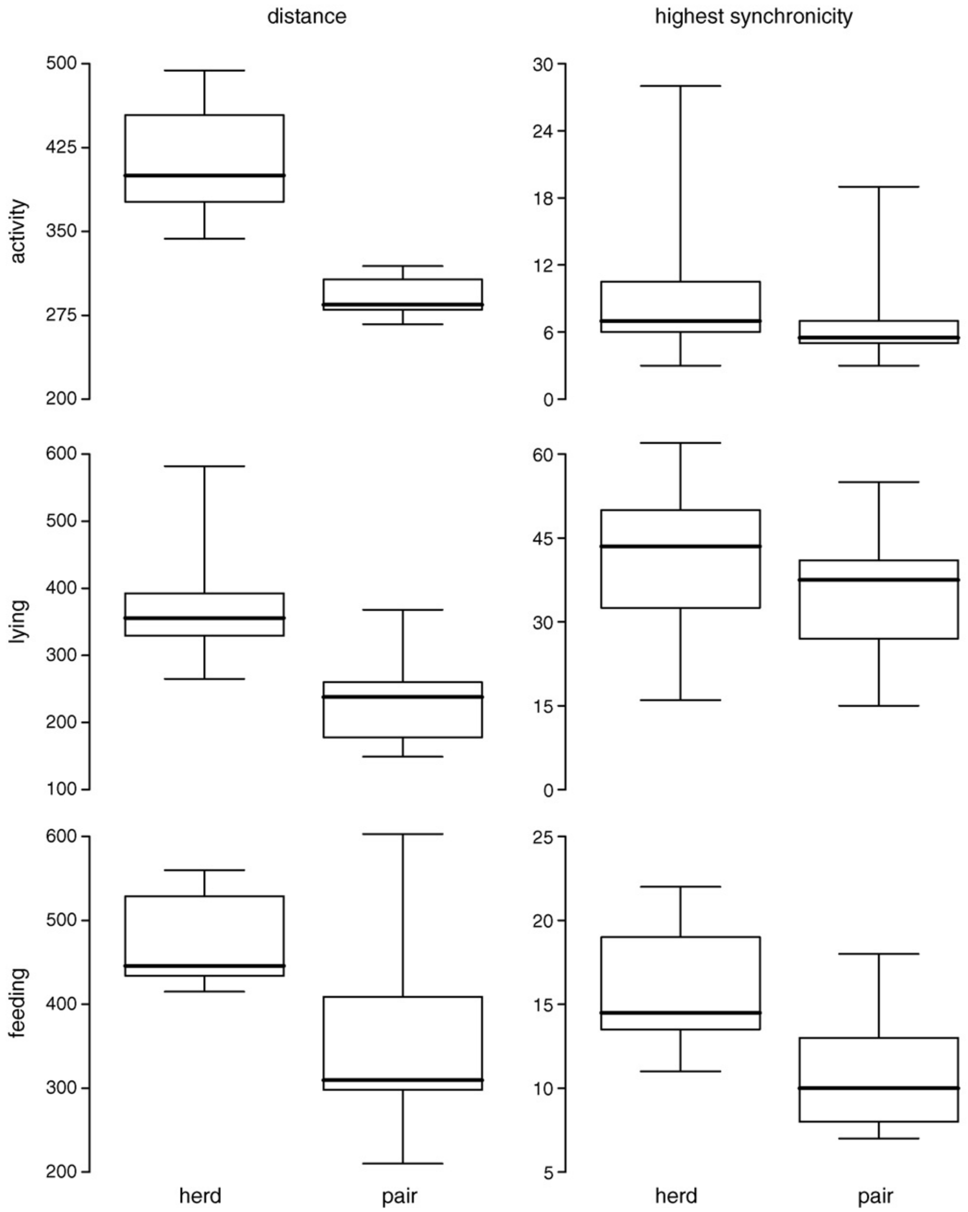

Fig. 3. Distance (cm) and highest synchronicity (%) among the heifers of the pair and between the heifers of the pair and the cows in the activity, lying and feeding areas.

heifers might otherwise try to avoid aggressive cows in time and space staying close to cows with a more welcoming response. In that case, synchronicity and distance may well serve to identify the start of what may develop into an affiliative relationship. To decide whether synchronicity and distances in the activity and feeding areas reflect the agonistic interactions after an introduction of heifers or the start of affiliative relationships, the aggressors need to be identified individually in a future study to see whether these are the animals synchronous and at short distances to the heifers. In keeping with our results on aggressions, Knierim (1999) observed a slight reduction in aggression if heifers were introduced in groups of three rather than singly, whereas Menke et al. (2000) found higher rates of agonistic interactions after the integration of three rather than a single heifer. The latter study may, however, suffer from a systematic bias, since groups of three heifers were always introduced shortly after the single animals. Both of these authors suspected that the introduced groups of three would present a strong opposition to the cows such that overall aggression was lower. It seems that in our study about the same absolute frequency of agonistic interactions was distributed among the two introduced heifers in a process that could be viewed as a pure dilution effect.

Munksgaard et al. (2005) found that lying has a higher priority than feeding and social contact for dairy cows, and that shorter feeding time will be compensated for by an increased feed intake/time. Thus, a possible disruption of feeding behaviour due to closer associations and a higher frequency of agonistic interactions of single heifers in the feeding area may have been compensated for. Lying, however, cannot be compensated for so easily, and thus the closer associations of the heifers of the pair in the lying area where no agonistic interactions took place may indeed be viewed as reflecting affiliative social behaviour which would then indicate an easier establishment of such relationships in the heifers of the pairs.

There was only a very small difference in favour of the introduction of a single rather than a pair of heifers in respect to changes in dyadic synchronicity and dyadic distances among the cows of the herd. Though consistent, this difference is unlikely to be of great relevance and is thus unlikely to reflect a difference in welfare of the cows of the herd.

Heifers of the pair tended to remain fairly close to each other in time and space but did not have uniquely close relationships, and could thus not be considered a clear-cut subgroup (Section 3.4). This contradicts the hypothesis of subgroup formation of several heifers introduced simultaneously as proposed by Knierim (1999) and Menke et al. (2000), though our introduced pairs may not have attained the same social momentum as the groups of three heifers introduced by these authors. Bearing in mind the relative high synchronicity and short distance between the heifers of the pair, they may well provide a degree of mutual social support in the process of integration into the dairy herds (Lazo, 1994; Sachser et al., 1998).

4.2. Methodological issues

In most of our analyses, we used both synchronicity and distance as a response each for all the three functional areas (activity, lying and feeding areas). Thus, although analogous patterns were tested repeatedly, not all six combinations necessarily reached significance. Nevertheless, patterns in the non-significant combinations were often consistent with those of the significant results. We therefore present and discuss consistent patterns that may not have attained significance in all possible combinations, in addition to single aspects that were significant. Some of the consistent patterns were rather weak as e.g. reflected by the differences in distances of singly introduced heifers and heifers introduced in pairs towards the cows (0.03–0.1 m). These differences were consistently reflected by other measures, though, i.e. by differences in synchronicity (0.9–3.6%) and distances and synchronicity towards the heifers based on the perspective of the cows (Fig. 2). It is currently difficult to assess whether such differences are biologically meaningful and worth of interpretation. We do think that what we observe are weak but consistent patterns and, as we are currently unaware of what such changes in dyadic values of synchronicity and distance signify for the animals, we have valued consistency more than the size of an effect. We think that only future studies can clarify this issue.

The free use of the exercise yard on three farms may have lead to varying stocking densities within the barn during the period of data collection. It was, however, not reasonable to restrict data collection to times when all cows are in the barn (as this happened only rarely). The yards were much smaller in area than the barns, though, and could thus not really serve as a route to escape proximity of other animals. We restricted our evaluation to data collected within one of the defined three functional areas and we do not think that the additional use of an exercise yard influenced the socio-spatial relationship of the cows given their presence in these specific functional areas.

In addition to the use of the exercise yard, external time keepers such as the presentation of fresh feed may have lead to different average absolute values in synchronicity and distance for the functional areas if e.g. cows went to feed together and thus acted synchronously as a herd. Nevertheless, we have quite a bit of variability in our data given a functional area (as seen in Fig. 2). Thus, cows seem to choose how synchronous and at what distances they stay in relation to specific other cows conditional on the functional area.

4.3. Conclusions

Our findings indicate that the welfare of heifers introduced in pairs was less at risk compared to singly introduced heifers. Given their temporal and spatial proximity, they seemed to provided some mutual security and support. Moreover, the halved rate of agonistic interactions directed towards a heifer of the pair indicated a dilution process. Finally, specific cow individuals seemed to play a general crucial role in the herds and while introducing heifers: they were central to the socio-spatial relationships in the herds and central in taking up such relationships with the introduced heifers. To summarise, we would recommend the introduction of pairs of heifers rather than single heifers into dairy herds similar to those of our study, i.e. herds composed of up to 50 dehorned cows kept in cubicle housing systems.

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