ABSTRACT: Recent evidence implicates fiber type proportions as playing a role in meat eating quality, and in pigs it has been suggested that the slow oxidative fibers contribute to both juiciness and tenderness. The fiber distribution in pigs is different from that found in most other species, in which the various types of skeletal muscle fiber are distributed in a “checkerboard” pattern, because in pigs the slow oxidative fibers have a clustered distribution. The initial processes leading to fiber clustering are likely to occur during myogenesis, but the precise mechanistic aetiology of this patterning and whether the slow oxidative fiber clusters occur in a random or ordered fashion is unknown. In the present study longissimus thoracis muscle from Large White crossbred pigs was sampled at the 10th rib, 48 h post-mortem. Transverse cryo-sections were cut and histochemically stained to allow the identification of the main muscle fiber types: slow oxidative, fast glycolytic, and fast oxidative glycolytic. Images of the sections were captured and analyzed using point processes and Voronoi Tesselations to examine the randomness and spatial distribution of the clusters of slow oxidative fibers found in pig longissimus thoracis muscle. The results showed that an assumption of complete spatial randomness can be rejected and that a mathematical model incorporating a minimum distance of 1.7 to 2.0 μm between cluster centers produced fiber patterns similar to those observed in the original transverse sections of the muscle. In addition, if it assumed that the central fiber in each cluster is derived from primary myoblast progenitors, these results suggest that there may be some degree of repulsion between the primary fibers during the initial stages of cluster formation. The mechanistic basis of such repulsion is not clear, but it is speculated that secreted factors, such as sonic hedgehog or myostatin may play a role.

Key Words: Meat Quality, Muscle Fibers, Simulation Models, Spatial Distribution, Stochastic Processes

Introduction

Muscle comprises different types of fiber that can be classified according to their contractile and metabolic nature. Various methods (Klont et al., 1998; Karlsson et al., 1999; Lefaucher et al., 2002), including immuno-cytochemistry, in situ hybridization, and combined myofibrillar ATPase and metabolic enzyme labeling are used to classify the different muscle fiber types. Using histochemistry, fiber types can be classified into three groups: slow twitch oxidative (SO), fast twitch oxidative glycolytic, and fast twitch glycolytic. In most species, the fiber type distributions give a “checkerboard” appearance in cross section, but in pigs, fiber patterns are typified by type grouping with SO fibers in clusters throughout the tissue (Beermann et al., 1978). The origin of fiber clustering is unknown, but it probably arises during myogenesis, a biphasic process (Beermann and Cassens, 1977a; Wigmore and Stickland, 1983; Wigmore and Dunglison, 1998) in which primary fibers, progenitors of adult SO fibers, form first acting as scaffolding for the later formation of secondary fibers. Secondary fibers are progenitors of adult fast fibers, although fiber type transformations occur during maturation (Lefaucher et al., 1995). In pigs the central cluster fiber is a primary fiber, so the number of SO clusters indicate the number of primary myofibers formed during myogenesis.
The determinants of meat quality remain unclear, but it is speculated that muscle fiber type composition is relevant, where the SO fiber proportions appear to contribute to juiciness and tenderness (Henckel et al., 1997; Maltin et al., 1997). Hence, knowledge of the factors that influence cluster pattern formation in the pig will contribute to the understanding of meat quality. This study evaluates the spatial distribution of clusters in pig muscle and develops a mathematical model to describe their formation as a first step towards unraveling the biological basis of cluster formation.

Materials and Methods

Animals

Thirty-one commercially available Large White crossbred pigs were raised in the U.K. under standard commercial production systems and subjected to normal husbandry practices. Briefly, the animals were weaned at 21 d of age, had ad libitum access to feed (using a conventional commercial diet) from 30 kg, and were slaughtered at a liveweight of between 85 and 95 kg. Slaughter, by electrical stunning and exsanguination, was carried out at a commercial abattoir in accordance with the U.K. Meat and Livestock Commission’s Blueprint for pork (MLC, 1992); the carcasses were chilled according to normal U.K. plant practice; that is, the muscle temperature did not fall below 10°C through the chain from carcasses to prepared cuts. Chop samples of longissimus thoracis muscles were recovered from the carcasses at the 10th rib, 48 h after slaughter and were prepared for histochemical analysis.

Histochemical Analysis

Blocks approximately 1 cm³ were cut from the center of the longissimus thoracis muscle. Care was taken to ensure that the same area was sampled from each of the samples. The cubes of muscle were oriented for transverse sectioning, mounted on a piece of cork with optimal cutting temperature compound, covered with more optimal cutting temperature and with unpreserved talcum powder and frozen in liquid nitrogen with constant agitation. Serial transverse sections were cut at 10 μm using a Frigocut 2800 cryostat (Leica, Milton Keynes, U.K.). No freezing artifacts were observed in the tissue. The sections were reacted to demonstrate the activities of Ca²⁺-activated myofibrillar adenosine triphosphatase (ATPase) at pH 9.5 (Hayashi and Freiman, 1966). Using this staining regimen the SO fibers appear light, while the fast twitch oxidative glycolytic and fast twitch glycolytic fibers appear dark. Thirty-one sections (one from each animal) were viewed in a Leica microscope and captured at magnification of ×2.5 as bitmap (bmp) files with window dimension 736 × 574 pixels.

Image Analysis

Captured images were converted into gray-scale images, thresholded to isolate bright areas, and manually edited to remove bright areas, such as extracellular spaces between fascicles, not associated with SO clusters. Cluster centers were obtained as the centroid of all the pixels in a cluster. Image manipulation and calculations were carried out using ImageJ software (NIH, 2001). Clusters touching image boundaries were discarded. While this will lead to bias in estimating image characteristics, the same bias will be present in estimates from the simulated images. Clusters containing just one fiber were considered to have a different origin and so were omitted in later analyses.

Seed Points and Point Processes

The starting point of the model is a set of points in a rectangular region. These points will be termed seed points or simply “seeds” although no botanical association is implied. A point process is a model for the generation of random seed positions. The simplest such model, termed the Poisson process or complete spatial randomness (CSR) is one in which each seed is placed at random independently of all other seeds. The number of seeds (n) is chosen from a Poisson distribution with a specified mean (the density of the process), although the term CSR is also used for the situation where n is specified directly.

Testing Randomness

Although SO fiber clustering is quite obvious, it was not known how clusters were distributed throughout the tissue. To investigate this, clusters were treated as individual objects in all 31 sampled images, and their centroid positions were considered to be their spatial location. Spatial randomness of the point process formed by those locations was tested as a first step in this analysis. Ripley’s K-function (Ripley, 1977) was used for this end, and it was computed using the software program STG (Stoyan et al., 1996; Stoyan, 1998). It is defined as

\[ K(d) = \frac{\text{Expected number of points within a distance } d \text{ of an arbitrary point}}{\lambda} \]

where \( \lambda \) is the density of points (number per unit area). For CSR, \( K(d) = \pi d^2 \). An empirical estimate of \( K(d) \) for a set of points is given by the quotient of the number of points in \( W \) (region of study) separated by a distance less than \( d \) and the area of \( W \).

Randomness was also tested by means of another two methods based on the nearest neighbor distances, which were recorded as an indicator of interaction between adjacent clusters.

King (1969) suggested that in a random pattern the nearest neighbor distance is approximately normally distributed. Using this and considering a stationary
Poisson process with density $\lambda$, the expected value and standard error of the nearest-neighbor distance is

$$d_E = \frac{1}{2\sqrt{\lambda}} \quad \text{and} \quad \sigma_E = 0.26136 \sqrt{\frac{\lambda}{n\lambda}},$$

respectively. The test proceeds by considering the test statistic

$$c = \frac{\overline{d} - d_E}{\sigma_E},$$

where $\overline{d}$ is the observed mean value of the nearest-neighbor distances. If CSR holds, $c$ is normally distributed with mean 0 and variance 1.

Another measure of randomness is given by $\overline{d}/d_E$. This ratio ($R$) is called the nearest-neighbor measure and is interpreted as follows; where $R < 1$ the pattern is aggregated, where $R = 1$ the pattern is random, and where $R > 1$ the pattern is considered regular.

As complete randomness was rejected (see results), a model was developed to simulate the formation of SO fiber clusters in pig muscle based on the spatial distribution of their centers. The elements used in this paper are now described. Full details are given in the Appendix.

**Voronoi Regions**

Each seed has an associated Voronoi region, which is the subset of the study region that is nearer to that seed than it is to any other seed. The Voronoi regions of all seed points form a Voronoi diagram (Okabe et al., 2000), which splits the region into “cells” (polygons) that cover the entire area. This can also be produced by letting the seeds “grow” at a constant rate until the entire area is filled. This is termed a “Voronoi growth model” for which many variations have been proposed. In both cases, the resultant diagram presents a pattern, which was used to model cross-sections of muscle fibers.

**Inhibition Processes**

As CSR was rejected as a model for SO clusters, the alternative model presented in this paper twice makes use of the idea of inhibition in a point process. Many models have been proposed for this. The simplest, termed simple sequential inhibition (SSI), is one in which points are sequentially placed at random, as in CSR, except that if the chosen position is within a distance $\delta$ of a seed point already placed, that position is rejected and further attempts are made until the position chosen is not within $\delta$ of a seed point. A discussion of this process and many related alternatives is presented by Cressie (1993).

**Cluster Size**

The number of fibers per cluster was used to assess the size of the clusters. According to previous analysis (Maltin et al., unpublished data) nearly 80% of SO clusters have three, four, and five fibers each. Therefore, in the proposed model, all generated clusters are equally likely to have three, four, or five fibers.

In addition, individual SO fibers, which can also be found in the samples, were discarded from analysis. Their origin is unknown, but it is possible that they are intrafascicular SO fiber endings, and therefore a structure that can not be incorporated in the model. Alternatively, they may be normal SO fibers that have become detached from the actual cluster as samples were sectioned.

**Cluster Growth**

The SO fiber clusters will be modeled by selecting a fiber (termed the primary) to start with and then growing the cluster by adding neighboring fibers. This will be done by a random process. There are many ways of doing this, and a comparison is not attempted. The model presented here does not consider any interaction between clusters while they are being formed; thus, new cluster fiber cells are attached to the seed in such a way that clusters can join together forming clusters with more than five fibers.

**Results**

**Complete Spatial Randomness**

Figure 1 shows the empirical $K$-function curves, $\hat{K}(d)$, (thin solid lines). They all appear below $K(d) = \pi d^2$ (bold solid line), the expected $K$-curve generated assuming complete spatial randomness. This clearly demonstrates that clusters are not completely randomly distributed. Instead, they appear to be more regularly distributed throughout the tissue.
Table 1. Testing randomness using NN measure ($R$) and normal assumption ($c$). $R$ is the ratio of the mean nearest neighbor distance to its expectation under complete spatial randomness; $c$ is the ratio of the difference between these two quantities and the expected standard deviation. Definitions of $d$, $d_E$, and $\sigma_E$ are given below the table.

<table>
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<th>Label</th>
<th>Size, n</th>
<th>$R = \frac{d}{d_E}$</th>
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The values obtained for the test statistic $c$ and for the nearest neighbor measure $R$ from the samples analyzed in this study are presented in Table 1. For all 31 samples, $R$ is greater than 1, which indicates uniform (regular) patterns, and the observed value for $c$ is large, which assures that the null hypothesis (randomness) is rejected at $P < 0.00001$. This confirms the regularity suggested by the $K$-function comparison. Figure 2 shows a histogram of minimum nearest neighbor distances over all of images derived from the transverse sections of the pig longissimus muscle. As expected, there are no clusters very close to each other nor particularly isolated. This minimum intercluster distance, which appears to be between 1.7 and 2 $\mu$m, can be interpreted as an indicator of negative interaction (repulsion) between clusters. Hence, these data indicate that SO clusters in the pig longissimus thoracis are not distributed completely at random but rather occur separated by a minimum intercluster distance.

Model

Mature muscle fibers are cylindrically shaped and roughly of similar size so that transverse sections present a reasonably regular grid of cells, which lack a well-defined shape. The centers of these cells are believed to be separated by a minimum distance. Let the coordinates of these centers be called seed points.

The model starts by considering a SSI point process, say, $SSI_1$, with parameter $\delta_1$, to generate seed points. The Voronoi regions of these seeds are assumed to define muscle fiber cells (Figure 3a). This Voronoi diagram will be used in all the simulations without any changes, as it is the framework of all the remaining procedures in the present model. Following muscle fiber development, a second SSI process, $SSI_2$ with parameter $\delta_2$, is generated, subsampling seed points generated in stage 1, and the corresponding fibers define the primary fibers for cluster generation (Figure 3b). Cluster size is determined by choosing a number at random from some distribution, being, in this case, a uniform distribution between three and five. Finally, SO clusters grow
Figure 3. A: Voronoi grid representing muscle fiber cells, B: Primary fibers, C: Generated cluster pattern, D: Transverse section of adult pig loin reacted to demonstrate the activity of Ca\(^{2+}\) activated myofibrillar ATPase at pH 9.4 in which SO fibers appear as pale fibers. The cluster patterns are clearly visible.

around the primary fibers by selecting the nearest neighbor of a randomly chosen fiber already in the cluster. This continues until the number of fibers in a cluster reaches between three and five (Figure 3c). In general the model provided a reasonably good approximation to the muscle fiber distribution seen in transverse sections of pig longissimus muscle (Figure 3d). A more mathematical description of the model is presented in the Appendix.

However, despite the similarity between the model muscle fiber patterns and those from ex vivo samples (Figures 3c and d), the Voronoi model does not provide a perfect fit for the full range of fiber sizes and shapes observed in adult porcine longissimus muscle. The model tends to overestimate the size of the smallest fibers while underestimating the size of the largest fibers as shown in Figure 4.

Two parameters were required—the number of primaries (\(m\)) and the minimum distance between them (\(\delta_2\)). The estimation of the two parameters in this model (\(m\) and \(\delta_2\)) was made by trial and error as shown below. The number of primaries (\(m\)) was chosen according to the total number of clusters usually observed in each sampled image, and \(\delta_2\) was set to be slightly greater than the minimum intercluster distance found in the samples (between 1.7 and 2 \(\mu\)m).

Three variations of the initial model are presented with parameters as follows: Model 1, \(m = 100\), \(\delta_2 = 2.5 \mu\)m; Model 2, \(m = 100\), \(\delta_2 = 2.7 \mu\)m; Model 3, \(m\) not fixed, \(\delta_2 = 2.7 \mu\)m. (Models 1 and 2 did not fail to allocate
Figure 4. Fitting a Voronoi diagram to a transverse section of pig muscle shows clearly that while the fit of the model is generally good, the full range of observed fiber sizes are not reproduced.

100 seeds: for model 3, \( m > 100 \) for all the simulations). These three models were compared relative to true cluster patterns by means of the \( K \)-function. One hundred cluster patterns for each of the models were generated and used to determine the envelopes of 95% confidence for the \( K \)-function. The lower (\( \hat{K}_L \)) and upper (\( \hat{K}_U \)) envelopes were calculated as

\[
\begin{align*}
\hat{K}_L(d) &= \frac{1}{2}(\hat{K}_{2:100}(d) + \hat{K}_{3:100}(d)) \\
\hat{K}_U(d) &= \frac{1}{2}(\hat{K}_{98:100}(d) + \hat{K}_{99:100}(d))
\end{align*}
\]

where \( \hat{K}_{i:100}(d) \) is the \( i \)th ordered value (out of 100) of \( \hat{K} \) at distance \( d \).

Empirical \( K \)-curves within \( \hat{K}_L \) and \( \hat{K}_U \) for Models 1, 2, and 3 are shown in Figures 5, 6, and 7, respectively. The percentage of times that an empirical sample is within the \( K \)-envelopes was taken as a measure of goodness of fit. Model 1 showed best fit, the observed \( K \)-function was within the 95% confidence envelope 74% of the times whereas for Models 2 and 3 the values were 68% and 63% respectively.

Hence, a reasonably simple model of cluster position and growth is able to reproduce the main features of pig muscle fiber patterning.

Discussion

The classification of muscle fibers into various types has been based on the identification of their contractile and/or metabolic properties. The simple histochemical methods based on reacting sections to demonstrate the activity of key enzymes of contraction, and oxidative or glycolytic metabolism give at least three different fiber types. The empirical \( K \) curves within \( \hat{K}_L \) and \( \hat{K}_U \) for model 1 is shown. The \( K \) envelope is shown in a solid thick line, the sample lines are thinner.
types: SO, fast twitch oxidative glycolytic, and fast twitch glycolytic. A more detailed and complex classification of fiber types on the basis of their myosin heavy chain (MHC) isoforms is achieved by immunochemical labeling of fibers using specific monoclonal antibodies against the MHC. This gives no information as to the metabolic nature of the fiber so the fiber type nomenclature is MHC based namely type I, type IIa, type IIx, and type IIb (Lefaucher et al., 1998). Unlike other muscle from other farm species, in the pig most muscles show fiber type grouping patterns in which SO fibers occur in clusters (Beermann et al., 1978) and are surrounded by type IIa or fast twitch oxidative glycolytic fibers and more peripherally by type IIb or fast twitch glycolytic fibers (Lefaucher et al., 1995). The present study has shown that the distribution of SO clusters in longissimus thoracis muscle is not random and that the cluster patterns can be approximated by the development of a stochastic process model.

Among the many spatial tessellations that might have been used to model muscle fiber patterns, the Voronoi growth model with constant growth rate was selected as it allows greater flexibility than the perturbations of a regular lattice described by Dryden et al. (1999). The muscle sections studied do not present an appearance of perturbed regularity. The issue of whether such regularity was present was not addressed in this study. However, the Voronoi model does not reproduce the full range of observed fiber sizes (Figure 3). A model based on a constant growth rate from seed points may be too restrictive. Generalizations, such as those described by Okabe et al. (2000), have not been explored here. The clustering of SO fibers was quite apparent, so an assessment of the significance of the spatial arrangement (Venema, 1995) was not needed.

The model used was based on a number of biological observations. In relation to myogenesis, it is known that during early myogenesis slow MHC-expressing primary fibers are formed and are found quite close to each other. Then, as secondary fibers form on the surface of primary myoblasts at the site of innervation and late in gestation express slow MHC (Lefaucher et al., 1995), the primaries are pushed apart (Maltin et al., 2001). In the pig, primary fibers form the center of each cluster that usually contains between three and five SO fibers (Maltin et al., unpublished data). This would suggest the model should address the development of the cluster from a central point and gave some indication of the final cluster size. In addition, the placing of the initial seeds was based on the observation that in adult animals, generally, and independent of muscle fiber type, the range of muscle fiber sizes in pig longissimus thoracis muscle is quite similar (Maltin et al., 1997; Wigmore and Dunglison, 1998), so the initial seeds were placed according to a regular point process.

Hence, the model included processes whereby \( m \) primaries are placed according to an SSI process that will keep them apart, with parameter \( \delta_2 \). For the model, the minimum distance between primaries was set to be slightly greater than the minimum intercluster distance, which was found to be mostly between 1.7 and 2 \( \mu \)m. This also allows for the fact that the center of the primary fiber around which a cluster has formed will deviate somewhat from the centroid of that cluster.

In addition, in order to reflect the development of the secondary fibers around the central primary fiber after the selection of primaries, a marked point process was created, and the mark variable was set to be a discrete random variable with values three, four, and five equally likely.

Since the presented model of the SO cluster patterning gave a reasonable fit, it may be assumed that the basic assumptions used to derive the model have biological relevance. The mechanistic basis of cluster formation is unknown, but it is clear that the grouping is not a result of subterminal axon sprouts innervating multiple fibers but rather appears to be a result of a unique motor unit topography (Beermann and Cassens, 1977b). This is consistent with the pattern of MHC expression seen in the superficial and deeper regions.
of porcine semitendinosus muscle (Lefaucheur et al., 1995) and the concepts of innervation-based fiber type determination (Chin et al., 1998). Together, these data and the present results suggest that the spatial organization of the central fibers in the cluster occurs before innervation has taken place and that subsequently, innervation and slow MHC expression in secondary fibers (Lefaucheur et al., 1995) occurs to form the observed pattern of motor units.

The present study shows that the distribution of SO clusters is not completely random but rather depends on some degree of spatial inhibition with a minimum intercluster distance. Given that evidence from the literature suggests that primary fiber numbers tend to be rather fixed and unaffected by most nonlethal manipulations to alter fiber number (Maltin et al., 2001), the present data may be speculatively interpreted as implying that some inhibitory element exists during myogenesis to limit both the number and spatial location of primary fibers. The origin of such an inhibitory element cannot be deduced from the present study. However, it may be speculated that such a proposed inhibitory element would be a secreted substance, and some indications as to possible inhibitory elements may be derived from published literature.

Myostatin, a member of the transforming growth factor β superfamily of growth factors, is an extracellular secreted factor, which has been shown to be a potent regulator of muscle growth (McPherron and Lee, 1997). Myostatin has been shown to negatively regulate muscle fiber number such that in double-muscled cattle, where a myostatin mutation compromises the biological action of the protein, muscle fiber numbers are significantly increased due to increased hyperplasia (McPherron and Lee, 1997). In the context of the present results, it is interesting to note that in double-muscled cattle it has been shown in vitro that there are higher levels of myoblast proliferation, which are associated with smaller proportions of primary fibers and higher proportions of secondary fibers than in normal animals (Deveaux et al., 2001). This suggests that myostatin may play some role in the differential regulation of both primary and secondary fiber numbers.

Studies on the origin of myoblasts may also provide some insights as to secreted factors affecting myogenesis. Using retroviral marking of rat myoblasts at embryonic d 15 in vivo, Dunglison et al. (1999) showed that embryonic myoblasts contribute only to the formation of primary fibers, all of which initially form SO fibers. In contrast, fetal and adult myogenic populations contribute to the formation of either fast or slow fibers. While the signal mechanisms controlling these processes are not fully understood, it appears that members of the hedgehog family of secreted glycoproteins are important regulators for tissue patterning and stem cell specification in many vertebrates (Lewis et al., 1999; Cann et al., 1999). Sonic hedgehog (Shh) in particular has been shown to mediate both long-range and short-range signaling responses in embryonic tissues. In mouse embryos it has been shown that Myf5 is the direct target of long range Shh signaling demonstrating that Shh has a direct inductive function in muscle cell lineage specification (Gustafsson et al., 2002). Moreover, in avian embryos, Shh appears to regulate the numbers of slow myosin-expressing primary fibers (Cann et al., 1999). This is supported by evidence from zebrafish, which suggests that hedgehog family proteins regulate a u-boot gene, which acts as a myogenic switch (Roy et al., 2001) to regulate the formation of slow fibers (Blagden et al., 1997; Norris et al., 2000). Clearly, then, the hedgehog family proteins and Shh, in particular, may be important in the SO fiber clustering in pigs.

Thus, since the present data suggest primary fiber formation involves a local inhibition of the formation of other primary fibers, it is tempting to speculate that during development long-range signaling or a local diffusion gradient affects the spatial organization of primary fibers. This type of signaling might be generated by an effector system, such as the secreted hedgehog family or myostatin proteins and/or their pathway regulators acting to regulate the specification and/or differentiation of primary fibers, hence, resulting in the minimum intercluster distance of 1.7 and 2.0 μm seen in the present study. If this were the case, then it is possible that the fiber clustering seen in pigs results from species-specific differences in the actions and interactions of secreted regulatory proteins.

The finding that distribution of SO clusters in porcine longissimus thoracis muscle is not random may have important consequences for meat quality. The precise factors which control tenderness are not defined, but it is clear that proteolysis and collagen content make contributions to tenderness, which is known to vary between muscles (Wheeler et al., 2000). Muscle fiber types also vary between muscles, and if the variation in fiber type proportions do contribute in some way to the variation in eating quality, as has been widely speculated, then the apparently structured nature of SO fiber distributions patterns may influence eating quality. It is likely that any effects on quality are related to the specific characteristics of SO fibers, namely their aerobic nature, lower glycolytic potential, and apparently higher calpain activity (Ouali, 1990). For example, the spatial organization of the SO clusters (and their size) may affect the overall measure of muscle toughness through the mechanical impact of islets of fibers with higher proteolytic activity leading to variations in eating quality characteristics between muscles. This speculation may be supported by data from Duroc pigs showing that in these animals, known for their eating quality benefits (Blanchard et al., 1999), cluster characteristics are different from those found in Large White pigs (Maltin et al., 1998). These and the present observations raise the question of whether cluster patterns are important determinants of eating quality and if they can be influenced by breed selection or other strategies, such as maternal feeding during gestation (Ji et
al., 1998). Further studies of the mechanisms of cluster formation in pig muscle will contribute to the understanding of the impact of cluster patterns on meat eating quality.

Implications

The findings that the distribution of the clusters in porcine longissimus thoracis muscle is not random and that there is a minimum intercluster distance has important implications for the understanding of cluster formation. The data allow speculation as to the existence of a secreted inhibitory factor regulating the proliferation and/or differentiation of the primary fibers during early myogenesis, prior to innervation. Moreover, it is possible that the spatial organization of clusters may relate to eating quality and that factors, such as breed or feeding during gestation may be important.

Literature Cited


Appendix

Model Algorithm

Modeling Muscle Fiber Cells. Consider a set of \( n \) seed points, \( G = \{x_1, x_2, \ldots, x_n\} \), outcome of a SSI process with parameter \( \delta_1 \), where \( x_i = (x_i, y_i) \in \mathbb{R}^2 \forall i \).

Assign to \( G \) a growth process with constant rate \( \rho \), which results in a set of \( n \) identical polygonal cells \( V(G) = \{V(x_1), V(x_2), \ldots, V(x_n)\} \), which we assume to be muscle fibers. Note that \( V(x_i) \) is the Voronoi region of the seed point \( x_i \).

Placing the Primary Fibers. Select \( S = \{s_1, s_2, \ldots, s_m\} \) from \( G \) according to a SSI process with parameter \( \delta_2 \). Then, \( V(S) = \{V(s_1), V(s_2), \ldots, V(s_m)\} \subset V(G) \) is the set of \( m \) primary fibers.

Generating the Clusters. We assume that each primary generates a cluster. Thus, assign to each primary, say \( V(s_j) \), a number \( z(s_j) \) to be the number of fibers per cluster according to a marked point process with \( Z(s_j) \) - \( U(ab) \).

\( Z(s_j)-1 \) cells are selected from \( V(G) \setminus \{V(S)\} \) and attached to \( V(s_j) \) to form the cluster \( j \) for \( j = 1, \ldots, m \).

The mechanism for cluster formation is mathematically explained in the following algorithm:

Cluster \( j \) formation starts by letting \( V(s_j) \) to be the first cell of the cluster \( j \),

\[
c_{1,j} = V(s_j)
\]

and

\[
c_{2,j} = \text{NN}(V^{-1}(c_{1,j}))
\]

For \( k = 3, \ldots, Z(s_j) \),

\[
c_{k,j} = \begin{cases} 
V(\text{NN}(V^{-1}(c_{1,j}))) & p_1 = \frac{1}{k - 1} \\
V(\text{NN}(V^{-1}(c_{2,j}))) & p_2 = \frac{1}{k - 1} \\
\vdots & \vdots \\
V(\text{NN}(V^{-1}(c_{k-1,j}))) & p_{k-1} = \frac{1}{k - 1}
\end{cases}
\]

where \( c_{k,j} \) represents the \( k^{th} \) cell of the cluster \( j \),

\( \text{NN}(x) \) gives the nearest neighbor point of the \( x \)

\( V^{-1}(y) \) gives the seed point of the cell \( y \)

Note that a selected cell is not picked again, i.e., \( c_{k,j} \neq c_{l,i} \quad \forall i \neq j, \forall k, \forall l \).

The NN function can be mathematically defined as:

\[
\text{NN}(V^{-1}(c_{k,j})) = s_l; D(V^{-1}(c_{k,j}), s_l) = \min_i \{D(V^{-1}(c_{k,j}), s_i) \mid i = 1, \ldots, m \}
\]

where \( D(.,.) \) is the Euclidean distance.

Spatial Point Process

Let \( D \) be a fixed subset of \( \mathbb{R}^2 \), \( s \in \mathbb{R}^2 \) the spatial location of a generic data and \( Z(s) \) a random quantity associated with \( s \); we can define a multivariate random process \( \Phi(D) = \{Z(s) : s \in D \subset \mathbb{R}^2 \} \) by allowing \( s \) to vary over \( D \).

Thus, a simple spatial point process is given by \( \{z(s_1), z(s_2), \ldots\} \), where each \( z(s_i) \) is a realization of \( \Phi(D) \) and \( Z(s) \) a degenerate variable,

\[
Z(s) = \begin{cases} 
1 \text{ if } s \in D \\
0 \text{ otherwise }
\end{cases}
\]

Marked Point Processes

Let \( \Phi(D) = \{Z(s) : s \in D \subset \mathbb{R}^2 \} \) be the spatial point process defined above. If we consider \( Z(s) \) to be a nondegenerate random variable then \( \Phi(D) \) becomes a marked point process with \( Z(s) \) a mark variable, which denotes some measurement, mark, characteristic of the event located at \( s \). It can either be continuous or discrete depending on the phenomenon we are looking at.

As a result, the spatial point process \( \Phi(D) \) is now defined as \( \psi(D \times L) = \{[s_1, u_1], [s_2, u_2], \ldots\} \) where \( u_i = Z(s_i) \in L \) is the mark of the event located at \( s_i \).

Planar Voronoi Diagram

General Definition. Given a finite set of distinct, isolated points in a continuous space, we associate all the locations in that space with the closest member of the point set. This results in a partition of the space into regions which we call Voronoi diagrams (Okabe et al., 2000).

Growth Model. A Voronoi diagram can be interpreted as the final result of a growth model—Voronoi growth model. Thus, consider a set points \( P = \{p_1, p_2, \ldots, p_n\} \) in a region \( A \subset \mathbb{R}^2 \).

Assumption 1) Each point \( p_i \) (\( i = 1, \ldots, n \)) is located simultaneously,

Assumption 2) Each point \( p_i \) remains fixed at \( x_i \) throughout the growth process,

Assumption 3) Once \( p_i \) is established, growth commences immediately and at the same rate, \( l_i \) in all the directions of \( p_i \),

Assumption 4) \( l_i \) is the same for all members of \( P \),

Assumption 5) Growth ceases whenever and wherever the region growing from \( p_i \) comes into contact with that growing from \( p_j, i \neq j \).

If points are regularly spread in the region \( A \), the described process results in a grid of equally sized polygons.
**Simple Sequential Inhibition Process**

Inhibitory processes are more regular than CSR, and they are expected in patterns in which events interact with each other. The most common way to impose regularity is to prohibit pairs of points from being closer than a certain fixed distance, say $\delta$. These processes are also known in the literature as hard-core models. In particular, we will refer to Diggle’s SSI process (Diggle et al., 1976).

It starts by considering a disk of radius $\delta$ at random in the region $D$. In each step a point is selected at random from a uniform distribution of these points, and it is kept in the process if the disk centered on it with radius $\delta$ does not overlap any of the ones placed before. The process stops when the number $n$ of discs required is achieved or if it is impossible to allocate more discs.

For inhibitory point processes a packing intensity $\tau$ is defined, representing the proportion of the plane covered by nonoverlapping discs of radius $\delta$. For the SSI process we have the following.

$$\tau = \frac{n \pi \delta^2}{\text{area } (D)}$$