"Excelling in equality: a new method to improve life-long uniformity and resilience of livestock via precision livestock breeding"

Rationale and positioning with regard to the state-of-the-art

Ranging from malpractices in abattoirs to the selling of expired meat: during the past year meat production has shown up in media in a very negative light. The elaborate selective breeding in livestock has produced animals which perform efficient both economically and environmentally. The downside, however, is that animals are pushed towardstheir biological limits and even beyond, for example with ever-increasing litter sizes in pigs[1]. This process of optimization is associated with an incompatibility of breeding robust and healthy animals and has led to several welfare issues[2]. In this research project a new and innovative method of breeding is proposed: precision livestock breeding. **Precision livestock breeding integratesreal-time data in new statistical genetic models. Ultimately, this will allow us to improve uniformity and resilience of animals by selection.** The methods presented in this proposal circumvent distress of both animals and farmers, and will benefit all actors in the meat production network.

First things first, a new selection method?

In classical selection, the genetic value of a breeding animal is estimated based upon its own performance and the performance of its relatives. Classical selection works well for most (re)production traits. However, it offers little opportunities to improve lowly heritable and hard to define traits like uniformity and resilience[3,4]. Shortcomings are the focus on a single measurement per animal (e.g. the end weight) and the use of pedigree data to account for genetic relationships. The emergent precision livestock farming (PLF) technologies, on the contrary, enable to phenotype individuals precisely, continuously and on a large scale. This collection of data throughout an animals' lifetime is called phenomics[5]. Additionally, genomic data becomes more and more available as a consequence of continuously decreasing genotyping costs[6]. These two new information sources open a window of opportunities, not in the least for breeding livestock animals. Combining phenomics and genomics enables us to improve selection accuracy. That means, a whole new range of traits such as uniformity and resilience becomes accessible for selection[4,6]. However, having the right equipment and gathering data is not sufficient for a successful selection process: many biological, statistical and practical questions still need to be answered.

This research project is the first attempt to investigate the quantitative genetics of uniformity and resilience throughout the lifespan of animals.

Resilience and uniformity: what's in the name?

Resilience is a complex trait that is hard to measure. Knap[7] defined robustness in pigs as '*the ability to combine a high production potential with resilience to stressors, allowing for unproblematic expression of a high production potential in a wide variety of environmental conditions*'. When facing an environmental challenge, more resilient animals will be less affected (small drop in production potential) and will recover more quickly afterwards[8] (Figure 1).

On the other hand, livestock *uniformity* can be divided in two subtypes: within-individual uniformity (e.g. symmetry of body composition) and within-family uniformity[9]. The focus in this research project will be on *within-family uniformity*: *differences in variation between individuals in the same family and the same environment*[9]. Evidence in livestock[1,3,4] and aquatic species[10] suggests that one generation of selection has the potential to improve uniformity by 20-60%.

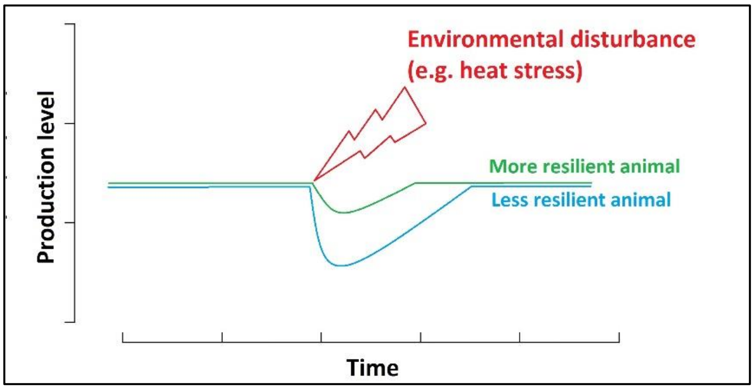


Figure 1: Visual representation of resilience in terms of maintaining production level. After an environmental disturbance, more resilient animals will be less affected and will recover more quickly[8].

Resilience and uniformity are not independent traits. Breeding more uniform animals is associated with improved robustness, vitality and health. This association was evidenced in both statistical studies on different livestock species[4] and selection experiments on litter size and birth weight variability in rabbits11 and mice[12,13].

Breeding more resilient and uniform growing animals has become a priority[2,14,15]. First, the average number of animals per farmer is increasing. As a consequence, farmers desire easy to manage animals which are also economically efficient[16]. Furthermore, breeding companies distribute their genetic resources worldwide. Hence, they desire breeding animals that perform well across borders and in different environmental conditions. Third, climate change increases the need for animals which are resilient to environmental changes[2,14]. Fourth, public concern considering animal welfare and health is gaining importance[2]. Last but certainly not least, uniform animals are required to cope with increasing automation and rising quality demands[17] (see further).

Growth curves: the key to the problem?

The corner stone of this research is phenomic growth data, which provide multiple measurements throughout an animals' lifetime. The goal is to develop a new statistical methodology to exploit this extra information for breeding more uniform and resilient animals. Longitudinal growth data are suitable to quantify and predict individual Gompertz growth curves[22], as different studies demonstrated in pigs[18,19]. **Growth curves are able to condense the complex weight-age relationship into three biologically interpretable parameters[20]** (Figure 2A).

Modelling the genetics of growth curves is a complex problem because non-linear longitudinal data – influenced by genetic and environmental effects – have to be fitted. Consequently, multiple statistical methods have been developed for estimating these genetic effects[18,21]. Ibáñez-Escriche and Blasco[21] showed how genomic data can be used to significantly improve the accuracy of selection on growth curves. **Breeding more uniform and resilient animals via precision livestock breeding is a completely new and innovative way of breeding** (Figure 2B).

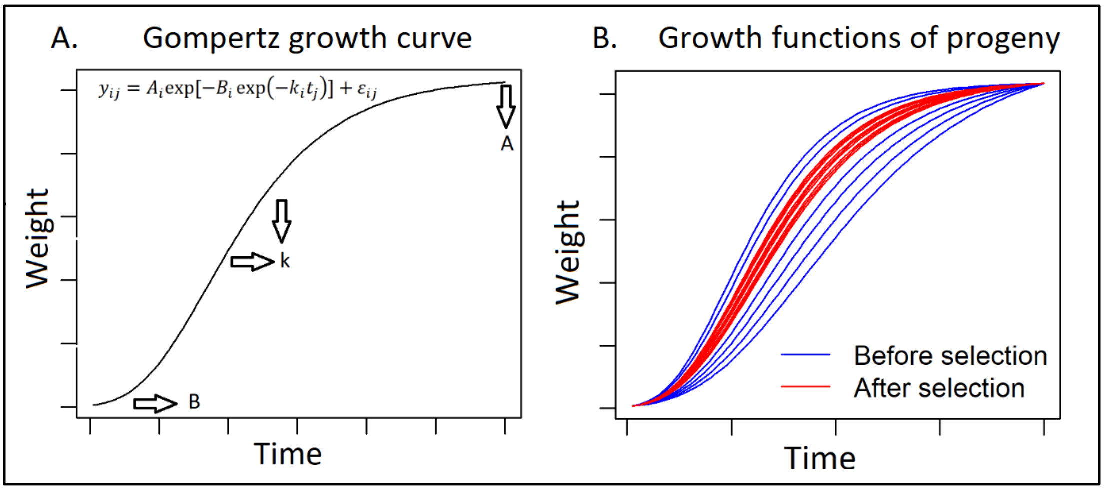


Figure 2: A. Gompertz growth curve for the weight (y_{ij}) of an individual i at time point j (t_j; age) with residual error ε_{ij}. Arrows indicate the 3 biologically interpretable parameters: asymptotic end weight (A), the ratio of mature weight to birth weight (B) and the rate of maturation (k). B. Illustration for increasing uniformity of growth rate parameter k by selection, with fixed parameters A and B. Blue lines indicate animals before selection, red lines indicate animals after selection.

Scientific research objectives

The main objective of this research proposal is to investigate the quantitative genetics of uniformity and resilience over time. New models, including features from growth curves in pigs, will be developed and enable the integration of phenomics and genomics.

By applying several state of the art methods and techniques – spread out across different work packages – the following **hypotheses** will be investigated:

1. Uniformity of growth curves can be analyzed by newly developed statistical methods (WP2)
2. Genetic parameters of uniform growth can be calculated over an animals lifetime and novel genomic breeding values (GBVs) can be estimated for these animals. The genetic correlation of uniform growth is high between purebreds and crossbreds (WP3)
3. Uniformity of growth and resilience are genetically closely related (WP4)
4. We can validate our findings and select more uniform and resilient pigs in practice (WP5)

Objective 1. Just in time: developing new methods to analyze genetics of uniformity of growth

Modelling the genetics of uniformity using growth curves is a completely new and innovative way of breeding more uniform and resilient animals. It is specifically challenging because of the non-linear character of longitudinal growth data and the complexity of modeling uniformity[3,21]. Presently, no methodology exists to model uniformity of growth over time. This study aims to develop such statistical methods for the first time. Consequently, this research will provide insight in the genetics of uniformity and resilience. Most importantly, it will provide farmers and animal breeders an efficient tool to increase the lifetime uniformity and resilience within their herds.

Outcome: *A scientific paper will be written about these new methods which go beyond the state of art. New statistical tools for livestock breeding are developed. These methods will be used in following objectives.*

Objective 2. Time will tell: quantifying the genetics of uniformity throughout an animals life and calculating genomic breeding values

By using the newly developed statistical methodologies, genetic parameters of uniformity of growth in time are calculated. This will be the first study to calculate genomic breeding values (GBVs) for uniformity of growth in time. Moreover, very little is known about the genetic correlation of uniformity between purebreds and crossbreds (r_{pc}), except for one study on laying traits in hen[23]. This correlation is of vital importance for pig breeders, since selection is mainly based on purebred data, while the goal is rather to increase the performance of the crossbred offspring[23,24]. A r_{pc} below 0.80 means that crossbred data would have to be implemented in breeding schemes to assure an efficient selection[24]. For these reasons, r_{pc} of uniform growth will be calculated.

Outcome: *Genetic parameters, GBVs and a calculation of* r_{pc} *of uniformity of growth in time will be estimated in this study, resulting in a scientific publication. Moreover, the GBVs will be a very useful tool for animal breeders to improve the uniformity of their herd genetically. The estimation of* r_{pc} *is vital for breeders to determine the amount of crossbred information in their breeding design.*

Objective 3. Great animals look alike? Quantifying the genetic correlation between lifetime uniformity of growth and resilience

Resilience of livestock animals is a hot topic in animal breeding. Hereby the goal is to breed animals which maintain a high production level and health, even in the presence of external stressors[2,4,8,25]. This is the first study using growth curves to investigate the genetic relationships between lifetime performance, uniformity and resilience. Our findings will improve the knowledge about these new traits and may result in an efficient way to simultaneously improve both traits via selection.

Outcome: *The genetic links between lifetime growth, uniformity and resilience will be uncovered in pigs. This will lead to new insights in the genetics of these complex traits and new ways to improve both uniformity and resilience. These findings will be presented in a third publication.*

Objective 4. Can we put it in practice? A selection experiment to validate our findings

The previous work will result into GBVs for uniform lifetime growth. A selection experiment will evaluate the progeny of two selected groups for uniformity based on these GBVs. The progeny will encounter several challenges at specific time points, providing more information about the relation between environmental changes, uniformity and resilience of animals. This would be the first selection experiment considering uniformity in pigs and will validate the findings in this project.

Outcome: *A selection experiment will validate the previous findings and will uncover more about the effect of environmental challenges on both uniformity and resilience of animals. These results will be reported in a publication.*

Research methodology and work plan

This research project is subdivided in three major parts (Figure 3). In a first part, longitudinal growth data and genomic data are collected in pigs (WP1). Simultaneously, a large simulation study will be performed (WP2). For the simulated growth trajectories new statistical methodologies for modeling uniformity of growth over time are developed and tested. In the second part, the new statistical methods are used to calculate genetic parameters of uniformity over time, GBVs and r_{pc} (WP3) and to investigate the relation between lifetime growth, uniformity and resilience (WP4). Part 3 is based on the outcome of the second part. Specific animals are selected to produce progeny for a selection experiment. In this experiment, previous findings are validated and the effect of environmental challenges on uniformity and resilience is investigated (WP5).

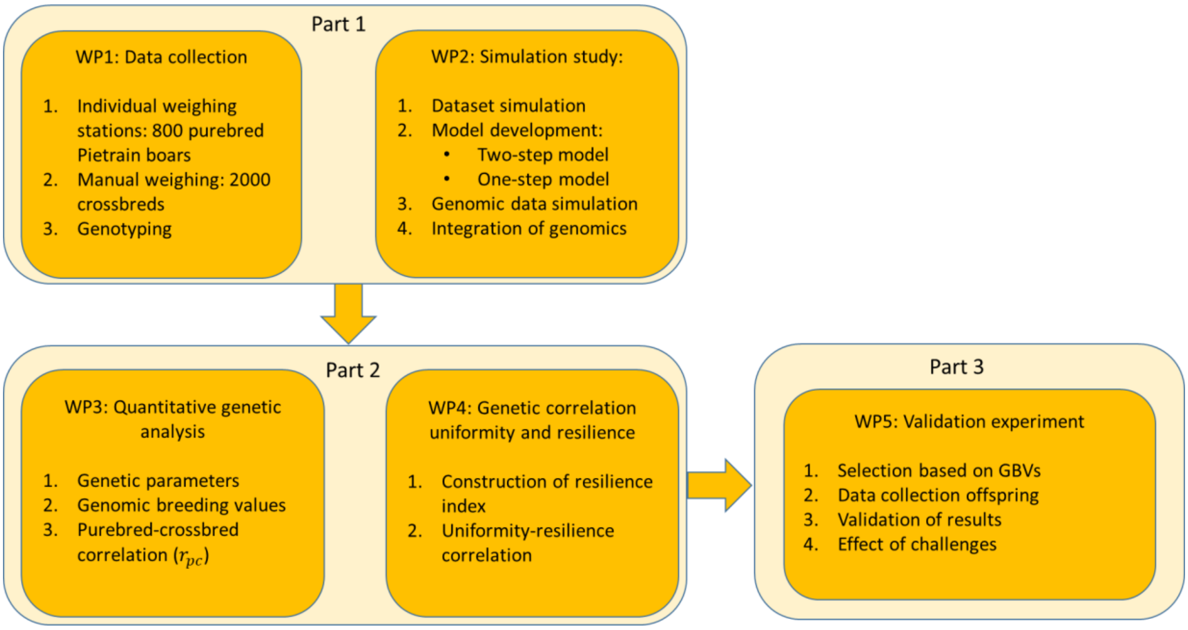


Figure 3: Workflow of the research methodology for this project. In general, this project consists of 3 main parts which are subdivided in 5 work packages (WP).

The majority of data for this research project will be obtained in cooperation with 'Vlaamse Piétrain Fokkerij vzw' (VPF). VPF is the leading organization in Flanders for breeding Pietrain boars. Currently, breeding values are based on the performance of crossbred offspring in experimental farms (18-24 offspring per Pietrain boar). Annually, 250 boars are tested based on approximately 5000 offspring. Pedigree data is available for all registered purebred Pietrains and about 650 Pietrain boars are genotyped at the start of this project.

In the following part, different work packages are discussed. The planned course of activities is shown in Figure 4.

Work package 1: Data collection

Gathering individual longitudinal growth data of pigs will be done in cooperation with the VPF. In general, experimental data are obtained from 2 sources.

First, data are collected in pig performance testing (PPT) units. These state of the art units measure and record the weight, the amount and duration of visits and the consumed feed per visit. Starting from May 2018, data are acquired continuously by nine PPT units, installed at 4 Pietrain breeders. Pigs are measured for three months, i.e. a growth trajectory of 25 to 115 kg. Growth and feeding trajectories of about 360 (9 units*4 rounds a year*8-12 pigs per unit) purebred Pietrain boars will be obtained per year. The goal is to obtain at least 1000 high precision phenotypes of growth trajectories of Pietrain boars by June 2021 (**Milestone 1**), with at least 400 of them genotyped using the GGP Porcine HD (70k) SNP-chip.

<u>The second source of data</u> will be obtained by manually weighing crossbred pigs at a testing farm of VPF. Presently, pigs are weighed individually at the beginning (±25kg and 75 days old) and at the end (±115kg and 182 days old) of a testing period. According to previous studies, a minimum of 5 individual measurements is sufficient to produce highly accurate Gompertz growth curves in pigs[18,19,26]. During their stay of about 100 days at the testing station, pigs will be weighed individually every two weeks, leading to a total of 9-10 weight measurements per pig. Weighing will be done via a run-through weighing scale. Weights are automatically annotated to individual pigs via electronic ear tags. The goal is to obtain measurements of 2000 crossbred pigs, originating from 100 genotyped Pietrain boars, over a period of 18 months (**Milestone 2**). Overall, about 1000-1200 Pietrain boars will be genotyped: 650 are already genotyped and 400 boars from PPT and 100 from test stations will be genotyped as well (**Milestone 3**).

Via a collaboration with the KU Leuven M3-Biores research group, cameras will be installed at the test farm. The M3-Biores group has algorithms and software for image analysis and to calculate pen-level measures of activity, aggression and visits to feeders and drinking places. The data obtained via this PLF-technique can be used as a proxy for resilience at a group level.

Risks: *Few risks are expected for the weight data, since data collection has already started. Budgets for genotyping individuals are established, PPT units are installed and VPF supports the manual weighing at the test farm. Obtaining data via image-analysis poses a risk. Camera settings and algorithms will have to be adapted to the specific settings of the farm. However, the M3-Biores group has substantial experience with installing and optimizing PLF technologies.*

To assure enough growth trajectory data is available for model development and genetic analyses, an agreement for data sharing was found with Topigs Norsvin (Eli Grindflek). The Topigs Norsvin data contain dailly weight and feed intake trajectories (±200 days) of Norwegian Landrace and Duroc boars from the period 1992-2018 (±40.000 animals in total).

Work package 2: Modeling uniformity of growth using genomic data: a simulation experiment

A quantitative genetic analysis of uniformity of growth over time requires specific statistical modeling, certainly when genomic information has to be implemented as well. Since these models are not developed yet, these models will be developed and tested in a simulation study.

Data and genetic parameters will be simulated based on growth analysis studies in pigs[18,19,26]. Simulations will be made for a growth trajectory of 200 days with daily weight measurements per pig. Two simulation data sets will be constructed (**Milestone 4**) based on a different simulation mechanism: one using hierarchical deterministic modelling and one using Bayesian Monte Carlo simulation. Simulations will be made for a population of 1000 boars with each 50 half-sib offspring, giving datasets of 2 million weight records. Different scenarios with differences in variability in growth of the offspring of boars will be tested.

Two different modeling methods will be developed. *In approach one, a hierarchical two-step model is constructed* (**Milestone 5**) which will integrate current state of the art statistical methodologies. Herein, growth trajectories and growth parameters are estimated for each animal by Gompertz growth curve modelling, to overcome the problem of non-linearity of the data. The parameters (A, B and k) of each animal are afterwards used as linear 'phenotypes' for the quantitative genetic modeling of uniformity. This will be done via double hierarchical generalized linear models[27] (DHGLM). This two-step approach is attractive because of its simplicity, yet it relies on normality assumptions. To circumvent this reliability, *a one-step model using the Bayesian framework will be constructed as well* (approach 2; **Milestone 6**). This will be done using the Bayz software[28] within the R-software. In this Bayesian framework, marginal posterior distributions will be estimated for the genetic parameters by Markov Chain Monte Carlo (MCMC) methods, such as Gibbs or Metropolis-Hastings sampling[29]. This research will be the first to develop these two models for quantifying the genetics of uniformity of growth over time.

After developing these models, genomic data will be simulated and integrated in these models (**Milestone 7**). Genomic data will be simulated for the 1000 simulated boars similar as done by Ibáñez-Escriche and Blasco[21]. Every parameter will be assumed to be regulated by a specific number of quantitative trait loci, for the mean (QTL) as for uniformity (vQTL). The number of (v)QTLs and their genetic correlations will be based on the few available genome wide association studies on uniformity in livestock animals[1,30]. Furthermore, mutation and drift are accounted for and chromosomal recombinations are modeled using a binomial map function[31]. Implementing genomic selection will be done via the most recent single-step genomic best linear unbiased prediction (ssGBLUP). This method combines phenotypic data, pedigree data and genomic data in one step. Hence, all available phenotypical and genomic data can be used in one step, reducing complexity and improving the accuracy[32]. Model testing will be done using cross-validation whereby predictive abilities and error rates are calculated.

Risks: *Some experience in simulating data and calculating genetic parameters of uniformity via DHGLM was gained in my master dissertation. Furthermore, two experts in the field of statistics and modeling in animal genetics – Han Mulder (Wageningen University) and Peter Goos (KU Leuven) – are assessors of this PhD project and fully support this project.*

Work package 3: Quantifying the genetics of uniformity throughout an animals lifetime

Different analyses will be performed using the field data from WP1 on the developed statistical models from WP2. *Firstly, genetic parameters will be calculated for growth trajectories and their uniformity* (**Milestone 8**). Novel genomic breeding values (GBVs) will be obtained for uniformity of growth (**Milestone 9**). Accuracies of GBVs will be compared to classical breeding values (only pedigree data) and the two-step model will be compared to the one-step model. This allows to determine which model performs optimal: the more simple and intuitive two-step model or the complex Bayesian one-step model.

Hereafter, the genetic purebred-crossbred correlation will be estimated (**Milestone 10**). The field data on crossbreds allow for this analysis because the crossbred animals are genetically closely related to the purebred animals (most of them are half-sibs). Moreover, a large amount of genomic data is available.

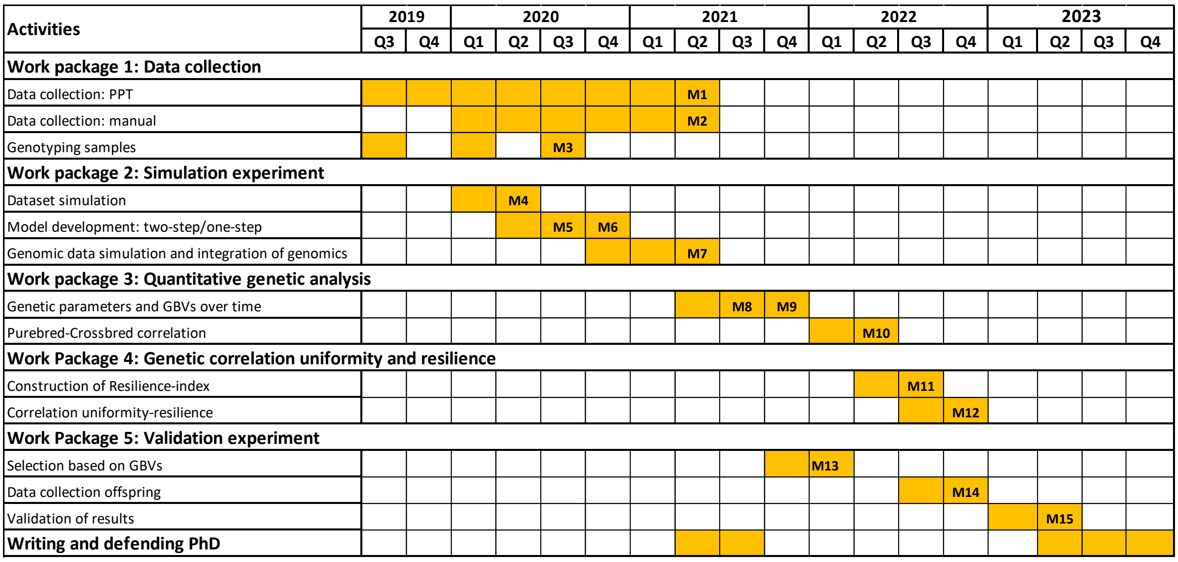
Risks: *Statistical methodologies need to be ready at the start of this work package and data need to be collected. The timing for these two requirements is ambitious but realistic.*

Work package 4: Quantifying the correlation between uniformity and resilience

The greatest challenge is to obtain a good measure for resilience. Breeding towards an increased resilience is hindered by the complexity of the data and a low heritability[2]. Different authors[25,33] suggest the use of performance, disease incidence and mortality rates to obtain measures of resilience which can be used in genetic analyses. Here, a reliable resilience-index will be constructed based on disease incidence, mortality and performance combined with image-analysis. Moreover, longitudinal data itself can be analyzed as a proxy for resilience. More resilient animals will tend to deviate less from their expected growth curve and will recover faster from deviations.

For every litter born the number of piglets born alive/dead is recorded and a vitality score is given by the farmer, as well as the number of remarkably small/large piglets. When transported to the slaughter house, pigs get a score for ease of loading. Use of medication is recorded. Furthermore, data from the PPT and image-analysis give information about a pigs health status. Pigs with no feeding visits during a whole day, decreased activity, weight loss,… are probably challenged by an external stressor. Based on these information sources, a <u>*resilience index will be constructed*</u> (**Milestone 11**). <u>*Afterwards, the genetic correlations between performance, uniformity and resilience in time will be calculated*</u> (**Milestone 12**).

Risks: *The construction of a resilience index poses the greatest risk in this work package. However, a significant amount of resilience-related traits are available and literature supports the use of these data as a proxy for resilience. Furthermore, image analysis will increase the data quality.*



Activities	**2019**		**2020**				**2021**				**2022**				**2023**			
	Q3	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Work package 1: Data collection																		
Data collection: PPT	🟧	🟧	🟧	🟧	🟧	🟧	🟧	**M1**										
Data collection: manual			🟧	🟧	🟧	🟧	🟧	**M2**										
Genotyping samples	🟧		🟧		**M3**													
Work package 2: Simulation experiment																		
Dataset simulation			🟧	**M4**														
Model development: two-step/one-step				🟧	**M5**	**M6**												
Genomic data simulation and integration of genomics						🟧	🟧	**M7**										
Work package 3: Quantitative genetic analysis																		
Genetic parameters and GBVs over time								🟧	**M8**	**M9**								
Purebred-Crossbred correlation											🟧	**M10**						
Work Package 4: Genetic correlation uniformity and resilience																		
Construction of Resilience-index												🟧	**M11**					
Correlation uniformity-resilience													🟧	**M12**				
Work Package 5: Validation experiment																		
Selection based on GBVs										🟧	**M13**							
Data collection offspring													🟧	**M14**				
Validation of results															🟧	**M15**		
Writing and defending PhD								🟧	🟧							🟧	🟧	🟧

Figure 4: Planned course of activities over the 4 years grant period. Milestones are indicated with 'Mx'.

Work Package 5: A validation experiment

To validate the findings of WP3 and WP4, a selection experiment is conducted. Two extreme groups will be selected based on GBVs for uniformity (**Milestone 13**). Per group, 5 boars will each be mated with 4 sows to produce 40-50 offspring per boar and at least 200 pigs per group (**Milestone 14**). Each litter will be kept in the same pens, giving 20 pens per condition. The offspring will be measured every 2 weeks (±12 weight measurements per animal). Resilience-related traits will be measured as in previous work packages, with an additional measure of respirational diseases using Pig Cough Monitors (PCM). PCM use audio recording to calculate cough frequencies, which are linked to respirational diseases. These devices will be installed in cooperation with the M3-Biores group to obtain cough frequencies at a group level.

To evaluate the resilience when faced with external stressors, environmental challenges will be presented at specific time points. These challenges are a rise in ambient temperature at several time points (heat distress) and a 24 hours feed-restriction. *Based on this experiment, previous results will be validated* (**Milestone 15**). Ethical approval will be obtained before experiment starts.

Risks: *The timing poses the greatest risk for WP5. Inseminating sows and raising their offspring until slaughter age roughly takes about 10 months of time. Therefore, it is important that accurate GBVs are available in time to start the selection experiment.*

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