Genetics of inherited variability

Increasing uniformity by reducing competition

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Abstract

Social interactions are common for all living organisms. In animal breeding, these interactions are of interest as they are often a source of indirect genetic effects (IGEs). An IGE is a heritable effect of an individual on the trait value of another individual. In aquaculture populations and some plants, social interactions have an additional consequence – interactions in the form of competition inflate variability of trait values among individuals. The phenotypic variability of a genotype has been studied as a quantitative trait in itself, and is often referred to as inherited variability. The main objective of this thesis was to study the genetics of inherited variability, with a focus on the relationship between competition (i.e., IGEs) and variability.

In the thesis, we used Nile tilapia as a model species. We found that variability of body weight and body size traits in Nile tilapia is heritable, and shows a large genetic coefficient of variation, which offers good opportunities for improvement of uniformity by means of genetic selection.

To study the genetic relationship between social interactions and variability, we developed a quantitative genetic model that integrates both phenomena. In this model, interactions between social partners lead to divergence (competition) or convergence (cooperation) of their phenotypes (e.g., body weight) over their life time. The effects of social interaction in the model are heritable and can evolve. These effects comprise direct genetic effect of the focal individual and IGE of its social partner. With a simulation study we showed that the model yields increased variability of body weight with increase of competition, similar to what is observed in real aquaculture populations. Selection for cooperation will therefore lead to decreased variability. These findings suggest that IGEs may be creating an entire level of genetic variation in variability, that has so far been overlooked. Using existing statistical models, we show that direct genetic effects of competition on variability could be captured with a direct model of inherited variability, and similarly, IGEs of competition could be captured with an indirect model of inherited variability.

According to kin selection theory individuals should show better social behavior, i.e., less competition, towards relatives, which should be reflected in their body weight and the variability thereof. We tested this hypothesis by comparing two treatments in an experiment, in which tilapia were reared in either kin or in non-kin groups. Individuals had significantly higher body weight in kin groups, however, there was no difference in variability of body weight between the two treatments.

Findings of this thesis demonstrate that variability of body weight in tilapia is heritable and that genetic variation in variability may comprise not only direct genetic effects but also IGEs. Studies focusing on evolution of variability/uniformity, therefore, should consider IGEs.

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General introduction

1.1 Social interactions

Many traits that are important for agriculture are complex quantitative traits. In animal breeding, it is desirable to improve these traits by means of genetic selection. A complete understanding of the potential of a trait to respond to selection requires identifying all sources of genetic variation underlying the trait. Traditional selection methods only consider the direct genetic effects (DGEs) of an individual's own genes on the phenotypic value of the individual. The environmental effects on a trait expression are generally assumed as non-heritable, and therefore not able to evolve by selection. In certain cases, however, the environment itself may have a genetic basis. This alters the genetic architecture and inheritance of a trait.

Animals are social beings who spend the majority of their lifetime engaged in interactions with conspecifics (Allee, 1927). These social interactions are often the most important part of the environment that individuals experience (Wolf, 2003; Frank, 2007). The environment created by social partners through actions such as competition or cooperation, is referred to as the social environment. Variation in the quality of the social environment can be attributed to traits expressed by social partners. Since these traits may reflect genetic variation, the socially provided environment can be heritable (Wolf *et al*., 1998; Bleakley and Brodie IV, 2009). The most extensively studied example of heritable environmental effects is the environment provided by a mother to her offspring (Dickerson, 1947; Willham, 1963; Falconer, 1965; Kirkpatrick and Lande, 1989; Cheverud, 2003; Bijma, 2011).

When the environment contains a genetic component, the phenotype of an individual may not only be influenced by its own genes (DGEs), but also by genes of its social partners. This heritable effect of a social partner on trait values of the focal individual is known as an indirect genetic effect (IGE; referred to as associative effects in Griffing, 1967). IGEs give rise to additional genetic (co)variation, which has consequences for trait values and fitness of individuals that interact, and subsequently for the direction and magnitude of response to selection (e.g. Hamilton, 1964; Moore et al., 1997; Wolf *et al*., 1998).

IGEs have been studied in animals (e.g. Ellen et al., 2014), plants (e.g. Mutic and Wolf, 2007; Brotherstone *et al*., 2011), and microorganisms (Crespi, 2001), and both in natural (e.g. Wilson *et al*., 2011) and in domestic populations (e.g. Muir, 1996; Khaw *et al.,* 2016). A number of studies have shown that social interactions can contribute substantially to heritable variation underlying a trait (reviewed by Ellen *et al*., 2014).

For animal breeders, social interactions with negative effect on trait values, health, and welfare, are especially of interest. Such interactions have been well-documented for laying hens, where cannibalistic behavior causes mortality (Muir, 1996; Ellen *et al*., 2008), and for pigs, where competition and tail biting leads to poorer growth and reduced animal welfare (Arango *et al*., 2005; Camerlink *et al*., 2013, 2014; Bergsma *et al*., 2013). In fish species, social interactions such as aggression and competition have been studied for their detrimental effect on growth of the population (medaka, Ruzzante and Doyle, 1991; Atlantic cod, Monsen *et al*., 2008; Nile tilapia, Khaw *et al*., 2016).

In summary, both empirical and theoretical work show that IGEs can considerably contribute to the potential of traits to respond to selection, and therefore need to be included in the genetic analysis of traits affected by social interactions.

1.2 Social interactions and inherited variability

So far, social interactions have been studied mainly in relation to their effects on fitness and trait values of individuals. However, in aquaculture populations, it has been observed that competition for feed and formation of social hierarchy also increases the variation of trait values among individuals (Jobling, 1995; Cutts *et al*., 1998; Hart and Salvanes, 2000). The variability of trait values of a genotype, measured either on the same individual multiple times, or on multiple individuals belonging to the same family, can be studied as a quantitative trait on its own. This phenomenon is often referred to as inherited variability, genetic variation in uniformity, or heritable variation in environmental variance (SanCristobal-Gaudy *et al*., 1998; Mulder *et al*., 2008; Hill and Mulder, 2010). Genetic variation in trait variability suggests that some individuals are less sensitive to small fluctuations in the environment, which allows them to maintain a stable phenotype.

The study of inherited variability has been an integral part of quantitative genetics for more than 70 years (Waddington, 1942), with growing interest in the topic over the last two decades, largely due to the development of methods to estimate genetic variance in variability (SanCristobal-Gaudy *et al*., 1998; Sorensen and Waagepetersen, 2003; Mulder *et al*., 2009; Rönnegård *et al*., 2010) and increasing empirical evidence for a genetic basis of variability in livestock, aquaculture, and laboratory populations (reviewed by Hill and Mulder, 2010). In addition, variability is an important economic trait in animal production, which further stimulated the research in this area.

In aquaculture, uniformity of body weight has recently been identified as one of the most important traits to be improved by selective breeding (Sae-Lim *et al*., 2012; Janssen *et al*., 2017; Omasaki *et al*., 2017). Studies in Atlantic salmon, rainbow trout, and Nile tilapia found a large genetic component in variability of body weight (Janhunen *et al*., 2012; Sonesson *et al*., 2013; Khaw *et al*., 2015; Sae-Lim, *et al*., 2015a; Sae-Lim, *et al*., 2015b; Marjanovic *et al*., 2016).

The relationship between competition and phenotypic variability is not unique for aquaculture, but can also be observed in plants. Plant breeders have successfully improved productivity of crops by selecting, partly unintentionally, less competitive phenotypes, which has resulted in more uniform crops (Donald, 1968; Austin *et al*., 1980; Denison *et al*., 2003).

These observations suggest that phenotypic variability may also be socially affected trait, with IGEs harboring genetic variation in variability that has so far been overlooked.

1.3 Models of IGE and inherited variability

The quantitative genetics of socially-affected traits have been studied in two modelling frameworks: variance component models and trait-based models (McGlothlin and Brodie, 2009; Bijma, 2014).

In variance component models, the phenotype of the focal individual *i* (P_i) who interacts with a single social partner *j*, is the sum of a direct genetic ($A_{D,i}$) and a direct environmental ($E_{D,i}$) component originating from the focal individual, and an indirect genetic ($A_{I,j}$) and an indirect environmental ($E_{I,j}$) component originating from its social partner *j* (Griffing, 1967):

$$P_i = A_{D,i} + E_{D,i} + A_{I,j} + E_{I,j} \quad (1)$$

In this approach, DGEs and IGEs are estimated as random effects using linear mixed models and information on genetic relationships between individuals (Muir, 2005; Bijma, Muir, Ellen, *et al.*, 2007). When all individuals are both donor and recipient of social interactions, each individual has a direct genetic effect $A_{D,i}$, i.e., a direct breeding value expressed in its own phenotype, and an indirect breeding value $A_{I,i}$, expressed in the phenotype of its social partner. The sum of $A_{D,i}$ and $A_{I,i}$, i.e., the total breeding value, represents the total heritable impact of an individual on the population mean trait value, and the genetic unit of interest in the selection of

individuals for socially affected traits (Moore *et al.*, 1997; Bijma, Muir, and Van Arendonk, 2007).

The second type of IGE models, i.e., the trait-based models, define IGEs on the phenotype of the focal individual as a function of trait values of its social partners (Moore *et al.*, 1997; Wolf *et al.*, 1998; Bijma, 2014). For example, the level of aggression displayed by focal individual is often affected by body weight of its social partner (Thornhill, 1984; Smith and Brown, 1986). Therefore, for empirical use of this model, the traits causing the indirect effects need to be identified. If we consider interaction of two individuals, where the target trait and the trait causing the IGE are the same, the trait-based model equals (Moore *et al.*, 1997)

$$P_i = A_i + E_i + \psi P_j \qquad (2)$$

where P_i is the phenotypic value of the focal individual *i*, A_i is the additive genetic effect and E_i the environmental effect originating from the focal individual, while P_j is the phenotypic value of its social partner *j*. The ψ is known as the "interaction coefficient", and it defines the strength of the social interaction. The ψ can take positive or negative value, and is assumed constant in the population.

Both types of IGE models, however, cannot fully make the connection between competition and variability observed in aquaculture and plant populations, since they model phenotypic variance as largely independent of the level of IGEs (for further explanation see General discussion - Chapter 6). In addition, observations from aquaculture suggest that behavior of a fish towards its social partners depends on its size relative to that of its partners. Therefore, to account for the competitive effect of body weight on growth rate in aquaculture, evolution of body weight needs to be modelled over the life of the interacting individuals. Current IGE models, however, are only applied to the final phenotype.

Quantitative genetics of inherited variability is most commonly studied using a class of models which allow for genetic effects on both the phenotypic mean and the environmental or residual variance of a trait. In the classical quantitative genetic model variation in a phenotype is defined as $\sigma_P^2 = \sigma_A^2 + \sigma_E^2$ (Falconer and Mackay, 1996), where σ_A^2 is the additive genetic variance affecting the mean trait value and σ_E^2 is the environmental variance, assumed to be constant for different genotypes. However, when phenotypic variability differs among genotypes, part of that difference may be attributed to genetic variation in environmental variance, i.e. $\sigma_E^2 = A_v + E_v$, where A_v is the breeding value for environmental variance and E_v is

the residual in environmental variance. Models for inherited variability, however, consider variability as a property of the focal individual, affected only by direct genetic effects, while the potential contribution of the social partner is ignored.

In terms of available quantitative genetic models, social interactions and variability are poorly connected. Therefore, there is a need for new models to understand the relationship between competition and variability observed in aquaculture and plants populations, and the potential of inherited variability to respond to selection.

1.4 Aim and outline of the thesis

The observed relationship between social interactions and variability on the phenotypic level (Jobling, 1995; Cutts *et al*., 1998; Hart and Salvanes, 2000; Denison *et al*., 2003) strongly suggests an underlying genetic relationship between the two phenomena, of which very little is known. The main objective of this thesis, therefore, was to study the genetics of inherited variability and possibilities for its genetic improvement, focusing primarily on the relationship between competition and variability.

Research presented in this thesis is a result of collaboration between Wageningen University & Research and Swedish University of Agricultural Sciences, in cooperation with WorldFish. WorldFish provided the data for Chapter 2 and the experimental facilities used to generate data for Chapter 5. Previous collaboration between Wageningen University & Research and WorldFish resulted in a PhD project which aimed to estimate direct and indirect genetic effects on growth rate in Nile tilapia (Khaw, 2015). This thesis builds on that knowledge, but primarily focuses on relationship between social interactions and variability. The large size differences related to competition for feed, together with the desire to reduce these differences by means of genetic selection (Ponzoni *et al*., 2005, 2011; Khaw *et al*., 2016), makes Nile tilapia an ideal species to study the relationship between social interactions and variability. Therefore, Nile tilapia was also used as a model species in this thesis.

In **Chapter 2** we investigate the potential for genetic improvement of inherited variability of harvest weight and body size traits in a domestic Nile tilapia population. We analyzed within-family variance of harvest weight, body length, depth, and width, by applying a double hierarchical generalized linear models (DHGLM) to individual trait values (Rönnegård *et al*., 2010). In addition to quantifying genetic variation in inherited variability of those traits, we also looked into possibilities of

simultaneous improvement of the level and the variance of the traits, by estimating the genetic correlation between these two components.

Observations from aquaculture and plant populations indicate that inherited variability and IGEs are related via competition (Donald, 1968; Austin *et al*., 1980; Jobling, 1995; Cutts *et al*., 1998; Hart and Salvanes, 2000; Denison *et al*., 2003). The absence of quantitative genetic models to study the effects of competition on variability, however, hinders further research. In **Chapter 3** we make a first step towards understanding the genetic relationship between social interactions and variability, by presenting a quantitative genetic model that integrates both phenomena. Furthermore, we use Monte Carlo simulation to demonstrate that the model produces the co-evolution of social interactions and variability observed in real populations.

To exploit genetic variation in inherited variability originating from IGEs, we need statistical models to capture this effect. To our knowledge, however, it is entirely unknown to what extent currently available statistical models capture the effect of competition on inherited variability. We address this issue in **Chapter 4** by investigating the ability of existing statistical models for inherited variability and for the phenotype itself, to capture the direct and indirect genetic effects of competition on variability.

According to kin selection theory, genetic relatedness should influence social behavior, because individuals able to interact differently with kin vs. non-kin would have higher inclusive fitness (Hepper, 1986). In many animal species, relatives show better social behaviors to each other than to unrelated conspecifics, such as food sharing and reduced aggressiveness (Kareem and Barnard, 1982; Hepper, 1986; Hiscock and Brown, 2000; Gerlach *et al*., 2007). Moreover, some studies have shown that rearing in kin groups can significantly increase growth of individuals (Brown and Brown, 1996; Gerlach *et al*., 2007). Since interaction with kin leads to evolution of less competition (Bijma and Wade, 2008), the effect should also be seen in the variability of trait values. However, little is known of the ability of Nile tilapia to recognize kin, and therefore, whether the evolutionary mechanism of kin selection can be used to increase yield and decrease variability in this species. In **Chapter 5** we conducted an experiment to investigate effects of relatedness in Nile tilapia, by comparing two treatments: rearing of fish in kin groups vs. rearing in non-kin groups. We investigated differences in average body weight and variability of body weight of individuals between both treatments.

The general discussion, **Chapter 6**, addresses several topics. First, I elaborate on integrating the two fields in quantitative genetics, social interactions and inherited variability. Second, I discuss benefits and downsides of selection for uniformity in domestic and natural populations. Finally, I give perspectives for selection for uniformity, future studies, and possible applications of the model developed in Chapter 3.

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2

Genetic parameters for uniformity of harvest weight and body size traits in the GIFT strain of Nile tilapia

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Abstract

Animal breeding programs have been very successful in improving the mean levels of traits through selection. However, in recent decades, reducing the variability of trait levels between individuals has become a highly desirable objective. Reaching this objective through genetic selection requires that there is genetic variation in the variability of trait levels, a phenomenon known as genetic heterogeneity of environmental (residual) variance. The aim of our study was to investigate the potential for genetic improvement of uniformity of harvest weight and body size traits (length, depth, and width) in the genetically improved farmed tilapia (GIFT) strain. In order to quantify the genetic variation in uniformity of traits and estimate the genetic correlations between level and variance of the traits, double hierarchical generalized linear models were applied to individual trait values. Our results showed substantial genetic variation in uniformity of all analyzed traits, with genetic coefficients of variation for residual variance ranging from 39 to 58 %. Genetic correlation between trait level and variance was strongly positive for harvest weight (0.60 ± 0.09), moderate and positive for body depth (0.37 ± 0.13), but not significantly different from 0 for body length and width. Our results on the genetic variation in uniformity of harvest weight and body size traits show good prospects for the genetic improvement of uniformity in the GIFT strain. A high and positive genetic correlation was estimated between level and variance of harvest weight, which suggests that selection for heavier fish will also result in more variation in harvest weight. Simultaneous improvement of harvest weight and its uniformity will thus require index selection.

Key words: genetic correlation, residual variance, Nile tilapia, additive genetic variance, trait level

2.1 Introduction

In animal breeding, particular attention is paid to improving the mean level of traits through selection and this has been successful for many breeding programs. One such successful example is the genetically improved farmed tilapia (GIFT) project, which was led at WorldFish (http://www.worldfishcenter.org) and resulted in a line of tilapia known as the GIFT-strain. For this strain, a substantial realized genetic gain (>100 %) was achieved through 12 generations of genetic improvement for body weight at harvest (Ponzoni *et al*., 2011; Khaw, 2015). However, it is often desirable not only to improve the level of a trait, but also to reduce its variability (Mulder *et al.*, 2008; Pun *et al.*, 2013), because significant variation around the optimal value of a trait can have a negative impact on production performance, both in livestock and aquaculture (Cutts *et al.*, 1998; SanCristobal-Gaudy *et al.*, 2001a; Mulder *et al.*, 2008). In fish farming, differences in size among individuals are generally associated with competition for food within a group and the resulting feeding hierarchy (Jobling, 1995; Cutts *et al.*, 1998; Hart and Salvanes, 2000). The phenotypic coefficient of variation (CV) for body weight, apart from indicating variation of the trait is also an indicator of competitive interactions within a population (Jobling, 1995). For the GIFT strain, the CV ranges from 40 to 60 %, which is considered a high value (Ponzoni *et al.*, 2005).

Although good management during the grow-out phase can help reduce the CV, as noted by Ponzoni *et al*. (2011), its average value across eight generations of GIFT remained at around 40 %. A common approach in fish farming to decrease phenotypic variation in body size and weight is to grade or sort fish into groups, according to size. If fish are not graded, the large variation in weight and size at harvest reduces their market value and has animal welfare consequences (Sae-Lim *et al.*, 2012; Khaw, Ponzoni, Yee, Aziz, Mulder *et al.*, 2016). From the point of view of fish farmers, uniformity of growth and body size is one of the key traits to be improved (Sae-Lim *et al.*, 2012). From the consumer's point of view, weight but also body size and appearance traits, play an important role in buying decisions (Kause *et al.*, 2003; Blonk *et al.*, 2010; Colihueque and Araneda, 2014).

An alternative approach to management procedures for reducing the variability of a trait is selective breeding. Selection for more uniform individuals requires that the variability of the trait itself has a genetic component, i.e., that there is genetic variation, which is also known as genetic heterogeneity of environmental (residual) variance (SanCristobal-Gaudy *et al*., 1998; Sorensen and Waagepetersen, 2003). In

this case, within a population, some animals will be less prone than others to phenotypic changes in response to small environmental fluctuations, and thus will have a more stable performance. Several studies on livestock and laboratory animals have demonstrated the existence of genetic differences in residual variance among genotypes and have quantified their magnitude (Ibáñez-Escriche *et al.*; SanCristobal-Gaudy *et al.*, 2001b; Damgaard *et al.*, 2002; Sorensen and Waagepetersen, 2003; Gutiérrez *et al.*, 2006; Rowe *et al.*, 2006; Ibáñez-Escriche *et al.*, 2008; Ibáñez-Escriche *et al.*, 2008; Garreau *et al.*, 2008; Mulder *et al.*, 2009; Waddington, 2009; Rönnegård *et al.*, 2013; Vandenplas *et al.*, 2013). In aquaculture species, evidence for substantial genetic heterogeneity of residual variance comes from three studies on body weight in salmonids (Janhunen *et al.*, 2012; Sonesson *et al.*, 2013; Sae-Lim *et al.*, 2015). A previous study on uniformity in Nile tilapia that analyzed the standard deviation of harvest weight using a traditional linear mixed model indicated a genetic basis for variability of harvest weight (Khaw, Ponzoni, Yee, Aziz, Mulder *et al.*, 2016). However, to date, variability of harvest weight in Nile tilapia has not been analyzed at the variance level using double hierarchical generalized linear models (DHGLM). The DHGLM is a novel approach which can be used to study uniformity of individual trait values. The advantage of DHGLM compared to analyzing a variance or the standard deviation of a group is that it can take into account systematic effects on the variance of the individual record level such as sex of the fish. Genetic basis of variability of body size traits has not been explored in any species, except in humans for height (Yang *et al.*, 2012).

The main objective of our study was to investigate the potential for genetic improvement of uniformity of harvest weight and body size traits in the GIFT strain. For this purpose, we analyzed within-family variance of harvest weight, body length, depth, and width, by applying a DHGLM to individual trait values (Rönnegård *et al*., 2010). To quantify the genetic relationship between the level and the variance of these traits, we also estimated the genetic correlation between these two components.

2.2 Materials and methods

2.2.1 Environment

We used data that were obtained from an experiment that was specifically designed to estimate indirect genetic effects (IGE) for growth rate in the GIFT strain (Khaw, Ponzoni, Yee, Aziz and Bijma, 2016). This experiment was carried out between 2009

and 2012 at the Jitra Aquaculture Extension Centre of the Department of Fisheries, which is managed by WorldFish and located at Kedah State of Malaysia. WorldFish complies with the Malaysian laws on animal experiments. During this experiment, four batches of fish were produced, i.e., one batch each year (batch named per year). However, for the last batch (2012), a high level of mortality occurred due to extreme weather conditions, which resulted in an insufficient number of records, and thus it was excluded from the analysis.

2.2.2 Experimental design

To produce families, the GIFT breeding program uses a nested-mating design, where one male is mated to two females. For this work, we used the same mating scheme to produce the experimental fish, and thus two full-sib families were obtained from each father. Each full-sib family contributed 80 offspring to the experiment. Fry that belonged to the same full-sib family were nursed together and separately from other families. During the grow-out phase, fish were kept in groups. Before placing each fish in a group, they were individually identified with a PIT (Passive Integrated Transporter) tag. Following the optimal design for the estimation of IGE (Bijma, 2010), families were assigned to groups so that each group consisted of members of two distinct, unrelated families. Both families contributed eight randomly selected individuals to each group to form groups of 16 members. Therefore, each family of 80 offspring contributed to 10 distinct groups (i.e. 80/10 members per group). Unique combinations of families in groups were created using a block design, with 11 families per block, where each family was combined only once with the other ten families in the same block. Hence, there were 55 family combinations i.e. groups, per block. Figure S2.1 (See Supplementary material) contains an example of the block design. If the number of available families for the last block was less than 11, an incomplete block was used with all remaining families. An outline of the various steps that were carried out for each batch is in Fig. 2.1.

The groups were kept in net-cages that were placed in earthen ponds in rows and columns. For each batch, two ponds were available. Due to the small number of fish available for batch 2010, only one pond was used. The groups for each block were distributed randomly and as evenly as possible over both ponds. Thus, the 55 groups of a block were split into 27 groups for pond 1, and 28 groups for pond 2.

During the grow-out phase, fish were fed with commercial dry pellets containing 32 % of protein; the amount of pellets (3 to 5 % of average live weight) and feeding frequency (twice a day) were the same as for the GIFT selective breeding population.

However, because the fish were kept in net-cages rather than in communal rearing, the feeding strategy differed from that in the standard GIFT program. Rather than spreading the food over the entire surface of a pond, it was placed in the corner of each net-cage so that the fish could express their competitive tendency (see Discussion). More details on the experiment are in Khaw *et al*. (Khaw, Ponzoni, Yee, Aziz and Bijma, 2016; Khaw, Ponzoni, Yee, Aziz, Mulder *et al.*, 2016). The GIFT technology manual provides a description of key husbandry procedures (Thodesen and Ponzoni, 2004).

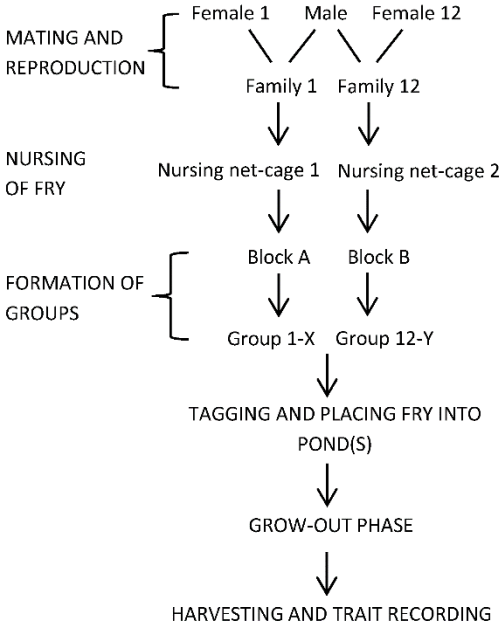

Figure 2.1 Outline of the experimental design for two paternal families. X represents any family from Block A, other than family 1; Y represents any family from Block B, other than family 12; an example of Block A is in Supplementary material (Figure S2.1).

2.2.3 Records

Fish were harvested 5 to 8 months after the grow-out period, when the average weight ranged from 200 to 250 g. At harvest, the following traits and parameters were recorded: live body weight (g), body measurements (length, depth, and width, in cm), tag number, sex, pond, and net-cage label. The age at harvest of each fish was computed from the recorded spawning and harvesting dates (Khaw, Ponzoni, Yee, Aziz and Bijma, 2016). Over three batches, phenotypic observations on body

weight and body measurements at harvest were available for 6,330 fish from 493 groups.

Ideally, each group should contain 16 individuals at harvest. However, due to mortality, some groups contained very few individuals, and a threshold was set for group and family size. Thus, groups that contained less than seven individuals in total or less than three fish per family were discarded, which reduced the number of groups to 446. With two families in each group, 892 family-by-group combinations and 6,090 individual records were available for each trait. Table 2.1 shows the number of observations at harvest (full dataset) and number of observations used in the analysis (edited or reduced dataset). The pedigree consisted of 34,517 records that traced the GIFT population back seven generations.

Table 2.1 Number of groups, families per group, and individuals at harvest (C-complete dataset) and after editing (R-reduced dataset)

Batch	Families		Groups		Families per group		Individuals	
	C	R	C	R	C	R	C	R
2009	66	66	209	188	418	376	2565	2461
2010	33	31	45	37	90	74	509	464
2011	68	68	239	221	478	442	3256	3165
Total	167	165	493	446	986	892	6330	6090

2.2.4 Statistical analysis

The environmental component in the phenotypic variation of a trait can be measured either on the same individual for which repeated observations are available or on the individuals belonging to the same family (Hill and Mulder, 2010). In our dataset, body weight and body measurements were recorded at harvest. Hence, only one record for each trait was available for each individual, but eight observations were recorded per family per group. To analyze the genetic heterogeneity of the environmental variance, different approaches have been proposed (Hill and Mulder, 2010) and we chose a DHGLM that models the residual variance of individual observations on the exponential scale, and can be interpreted as a multiplicative model (SanCristobal-Gau*dy et al*., 1998). On the level of the natural logarithm, the multiplicative model becomes additive.

Sire and dam, group, kin, and social maternal effect were included as random effects.

A group effect was included to account for non-heritable indirect effects, which create a non-genetic covariance among individuals within the same group (Bergsma *et al*., 2008). If this covariance is present but not accounted for, it can cause bias in the estimated genetic parameters (Bijma *et al.*, 2007). According to the kin selection theory, relatives can cooperate with each other (Hamilton, 1964; File *et al.*, 2012), thus a non-genetic covariance between group mates belonging to the same family can arise. Therefore, we included a kin effect to account for this source of non-genetic covariance, i.e., between group mates of the same family compared to group mates of the other family within a group (Khaw, Ponzoni, Yee, Aziz and Bijma, 2016).

Finally, a social maternal effect was included that accounts for the non-genetic effect of the common maternal environment of one full-sib family on the performance of the other full-sib family in the group (Khaw, Ponzoni, Yee, Aziz, Mulder *et al.*, 2016). In other words, we fitted a non-genetic effect of the mother of a full-sib family on the trait values of the other full sib family kept in the same group. Hence, we termed this effect "social", because it is expressed in the trait values of the social partners of the offspring of a mother, rather than in her offspring themselves.

2.2.4.1 Double hierarchical generalized linear models (DHGLM)

Lee and Nelder (2006) developed a framework for the DHGLM, where level and residual variance of a trait can be modeled jointly with specified random effects. This approach has been applied in animal breeding by Rönnegård *et al.* (2010) who implemented the DHGLM in the statistical software SAS (Rönnegård *et al.*, 2009) and ASReml 2.0 (Rönnegård *et al*., 2010). The DHGLM algorithm iterates between two sets of mixed model equations, i.e., a linear mixed model for the phenotypic records and a generalized linear mixed model for the response variable ϕ_i. ϕ_i is defined as $\phi_i = E(\hat{e}_i^2/(1-h_i))$, where $\hat{e}_i^2$ is the squared residual for the i^{th} observation and h_i is the diagonal element of the hat matrix of y, corresponding to the same individual (Rönnegård *et al.*, 2010; Mulder *et al.*, 2013). As ϕ follows a χ^2 distribution, $\hat{e}_i^2/(1-h_i)$ can be linearized using a log link function so that $\log(\phi) = \log[\hat{e}_i^2/(1-h_i)]$ (Rönnegård *et al.*, 2010). Instead of using a log link function, $\log[\hat{e}_i^2/(1-h_i)]$ can be linearized using a first order Taylor-series expansion as shown by Felleki *et al.* (2012), which results in the response variable $\psi_i = \log(\hat{\sigma}_{e_i}^2) + (\{[e_i^2/(1-h_i)] - \hat{\sigma}_{e_i}^2\}/\hat{\sigma}_{e_i}^2)$, where $\hat{\sigma}_{e_i}^2$ denotes the predicted residual variance for observation i, and e_i is the residual for individual *i*. Due to linearization, a bivariate DHGLM can then be used:

$$\begin{bmatrix}\mathbf{y}\\ \boldsymbol{\psi}\end{bmatrix} = \begin{bmatrix}\mathbf{X} & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_\mathrm{v}\end{bmatrix}\begin{bmatrix}\mathbf{b}\\ \mathbf{b}_\mathrm{v}\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_\mathrm{Par} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{\mathrm{Par}_\mathrm{v}}\end{bmatrix}\begin{bmatrix}\mathbf{a}\\ \mathbf{a}_\mathrm{v}\end{bmatrix}$$

$$+\begin{bmatrix}\mathbf{V} & \mathbf{0}\\ \mathbf{0} & \mathbf{V}_\mathrm{v}\end{bmatrix}\begin{bmatrix}\mathbf{g}\\ \mathbf{g}_\mathrm{v}\end{bmatrix} + \begin{bmatrix}\mathbf{S} & \mathbf{0}\\ \mathbf{0} & \mathbf{S}_\mathrm{v}\end{bmatrix}\begin{bmatrix}\mathbf{k}\\ \mathbf{k}_\mathrm{v}\end{bmatrix}$$

$$+\begin{bmatrix}\mathbf{U} & \mathbf{0}\\ \mathbf{0} & \mathbf{U}_\mathrm{v}\end{bmatrix}\begin{bmatrix}\mathbf{m}\\ \mathbf{m}_\mathrm{v}\end{bmatrix} + \begin{bmatrix}\mathbf{e}\\ \mathbf{e}_\mathrm{v}\end{bmatrix},$$

where $\mathbf{y}$ is the vector of individual trait records (harvest weight, body length, depth, and width) and $\boldsymbol{\psi}$ is the vector of response variables for the variance part of the model, expressed per individual (ψ_i as defined above). $\mathbf{b}$ and $\mathbf{b}_\mathrm{v}$ are the vectors of fixed effects, while $\mathbf{a}$ and $\mathbf{a}_\mathrm{v}$ are the vectors of additive genetic effects of the sire and dam of each individual, with $\begin{pmatrix}\mathbf{a}\\ \mathbf{a_v}\end{pmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix}\sigma^2_\mathrm{a} & \sigma_{\mathrm{a,a_v}}\\ \sigma_{\mathrm{a,a_v}} & \sigma^2_{\mathrm{a_v}}\end{bmatrix} \otimes \mathbf{A}\right)$, where the sire and dam variance are a quarter of the additive genetic variance: $\sigma^2_{\mathrm{a_{(v)}}} = \frac{1}{4}\sigma^2_{\mathrm{A_{(v)}}}$, $\sigma^2_{\mathrm{A_{(v)}}}$ denoting the ordinary additive genetic variance. Note that we assume equal additive genetic variance for sire and dam, i.e., $\sigma^2_{\mathrm{sire_{(v)}}} = \sigma^2_{\mathrm{dam_{(v)}}} = \sigma^2_{\mathrm{a_{(v)}}}$. $\mathbf{g}$ and $\mathbf{g}_\mathrm{v}$ are the vectors of random group effects, with $\begin{pmatrix}\mathbf{g}\\ \mathbf{g}_\mathrm{v}\end{pmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix}\sigma^2_\mathrm{g} & \sigma_{\mathrm{g,g_v}}\\ \sigma_{\mathrm{g,g_v}} & \sigma^2_{\mathrm{g_v}}\end{bmatrix} \otimes \mathbf{I}\right)$; $\mathbf{k}$ and $\mathbf{k}_\mathrm{v}$ are the vectors of random kin effects, with $\begin{pmatrix}\mathbf{k}\\ \mathbf{k}_\mathrm{v}\end{pmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix}\sigma^2_\mathrm{k} & \sigma_{\mathrm{k,k_v}}\\ \sigma_{\mathrm{k,k_v}} & \sigma^2_{\mathrm{k_v}}\end{bmatrix} \otimes \mathbf{I}\right)$; $\mathbf{m}$ and $\mathbf{m}_\mathrm{v}$ are the vectors of social maternal effects, with $\begin{pmatrix}\mathbf{m}\\ \mathbf{m}_\mathrm{v}\end{pmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix}\sigma^2_\mathrm{m} & \sigma_{\mathrm{m,m_v}}\\ \sigma_{\mathrm{m,m_v}} & \sigma^2_{\mathrm{m_v}}\end{bmatrix} \otimes \mathbf{I}\right)$; and $\mathbf{e}$ and $\mathbf{e}_\mathrm{v}$ are the vectors of random residuals that are assumed to be independent and normally distributed $\begin{pmatrix}\mathbf{e}\\ \mathbf{e}_\mathrm{v}\end{pmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix}\mathbf{W}^{-1}\sigma^2_\mathrm{e} & 0\\ 0 & \mathbf{W}_\mathrm{v}^{-1}\sigma^2_{\mathrm{e_v}}\end{bmatrix} \otimes \mathbf{I}\right)$ with scaling variances σ^2_e and $\sigma^2_{\mathrm{e_v}}$. The expectations for the scaling variances σ^2_e and $\sigma^2_{\mathrm{e_v}}$ are equal to 1, because $\mathbf{W}$ and $\mathbf{W}_\mathrm{v}$ already contain the reciprocals of the estimated residual variances per record. The $\mathbf{X}(\mathbf{X}_\mathrm{v})$, $\mathbf{Z}(\mathbf{Z}_\mathrm{v})$, $\mathbf{V}(\mathbf{V}_\mathrm{v})$, $\mathbf{S}(\mathbf{S}_\mathrm{v})$ and $\mathbf{U}(\mathbf{U}_\mathrm{v})$ are known design matrices assigning observations to the level of fixed, sire and dam, group, kin, and social maternal effects for $\mathbf{y}(\boldsymbol{\psi})$, respectively. The weights, $\mathbf{W} = \mathrm{diag}\left(\widehat{\psi}\right)^{-1}$ and $\mathbf{W}_\mathrm{v} = \mathrm{diag}((1-h)/2)$, are, together with vector $\boldsymbol{\psi}$, updated in each iteration until convergence (Mulder *et al*., 2013). The social maternal effect was excluded for body width because the model did not converge, and for body length because it was not significant ($\chi^2_{1DF} = 2.66, p = 0.264$). The fixed effects included for trait level and

the variance part of the model were interaction of batch (2009, 2010, and 2011), sex (male and female), pond (1 and 2) and the linear covariate 'age at harvest'.

To facilitate interpretation in the Results section, the group effect for trait level is presented as $g^2 = \hat{\sigma}_g^2/\hat{\sigma}_P^2$, where σ_P^2 is the phenotypic variance, and the kin effect as $k^2 = \hat{\sigma}_k^2/\hat{\sigma}_P^2$. Moreover, for the genetic estimates, the genetic coefficient of variation (GCV) for trait level and its residual variance (GCV_{Ve}) are provided. These are defined as, $GCV = \sigma_A/\mu$, where σ_A is the genetic standard deviation in trait level while μ is the population mean level of the trait (Houle, 1992), and, $GCV_{Ve} = \sigma_{A_V}/\sigma_E^2$, where σ_{A_V} is the genetic standard deviation in the residual variance and σ_E^2 is the mean residual variance from the additive model (Mulder *et al*., 2007; Hill and Mulder, 2010). When $\sigma_{A_V}^2$ is on the exponential scale, as is the case for the residual variance in our analysis, GCV_{Ve} is close to $\sqrt{\sigma_{A_V}^2}$ (Mulder *et al.*, 2007; Hill and Mulder, 2010).

2.3 Results

2.3.1 Genetic parameters for trait levels

Estimated genetic parameters for levels of harvest weight, body length, depth, and width are in Table 2.2. The estimated heritability for individual harvest weight (estimated by using the average residual variance across all observations) was equal to 0.25 (0.04) and the same value was obtained with a univariate model assuming a homogeneous residual variance (results not shown). The log-likelihood ratio tests indicated that both group and kin effects were highly significant ($p < 0.0001$). The group effect explained 13 % of the phenotypic variance, which shows that individuals within the same group are more similar to each other than to members of other groups. The kin effect explained 10 % of the phenotypic variance, which indicates that individuals within the same family are more alike compared to individuals of the other family in the group, in addition to their genetic similarity. We tested the model for harvest weight when group and kin effects were not included and found that removing one or both effects created an upward bias in the estimated variances for both the level and variance of the trait (results not shown). The social maternal effect was significant ($p < 0.001$) but small and explained 2 % of the phenotypic variance.

Heritabilities of harvest weight and body width were similar (0.25 ± 0.05), while heritabilities of body length and body depth were a little higher (~0.30 ± 0.05). The group effect explained ~15 % of the phenotypic variance for length and depth, and

27 % for width. The kin effect explained ~10 % of the phenotypic variance for all three body size traits.

Table 2.2 Genetic parameters for level of harvest weight, length, depth, and width

Parameter	Harvest weight	Length	Depth	Width
[a]σ_A^2	573.46 (115.80)	0.732 (0.136)	0.202 (0.037)	0.034 (0.007)
σ_e^2	1426.3 (27.99)	1.443 (0.028)	0.365 (0.007)	0.067 (0.001)
σ_g^2	300.26 (42.81)	0.354 (0.047)	0.104 (0.012)	0.037 (0.004)
σ_k^2	240.29 (35.45)	0.235 (0.035)	0.047 (0.008)	0.013 (0.002)
σ_m^2	43.64 (20.83)	_	0.013 (0.006)	_
σ_P^2	2297.2 (70.78)	2.418 (0.081)	0.631 (0.022)	0.136 (0.005)
h^2	0.25 (0.04)	0.30 (0.05)	0.32 (0.05)	0.25 (0.05)
[b]g^2	0.13 (0.02)	0.15 (0.02)	0.16 (0.02)	0.27 (0.02)
[c]k^2	0.10 (0.02)	0.10 (0.01)	0.08 (0.01)	0.10 (0.01)
[d]m^2	0.02 (0.01)	_	0.02 (0.01)	_
[e]GCV	0.14	0.05	0.06	0.06

*Standard errors are indicated between brackets.
[a]Additive genetic variance was calculated as 4 times the sire-dam variance; [b]Group effect, calculated as $g^2 = \hat{\sigma}_g^2/\hat{\sigma}_P^2$; [c]Kin effect, calculated as $k^2 = \hat{\sigma}_k^2/\hat{\sigma}_P^2$; [d]Social maternal effect, calculated as $m^2 = \hat{\sigma}_m^2/\hat{\sigma}_P^2$.

2.3.2 Genetic parameters for the variance of traits

Estimated genetic parameters for the variance of harvest weight, body length, depth, and width are in Table 2.3. For all traits, the contribution of genetic effects to their variance was highly significant ($p < 0.0001$). Estimated GCV_{Ve} for harvest weight was high and equal to 0.58, whereas for body size traits, GCV_{Ve}were lower, i.e., 0.39, 0.42, and 0.45 for length, depth and width, respectively. These estimates indicate that there is substantial genetic variation in the residual variance compared to the average value of the residual variance, for all analyzed traits.

Table 2.3 Genetic parameters for the variance of harvest weight, length, depth, and width

Parameter	Harvest	Length	Depth	Width
[a]σ^2_A	0.343 (0.068)	0.156 (0.041)	0.184 (0.042)	0.203 (0.048)
σ^2_e	1.747 (0.034)	1.924 (0.038)	1.862 (0.036)	1.696 (0.033)
[b]σ^2_g	0.040 (0.021)	0.031 (0.021)	0.031 (0.018)	0.073 (0.020)
[c]σ^2_k	0.078 (0.027)	0.098 (0.029)	0.022 (0.023)	0.062 (0.023)
[d]σ^2_m	0.009 (0.009)	_	0.023 (0.011)	_
[e]GCV_{V_e}	0.58	0.39	0.42	0.45

*Standard errors are indicated between brackets

[a]Additive genetic variance was calculated as 4 times the sire-dam variance; [b]Group variance; [c]Kin variance; [d]Social maternal variance; [e]Genetic coefficient of variation at variance level.

2.3.3 Genetic correlations between level and variance of traits

Estimated genetic correlations between level and variance for harvest weight and body size traits are in Table 2.4. The genetic correlation between level and variance for harvest weight was high and positive (0.60 ± 0.09), which implies that selection for increased harvest weight will also yield more variation in the level of this trait. For body size traits, genetic correlations between level and variance were lower than for harvest weight, and were not significantly different from 0 for length and width, but moderate and positive for depth (0.37 ± 0.13).

Table 2.4 Genetic correlations between level and the variance for harvest weight, length, depth, and width

Harvest weight	Length	Depth	Width
0.60 (0.09)	0.11 (0.16)	0.37 (0.13)	0.20 (0.15)

*Standard errors are indicated between brackets

2.4 Discussion

In this study, we used a DHGLM to estimate genetic variation in uniformity of harvest weight and three body size traits, i.e., length, depth, and width. Our results showed substantial genetic variation in uniformity of all analyzed traits, with GCV_{Ve} ranging from 39 to 58 %, while GCV for trait levels ranged from 5 to 15 %. A strong genetic correlation of 0.60 was found between trait level and variance, which suggests that

selection for increased body weight at harvest will also result in more variation in the level of this trait.

2.4.1 Heritability of individual harvest weight and body size traits

Estimated heritability for individual harvest weight was moderate (0.25 ± 0.04), which is similar to results from previous studies on Nile tilapia (Khaw *et al*., 2009, 2012; Ponzoni *et al*., 2011). To date, the GIFT strain has undergone 14 generations of selection for harvest weight. Our findings, together with the small average inbreeding coefficient of 3.1 % in the analyzed GIFT population, suggest that there is still a considerable amount of genetic diversity available for further selection, which is also in agreement with the positive genetic trend observed in the GIFT strain (Khaw, 2015).

Heritabilities for individual body size traits were also moderate (0.25 to 0.32), which provide opportunities to improve body size traits in Nile tilapia. Body size traits could become traits of interest in future breeding programs since selection for heavier fish may lead to body shapes that deviate from the natural shape, the latter being favored by consumers (Nguyen *et al*., 2007; Trọng *et al*., 2013; Colihueque and Araneda, 2014).

2.4.2 Genetic variance in uniformity of harvest weight

Variance components that are estimated using the exponential model, as in this study, are independent of the scale of the trait, and thus, are comparable across traits and species (Sonesson *et al*., 2013; Rönnegård *et al*., 2013). We found a substantial additive genetic variance for uniformity of harvest weight (0.34 ± 0.07; Table 2.3), which is larger than that in a similar study on Atlantic salmon by Sonesson *et al*. (2013), who reported an additive genetic variance in the residual variance of 0.17 on the exponential scale. Our estimates are also higher than those reported for livestock traits (Hill and Mulder, 2010; Rönnegård *et al.*, 2013; Vandenplas *et al.*, 2013; Sell-Kubiak *et al.*, 2015; Mulder *et al.*, 2015). These findings suggest that the observed phenotypic variability of harvest weight in the GIFT strain has a substantial genetic component.

Regardless of the underlying model, comparison of additive genetic parameters for uniformity across different studies can also be done by using the genetic coefficient of variation for residual variance ($\mathrm{GCV_{Ve}}$) (Mulder *et al*., 2007; Hill and Mulder, 2010). $\mathrm{GCV_{Ve}}$ describes the change in residual variance when a genetic standard deviation of 1 is achieved in response to selection, relative to the mean of the residual

variance. In our study, GCV_{Ve} for harvest weight was large, i.e., 0.58. The proportional change in phenotypic variance can be calculated as $GCV_{Ve}(\sigma_E^2/\sigma_P^2)$, which in the case of harvest weight would be equal to 0.36. In the literature, GCV_{Ve} for variability of traits in livestock and laboratory animals usually ranges from 0.2 to 0.6 (Hill and Mulder, 2010). For uniformity of body weight in rainbow trout, GCV_{Ve} of 0.37 and ~0.2 were reported by Janhunen *et al.* (2012) and Sae-Lim *et al.* (2015), respectively, which are lower than the values found in our study. The estimated GCV_{Ve} for harvest weight suggests that there is sufficient genetic variation to allow a substantial change in the residual variance of this trait compared to its average value within a single generation of selection, which would be much larger than that for harvest weight level (Table 2.2). However, it should be noted that the accuracy of selection for uniformity tends to be lower than for trait levels (Mulder *et al.*, 2009), and that expressions for response to selection on environmental variability do not depend on GCV_{Ve} only (SanCristobal-Gaudy *et al.*, 1998, 2001a; Mulder *et al.*, 2007; Ibáñez-Escriche *et al.*, 2008).

2.4.3 Effect of data distribution

The estimated level and variance for harvest weight could be influenced by the non-normal distribution of harvest weight. In data on aquaculture species, skewness is not unusual (Jobling, 1986; Cutts *et al.*, 1998). A skewed distribution can result from inter-individual competition and subsequent feeding hierarchy, with a few individuals dominating the rest of the group. However, in many statistical inferences, normality is assumed and this is especially important in the analysis of the genetic heterogeneity of environmental variance (Yang *et al.*, 2011). To test whether genetic variation in residual variance is merely an artifact of a non-normal distribution, we applied a Box-Cox transformation to harvest weight. The transformation resulted in a normally distributed trait, which was then analyzed with the DHGLM. Results of the analysis (See Supplementary material, Table S2.1) showed that this transformation had only a minor effect on the estimated genetic parameters for trait level, but decreased the variance of the residual variance. Similar results were found in other studies that analyzed transformed traits (Yang *et al.*, 2011; Sonesson *et al.*, 2013; Sae-Lim *et al.*, 2015). Although the additive genetic variance of uniformity decreased somewhat after the Box-Cox transformation, this difference was not significant ($p = 0.22$). Thus, our results indicate that there is genetic variation in uniformity of harvest weight, irrespective of the scale of measurement of the trait. Unlike harvest weight, body length, depth, and width were normally distributed.

2.4.4 Genetic correlation between level and variance of harvest weight and body size traits

Our results imply that the observed variation in harvest weight in the GIFT strain could be reduced by selective breeding. However, selection for more uniform fish may result in a trade-off on improvement of harvest weight. The genetic correlation between level and variance of harvest weight was high and positive, 0.60 (Table 2.4), which means that single-trait selection for heavier fish will increase the variation in harvest weight among individuals. Similar correlations were obtained by Sae-Lim *et al.* (2015). Simultaneous improvement of harvest weight and its uniformity will therefore require index selection.

To maximize profit, not only uniformity of weight but also uniformity of size, may play an important role in fish farming, especially for markets where fish are sold as whole. The magnitudes of GCV for uniformity of body size traits and harvest weight were similar but improvement of body size traits based on the estimated correlations (Table 2.4) is expected to have a limited effect on the level of these traits.

2.4.5 Factors affecting magnitude of variability and genetic variance in variability

In our analyses we used a sire and dam model, which fits the additive genetic mid-parent mean, while the Mendelian sampling deviation is part of the residual. This can potentially inflate the size of the estimated genetic variation in residual variance in case of heterogeneous Mendelian sampling variation, which is then confounded with the genetic part of the residual variance (Sonesson *et al*., 2013). A Mendelian sampling variance that is heterogeneous among families can result from differential inbreeding coefficients of parents, or from the presence of a major gene that is segregating in some families but not in others (Rowe *et al.*, 2006).

In aquaculture species, maternal common environmental effects can have an important role in explaining differences among families. These effects can be included in the estimation of genetic parameters as non-genetic effects that account for covariances between full-sibs due to a shared environment. In this study, maternal common environmental effects were excluded from the models because of convergence problems, which arose when those effects were included. The same issue was observed in other studies that used the same dataset and for which the results showed confounding of maternal common environmental effects and direct genetic effects (Khaw, Ponzoni, Yee, Aziz and Bijma, 2016; Khaw, Ponzoni, Yee, Aziz, Mulder *et al.*, 2016). The main difficulty that occurs when disentangling the two

effects is due to the mating of one male with two females. Moreover, in our experiment, mating was often partly unsuccessful and resulted in 1x1 mating instead. However, even a perfect 2x2 mating design results in limited power to separate genetic and maternal common environmental effects, at least at the variance level, as reported by Sonesson *et al*. (2013). Previous studies on a larger GIFT population for which 1x2 mating was more successful, detected significant maternal common environmental effects (0.34) for individual harvest weight (Ponzoni *et al.*, 2011). Thus, our estimates of the genetic variance of uniformity may be inflated by the inability to fit maternal common environmental effects.

A recent study on birth weight of mice treated environmental variability as a maternal trait, and found a positive response to selection (Formoso-Rafferty *et al*., 2015). In an earlier study, the same authors found evidence that environmental variability of birth weight was more likely to be a maternal genetic trait than a trait due to direct genetic effects (Pun *et al.*, 2013). In the study by Rutten *et al.* (2005), the variance of body weight due to common environmental effects, which include maternal genetic and non-additive genetic effects, decreased with age. Since in our study, traits were measured at harvest, maternal genetic or common environmental effects probably explain only a small proportion of the heterogeneity of residual variance.

In Table S2.2 (Supplementary material), we present estimates of the fixed effects included in the model. All fixed effects had a significant impact on the magnitude of the observed variability. The effect of sex was especially large with males showing ~1.3 times greater residual variance compared to females. This finding may be related to the competitive behavior expressed primarily by males. Mulder *et al.* (2009) showed that the estimated genetic correlation between residual variances for body weight of both sexes was only 0.11, which suggests that they are different traits. A similar analysis could be conducted on our data, to investigate whether the large effect of sex is associated with a genetic correlation for variability between sexes less than 1. Ponzoni *et al*. (Ponzoni *et al.*, 2011) recorded the CV of body weight in the GIFT strain across eight generations and observed that good breeding management contributed to reduce the CV, although its average value remained at around 40 %. Thus, reducing uniformity will require both genetic and management interventions.

2.4.6 CV for harvest weight

In our experiment, the feeding strategy differed from that in the ordinary GIFT breeding program. Instead of spreading food on the surface of the pond as in the GIFT breeding population, we placed it in the corner of the net-cages so that the fish showed their competitive tendency. The CV for harvest weight in our study (35 %) was lower than the values found in previous studies on the GIFT strain where fish were communally reared (48 to 59 %) (Ponzoni *et al*., 2005; Nguyen *et al*., 2007). Thus, there is no evidence that the level of competition between individuals was higher in our conditions than in the communal rearing conditions of these studies. In communal rearing, the feed is not spread over the entire pond's surface because auto-feeders are not available, which may cause some competition. In addition, the fish in our experiment were kept in small net-cages and stocked at low density, while in commercial ponds all fish are kept together at high density. Because of the differences in rearing conditions, the question of whether our results can be extended to commercial situations remains open. A selection experiment, in which parents are kept in many small groups and selected for uniformity while offspring are evaluated under commercial conditions, would constitute the ideal proof.

2.4.7 Future prospects

Although studies on the genetic heterogeneity of environmental variance date as far back as 1942 (Waddington, 1942), selection experiments to improve uniformity in livestock are scarce. Still, some experiments (Rendel *et al*., 1966; Kaufman *et al*., 1977; Argente *et al*., 2008; Boldin *et al*., 2012) that were based on divergent selection for phenotypic variance, provided evidence for a genetic component in the phenotypic variability and suggested the possibility that this variability could be reduced by selective breeding. To our knowledge, selection for uniformity has never been performed in aquaculture species. Nevertheless, the high $\mathrm{GCV_{Ve}}$ found in our and other studies on aquaculture species suggest that aquaculture populations are suitable to validate the estimated genetic parameters by a selection experiment. Selection for uniformity of body weight or size could lead to increased profit by producing more fish in the size range that is favored by the consumers. Moreover, from the point of view of animal welfare, uniformity of fish body weight and size could reduce competition, and thus possible stress, injuries, and even mortality.

We studied the genetic variance of the residual variability. However, the total phenotypic variability also depends on other factors (Moreno *et al*., 2012), as shown by the significant fixed effects on variability, for example sex effect (see above). Hence, decreasing the total phenotypic variability even more would require reducing

the magnitude of these fixed effects. When the genetic correlation between growth rate in males and females differs from 1, it is possible, in principle, to remove the variability due to a difference in mean body weight between sexes. The magnitude of environmental effects, such as group and batch effects, is related to environmental sensitivity (and thus to genotype by environment interactions). Evaluating the prospects of reducing these components by genetic selection will require further research.

An interesting property of the specific design of our experiment is that it allows the simultaneous study of uniformity and social effects such as group and kin effects in our study and indirect genetic effects, which were analyzed in other studies on the same data (Khaw, Ponzoni, Yee, Aziz and Bijma, 2016; Khaw, Ponzoni, Yee, Aziz, Mulder *et al.*, 2016). However, the experimental setting and feeding strategy that we applied differed from those in a commercial setting. Thus, genotype by environment interactions may be present and our results may not represent uniformity in the case of commercial tilapia farms. The DHGLM approach could be used to test whether the genetic background of uniformity differs between both environments. Results from such a study would be a useful addition to our findings.

2.5 Conclusions

Our study revealed substantial genetic variation in uniformity of harvest weight and body size traits, which opens promising prospects for the genetic improvement of uniformity by selective breeding of the GIFT strain. The genetic correlation between level and variance of harvest weight was high and positive, which indicates that selection for heavier fish may also result in more variation in harvest weight. Simultaneous improvement of harvest weight and its uniformity will thus require index selection.

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Supplementary material

Family ID	2	3	4	5	6	7	8	9	10	11
1	1-2	1-3	1-4	1-5	1-6	1-7	1-8	1-9	1-10	1-11
2		2-3	2-4	2-5	2-6	2-7	2-8	2-9	2-10	2-11
3			3-4	3-5	3-6	3-7	3-8	3-9	3-10	3-11
4				4-5	4-6	4-7	4-8	4-9	4-10	4-11
5					5-6	5-7	5-8	5-9	5-10	5-11
6						6-7	6-8	6-9	6-10	6-11
7							7-8	7-9	7-10	7-11
8								8-9	8-10	8-11
9									9-10	9-11
10										10-11

Figure S2.1 Example of the block design used to allocate two families to each group. All families in the block are unrelated to each other.

Table S2.1. Genetic parameters for the mean and the variance of Box-Cox transformed harvest weight

Parameter	Mean	Variance
[a]σ_A^2	0.100 (0.019)	0.239 (0.052)
σ_e^2	0.184 (0.004)	1.804 (0.035)
σ_g^2	0.050 (0.007)	0.035 (0.019)
σ_k^2	0.032 (0.004)	0.048 (0.025)
σ_m^2	0.008 (0.003)	0.014 (0.009)
σ_P^2	0.323 (0.011)	_
h^2	0.31 (0.05)	_
[b]g^2	0.15 (0.02)	_
[c]k^2	0.10 (0.02)	_
[d]m^2	0.02 (0.01)	_
[e]GCV	0.06	0.49

*Standard errors are indicated between brackets

[a]Additive genetic variance was calculated as 4 times the sire-dam variance; [b]Group effect, calculated as $g^2 = \sigma_g^2/\sigma_P^2$; [c]Kin effect, calculated as $k^2 = \sigma_k^2/\sigma_P^2$; [d]Social maternal effect, calculated as $m^2 = \sigma_m^2/\sigma_P^2$.

Table S2.2 Estimates of fixed effects for variance level of harvest weight, length, depth, and width, obtained from the reduced model with main fixed effects (not the interactions) and random effects

Effect		Variance level of HW			Variance level of length			Variance level of depth			Variance level of width		
		Estimate	SE	Fraction[a]	Estimate	SE	Fraction	Estimate	SE	Fraction	Estimate	SE	Fraction
Sex	F	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0
	M	0.558	0.036	1.747	0.224	0.078	1.252	0.229	0.036	1.257	0.181	0.035	1.198
Batch	2009	-0.605	0.231	0.546	-0.033	0.192	0.968	-0.318	0.191	0.728	-0.353	0.195	0.703
	2010	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0
	2011	0.255	0.115	1.290	0.081	0.103	1.084	0.017	0.103	1.017	0.095	0.104	1.099
Pond	1	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0
	2	-0.376	0.049	0.687	-0.163	0.047	0.791	-0.112	0.045	0.894	-0.167	0.048	0.846
Age		0.008	0.001	1.008	0.001	0.001	1.001	0.003	0.001	1.003	0.004	0.001	1.004

[a] Exponent of the estimate

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Modelling the co-evolution of indirect genetic effects and inherited variability

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Abstract

When individuals interact, their phenotypes may be affected not only by their own genes but also by genes in their social partners. This phenomenon is known as Indirect Genetic Effects (IGEs). In aquaculture species and some plants, however, competition not only affects trait levels of individuals, but also inflates variability of trait values among individuals. In the field of quantitative genetics, the variability of trait values has been studied as a quantitative trait in itself, and is often referred to as inherited variability. Such studies, however, consider only the genetic effect of the focal individual on trait variability and do not make a connection to competition. Although the observed phenotypic relationship between competition and variability suggests an underlying genetic relationship, the current quantitative genetic models of IGE and inherited variability do not allow for such a relationship. The lack of quantitative genetic models that connect IGEs to inherited variability limits our understanding of the potential of variability to respond to selection, both in nature and agriculture. Models of trait levels, for example, show that IGEs may considerably change heritable variation in trait values. Currently, we lack the tools to investigate whether this result extends to variability of trait values. Here we present a model that integrates IGEs and inherited variability. In this model, the target phenotype, say growth rate, is a function of the genetic and environmental effects of the focal individual and of the difference in trait value between the social partner and the focal individual, multiplied by a regression coefficient. The regression coefficient is a genetic trait which is a measure of cooperation; a negative value indicates competition, a positive value cooperation, and an increasing value due to selection indicates the evolution of cooperation. In contrast to existing quantitative genetic models, our model allows for co-evolution of IGEs and variability, as the regression coefficient can respond to selection. Our simulations show that the model results in increased variability of body weight with increasing competition. When competition decreases, i.e., cooperation evolves, variability becomes significantly smaller. Hence, our model facilitates quantitative genetic studies on the relationship between IGEs and inherited variability. Moreover, our findings suggest that we may have been overlooking an entire level of genetic variation in variability, the one due to IGEs.

Key words: inherited variability, uniformity, social interactions, competition, cooperation, indirect genetic effects, IGE, aquaculture

3.1 Introduction

Social interactions are common in nature, and other individuals are usually the most important part of the environment experienced by an individual (Wolf, 2003; Frank, 2007). The environment created by social partners through actions such as competition or cooperation, is referred to as the social environment. Variation in the quality of the social environment may originate partly from genetic variation in the social partners, which would make the social environment heritable (Wolf *et al.*, 1998). The classical example of a heritable environment is the one provided by a mother to her offspring in mammals (Dickerson, 1947; Willham, 1963; Falconer, 1965; Kirkpatrick and Lande, 1989; Cheverud, 2003).

In the traditional quantitative genetic model, the phenotype of an individual is the sum of the direct effect of its own genes (DGE) and an environmental effect. However, because the environmental effect includes a component due to the social environment, the phenotype of an individual is also a function of the genes of its social partners. The heritable effect of a social partner on the trait value of the focal individual is known as an Indirect Genetic Effect (IGE) (Griffing, 1967). IGEs have consequences for trait values and fitness of individuals that interact, and subsequently for the course of the evolutionary processes (e.g. Hamilton, 1964; Moore *et al.*, 1997; Wolf *et al.*, 1998).

In the field of animal breeding, interest in social interactions has increased in recent decades, as both theoretical and empirical studies show that not only fitness but also trait values of individuals can be affected by genes of other individuals (Muir, 2005; Bijma, Muir, and van Arendonk, 2007; Bijma *et al.*, 2007). IGEs have been studied in both animal and plant populations, and in a number of those studies social interactions contributed substantially to heritable variation in the trait (reviewed by Ellen *et al.*, 2014). Well known cases of IGEs in domestic animals include cannibalistic behavior in laying hens, which causes mortality (Muir, 1996; Ellen *et al.*, 2008), competition and tail biting in pigs, which is associated with poorer growth (Arango *et al.*, 2005; Camerlink *et al.*, 2013, 2014; Bergsma *et al.*, 2013), and aggression and competition in fish species such as Nile tilapia and Atlantic cod, which reduces growth (Nielsen *et al.*, 2014; Khaw *et al*., 2016).

In addition to the effects of social interactions on trait values, it has been observed in aquaculture populations that competition for feed and formation of social hierarchies also inflates trait variability (Jobling, 1995; Cutts *et al.*, 1998; Hart and

Salvanes, 2000). Because this pattern is so evident, variability in body weight among individuals has become a standard measure of the degree of competition in aquaculture; the degree of competition is measured by the coefficient of variation (CV) of body weight, where a high CV indicates strong inter-individual competition (Jobling, 1995). In farmed fish populations, the CV is usually between 20-60 % (Gjedrem, 2000; Ponzoni *et al.*, 2005; Gjedrem and Baranski, 2009), which suggests moderate to strong competition.

Indications of a close relationship between competition and variability are also coming from the field of plant breeding, where breeders have successfully improved productivity of crops by selecting, partly unintentionally, less competitive phenotypes, which also resulted in more uniform crops (Donald, 1968; Austin *et al.*, 1980; Denison *et al.*, 2003). Moreover, the connection between yield, competition, and variability has also been made in game theory, where it was shown that the lowest competition and highest yield is achieved when plants are phenotypically uniform (Zhang *et al.*, 1999). Hence, in plants, there is clear evidence of a genetic relationship, where reduced competition leads to less variability and higher yield.
The variability of trait values of a genotype, measured either repeatedly on the same individual, or on multiple individuals belonging to the same family, has been studied as a quantitative trait in its own right. This trait is often referred to as "inherited variability" or "heritable variation in environmental variance" (SanCristobal-Gaudy *et al.*, 1998; Mulder *et al.*, 2007; Hill and Mulder, 2010). The study of variability has been a part of quantitative genetics for several decades already, but it has gained particular attention in recent years due to the development of new methods to estimate genetic variance in variability (SanCristobal-Gaudy *et al.*, 1998; Sorensen and Waagepetersen, 2003; Mulder *et al.*, 2009; Rönnegård *et al.*, 2010) and substantial empirical evidence for a genetic basis of variability in livestock, aquaculture, and laboratory populations (reviewed by Hill and Mulder, 2010). In several fish populations, for example, it has been found that variability of body weight has a large genetic component (Janhunen *et al.*, 2012; Sonesson *et al.*, 2013; Khaw *et al.*, 2015; Sae-Lim, Gjerde, *et al.*, 2015; Sae-Lim, Kause, *et al.*, 2015; Marjanovic *et al.*, 2016). However, despite the clear relationship between competition and variability observed at the phenotypic level, inherited variability has not been connected to competition in quantitative genetic models.

As social interactions are often a source of IGEs, the observed relationship between competition and variability on the phenotypic level (Jobling, 1995; Cutts *et al.*, 1998; Hart and Salvanes, 2000; Denison *et al.*, 2003) strongly suggests an underlying

genetic relationship between the two phenomena. At present, little is known of this genetic relationship, both in plants and animals, which may be due to a lack of quantitative genetic models that connect both phenomena. On the one hand, current quantitative genetic models of inherited variability ignore social interactions, since they treat variability as a trait of the focal individual only, ignoring the contribution of social partners. On the other hand, standard IGE-models cannot explain the relationship between competition and variability, since phenotypic variance is independent of the level of IGEs in those models. However, by ignoring IGEs, we may be overlooking an important component of heritable variation in trait variability.

The joint study of IGEs and inherited variability could help us understand observations from animal and plant breeding, and possibly enable utilization of genetic variation that has so far been untapped. In addition, it may bring new insight in mechanisms of canalization or insensitivity of individuals to genetic and environmental changes (Waddington, 1942), and broaden our understanding of phenotypic evolution. Therefore, a joint study of IGEs and variability could make a significant contribution to the field of quantitative genetics, and its applications in animal and plant breeding and in evolutionary biology.

As a first step towards unraveling the genetic relationship between social interactions and inherited variability, we present a quantitative genetic model that integrates both phenomena. We use Monte Carlo simulation to evaluate the behavior of the model, and demonstrate that the model mimics the co-evolution of social interactions and variability observed in phenotypic studies.

3.2 Theory

3.2.1 Model

The genetics of socially-affected traits can be studied using two approaches; variance component models or trait-based models (McGlothlin and Brodie, 2009; Bijma, 2014). In variance component models, the individual phenotype is divided into a direct genetic component originating from the focal individual, and an indirect genetic component originating from its social partner (Griffing, 1967). In this approach, it is not needed to know which traits are causing the IGE. Instead, DGEs and IGEs are estimated as random effects using linear mixed models and information on genetic relationships between individuals (Muir, 2005; Bijma, Muir, Ellen, *et al.*, 2007). See Table 3.1 for notation.

Table 3.1 Notation key

Symbol	Meaning
DGE, IGE	Direct genetic effect, indirect genetic effect
i, j	Focal individual, group mate of individual *i*
$P_{t,GR}$	Body weight in the current time point
$P_{t-1,GR}$	Body weight in the previous time point
μ_{GR}	Mean growth rate
A_{GR}	Breeding value for growth rate
A_D	Direct breeding value for *b* – genetic resistance to competition
A_I	Indirect breeding value for *b* – genetic cooperation effect
$E_{p,GR}$, $E_{t,GR}$	Permanent and temporary environmental effects on growth rate
E_D, E_I	Direct and indirect environmental effects for *b*
b	Regression coefficient
$\bar{b}$	Average regression coefficient
b-value	Regression coefficient which affects the phenotype of the focal individual
$\sigma^2_{A_{GR}}$, $\sigma^2_{A_D}$, $\sigma^2_{A_I}$	Genetic variance for growth rate, direct and indirect genetic variance for *b*
$\sigma^2_{E_{p,GR}}$, $\sigma^2_{E_{t,GR}}$	Permanent and temporary environmental variance for growth rate
$\sigma^2_{E_D}$, $\sigma^2_{E_I}$	Direct and indirect environmental variance for *b*
$\sigma^2_{P_{GR}}$	Phenotypic variance of growth rate
h^2	Heritability of growth rate

The trait-based models, in contrast, define IGEs on the phenotype of the focal individual as a function of trait values of its social partners (Moore *et al.*, 1997; Wolf *et al.*, 1998; Bijma, 2014). In this case, the traits causing the indirect effects need to be identified. When interaction is between two individuals, and the target trait and the trait causing the IGE, also known as the "effector trait", are the same, the trait-based model can be written as (Moore *et al.*, 1997)

$$P_i = A_i + e_i + \psi P_j \quad (1)$$

where P_i is the phenotypic value of the focal individual *i*, A_i is the additive genetic effect originating from the focal individual, P_j is the phenotypic value of its social partner *j*, ψ is the "regression coefficient" of P_i on P_j, and e_i is a residual. (With feedback, i.e., when trait levels of interacting individuals are reciprocally affected, ψ is not a true regression coefficient; see Bijma, 2014). We will use this model and observations from aquaculture as a starting point to draw a connection between IGEs and inherited variability.

Phenotypic studies in aquaculture suggest that the behavior of a fish towards its social partners depends on its size relative to that of its partners, where larger fish are usually dominant and aggressive, while smaller fish are subordinate and submissive (Doyle and Talbot, 1986; Huntingford *et al.*, 2012). In anemonefish, for example, large individuals are dominant members of social groups and display aggressive behavior towards subordinates (Fricke and Fricke, 1977; Iwata *et al.*, 2008). Similarly, Oscars (cichlid fish, *Astronotus ocellatus*) chase and attack smaller conspecifics, but avoid larger individuals (Beeching, 2010). Difference in body weight, therefore, affects phenotypes of the interacting individuals, with higher body weight giving a competitive advantage to the individual in terms of growth rate. Thus, to account for the competitive effect of body weight on growth rate, we need to model the evolution of body weight over the life of the interacting individuals.

Therefore, we developed a basic quantitative genetic model involving interactions of two individuals. In this model, our target trait is growth rate between time point *t*-1 and *t*, while the effector trait is the difference in body weight between the individuals that interact at the previous time point *t-1*. The change in body weight, i.e., growth rate, of the focal individual is a function of genetic and environmental effects of the focal individual itself on its growth rate, and of the difference in body weight between the social partner and the focal individual, multiplied by a regression coefficient,

$$P_{t,i} - P_{t-1,i} = \mu_{GR} + A_{GR,i} + E_{p,GR,i} + E_{t,GR,i} + b_{ij}\left(P_{t-1,j} - P_{t-1,i}\right) \quad (2)$$

where $P_{t,i}$ is the body weight of focal individual *i* at time point *t*, $P_{t-1,i}$ is body weight of *i* at the previous time point, μ_{GR} is the mean growth rate of the population, $A_{GR,i}$ is a (direct) breeding value for growth rate of individual *i*, $E_{p,GR,i}$ and $E_{t,GR,i}$ are

permanent and temporary non-heritable ("environmental") effects of individual *i*, and b_{ij} is a regression coefficient.

3.2.2 The meaning of b_{ij}

The b_{ij} in our model measures the effect of a difference in body weight between the social partner and the focal individual on the growth rate of the focal individual. Hence, the absolute value of b_{ij} reflects the strength of the social interaction. When b_{ij} is negative, growth rate of individual *i* is reduced when *j* has higher body weight than *i*, indicating competition. Conversely, when b_{ij} is positive, growth rate of *i* is increased when *j* has higher body weight than *i*, indicating cooperation, i.e., "helping the one who lags behind" (Box 1). Thus, *b* is a measure of cooperation; negative *b* indicates competition, positive *b* cooperation, and an increase in *b* an increase of cooperation (i.e., less competition). The model described by Equation 2 can be written in matrix form for both individuals simultaneously, which may facilitate analysis of the behavior of the model (see Appendix A).

3.2.3 Genetic variation in *b*

Trait-based IGE models usually assume that the "regression coefficient" ψ is constant within a population (Equation 1). However, several empirical studies that were able to estimate ψ, show that it may differ between genotypes (Kent *et al.*, 2008; Bleakley and Brodie IV, 2009; Chenoweth *et al.*, 2010). Hence, empirical studies suggest that ψ shows genetic variation, and can thus respond to selection. Following this evidence, we allow *b* to evolve. Therefore, *b* is not a fixed parameter, but specific for every interacting couple. We propose that heritable variation in *b* is a result of a direct genetic effect of the focal individual ($A_{D,i}$), representing resistance to competition, and an indirect genetic effect of its social partner, representing cooperative effect ($A_{I,j}$). While *b* is a property of both the focal individual and its social partner, it affects the phenotype of the focal individual only; we will therefore refer to this *b* as "the *b*-value of the focal individual". Thus, for focal individual *i* with social partner *j*, the regression coefficient b_{ij}, i.e., the *b*-value of the focal individual, is given by

$$b_{ij} = \bar{b} + A_{D,i} + E_{D,i} + A_{I,j} + E_{I,j} \qquad (3)$$

where $\bar{b}$ represents the average regression coefficient, which is a population parameter that is negative under competition and positive under cooperation. The $A_{D,i}$ and $E_{D,i}$ are the direct genetic and environmental effect of individual *i* on b_{ij},

while $A_{I,j}$ and $E_{I,j}$ are the indirect genetic and environmental effect of individual *j* on b_{ij}. Appendix B contains extension of Equation 2 to accommodate larger group size.

3.2.4 Inherited variability

Note that our model does not include an explicit breeding value for inherited variability. Instead, as shown in the section "Simulation" below, genetic variation in variability is an emerging property of the model, resulting from genetic effects of competition, i.e., the direct and indirect breeding values for *b.* In other words, our model shows that heritable effects on competition result in inherited variability. In the Discussion section, we further investigate how breeding values for *b* correlate with direct and indirect breeding values for inherited variability (see section "Estimating *b*" below; see also section "Breeding values for *b* and variability").

3.2.5 Competition, cooperation, and the sign of $\bar{b}$

We use the term "competition" to describe the situations where the larger individual continues to increase in size, while the smaller individual lags behind, leading to divergence of their body weights through time (Figure 3.1A). This is typical for populations where $\bar{b} < 0$. We use the term "cooperation" to describe the situation where individuals become increasingly similar in body weight over time (Figure 3.1B). This occurs when growth rate of the larger individual decreases, while the smaller one catches up. This is typical for populations where $\bar{b} > 0$.

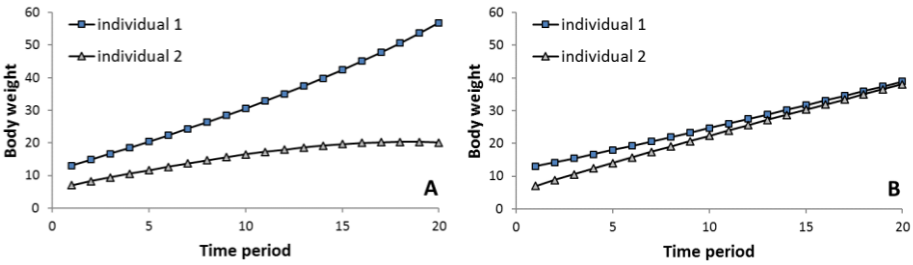

Figure 3.1 Expected growth curves of two individuals in a group under competition (A) and cooperation (B).

3.2.6 Asymmetry: b_{ij} vs b_{ji}

Note that we distinguish between resistance to competition (A_D) and cooperativeness (A_I), as these may be different properties of an individual. For example, consider the pair *i* and *j* in a population showing competition ($\bar{b} < 0$).

Box 1: Direct and indirect breeding values for *b*

Direct breeding value (A_D) is the additive genetic effect of the focal individual on its own *b* and is referred to as a "resistance to competition". Negative A_D would mean that the individual is sensitive to competition, while the individual with positive A_D is resistant to competition.

Indirect breeding value (A_I) refers to additive genetic effect of a social partner on *b* of a focal individual. It is also referred to as "cooperativeness". The social partner with negative A_I is competitive, while the one with positive A_I is cooperative.

Each individual, therefore, has two breeding values for *b* - one that affects their own *b* and one that affects their social partner's *b.*

If we consider two individuals, *i* and *j*, that differ in their body size in the previous time period by 2 grams, such as that *j* is the larger individual, i.e., $P_{t-1,j} - P_{t-1,i} = 2$ g and $P_{t-1,i} - P_{t-1,j} = -2$ g, then the change in phenotype for individual *i* from time *t*-1 to *t* is given as $\Delta P_{t,i} = b_{ij}(P_{t-1,j} - P_{t-1,i}) = 2b_{ij}$, and similarly $\Delta P_{t,j} = b_{ji}(P_{t-1,i} - P_{t-1,j}) = -2b_{ij}$ (Equation 2, assuming no effect of breeding value for growth and no environmental effects).

As given in Equation 3, $b_{ij} = \bar{b} + A_{D,i} + A_{I,j}$, **where $\bar{b}$ is population parameter, negative with competition and positive with cooperation**, 0 when neutral. Correspondingly, $b_{ji} = \bar{b} + A_{D,j} + A_{I,i}$.

Competitive environment
In the competitive environment, where, for example, $\bar{b}$ = - 0.05, and both individuals are cooperative and resistant to competition, with breeding values of 0.03, i.e., $A_{D,i} = A_{D,j} = A_{I,j} = A_{I,i} = 0.03$, the change in growth for individual *i* $(\Delta P_{t,i})$ is 0.02 g , while $\Delta P_{t,j}$ = - 0.02 g. However, if both individuals are competitive and sensitive to competition, $A_{D,i} = A_{D,j} = A_{I,j} = A_{I,i}$ = - 0.03, then $\Delta P_{t,i}$= - 0.22 g, while $\Delta P_{t,j} = 0.22$ g. Hence, in a competitive environment, when both $\bar{b}$ and individual breeding values for *b* are negative, the larger individual grows fast, while the growth of smaller one is slowed down. Positive breeding values in a competitive environment lead to small increase in growth for the smaller individual, and a small decrease for the larger one.
For explanation on chosen values see Simulation and Appendix C.

Cooperative environment
In the cooperative environment, where $\bar{b}$ is, for example, 0.05, and individuals have positive breeding values of 0.03, $\Delta P_{t,i} = 0.22$ g , while $\Delta P_{t,j}$ = - 0.22 g. If both individuals have all negative breeding values of - 0.03, then $\Delta P_{t,i}$= - 0.02 g, while $\Delta P_{t,j} = 0.02$ g. Therefore when both $\bar{b}$ and individual breeding values for *b* are positive, the growth of the larger individual slows down, allowing the smaller individual to catch up. Negative breeding values in a cooperative environment lead to small increase in growth for the larger individual, and a small decrease in growth for the smaller individual.
For more scenarios and effects of combining positive and negative breeding values for *b*, see Supplementary material (Tables S3.1 – S3.3).

Suppose that *i* is very competitive ($A_{I,i} < 0$) and also resistant to competition ($A_{D,i} > 0$), while *j* is very cooperative ($A_{I,j} > 0$) but very sensitive to competition ($A_{D,j} < 0$). Then the effect of *j* on *i* will be small, while the effect of *i* on *j* will be large (Supplementary material grey cells in Table S3.2). In other words, an individual that is strongly affected by its social partner, does not necessarily also have a strong effect on its social partner. Hence, *b* is non-symmetric, i.e., $b_{ij} \neq b_{ji}$.

3.3 Simulation

We used Monte Carlo simulation to investigate whether our model (Equation 2) predicts the empirically observed relationship between competition and variability, and whether methods for selection against competition (e.g. group selection) also result in a reduction of variability. We considered five values for the average value of *b* ($\bar{b}$), to which we refer as scenarios (Table 3.2).

Table 3.2 Parameters used in simulation

Parameters	**Scenarios**				
	Competition		Neutral	Cooperation	
	1	2	3	4	5
Mean growth rate, μ_{GR}	10 g				
Genetic variance for growth rate, $\sigma^2_{\mathrm{A_{GR}}}$	1 g^2				
Permanent environmental variance, $\sigma^2_{\mathrm{E_{p,GR}}}$	0.4 g^2				
Temporary environmental variance, $\sigma^2_{\mathrm{E_{t,GR}}}$	0.6 g^2				
Cooperation effect, $\bar{b}$	-0.08	-0.05	0	0.05	0.08
Direct and indirect genetic and environmental variance, $\sigma^2_{\mathrm{A_D}} = \sigma^2_{A_I} = \sigma^2_{E_D} = \sigma^2_{E_I}$	0.225 x 10^{-3}				
Phenotypic variance, $\sigma^2_{P_{GR}}$	2 g^2				

*$\sigma^2_{P_{GR}}$ is calculated excluding *b* i.e. as $\sigma^2_{P_{GR}} = \sigma^2_{\mathrm{A_{GR}}} + \sigma^2_{\mathrm{E_{p,GR}}} + \sigma^2_{\mathrm{E_{t,GR}}}$

Negative values of $\bar{b}$ correspond to competition (Scenario 1 – strong competition; Scenario 2 – moderate competition), while positive values reflect cooperation (Scenario 4 – moderate cooperation; Scenario 5 – strong cooperation). Scenario 3 represents a neutral environment with $\bar{b}$ = 0.

The genetic values of all individuals in the population were simulated as inherited from their parents (base population), assuming Mendelian inheritance, while their environmental values were sampled from independent normal distributions. All individuals were randomly assigned to groups of 2 members. Phenotypes were constructed for 10 time points using Equations 2 & 3. Average starting weight was 10 grams, and average growth rate between time points was also 10 grams. Hence, to illustrate the behavior of our model as simple as possible, we considered absolute growth. Obviously, for the analysis of real data, a more biologically realistic growth model, such as relative growth, may be used. For each scenario, there were 100 replicates. Table 3.2 contains parameter values used in the simulation. Appendix C contains a detailed description of the simulation procedure.

3.4 Relationship between $\bar{b}$ and variability

The relationship between competition and variability generated by our model was assessed at two levels. First, we considered the average within-group variance of body weight at the last time point. Second, we considered the overall phenotypic variance in the entire population. Results are presented in Figure 3.2 as averages over 100 replicates.

Across the five scenarios both average within-group variance and phenotypic variance decreased curvilinear with increasing $\bar{\boldsymbol{b}}$, i.e., with increasing cooperation (Figure 3.2). The average within-group variance ranged from 376.4 g^2 (sd, ±14.4 g^2) to 20.9 g^2 (sd, ±0.7 g^2), which is an 18-fold difference in variability of body weight between scenarios 1 and 5. The phenotypic variance ranged from 457.3 g^2 (sd, ± 15.7 g^2) to 95.1 g^2 (sd, ± 2.6 g^2), showing a 5-fold difference in variability between scenarios 1 and 5. These results show that our model results in a relationship between competition ($\bar{\boldsymbol{b}}$) and variability that is also found in real data.

The difference between the average within-group variance and the phenotypic variance is related to the similarity of group mates. Total phenotypic variance is the sum of between- and within-group variance. When group mates are independent and group size equals two, the average within-group variance is half of the

phenotypic variance. Average within-group variance, however, was much larger than half of the phenotypic variance in scenarios with negative *b*, but much smaller in scenarios with positive *b*. The correlation between group mates is calculated as $\rho = \frac{\sigma_b^2 - \sigma_w^2}{\sigma_b^2 + \sigma_w^2}$ where σ_b^2 is between group variance and σ_w^2 is within-group variance. In scenarios with negative *b*, the correlation between group mates was negative, which means that group mates were dissimilar in the competitive environment (Figure 3.2). When *b* was positive, correlation between group mates was positive, indicating higher similarity of group mates in the cooperative environment (Figure 3.2). For $\bar{b}$=0, the average within-group variance was approximately one half of the phenotypic variance.

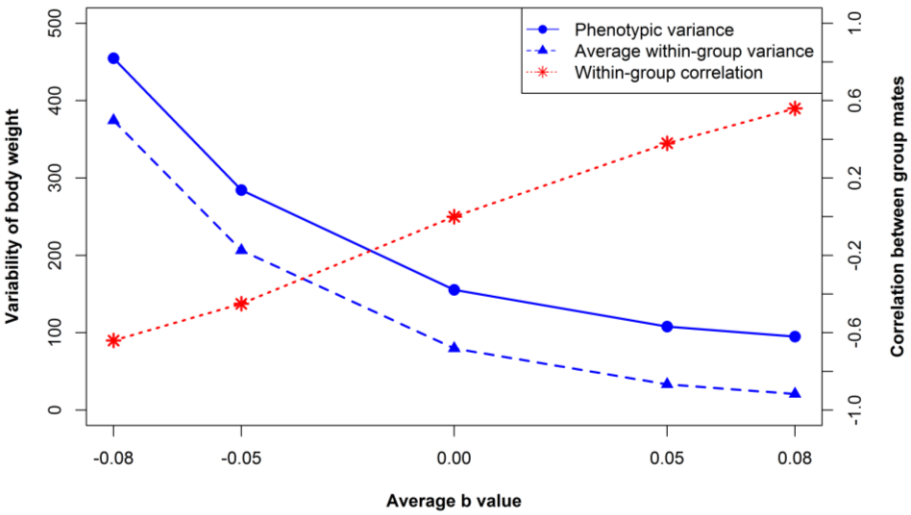

Figure 3.2 Variability of body weight in a population and correlation between group mates across five scenarios i.e. five average *b* values ($\bar{b}$). Variability is expressed as the average within-group variance of body weight of two group mates and as overall phenotypic variance in the whole population.

3.5 Growth curve patterns in relation to *b* values

In this section, we look into how variation in *b* around its average, affects the variability among group mates. Within every scenario (Table 3.2) $\bar{b}$ was the same for all individuals; however, variation in *b*-values of individuals existed due to variation in direct and indirect genetic and environmental components that make up *b* (Equation 3). Therefore, in every scenario some groups would have individuals that

both have high *b*-values, some groups would have individuals with low *b*-values, and variations in between. We hypothesize that group mates that both have high *b*-values, i.e., that are both cooperative and resistant to competition, grow more uniform compared to those with low *b*-values, i.e., group mates that are both competitive and sensitive to competition.

To illustrate this, we selected groups that have individuals with the highest and the lowest *b*-values for each of the scenarios. An additional condition when selecting groups was that individuals have an initial difference in their body weight of ~2 sd. The growth curves in relation to the level of *b*-values within a group are illustrated in panels A and D of Figure 3.3 for scenario 1 ($\bar{b}$ = -0.08, strong competition) and 5 ($\bar{b}$= +0.08, strong cooperation). Results for scenarios 2-4 are presented in Supplementary material (Figures S3.1 - S3.3). Tables S3.4 and S3.5 in Supplementary material contain *b*-values of individuals from all the scenarios.

In both scenarios 1 and 5, individuals in a group with the low *b*-values differed substantially in their final body weight (A panels, Figure 3.3). Individuals with the high *b*-values, however, maintained a similar body weight through time (D panels, Figure 3.3), which is in agreement with our hypothesis.

We also looked into groups that had individuals with positive/negative combinations of *b*-values. When the initially larger individual had a negative *b*-value, its body weight increased over time, resulting in a larger size difference between the two group mates, unless the smaller individual had a positive *b*-value, which allowed it to catch up (Panels B, Figure 3.3). Similarly, the size difference decreased when the larger individual had a positive *b*-value, even when the smaller individual had a negative *b*-value (Panels C, Figure 3.3). It was also possible to get re-ranking of the individuals, i.e., the smaller individual can become the larger one. This can happen for example when the smaller individual has a high positive *b*-value, while the larger individual has a low negative *b*-value (Scenario 5, Panel B, Figure 3.3).

Expressions (see Appendix A) for the expectation of the difference in the phenotypic values and the variance of this difference at time point *T*, i.e., $E\left(P_{T,i} - P_{T,j}|b_{ij}, b_{ji}\right)$ and $V\left(P_{T,i} - P_{T,j}|b_{ij}, b_{ji}\right)$, demonstrate that the phenotypic variance within a group is directly related to the sum of *b*-values within the group. The expressions show that the expected difference is zero if there is no initial difference at *T*=0, while the

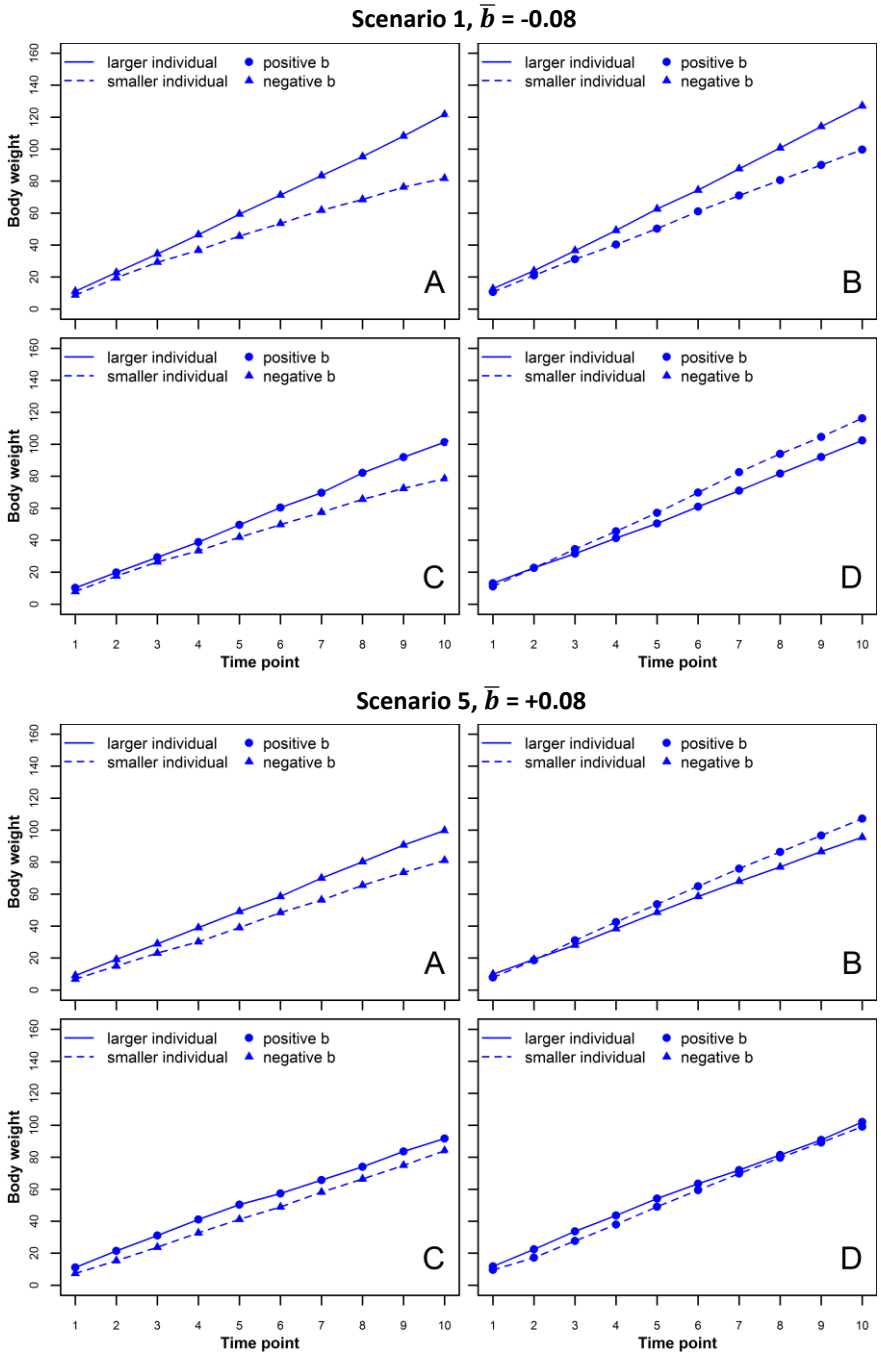

Figure 3.3 Growth curves of two group mates (one larger than the other) that have lowest sum of *b*'s (A); the initially larger individual has negative *b*, the smaller one has positive *b* (B); the initially larger individual has positive *b*, the smaller one has negative *b* (C); lowest sum of *b*'s (D), for scenarios 1 and 5. Each panel shows one typical replicate.

variance depends directly on the sum of b_{ij} and b_{ji}. More details can be found in Appendix A.

3.6 Breeding values for b and variability

If a connection between competition and variability exists not only on the phenotypic level but also on genetic level, we should see less variation in body size among the offspring of sires that have positive direct breeding values (A_D) for *b*, as these individuals should be more resistant to competition. This links our model to the definition of inherited variability, where parents with low breeding values for variability have offspring with lower phenotypic variance. Figure 3.4A indeed shows that the correlation between A_D of sires for *b* and variability of body weight of their offspring is negative, ranging from -0.55 (sd, ±0.07) to -0.20 (sd, ±0.09) across scenarios. This suggests that individuals that are genetically more resistant to competition are less variable. Moreover, offspring of sires with positive indirect breeding values (A_I) for *b* should be less competitive. The group mates of these "social" individuals should therefore show less variability compared to group mates of individuals with negative indirect breeding values for *b*. In other words, A_I of a sire affects the variability of phenotypes of the group mates of his offspring. As expected, Figure 3.4B shows negative correlations between A_I of sires and variability of the group mates of their offspring. Figures 3.4A and 3.4B also show a small negative correlation between A_I of a sire and variability of his offspring, and between A_D of a sire and variability of the group mates of its offspring. This result suggests a second-order effect; for the direct effect, for example, the A_D of a sire first affects the trait values of its own offspring, which subsequently affects the variability of their groups mates in the next time period. For standard errors of the correlations see Supplementary material (Table S3.6).

3.7 Selection

Individual selection has often been used with great success for improvement of livestock and aquaculture traits. However, this type of selection ignores the contribution of IGE which may hamper the improvement of socially affected traits. An alternative strategy is a group selection, which takes indirect genetic effects into account (Griffing, 1976).

To see how variability responds to selection, and whether we can capture direct (A_D) and indirect genetic effects (A_I) for *b* with existing selection methods, we performed three types of selection: individual selection for body weight, group selection for

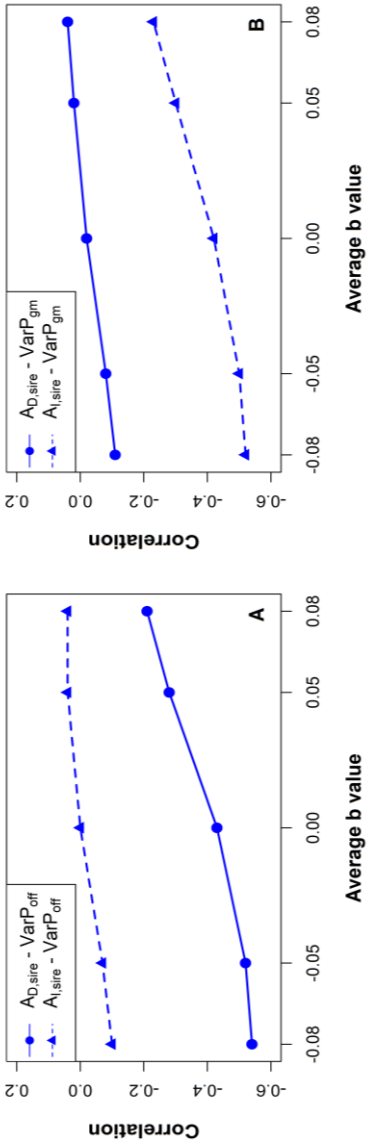

Figure 3.4 A. Correlation (r) between direct (A_D) and indirect (A_I) breeding value of a sire and variance of body weight of his offspring ($VarP_{off}$) for each of the five scenarios, i.e. average *b* values ($\bar{b}$). B. Correlation (r) between direct (A_D) and indirect (A_I) breeding value of a sire and variance of body weight of the group mates of its offspring ($VarP_{gm}$), for each of the five scenarios, i.e. average *b* values ($\bar{b}$).

body weight, and group selection for lower variance of body weight. In all three cases, selection was done using observations from time point 10. With individual selection, the 11 % of the heaviest individuals were selected as parents of the next generation. With group selection for body weight, the individuals from the 11 % of groups with the highest average body weight were selected. With group selection

for lower variance, the individuals from the 11 % of groups with the lowest variance in body weight were selected. We illustrate the effect of selection by using base population with $\bar{b}$ = -0.08 (Scenario 1 – strong competition, Table 3.2). Selections were performed for 10 generations. Correlations between A_D and A_I, A_D and A_{GR}, and A_I and A_{GR}, were all set to 0. See Appendix C for further details. Figure 3.5 presents the results as averages over 100 replicates. For standard errors see Supplementary material (Tables S3.7 - S3.9).

Individual selection increased mean body weight (Figure 3.5F), but also decreased A_D (Figure 3.5C) and A_I (Figure 3.5D), causing an increase in variability in the population (Figure 3.5A). In other words, individual selection increased variability.

Both types of group selection increased A_I (Figure 3.5D), suggesting that group selection at least partially exploited genetic differences in indirect genetic effects on *b*. Variability of body weight decreased when group selection was made on variance, but increased slightly when group selection was for average body weight, however much less compared to individual selection (Figure 3.5A). This increase in variability with group selection for average body weight originated from a decrease in A_D. With group selection on variance, in contrast, A_D increased (Figure 3.5C). Group selection on the variance, therefore, captured direct and indirect genetic effects on *b* better than group selection on the average body weight. Group selection on the variance did not change mean body weight, because the correlations between A_D and A_{GR}, and A_I and A_{GR} were zero (Figure 3.5F). Group selection for average body weight, on the other hand, increased mean body weight in magnitude similar to individual selection (Figure 3.5F).

3.8 Discussion

We have proposed a quantitative genetic model that integrates competition and variability, and have shown through simulation that our model mimics the observation in real populations, where competition for resources increases phenotypic variability among individuals. In our model an improvement of the social environment through an increase in *b*, which was modelled as a heritable trait in itself, resulted in reduced variability.

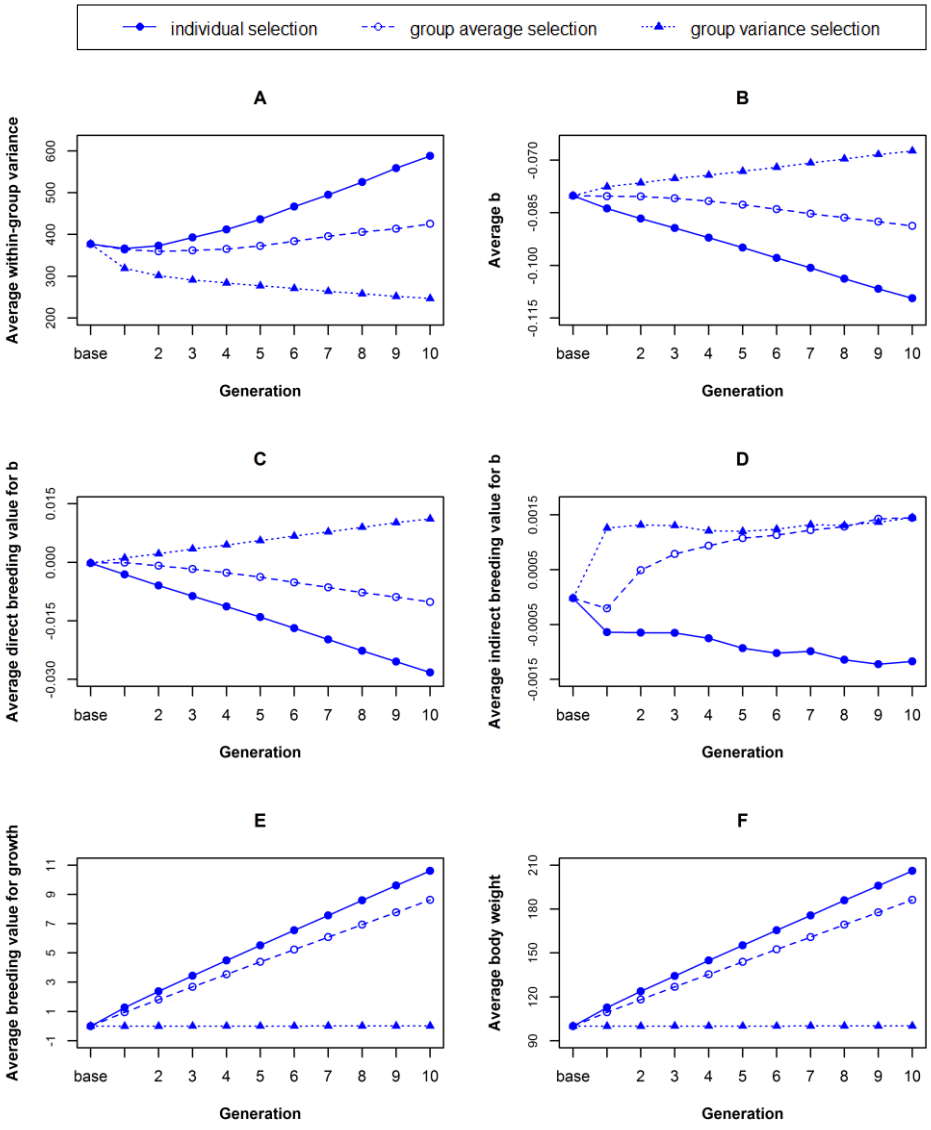

Figure 3.5 Effect of three types of selection on average within-group variance (A), average b ($\bar{b}$) (B), average direct breeding value for b (C), average indirect breeding value for b (D), average breeding value for growth (E), and average body weight (F), in the population.

3.8.1 Estimating *b*

The key parameter in our model is the regression coefficient *b*, which comprises both direct and indirect genetic effects. In other words, *b* is heritable and can respond to selection. Application of our model requires methods to estimate *b* and its genetic components. In the following, we discuss data requirements and propose models that could be used as a first step to estimate the average *b* and its direct and indirect genetic variance.

Our *b* connects the difference in trait values between the group mate and the focal individual at the previous time point to the target phenotype of the focal individual at the current time point. Estimating *b*, therefore, requires data on group-structured populations, where competition occurs within groups, and repeated observations on the phenotypes of the group members (*i.e.*, time-series data).

First, to estimate the overall average level of competition, one could fit single fixed *b* for all groups, using the model

$$y_{t,i} = P_{t+1,i} - P_{t,i} = \mu + b\left(P_{t,j} - P_{t,i}\right) + e_{t,i}.$$

In genetic analysis of outbred populations, interest is in the genetic (co)variances of growth and the direct and indirect effects on *b* (A_{GR}, A_D, and A_I in Equations 2 & 3). In animal and plant breeding, for example, knowledge of those parameters would indicate prospects for genetic selection against competition and variability. In outbred populations, the following mixed model may serve as starting-point to estimate genetic variance components (ignoring non-genetic terms for simplicity),

$$\mathbf{y}_{t,i} = \mu_t + \bar{b}\Delta\mathbf{y}_{t-1,ij} + \mathbf{Z}\mathbf{a}_{GR} + \mathbf{Z}_{D,\Delta y_{t-1,ij}}\mathbf{a}_D + \mathbf{Z}_{I,\Delta y_{t-1,ij}}\mathbf{a}_I + \mathbf{e}$$

where matrices and vectors are in bold and scalars are in italic. $\mathbf{y}$ is a vector of phenotypic observations, with elements $\mathbf{y}_{t,i} = P_{t,i} - P_{t-1,i}$, μ_t is an overall mean that may be specific to each time-point. The term $\bar{b}\Delta\mathbf{y}_{t-1,ij}$ accounts for the average competition in the population, and $\Delta\mathbf{y}_{t-1,\mathrm{ij}}$ is a vector of phenotypic differences between the group-mate and the focal individual at the previous time point, with elements $\Delta\mathbf{y}_{t-1,ij} = P_{t-1,j} - P_{t-1,i}$. The $\mathbf{Z}\mathbf{a}_{GR}$ are the ordinary (random) additive genetic effects on growth rate. The $\mathbf{Z}_{D,\Delta y_{t-1,ij}}\mathbf{a}_D$ accounts for the direct genetic effects in *b*, where $\mathbf{a}_D$ is a vector of random direct genetic effects on *b*, and $\mathbf{Z}_{D,\Delta y_{t-1,ij}}$ an incidence matrix for direct effects, with elements $P_{t-1,j} - P_{t-1,i}$ in the row and

column for focal individual *i*. The $\mathbf{Z}_{I,\Delta y_{t-1,ij}}\,\mathbf{a}_I$ accounts for the indirect genetic effects in *b*, where $\mathbf{a}_I$ is a vector of random indirect genetic effects on *b*, and $\mathbf{Z}_{I,\Delta y_{t-1,ij}}$ is an incidence matrix for indirect effects, with elements $P_{t-1,j} - P_{t-1,i}$ in the row for the focal individual *i* and column for its group mate *j*. Hence, direct and indirect effects on *b* are so-called random regressions. Note that the above expression merely serves as starting point, and will have to be extended with non-genetic random effects, such random group effects and permanent individual effects ($E_{p,GR,i}$ in Equation 2). Moreover, there may be issues with the identifiability of the genetic variance components, which will depend on the family relationships within and between groups (see e.g. Appendix of Bijma *et al.*, 2007).

When time series data are not available, which may often be the case, another approach could offer a solution. Quantitative genetic models for inherited variability can be used to estimate genetic variance in variability from records on within-family variance. Figure 3.4A shows that variability of sire offspring is correlated with the direct breeding value for *b* of the sire. Figure 3.4B shows that variability of the group mates of the offspring is correlated with the indirect breeding value for *b* of the sire. Therefore, it may be possible to capture direct and indirect effects on *b* by fitting linear mixed models to the within-family variance, and to the variance of the group mates of a family, with sire as random effect. This analysis requires an appropriate family and group structure, but not time series data. More research is needed to see how breeding values for inherited variability correlate with direct and indirect effects on *b*, and how those effects can be fully captured.

3.8.2 Evidence for genetic variation in b

To the best of our knowledge, there are no estimates of *b* available in the literature. However, some indications for variation in *b* may come from estimates of ψ (psi) in so-called trait-based models of IGE (Moore *et al*., 1997). When data are available on multiple discrete genotypes, such as inbred lines, fixed *b*-values could be estimated for each genotype, similar to the approach of Bleakley and Brodie IV (2009), who estimated ψ in guppies (see Equation 1).

This empirical study involved five inbred strains of guppies that differed genetically in their antipredator behavior. One individual from each (focal) strain was paired with three individuals from a different, unrelated strain i.e. social strain. In that way, each focal genotype was tested against different social environments. The results of the study show that the level of ψ differed between the focal strains and in some

cases also depended on the social strain, suggesting genetic variation in ψ. In a similar experimental design, where the focal genotype was held constant while social groups varied, the social group effects were estimated for chemical signaling in *D. melanogaster* (Kent *et al.*, 2008) and sexual display traits in *D. serrata* (Chenoweth *et al.*, 2010), and both studies estimated and found variation in ψ.

3.8.3 Implications for animal and plant breeding

Phenotypic uniformity is an important trait in animal breeding. In the pig industry, for example, it is desirable to deliver animals within a preferred range to the slaughter house, while deliveries outside that range result in penalties for the farmer (Hennessy, 2005; Mulder *et al.*, 2008). In aquaculture, fish that deviate too much from the average size are usually not sold, which reduces revenues. In addition, large size differences in fish populations stimulate competition, which reduces welfare and health of the animals. Better understanding of inherited variability, therefore, is interesting from an economic and animal welfare point of view. In plants, variability may also emerge as a commercially important trait, as some studies suggest that higher uniformity is related to higher productivity (Zhang *et al.*, 1999; Denison *et al.*, 2003).

There is substantial evidence of a genetic basis of variability, which has been obtained through selection experiments and by quantifying genetic variation in variability (reviewed by Hill and Mulder, 2010). Recently, methods have been developed to detect QTLs that control variability, so-called vQTLs (see Rönnegård and Valdar, 2011; Ronnegard and Valdar, 2012), and these have been found in studies of litter size in pigs (Sell-Kubiak *et al.*, 2015), several morphological traits and days to flowering in maize (Ordas *et al.*, 2008), and locomotor behavior in fruit flies (Ayroles *et al.*, 2015). Furthermore, several mechanisms resulting in vQTL effects have been proposed (Rönnegård and Valdar, 2011; Ronnegard and Valdar, 2012) including: epistatic gene interaction, gene-by-environmental interaction, multi-allelic additive effects underlying a QTL and scale of measurement for the observed phenotype. However, until now variability has been studied only in relation to direct genetic effects of the focal individual. Here, we considered an alternative mechanism that gives rise to genetic variation in variability, which does not only involve the genotype of the focal individual, but also a genetic effect of the social partner, and hence adds another layer to the complexity of inherited variability. The genetic contribution of the social partner is ignored in current QG models for inherited variability, which may reduce accuracy of estimated breeding values and response to selection. When traits are affected by social interactions, selection strategies that

accounts for both direct and indirect genetic effects can result in higher response (for example, Griffing, 1976; Muir, 1996; Bijma, Muir, Ellen, *et al.*, 2007). Our findings suggest that future breeding programs aiming to reduce variability may also need to consider increasing *b*.

3.8.4 Implications for evolutionary biology

In evolutionary biology, the study of canalization focuses on the absence or suppression of phenotypic variation. Hence, breeding for uniformity can be seen as an analogue of the evolution of canalization. Results of our model suggest that canalization may have a social genetic component. Evolution of canalization, therefore, could also be studied in the light of the regression coefficient *b*. A better understanding of the genetic mechanisms affecting variation may also increase our understanding of the potential for evolutionary change (Flatt, 2005). For example, traits may show less variability in some populations than in others, which is often attributed to low genetic variation. With canalization, however, phenotypic variation may be low while the underlying genetic variation is high, which can hinder phenotypic evolution (Flatt, 2005).

Mulder *et al.* (2016) showed that within-nest variability of fledging weight in a natural population of Great Tit (*Parus major*) has a genetic component and is under stabilizing selection. In that study, phenotypic variability was considered either a trait of the individual, or a trait of its parents, and it was discussed how this view would change the interpretation of the genetic parameters. Here we focused at connecting differences in phenotypic variability between individuals to the level of competition, which may be useful for future studies on variability in natural populations.

Kin selection theory predicts that individuals should interact differentially with kin *vs.* non-kin, because this increases their inclusive fitness (Hamilton, 1964b). Together with results of our model, this prediction suggests that related individuals should show less variability. In other words, groups consisting of relatives should have higher $\overline{\boldsymbol{b}}$ than groups of unrelated individuals. Hence, our findings suggest that canalization may partly evolve by kin-selection.

3.9 Conclusion

We presented a quantitative genetic model in which direct and indirect genetic effects lead to inherited variability of trait values on the phenotypic level. The *b* from our model can respond to selection, and changes in *b* resulted in changes in

variability, indicating the co-evolution of social interactions and inherited variability. Selection results showed that the effect of IGEs on *b* is ignored in classical mass selection, but can be partly captured by group selection on the mean or the variance. The latter also resulted in a decrease of variability. These findings suggest that we may have been overlooking an entire level of genetic variation in variability, the one due to IGEs. Genetic improvement of social effects, therefore, may be a promising route to reduce variability.

Acknowledgement

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Appendix A

In this appendix, explicit expressions for the expectation and the variance of the difference between the phenotypic values for individual *i* and *j* at time point *T* are derived, i.e. $E\left(P_{T,i} - P_{T,j} | b_{ij}, b_{ji}\right)$ and $V\left(P_{T,i} - P_{T,j} | b_{ij}, b_{ji}\right)$ respectively. By deriving these formula, given the parameters b_{ij} and b_{ji}, it is possible to study the effect of these parameter values on the expectation and variance of the phenotypic difference. The derived formulae show that the expected difference is 0 if there is no initial difference at *T*=0 (i.e. $\Delta P_0 = 0$), whereas the variance depends directly on the sum of b_{ij} and b_{ji}.

The model used throughout the paper for individuals *i* and *j* is

$$P_{t,i} = P_{t-1,i} + \mu_{GR} + A_{GR,i} + E_{p,GR,i} + E_{t,GR,i} + b_{i,j}\left(P_{t-1,j} - P_{t-1,i}\right)$$

$$P_{t,j} = P_{t-1,j} + \mu_{GR} + A_{GR,j} + E_{p,GR,j} + E_{t,GR,j} + b_{j,i}\left(P_{t-1,i} - P_{t-1,j}\right)$$

Let $\Delta P_t = P_{t,i} - P_{t,j}$, $\Delta A_{GR} = A_{GR,i} - A_{GR,j}$, $\Delta E_{p,GR} = E_{p,GR,i} - E_{p,GR,j}$ and $\Delta E_{t,GR} = E_{t,GR,i} - E_{t,GR,j}$. Then we have the recursive formula:

$$\Delta P_{t+1} = \left(1 - \left(b_{ij} + b_{ji}\right)\right)\Delta P_t + \Delta A_{GR} + \Delta E_{p,GR} + \Delta E_{t,GR}$$

which can be written in explicit form for time *T* (in our simulations *T*=10):

$$\Delta P_T = \lambda^T \Delta P_0 + \sum_{t=0}^{T-1} \lambda^t \left(\Delta A_{GR} + \Delta E_{p,GR}\right) + \sum_{t=0}^{T-1} \lambda^t \Delta E_{t,GR}$$

where $\lambda = 1 - \left(b_{ij} + b_{ji}\right)$.

Noting that the first sum is a geometric series multiplied by a constant the formula can be simplified:

$$\Delta P_T = \lambda^T \Delta P_0 + \frac{\lambda^T - 1}{\lambda - 1}\left(\Delta A_{GR} + \Delta E_{p,GR}\right) + \sum_{t=0}^{T-1} \lambda^t \Delta E_{t,GR}$$

Thus, the expected difference given the parameters b_{ij} and b_{ji} is:

$$\mathrm{E}(\Delta P_T | b_{ij}, b_{ji}) = \lambda^T \Delta P_0 + \frac{\lambda^T - 1}{\lambda - 1}\mathrm{E}\left(\Delta A_{GR} + \Delta E_{p,GR}\right) + \sum_{t=0}^{T-1} \lambda^t \mathrm{E}(\Delta E_{t,GR})$$
$$= \lambda^T \Delta P_0 + \frac{\lambda^T - 1}{\lambda - 1}\mathrm{E}\left(\Delta A_{GR} + \Delta E_{p,GR}\right) + \frac{\lambda^T - 1}{\lambda - 1}\mathrm{E}(\Delta E_{t,GR})$$

and is equal to 0 for $\Delta P_0 = 0$.

The variance of the difference in phenotypes given the parameters b_{ij} and b_{ji} is:

$$V\left(\Delta P_T \middle| b_{ij}, b_{ji}\right) = \left(\frac{\lambda^T - 1}{\lambda - 1}\right)^2 V\left(\Delta A_{GR} + \Delta E_{p,GR}\right) + \sum_{t=0}^{T-1} (\lambda^t)^2 V\left(\Delta E_{t,GR}\right)$$
$$= \left(\frac{\lambda^T - 1}{\lambda - 1}\right)^2 V\left(\Delta A_{GR} + \Delta E_{p,GR}\right) + \frac{\lambda^{2T} - 1}{\lambda - 1} V\left(\Delta E_{t,GR}\right)$$

For the special case $b_{ij} + b_{ji} = 0$, $V\left(\Delta P_T \middle| b_{ij}, b_{ji}\right) = T^2 \times V\left(\Delta A_{GR} + \Delta E_{p,GR}\right) + T \times V\left(\Delta E_{t,GR}\right)$.

Furthermore, $\frac{\lambda^T - 1}{\lambda - 1} < T$ and $\frac{\lambda^{2T} - 1}{\lambda - 1} < T$ for $\lambda < 1$, and for $\lambda > 1$ we have $\frac{\lambda^T - 1}{\lambda - 1} > T$ and $\frac{\lambda^{2T} - 1}{\lambda - 1} > T$. Recall that $\lambda = 1 - \left(b_{ij} + b_{ji}\right)$. Thus the variance of the phenotypic difference will be smaller than the variance for a model without social interaction

effects (i.e. $P_{t,i} = P_{t-1,i} + \mu_{GR} + A_{GR,i} + E_{p,GR,i} + E_{t,GR,i}$) if $b_{ij} + b_{ji} > 0$, and larger if $b_{ij} + b_{ji} < 0$.

Matrix version of the model

In the following part of the appendix, it is shown how the model can be written in matrix form and the variance of the individual phenotypes (given b_{ij} and b_{ji}) can be derived. Hence, an advantage of writing the model in matrix form is that we can derive an expression for the variance of the individual phenotypes, whereas in the previous derivations the variance of the phenotypic difference was derived. Furthermore, by studying the eigenvalues of the matrices in the model, the sensitivity to stochastic environmental effects can be assessed. The matrix notation can also be a tool to simplify computations in simulation studies.

An important result derived below is that the phenotypic values at the final time point *T* are sensitive to the simulated environmental variables (error terms) if $b_{ij} + b_{ji} < 0$.

Equation 2 can be written in matrix form for the two individuals *i* and *j* simultaneously as:

$$P_{t+1} = BP_t + \Delta + \varepsilon_t$$

$$P_t = \begin{pmatrix} P_{t,i} \\ P_{t,j} \end{pmatrix}$$

$$B = \begin{pmatrix} 1 - b_{ij} & b_{ij} \\ b_{ji} & 1 - b_{ji} \end{pmatrix}$$

$$\Delta = \begin{pmatrix} \mu_{GR} + A_{GR,i} + E_{P,GR,i} \\ \mu_{GR} + A_{GR,j} + E_{P,GR,j} \end{pmatrix}$$

$$\varepsilon_t = \begin{pmatrix} E_{t,GR,i} \\ E_{t,GR,j} \end{pmatrix}$$

with expectation and variance for Δ and ε_t

$$E(\Delta) = \begin{pmatrix} \mu_{GR} \\ \mu_{GR} \end{pmatrix}, V(\Delta) = (\sigma^2_{A_{GR}} + \sigma^2_{E_{p,GR}})I$$

$$E(\varepsilon_t) = \begin{pmatrix} 0 \\ 0 \end{pmatrix}, V(\varepsilon_t) = \sigma_\varepsilon^2 I$$

In the formula for $V(\Delta)$, the two individuals are assumed to be unrelated.

At time *T* (with *T*=10 in our simulations) we then have

$$P_T = B^T P_0 + \sum_{k=0}^{T-1} B^k \Delta + \sum_{k=0}^{T-1} B^k \, \varepsilon_k$$

In the simulations, the initial phenotypes were set to zero, i.e. $P_0 = 0$. Hence, the first term can be ignored and we can focus on the second and third terms, i.e. the sums, in the above formula.

Let $B = \Gamma \Lambda \Gamma^{-1}$ be the spectral decomposition of B, with eigenvalues λ_1 =1 and $\lambda_2 = 1 - (b_{ij} + b_{ji})$. One can note that the phenotypes at time *T*, i.e. P_T, will be sensitive to the simulated environmental variables (error terms) if $b_{ij} + b_{ji} < 0$ because the dominating eigenvalue will then be greater than 1.

Furthermore, the first sum in the above formula is a geometric series and can be written as:

$$\sum_{k=0}^{T-1} B^k \Delta = \Gamma \begin{pmatrix} T & 0 \\ 0 & \dfrac{\lambda_2^T - 1}{\lambda_2 - 1} \end{pmatrix} \Gamma^{-1} \Delta$$

with expectation

$$E\left(\sum_{k=0}^{T-1} B^k \Delta \right) = \Gamma \begin{pmatrix} T & 0 \\ 0 & \dfrac{\lambda_2^T - 1}{\lambda_2 - 1} \end{pmatrix} \Gamma^{-1} E(\Delta)$$

The variance of the sum is

$$V\left(\sum_{k=0}^{T-1} B^k \Delta \right) = \Gamma \begin{pmatrix} T & 0 \\ 0 & \dfrac{\lambda_2^T - 1}{\lambda_2 - 1} \end{pmatrix} \Gamma^{-1} (\Gamma^{-1})' \begin{pmatrix} T & 0 \\ 0 & \dfrac{\lambda_2^T - 1}{\lambda_2 - 1} \end{pmatrix} \Gamma' V(\Delta)$$

The variance of the second sum is

$$V\left(\sum_{k=0}^{T-1} B^k\,\varepsilon_k\right) = \sum_{k=0}^{T-1} B^k\,(B^k)'V(\varepsilon_k)$$

With

$$V(\varepsilon_k) = I\sigma_\varepsilon^2$$

and

$$\sum_{k=0}^{T-1} B^k\,(B^k)' = \Gamma\begin{pmatrix} A_{11}T & A_{12}\dfrac{\lambda_2^T-1}{\lambda_2-1} \\ A_{21}\dfrac{\lambda_2^T-1}{\lambda_2-1} & A_{22}\dfrac{\lambda_2^{2T}-1}{\lambda_2^2-1}\end{pmatrix}\Gamma'$$

Where A_{kl} is the element on row *k* and column *l* in the matrix $A = \Gamma^{-1}(\Gamma^{-1})'$
Thus

$$V\left(\sum_{k=0}^{T-1} B^k\,\varepsilon_k\right) = \Gamma\begin{pmatrix} A_{11}T & A_{12}\dfrac{\lambda_2^T-1}{\lambda_2-1} \\ A_{21}\dfrac{\lambda_2^T-1}{\lambda_2-1} & A_{22}\dfrac{\lambda_2^{2T}-1}{\lambda_2^2-1}\end{pmatrix}\Gamma'\sigma_\varepsilon^2$$

with $A_{11} = A_{22} = \frac{2(b_{ij}^2+b_{ji}^2)}{(b_{ij}+b_{ji})^2}$ and $A_{12} = A_{21} = \frac{b_{ji}-b_{ij}}{(b_{ij}+b_{ji})^2}\sqrt{2(b_{ij}^2+b_{ji}^2)}$.

Using the matrix of eigenvectors

$$\Gamma = \begin{pmatrix} \sqrt{\dfrac{1}{2}} & \dfrac{b_{ij}}{\sqrt{b_{ij}^2+b_{ji}^2}} \\ \sqrt{\dfrac{1}{2}} & \dfrac{-b_{ji}}{\sqrt{b_{ij}^2+b_{ji}^2}}\end{pmatrix}$$

and its inverse

$$\Gamma^{-1} = \begin{pmatrix} \frac{b_{ji}}{\sqrt{b_{ij}^2 + b_{ji}^2}} & \frac{b_{ij}}{\sqrt{b_{ij}^2 + b_{ji}^2}} \\ \sqrt{\frac{1}{2}} & -\sqrt{\frac{1}{2}} \end{pmatrix} \times \frac{\sqrt{2(b_{ij}^2 + b_{ji}^2)}}{b_{ij} + b_{ji}}$$

Using the relationship $V(P_{T,i} - P_{T,j}) = (1 \quad -1)V(P_T)\begin{pmatrix} 1 \\ -1 \end{pmatrix}$ and applying several steps of straightforward derivations, the same expression for the variance of the phenotypic difference is derived as above

$$V(P_{T,i} - P_{T,j}|b_{ij}, b_{ji}) = 2D_1\left(\sigma_{A_{GR}}^2 + \sigma_{E_{p,GR}}^2\right) + 2D_2\sigma_\varepsilon^2$$

with $D_1 = \left(\frac{\lambda_2^T - 1}{\lambda_2 - 1}\right)^2$ and $D_2 = \frac{\lambda_2^{2T} - 1}{\lambda_2^2 - 1}$ for $b_{ij} + b_{ji} \neq 0$, whilst for $b_{ij} + b_{ji} = 0$ we have $D_1 = T^2$ and $D_2 = T$. Hence, we have derived the variance for the difference between phenotypes of two group members, i.e., $V(P_{T,i} - P_{T,j}|b_{ij}, b_{ji})$.

Furthermore, the above derivations give an explicit expression for the variances (and covariances) of the individual phenotypes for the two group members, i.e., $V\left(\begin{pmatrix} P_{T,i} \\ P_{T,j} \end{pmatrix}|b_{ij}, b_{ji}\right)$. Using this expression one can assess how the variances (and covariances) at time *T* depend on the values of b_{ij} and b_{ji} .

Appendix B

Larger group size

We presented a model for interaction between 2 individuals. To accommodate interactions among more individuals, Equation 2 could be extended as follows

$$P_{t,i} - P_{t-1,i} = \mu_{GR} + A_{GR,i} + E_{p,GR,i} + E_{t,GR,i} + \sum_{j=1}^{n} b_{ij}\left(P_{t-1,j} - P_{t-1,i}\right) \qquad (5)$$

so that the effect of a difference in trait value between a group mate *j* and focal individual *i* is summed over the *n* group mates.

Appendix C

Simulation description

Population structure

Monte Carlo simulations were conducted using the R software (R Development Core Team, 2011). We first simulated a base population of 100 sires and 10,000 dams, all unrelated. Each animal was assigned a breeding value for growth rate and direct and indirect breeding value for *b*, drawn from a multivariate normal distribution. Next, we created the offspring population by mating each sire with 100 randomly chosen dams. Each sire had 100 offspring. The total number of individuals in the offspring population was 10,000. The breeding values for growth rate and direct and indirect breeding values for *b* in the offspring population were simulated as the average breeding value of sire and dam, plus a Mendelian sampling term drawn from $N\left(\begin{bmatrix}0\\0\\0\end{bmatrix}, \frac{1}{2}\begin{bmatrix}\sigma^2_{A_{GR}} & 0 & 0\\ 0 & \sigma^2_{A_D} & 0\\ 0 & 0 & \sigma^2_{A_I}\end{bmatrix}\right)$. Each offspring was also given the permanent and temporary environmental effect on body weight, as well as direct and indirect environmental effects on *b*. These were sampled from $N, \left(\begin{bmatrix}0\\0\\0\\0\end{bmatrix}, \begin{bmatrix}\sigma^2_{E_{p,GR}} & 0 & 0 & 0\\ 0 & \sigma^2_{E_{t,GR}} & 0 & 0\\ 0 & 0 & \sigma^2_{E_D} & 0\\ 0 & 0 & 0 & \sigma^2_{E_I}\end{bmatrix}\right)$. All genetic and environmental covariances were set to zero. Individuals from offspring population were randomly assigned to groups of 2 members, creating 5000 groups in total. Finally, growth curves of individuals were simulated by creating phenotypes for 10 time points using Equation 2. Therefore, each individual had repeated observations.

For the selection part, we used a simulated population of individuals (offspring population) as explained in previous paragraph to be the base population. Three types of selections were performed, individual and 2 group selections, using phenotypes from the last time point i.e. time point 10. Individual selection was made on body weight, by selecting 11% of best individuals. First group selection was made on average body weight of the pair making up a group, while second group selection was performed on the squared difference in body weight within a pair, i.e. the variance among the two group mates. In both group selections 11% of best groups were selected. Selections were performed for 10 generations. To maintain the same

number of individuals through selection (10 000), sex ratio and mating was performed differently in the selection generations compared to the base. Sex was randomly assigned to 1100 selected individuals in 1 male : 10 females probability, and 1 male was mated with 10 randomly chosen females. The genetic and environmental values of offspring, group assignment and phenotype construction was done in the same manner as described in the previous paragraph.

Parameters

Table 3.2 contains parameters used in the simulation. In farmed aquaculture species, for example Nile tilapia, fish weights around 10 grams (g) when it is first stocked into the pond, and between 100-200 grams at the end of the growth period. To connect our results somewhat to aquaculture species, we have set 10 g as a mean starting weight and assumed that in every time period individuals gain on average 10 g. Therefore, mean growth rate, (μ_{GR}) was 10 g. The genetic standard deviation of growth rate ($\sigma_{\mathrm{A_{GR}}}$) was 10% of μ_{GR}, which was 1 g, therefore $\sigma^2_{\mathrm{A_{GR}}}$ was 1 g^2.

The range of $\bar{b}$ values was from -0.08 to 0.08. Standard deviation of $\bar{b}$ was set as 60% of $\bar{b}$= -0.05. Therefore, standard deviations of genetic and environmental components of *b* were calculated as $\sqrt{\sigma^2_{A_D} + \sigma^2_{A_I} + \sigma^2_{E_D} + \sigma^2_{E_I}} = 0.6\bar{b}$, and since all variances were assumed equal, each of them had value of 0.225 x 10^{-3} (Table 3.2). Repeatability was set to 0.7 and heritability of growth rate to 0.5, in absence of social interactions (*b*=0). Phenotypic variance was calculated as $\sigma^2_P = \sigma^2_{\mathrm{A_{GR}}}/h^2$ and was equal to 2 g^2, permanent environmental effect on growth ($\sigma^2_{\mathrm{E_{p,GR}}}$) as $0.2\sigma^2_P$ = 0.4 g^2 and temporary environmental effect ($\sigma^2_{\mathrm{E_{t,GR}}}$) as $0.3\sigma^2_P$ = 0.6 g^2 (Table 3.2).

Five simulated scenarios were based on 5 different values of $\bar{\boldsymbol{b}}$ (Table 3.2). For the selection part, only a base population was used with $\bar{\boldsymbol{b}}$ of -0.08 to test the effect of selection on variability.

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Supplementary material

Table S3.1 Effect of *b x difference in body weight* on change in body weight of the focal individual when $\bar{b}$ = 0 (no competition or cooperation)

$P_{t-1,j} - P_{t-1,i} = 2$ g	$\Delta P_{t,i}$		$P_{t-1,i} - P_{t-1,j} = -2$ g	$\Delta P_{t,j}$	
Focal individual *i*	**Social partner *j***		**Focal individual *j***	**Social partner *i***	
	$A_{I,j}$ = 0.03	$A_{I,j}$ = - 0.03		$A_{I,i}$ = 0.03	$A_{I,i}$ = - 0.03
$A_{D,i}$ = 0.03	0.12 g	0 g	$A_{D,j}$ = 0.03	- 0.12 g	0 g
$A_{D,i}$ = - 0.03	0 g	- 0.12 g	$A_{D,j}$ = - 0.03	0 g	0.12 g

Table S3.2 Effect of *b x difference in body weight* on change in body weight of the focal individual when $\bar{b}$ = - 0.05 (competition)

$P_{t-1,j} - P_{t-1,i} = 2$ g	$\Delta P_{t,i}$		$P_{t-1,i} - P_{t-1,j} = -2$ g	$\Delta P_{t,j}$	
Focal individual *i*	**Social partner *j***		**Focal individual *j***	**Social partner *i***	
	$A_{I,j}$ = 0.03	$A_{I,j}$ = - 0.03		$A_{I,i}$ = 0.03	$A_{I,i}$ = - 0.03
$A_{D,i}$ = 0.03	0.02 g	-0.1 g	$A_{D,j}$ = 0.03	-0.02 g	0.1 g
$A_{D,i}$ = - 0.03	-0.1 g	-0.22 g	$A_{D,j}$ = - 0.03	0.1 g	0.22 g

Table S3.3 Effect of *b x difference in body weight* on change in body weight of the focal individual when $\bar{b}$ = 0.05 (cooperation)

$P_{t-1,j} - P_{t-1,i} = 2$ g	$\Delta P_{t,i}$		$P_{t-1,i} - P_{t-1,j} = -2$ g	$\Delta P_{t,j}$	
Focal individual *i*	**Social partner *j***		**Focal individual *j***	**Social partner *i***	
	$A_{I,j}$ = 0.03	$A_{I,j}$ = - 0.03		$A_{I,i}$ = 0.03	$A_{I,i}$ = - 0.03
$A_{D,i}$ = 0.03	0.22 g	0.1 g	$A_{D,j}$ = 0.03 g	-0.22 g	-0.01 g
$A_{D,i}$ = - 0.03	0.1 g	-0.02 g	$A_{D,j}$ = - 0.03	-0.01 g	0.02 g

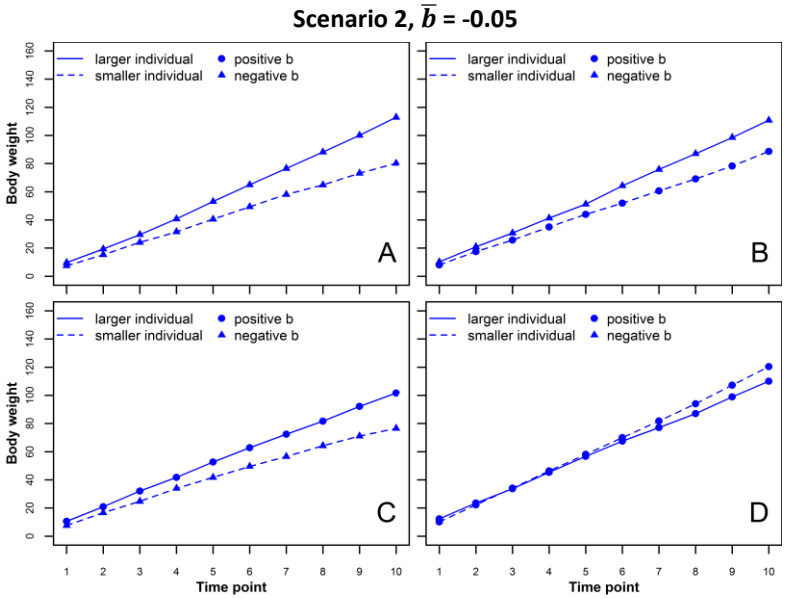

Figure S3.1 Growth curves of two group mates (one larger than the other) that have lowest sum of *b*'s (A); the initially larger individual has negative *b*, the smaller one has positive *b* (B); the initially larger individual has positive *b*, the smaller one has negative *b* (C); lowest sum of *b*'s (D), for scenario 2. Each panel shows one typical replicate.

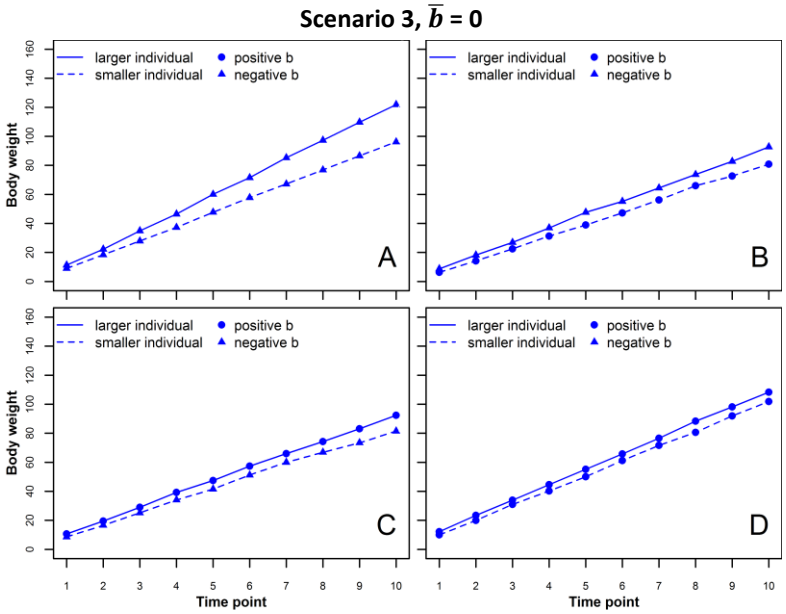

Figure S3.2 Growth curves of two group mates (one larger than the other) that have lowest sum of *b*'s (A); the initially larger individual has negative *b*, the smaller one has positive *b* (B); the initially larger individual has positive *b*, the smaller one has negative *b* (C); lowest sum of *b*'s (D), for scenario 3. Each panel shows one typical replicate.

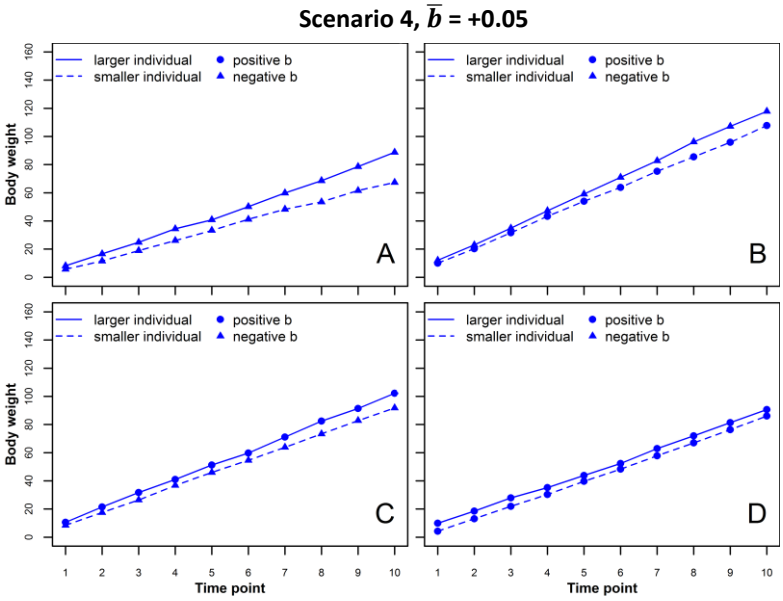

Figure S3.3 Growth curves of two group mates (one larger than the other) that have lowest sum of b's (A); the initially larger individual has negative b, the smaller one has positive b (B); the initially larger individual has positive b, the smaller one has negative b (C); lowest sum of b's (D), for scenario 4. Each panel shows one typical replicate.

Table S3.4 Maximum and minimum sum of *b*'s for two individuals in a group, averaged over 100 replicates

Scenario	1	2	3	4	5
Min	-0.32 (0.01)	-0.26 (0.01)	-0.16 (0.01)	-0.06 (0.01)	0.003 (0.01)
Max	-0.01 (0.01)	0.05 (0.01)	0.15 (0.01)	0.25 (0.01)	0.31 (0.01)

Table S3.5 Values of *b*, sum of *b*'s and squared difference of two individuals in a group for five scenarios

Panel	*b*		Sum of *b*'s	Squared difference
	Larger individual	Smaller individual		
Scenario 1 (Figure 3.3)				
A	-0.16	-0.14	-0.31	1983.7
B	-0.10	0.005	-0.09	908.6
C	0.007	-0.07	-0.06	1377.5
D	0.01	0.004	0.02	243.4
Scenario 2 (Figure S3.2)				
A	-0.13	-0.11	-0.25	1026.7
B	-0.08	0.05	-0.03	272.4
C	0.04	-0.11	-0.07	646.1
D	0.03	0.009	0.04	131.8
Scenario 3 (Figure S3.3)				
A	-0.09	-0.06	-0.15	328
B	-0.09	0.01	-0.08	194.4
C	0.07	-0.03	0.05	64
D	0.05	0.01	0.15	8.7
Scenario 4 (Figure S3.4)				
A	-0.04	-0.01	-0.05	117.3
B	-0.04	0.06	0.02	81.8
C	0.14	-0.01	0.13	81.6
D	0.10	0.15	0.25	2.74
Figure 5 (Figure 3.3)				
A	-0.002	-0.004	-0.006	232.03
B	-0.02	0.1	0.08	25.7
C	0.08	-0.001	0.08	33.3
D	0.13	0.18	0.3	1.4

Table S3.6 Correlations with standard errors

Scenario	$A_D - VarP_{off}$	$A_D - VarP_{gm}$	$A_I - VarP_{off}$	$A_D - VarP_{gm}$
1	-0.54 (0.08)	-0.11 (0.09)	-0.10 (0.1)	-0.52 (0.06)
2	-0.52 (0.08)	-0.08 (0.09)	-0.07 (0.1)	-0.50 (0.07)
3	-0.43 (0.08)	-0.02 (0.09)	0.00 (0.1)	-0.42 (0.09)
4	-0.28 (0.09)	0.02 (0.09)	0.04 (0.1)	-0.30 (0.08)
5	-0.21 (0.09)	0.04 (0.09)	0.04 (0.1)	-0.23 (0.09)

Table S3.7 Individual selection on body weight

Generation	Variability	Average A_D	Average A_I	Average A_{GR}	Average b	Average body weight
base	376.89 (29.15)	-0.0001 (0.0003)	-0.00002 (0.0003)	0.01 (0.002)	-0.08 (0.0003)	100.1 (0.38)
1	362.70 (18.82)	-0.0031 (0.001)	-0.0008 (0.001)	1.25 (0.08)	-0.08 (0.002)	112.48 (0.81)
2	368.87 (23.54)	-0.0059 (0.001)	-0.001 (0.002)	2.35 (0.10)	-0.08 (0.002)	123.53 (1.03)
3	386.93 (28.01)	-0.0087 (0.002)	-0.001 (0.002)	3.41 (0.12)	-0.09 (0.003)	134.09 (1.16)
4	406.13 (32.79)	-0.0115 (0.002)	-0.0011 (0.002)	4.44 (0.13)	-0.09 (0.004)	144.42 (1.34)
5	432.55 (40.38)	-0.0141 (0.002)	-0.0012 (0.003)	5.48 (0.15)	-0.10 (0.004)	154.75 (1.49)
6	455.99 (42.47)	-0.0169 (0.003)	-0.0013 (0.003)	6.50 (0.17)	-0.10 (0.004)	165.04 (1.67)
7	483.93 (49.42)	-0.0196 (0.003)	-0.0014 (0.003)	7.53 (0.17)	-0.10 (0.005)	175.26 (1.78)
8	512.26 (55.38)	-0.0224 (0.003)	-0.0014 (0.004)	8.54 (0.19)	-0.10 (0.005)	185.40 (1.94)
9	546.22 (62.66)	-0.0252 (0.003)	-0.0015 (0.004)	9.56 (0.21)	-0.11 (0.005)	195.59 (2.07)
10	580.14 (67.69)	-0.0281 (0.003)	-0.0015 (0.004)	10.58 (0.21)	-0.11 (0.005)	205.79 (2.12)

Table S3.8 Group selection on average body weight

Generation	Variability	Average A_D	Average A_I	Average A_{GR}	Average *b*	Average body weight
base	376.89 (29.15)	-0.0001 (0.0003)	-0.00002 (0.0003)	0.01 (0.002)	-0.08 (0.0003)	100.10 (0.38)
1	363.49 (16.49)	-0.0002 (0.001)	-0.0002 (0.001)	0.95 (0.07)	-0.08 (0.001)	109.59 (0.67)
2	354.95 (21.38)	-0.0010 (0.002)	0.0003 (0.001)	1.81 (0.09)	-0.08 (0.002)	118.21 (0.89)
3	361.34 (25.35)	-0.0017 (0.002)	0.0004 (0.002)	2.67 (0.09)	-0.08 (0.003)	126.73 (0.98)
4	370.77 (29.09)	-0.0029 (0.002)	0.0006 (0.002)	3.51 (0.11)	-0.08 (0.003)	135.19 (1.01)
5	375.98 (29.89)	-0.0039 (0.002)	0.0006 (0.0020	4.37 (0.13)	-0.08 (0.003)	143.80 (1.33)
6	385.48 (34.45)	-0.0050 (0.003)	0.0008 (0.003)	5.23 (0.14)	-0.08 (0.003)	152.35 (1.42)
7	394.27 (39.34)	-0.0062 (0.003)	0.0007 (0.003)	6.08 (0.15)	-0.09 (0.004)	160.89 (1.40)
8	404.00 (43.31)	-0.0074 (0.003)	0.0006 (0.003)	6.93 (0.16)	-0.09 (0.004)	169.42 (1.63)
9	415.67 (46.07)	-0.0086 (0.003)	0.0007 (0.003)	7.79 (0.18)	-0.09 (0.004)	178.02 (1.78)
10	429.29 (50.59)	-0.0097 (0.004)	0.0006 (0.003)	8.64 (0.19)	-0.09 (0.005)	186.46 (1.88)

Table S3.9 Group selection on variance of body weight

Generation	Variability	Average A_D	Average A_I	Average A_{GR}	Average *b*	Average body weight
base	376.89 (29.15)	-0.0001 (0.0003)	-0.00002 (0.0003)	0.01 (0.002)	-0.08 (0.0003)	100.1 (0.38)
1	321.72 (15.96)	0.001 (0.001)	0.0010 (0.001)	-0.007 (0.07)	-0.08 (0.001)	99.9 (0.70)
2	301.54 (15.27)	0.002 (0.001)	0.0010 (0.001)	0.001 (0.08)	-0.08 (0.002)	100.0 (0.84)
3	291.06 (14.36)	0.003 (0.002)	0.0011 (0.001)	0.004 (0.09)	-0.08 (0.002)	100.1 (0.95)
4	284.19 (15.29)	0.004 (0.002)	0.0011 (0.002)	0.008 (0.09)	-0.07 (0.002)	100.1 (0.90)
5	277.10 (15.02)	0.005 (0.002)	0.0011 (0.002)	0.007 (0.11)	-0.07 (0.003)	100.0 (1.06)
6	272.18 (17.24)	0.006 (0.002)	0.0011 (0.002)	0.004 (0.11)	-0.07 (0.002)	100.1 (1.13)
7	268.64 (17.91)	0.008 (0.002)	0.0011 (0.002)	0.006 (0.12)	-0.07 (0.003)	100.0 (1.19)
8	262.72 (18.48)	0.009 (0.002)	0.0011 (0.003)	0.002 (0.12)	-0.07 (0.003)	100.1 (1.23)
9	255.94 (18.99)	0.010 (0.002)	0.0010 (0.003)	0.009 (0.13)	-0.06 (0.003)	100.1 (1.34)
10	250.18 (21.47)	0.011 (0.003)	0.0011 (0.003)	0.008 (0.13)	-0.06 (0.003)	100.1 (1.36)

4

Capturing indirect genetic effects on phenotypic variability: Competition meets canalization

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Abstract

Phenotypic variability of a genotype is relevant in natural and domestic populations. In the past two decades, variability has been studied as a genetic trait in its own right. This trait is often referred to as inherited variability, heritable variation in environmental (residual) variance, or environmental canalization. So far, studies on inherited variability have only considered genetic effects of the focal individual, i.e., direct genetic effects on inherited variability. Observations from aquaculture populations and some plants, however, suggest that an additional source of genetic variation in inherited variability may be generated through competition. Social interactions, such as competition, are often a source of Indirect Genetic Effects (IGE). An IGE is a heritable effect of an individual on the trait value of another individual. Studies have shown that IGEs may substantially affect heritable variation underlying the trait and the direction and magnitude of response to selection. To understand the contribution of IGEs to evolution of environmental canalization in natural populations, and to exploit such inherited variability in animal and plant breeding, we need statistical models to capture this effect. However, to our knowledge it is unknown to what extent the current statistical models that are commonly used for IGE and inherited variability capture the effect of competition on inherited variability. Here we investigate the potential of current statistical models for inherited variability and trait values, to capture the direct and indirect genetic effects of competition on variability. Our results show that a direct model of inherited variability almost entirely captures the direct genetic effect of competition on variability, as illustrated by high correlations between estimated genetic effects and simulated direct breeding values. Similarly, an indirect model of inherited variability captures indirect genetic effects of competition. Models for trait levels, however, capture only a small part of the genetic effects of competition. Capturing genetic effects of competition, therefore could be possible with direct and indirect models of inherited variability but may require a two-step analysis.

Key words: inherited variability, canalization, competition, indirect genetic effects, IGE, statistical models

4.1 Introduction

Some genotypes may produce less variable phenotypes compared to others in response to perturbations in both genome and environment. The genetic mechanism that leads to insensitivity of a phenotype to genetic and non-genetic perturbations is known as "canalization" (Waddington, 1942). Evolution of canalization is often associated with stabilizing natural selection for an optimal phenotype, as such selection favors mechanisms that reduce variance around the optimum (Waddington, 1942; Wagner *et al.*, 1997; Flatt, 2005; Edgell *et al.*, 2009). Long-term stabilizing selection of a trait is therefore expected to reduce phenotypic variation.

Depending on the source of perturbation, canalization can be either genetic or environmental. In the following we refer only to environmental canalization. Environmental canalization is commonly inferred from size of the environmental variance (V_E) of a genotype. In other words, genotypes that produce more stable phenotypes have lower V_E, and a decrease of V_E due to selection indicates canalization (Gibson and Bradley, 1974; Wagner *et al.*, 1997; Flatt, 2005).

Phenotypic variability of a genotype is relevant not only in natural populations, but also in agriculture. In animal and crop production, uniformity of traits is often of economic importance. In the pig industry, for example, excessive variability in size and weight of animals is penalized by slaughterhouses, so that delivering animals within a preferred range has an economic benefit (Hennessy, 2005; Mulder et al., 2008). In aquaculture, fish that deviate too much from the average size are usually not sold, which reduces revenues (Khaw *et al.*, 2016; Marjanovic *et al.*, 2016). Low V_E in crops is desirable, as it indicates stability against unpredictable conditions (Edwards and Jannink, 2006). Selection for trait uniformity in animal and plant breeding is an analogy of evolution of canalization in natural populations.

The phenotypic variability of a genotype, measured either repeatedly on the same individual, or on multiple individuals belonging to the same family, has been studied as a quantitative trait in its own right. This concept was first introduced by Waddington (Waddington, 1942), and has been an integrative part of quantitative genetics ever since, with the growing interest in the topic over the last two decades, largely due to the development of methods to estimate genetic variance in variability (SanCristobal-Gaudy *et al.*, 1998; Sörensen and Waagepetersen, 2003; Mulder *et al.*, 2009; Rönnegård *et al.*, 2010). Inheritance of the phenotypic variability of a genotype

is often referred to as “inherited variability” or “heritable variation in environmental variance” (SanCristobal-Gaudy *et al.*, 1998; Mulder *et al.*, 2007; Hill and Mulder, 2010). There is strong evidence of genetic variation in V_E. The study by Mackay and Layman (2005), who compared bristle number of different isofemale lines of *Drosophila*, is one of the best evidences that genotypes differ in V_E, i.e., that environmental canalization has a genetic component. A number of studies in plant and animal populations also showed that variability often has a substantial genetic component (reviewed by Hill and Mulder, 2010).

Research on inherited variability has focused primarily on quantifying genetic variation in V_E, and some selection experiments have been performed to investigate how variability responds to selection (Hill and Mulder, 2010). With the availability of genomic data, scientists started to look for QTLs that control variability of trait values, known as vQTL (Ordas *et al.*, 2008; Mulder *et al.*, 2013; Sell-Kubiak *et al.*, 2015; Ayroles *et al.*, 2015). All studies so far have only considered genetic effects of the focal individual, i.e., direct genetic effects on inherited variability. Observations from aquaculture populations, and some plants, however, suggest that an additional source of genetic variation in inherited variability may be generated through competition. Hence, we may currently be overlooking a component of inherited variability.

In aquaculture populations, competition for feed not only decreases productivity and survival of the animals, but also inflates variation in trait values among individuals (Jobling, 1995; Cutts *et al.*, 1998; Hart and Salvanes, 2000). Phenotypic studies show that populations displaying less competition tend to grow more uniform and have higher average performance (Jobling, 1995; Cutts *et al.*, 1998; Hart and Salvanes, 2000). Plants also express competitive behaviors, for example by increasing their leaf area, height, and branching of stem and roots (Zhang *et al.*, 1999; Denison *et al.*, 2003; File *et al.*, 2012). In plant breeding, kin-group selection for higher plot performance in crops has resulted in less competitive phenotypes of individual plants and a more uniform appearance of crop fields (Donald, 1968; Austin *et al.*, 1980; Denison *et al.*, 2003). These results clearly suggest a close relationship between competition and V_E.

In quantitative genetics, the effects of social interactions, such as competition, on trait values of individuals is usually modelled within the framework of Indirect Genetic Effects (IGE). An IGE is a heritable effect of an individual on the trait value of another individual (Griffing, 1967; Moore *et al.*, 1997). Several studies have shown

that IGEs may substantially affect heritable variation underlying the trait and the direction and magnitude of response to selection (Hamilton, 1964a, 1964b; Griffing, 1976; Ellen *et al.*, 2007; Bijma *et al.*, 2007; Bijma, 2011). Because of IGEs, heritable variance of a trait may exceed the phenotypic variance, while in other cases IGEs can completely remove heritable variance (Bijma, 2011; Costa e Silva *et al.*, 2017). Therefore, it has become widely accepted that inclusion of IGEs in trait models is important for understanding the potential of trait levels to respond to selection.

Until recently we lacked the tools to investigate whether IGEs also contribute to genetic variation in variability. IGE-models come in two types; variance-component models and trait-based models (Griffing, 1967; Moore *et al.*, 1997; reviewed by McGlothlin and Brodie, 2009, and Bijma, 2014). Variance component models cannot explain the observed relationship between competition and variability, because phenotypic variance is independent of the average level of the IGE. Trait-based models lead to a relationship between competition and variability, but on the population level this relationship is identical for competition and cooperation, which does not reflect the pattern observed in real populations. On the other hand, current models of inherited variability treat variability as a property of a single individual, ignoring the component due to competition.

We recently proposed a quantitative genetic model that allows for a relationship between IGEs and inherited variability (Marjanovic *et al.,* in press). In this model, competition between social partners leads to divergence of their phenotypes (*e.g.,* body weight) over their life time. Hence, the model allows for indirect genetic effects to lead to differences in variability of trait values, on both group and population level, similar to observations in real populations.

To understand the contribution of IGEs to evolution of environmental canalization in natural populations, and to exploit such inherited variability in animal and plant breeding, we need statistical models to capture this effect. The model of Marjanovic *et al.* (submitted) can be used to estimate effects of competition, but it requires time-series data, which are often not available. The use of existing statistical models for IGE and inherited variability applied to a final phenotype would be an easier approach. However, to our knowledge it is unknown to what extent such models capture the effect of competition on inherited variability.

Here we investigate the potential of existing statistical models for inherited variability and trait values, to capture the direct and indirect genetic effects of

competition on variability. To address this issue, we conducted a simulation study in which competition between social partners (i.e., IGEs) leads to inherited variability of trait values, using the model of Marjanovic *et al.* (in press). Subsequently, we analyzed these data with four models. The ability of those models to capture direct and indirect genetic effects on variability was tested by comparing estimated genetic effects from each of the models with simulated direct breeding values for trait level, and with direct and indirect breeding values for competition.

4.2 Materials and Methods

4.2.1 Quantitative genetic model

In this section, we summarize the quantitative genetic model of Marjanovic *et al.* (*in press*) that integrates IGEs and inherited variability. The parameters were chosen to represent growth of fish as an example.

In this model, we consider groups of two individuals. Each individual is both a focal individual in the model for its own phenotype, and a social partner in the model for the phenotype of its group mate. Our target phenotype is individual growth rate, modelled over time. In aquaculture, growth of individuals is affected by the difference in body weight between interacting individuals, with higher body weight giving a competitive advantage to an individual in terms of growth (Doyle and Talbot, 1986). Therefore, the phenotypic value for growth rate of the focal individual is affected by the ordinary direct genetic and environmental effects of the focal individual itself, and by the difference in body weight between the focal individual and its social partner. The degree to which the difference in body weight affects the phenotype of an individual is measured by a regression coefficient *b*,

$$P_{t,i} - P_{t-1,i} = \mu_{GR} + A_{GR,i} + E_{p,GR,i} + E_{t,GR,i} + b_{ij}\left(P_{t-1,j} - P_{t-1,i}\right) \quad (1)$$

where $P_{t,i}$ is the body weight of focal individual *i* at time point *t*, $P_{t-1,i}$ is body weight of *i* at the previous time point, μ_{GR} is the mean growth rate of the population, $A_{GR,i}$ is a (direct) breeding value for growth rate of individual *i*, $E_{p,GR,i}$ and $E_{t,GR,i}$ are permanent and temporary non-heritable ("environmental") effects of individual *i*, and b_{ij} is a regression coefficient.

The absolute value of b_{ij} describes the strength of the social interaction. The sign of *b* is a measure of cooperation, where a negative *b* indicates competition, while a positive *b* indicates cooperation.

In this model, *b* is not a fixed parameter, but a composite genetic trait that can evolve over generations. The *b* exhibits genetic variation due to a direct genetic effect of the focal individual ($A_{D,i}$), representing genetic resistance to competition, and an indirect genetic effect of its social partner, representing the genetic cooperative effect ($A_{I,j}$). Hence, the model allows for variation among individuals in sensitivity to competition, so that some individuals may suffer less than others from the presence of a large social partner. Similarly, the model allows for variation among individuals in competitive effect. Some individuals may be large at the expense of their group mate, whereas other large individuals may not suppress growth of their social partner. Thus, for focal individual *i* with social partner *j*, the regression coefficient b_{ij} is given by

$$b_{ij} = \bar{b} + A_{D,i} + E_{D,i} + A_{I,j} + E_{I,j} \quad (2)$$

where $\bar{b}$ represents the average regression coefficient, which is a population parameter that is negative under competition and positive under cooperation. The $A_{D,i}$ and $E_{D,i}$ are the direct genetic and the environmental effect of individual *i* on b_{ij}, while $A_{I,j}$ and $E_{I,j}$ are the indirect genetic and environmental effect of individual *j* on b_{ij}. Negative values of A_D indicate that individual is sensitive to competition (as compared to an average individual), while an individual with positive A_D is resistant to competition. Similarly, an individual with negative A_I is competitive, while an individual with positive A_I is cooperative. Note that *b* is non-symmetric, i.e., $b_{ij} \neq b_{ji}$, as individuals may differ in their breeding values for *b*. In other words, an individual that is strongly affected by its social partner, does not necessarily also have a strong effect on its social partner.

Therefore, in the total model (Equations 1 & 2) there are three breeding values – a direct breeding value for growth (A_{GR}), a direct breeding value for *b* (A_D), and an indirect breeding value for *b* (A_I).

4.2.2 Simulation

4.2.2.1 Population structure

We simulated a family-structured population using the model proposed above (Equations 1 and 2). Our objective was to test whether currently available models for IGE and inherited variability capture the effect of IGE on variability, rather than to investigate statistical power of those models. For this reason, we simulated large data sets, so as to avoid that limited power would blur the results.

We first simulated a base population of 100 sires and 10,000 dams, all unrelated. Each animal in the base population was assigned a breeding value for growth rate, and a direct and indirect breeding value for *b*, drawn from a multivariate normal distribution. Next, the offspring population was created by mating each sire with 100 randomly chosen dams. To create records on body weight for the analysis of trait levels, each dam produced 10 offspring, resulting in 1,000 offspring per sire, and a total of 100,000 offspring. Because analysis of variability was performed on records grouped by family (see below), we simulated a larger data set for the analysis of variability, so as to increase precision of estimates. Thus, to create records on variability of body weight, each dam produced 100 offspring, resulting in 10,000 offspring per sire, and a total of 1 million offspring.

The breeding values for growth rate and direct and indirect breeding values for *b* of the offspring were simulated as the average breeding value of the sire and dam, plus a Mendelian sampling term drawn from $N\left(\begin{bmatrix}0\\0\\0\end{bmatrix}, \frac{1}{2}\begin{bmatrix}\sigma^2_{A_{GR}} & 0 & 0\\ 0 & \sigma^2_{A_D} & 0\\ 0 & 0 & \sigma^2_{A_I}\end{bmatrix}\right)$. In addition, each offspring was assigned a permanent and temporary environmental effect on body weight, and direct and indirect environmental effects on *b*. These were drawn from $N\left(\begin{bmatrix}0\\0\\0\\0\end{bmatrix}, \begin{bmatrix}\sigma^2_{E_{p,GR}} & 0 & 0 & 0\\ 0 & \sigma^2_{E_{t,GR}} & 0 & 0\\ 0 & 0 & \sigma^2_{E_D} & 0\\ 0 & 0 & 0 & \sigma^2_{E_I}\end{bmatrix}\right)$. The genetic and environmental covariances were all set to 0. Groups of two members were created by randomly assigning a social partner to each individual, which resulted in 50,000 groups for the analysis of trait levels, and 500,000 groups for the analysis of trait variability.

Subsequently, phenotypes for 10-time points were obtained for all individuals by using Equations 1 & 2. Body weight at the last time point was used as the trait of interest and may for example reflect harvest weight in fish. Simulations were conducted using the R software (R Development Core Team, 2011).

4.2.2.2 Parameters

Table 4.1 shows the parameters used in the simulations. Starting weight of the individuals was set to 10 g. Mean growth rate (μ_{GR}) was also 10 g. The genetic standard deviation of growth rate ($\sigma_{A_{GR}}$) was set to 1 g. (See Marjanovic *et al.*, in press) for examples of the typical behavior of populations for these parameter values).

The $\bar{b}$ values used in the simulation were -0.05 (competition), 0 (no social interaction), 0.05 (cooperation). Standard deviation of $\bar{b}$ was set as 60% of 0.05; $\sigma_b = 0.03$. Therefore, standard deviations of genetic and environmental components of *b* had to satisfy $\sqrt{\sigma_{A_D}^2 + \sigma_{A_I}^2 + \sigma_{E_D}^2 + \sigma_{E_I}^2} = 0.03$. All standard deviations were assumed equal, hence each of them had a value of 0.015 (Table 4.1). Repeatability was set to 0.7 and heritability of growth rate to 0.5, in the absence of social interactions (*b*=0). Phenotypic variance was calculated as $\sigma_P^2 = \sigma_{\mathrm{A_{GR}}}^2/h^2$ and was equal to 2 g^2, permanent environmental effect on growth ($\sigma_{\mathrm{E_{p,GR}}}^2$) as $0.2\sigma_P^2$ = 0.4 g^2 and temporary environmental effect ($\sigma_{E_{t,GR}}^2$) as $0.3\sigma_P^2$ = 0.6 g^2 (Table 4.1).

In addition to default values of σ_{A_D}, σ_{A_I}, and $\sigma_{A_{GR}}$, we also simulated data where these values were 3x larger or 3x smaller (Table 4.1). These values were used to test the effect of magnitude of genetic variance on the estimates. In total, we tested 21 scenarios with different values of σ_{A_D}, σ_{A_I}, $\sigma_{A_{GR}}$, and *b* (Table 4.2).

Finally, we investigated how a non-zero genetic correlation affects estimated correlations, by simulating data with correlations of -0.5 or +0.5 between σ_{A_D}, σ_{A_I}, $\sigma_{A_{GR}}$, and default values for the other parameters.

For the analysis of inherited variability, each scenario had 100 replicates. For the analysis of levels of a trait, each scenario had 10 replicates.

Table 4.1 Parameters used in the simulation

Parameters	Default values	Alternate values
Mean growth rate, μ_{GR}	10 g	
Starting weight	10 g	
Genetic standard deviation for growth rate, $\sigma_{\mathrm{A_{GR}}}$	1 g	3 g or 0.3 g
Cooperation effect, $\bar{b}$	-0.05, 0, or 0.05	
Direct and indirect genetic standard deviation, $\sigma_{A_D} = \sigma_{A_I}$	0.015	0.045 or 0.005
Direct and indirect environmental standard deviation, $\sigma_{E_D} = \sigma_{E_I}$	0.015	
Phenotypic variance, $\sigma^2_{P_{GR}}$*	2 g	18 g or 0.18 g
Permanent environmental variance, $\sigma^2_{\mathrm{E_{p,GR}}}$	0.4 g	3.6 g or 0.036 g
Temporary environmental variance, $\sigma^2_{\mathrm{E_{t,GR}}}$	0.6 g	5.4 g or 0.054 g

*$\sigma^2_{P_{GR}}$ was calculated assuming b = 0 i.e. as $\sigma^2_{P_{GR}} = \frac{\sigma^2_{\mathrm{A_{GR}}}}{h}$, where $h = 0.5$.
**$\sigma^2_{E_{p,GR}}$ was calculated as $0.2\sigma^2_{P_{GR}}$, and $\sigma^2_{E_{t,GR}}$ as $0.3\sigma^2_{P_{GR}}$.

Table 4.2. Scenarios

	Scenario	$\bar{b}$ effect	σ_{A_D}	σ_{A_I}	$\sigma_{A_{GR}}$
Default scenario	1	Competition	0.015	0.015	1
	2	Neutral	0.015	0.015	1
	3	Cooperation	0.015	0.015	1
Different σ_{A_D}	4	Competition	**0.045**	0.015	1
	5	Neutral	**0.045**	0.015	1
	6	Cooperation	**0.045**	0.015	1
	7	Competition	**0.005**	0.015	1
	8	Neutral	**0.005**	0.015	1
	9	Cooperation	**0.005**	0.015	1
Different σ_{A_I}	10	Competition	0.015	**0.045**	1
	11	Neutral	0.015	**0.045**	1
	12	Cooperation	0.015	**0.045**	1
	13	Competition	0.015	**0.005**	1
	14	Neutral	0.015	**0.005**	1
	15	Cooperation	0.015	**0.005**	1
Different $\sigma_{A_{GR}}$	16	Competition	0.015	0.015	**3**
	17	Neutral	0.015	0.015	**3**
	18	Cooperation	0.015	0.015	**3**
	19	Competition	0.015	0.015	**0.3**
	20	Neutral	0.015	0.015	**0.3**
	21	Cooperation	0.015	0.015	**0.3**

* Parameter values that differ from those in default scenario are given in bold. **Competition corresponds to $\bar{b}$ of -0.05; Neutral corresponds to $\bar{b}$ of 0; Cooperation corresponds to $\bar{b}$ of +0.05.

4.2.3 Statistical models

We estimated genetic effects for the target trait and its variability using two models each. These were i) a direct sire model for inherited variability, ii) an indirect sire model for inherited variability, iii) a direct sire-dam model for the trait, iv) and an indirect sire-dam model for the trait. For all four models, genetic effects were estimated using residual maximum likelihood (REML) implemented in ASReml 4.1 software (Gilmour *et al.*, 2015). Subsequently, we estimated Pearson correlations between the estimated genetic effects from each model and each of the simulated breeding values. Estimated genetic effects from sire models were correlated with simulated breeding values of sires, while estimated genetic effects from sire-dam

models were correlated with simulated breeding values of both sires and dams. Table 4.3 gives an overview of calculated correlations. Models are explained in detail below.

Table 4.3 Overview of estimated correlations between estimated and simulated

Model	**Estimated genetic effects**	**Simulated breeding values**		
Analysis of the variability		$A_{GR}{}^{*}$	A_{D_b}	A_{I_b}
Direct sire model	$\mathbf{s}_D$			
Indirect sire model	$\mathbf{s}_I$		$\boldsymbol{r}$	
Analysis of the trait				
Direct sire and dam model	$\mathbf{p}_D$			
Indirect sire and dam model	$\mathbf{p}_I$			

*****Estimated genetic effects from sire models were correlated with simulated breeding values of sires, while estimated genetic effects from sire and dam models were correlated with simulated breeding values of sires and dams.

4.2.3.1 Direct sire model for inherited variability

As a measure of the direct component of inherited variability, we used the log-transformed within-family variance of body weight. Log transformed within-family variance of one full-sib family was treated as a trait of the sire, so that each sire had 100 observations of within-family variance, each based on 100 offspring per sire-dam combination. This model corresponds to an ordinary model for inherited variability (Rowe *et al.*, 2006), and gives estimates of half of the direct breeding values of a sire for inherited variability ($\mathbf{s}_D$). The model was:

$$\mathbf{y}_{v,D} = \mu + \mathbf{Z}_{D_s}\mathbf{s}_D + \mathbf{e},$$

where $\mathbf{y}_{v,D}$ is the vector of log-transformed within-family variance of body weight, μ is the overall mean, $\mathbf{s}_D$ is a vector of direct random genetic effects of sires, with $\mathbf{s}_D \sim N(\mathbf{0}, \sigma^2_{S_D})$, where $\sigma^2_{S_D}$ is the direct sire variance, $\mathbf{Z}_{D_s}$ is an incidence matrix linking observations to sires, and $\mathbf{e}$ is the vector of random residuals, with $\mathbf{e} \sim N(\mathbf{0}, \sigma^2_e)$.

4.2.3.2 Indirect sire model for inherited variability

Indirect genetic effects are expressed in the phenotypes of social partners. Therefore, to estimate indirect random genetic effects of sires for variability ($\mathbf{s}_I$), we used the log-transformed variance of body weight of the group mates of full-sib families descending from the sire. Thus, each sire had 100 records, which were the log-transformed variance of body weight of the group mates of each of the 100 families produced by a sire. The model was:

$$\mathbf{y}_{v,I} = \mu + \mathbf{Z}_{I_s}\mathbf{s}_I + \mathbf{e},$$

where $\mathbf{y}_{v,I}$ is the vector of log-transformed variance of body weight of group mates of full-sib families descending from the sire, μ is the overall mean, $\mathbf{s}_I$ is the vector of indirect random genetic effects of a sire, with $\mathbf{s}_I \sim N(\mathbf{0}, \sigma^2_{s_I})$, where $\sigma^2_{s_I}$ is the indirect sire variance for variability, $\mathbf{Z}_{I_s}$ is an incidence matrix linking observations to sires, and e is the vector of random residuals, with $\mathbf{e} \sim N(\mathbf{0}, \sigma^2_e)$.

4.2.3.3 Direct sire-dam model for the trait

Here we use an ordinary sire-dam model, which assumes equal genetic variance for sires and dams. The model was as follows:

$$\mathbf{y}_{t,D} = \mu + \mathbf{Z}_{D_p}\mathbf{p}_D + \mathbf{e},$$

where $\mathbf{y}_{t,D}$ is the vector of individual body weight records of offspring, μ is the overall mean, $\mathbf{p}_D$ is the vector of direct random genetic effects of sires and dams ("parents"), with $\mathbf{p}_D \sim N(\mathbf{0}, \sigma^2_{p_D})$, where $\sigma^2_{p_D}$ is the direct sire-dam variance, $\mathbf{Z}_{D_p}$ is an incidence matrix linking observations to parents, and has "1" in the column for the sire and in the column for the dam of the offspring producing the record, and $\mathbf{e}$ is the vector of random residuals, with $\mathbf{e} \sim N(\mathbf{0}, \sigma^2_e)$.

4.2.3.4 Indirect sire-dam model for the trait

In this model, we link individual observations on individual to the sire and dam of the group mate of an individual. The model was:

$$\mathbf{y}_{t,I} = \mu + \mathbf{Z}_{I_p}\mathbf{p}_I + \mathbf{e},$$

where $\mathbf{y}_{t,I}$ is the vector of individual body weight records of individuals, μ is the overall mean, $\mathbf{p}_I$ is the vector of indirect random genetic effects of the parents of

the group mate of the focal individual, with $\mathbf{p}_I \sim N(\mathbf{0}, \sigma^2_{p_I})$, where $\sigma^2_{p_I}$ is the indirect sire-dam variance, $\mathbf{Z}_{I_p}$ is an incidence matrix with "1" in the column for the sire and in the column for the dam of the group mate of the focal individual, and **e** is the vector of random residuals, with $\mathbf{e} \sim N(\mathbf{0}, \sigma^2_e)$.

4.3 Results

4.3.1 Variability models

Both direct and indirect estimated sire effects for variability showed near zero correlations with simulated breeding values of sire for growth (A_{GR}, Tables 4.4 & 4.5). Therefore, variability models do not capture trait level, which is expected.

Direct effects: The estimated direct sire effects on variability showed strongly negative correlations with simulated direct breeding values for *b* (A_{D_b}), under competition, cooperation and for neutral *b* (Table 4.4). Therefore, offspring of sires that are resistant to competition (i.e., have higher *b*) show lower variability of body weight. Correlations between estimated sire effects and simulated indirect breeding values for *b* (A_{I_b}), on the other hand, were near zero, under competition, cooperation, and for neutral *b*. These results indicate that cooperative effects of sires (A_{I_b}) have negligible effect on within-family variance. In conclusion, these results suggest that current (i.e., direct) models of inherited variability capture mostly the direct genetic effects (A_{D_b}) of competition, but not the indirect effect (A_{I_b}). In other words, they capture the sensitivity of individuals to competition, but not the competitive effects of individuals on the phenotypes of their group mates.

With higher direct genetic variation in *b* (σ_{A_D}; compared to the default value), or lower indirect genetic variation in *b* (σ_{A_I}), estimated correlations between estimated direct sire effects and simulated direct breeding values for *b* (A_{D_b}) were slightly more negative. The opposite was true for lower direct genetic variation in *b* (σ_{A_D}) and higher indirect genetic variation in *b* (σ_{A_I}). When direct genetic variation in *b* was small, or when indirect genetic variation in *b* was large, the direct model for inherited variability captured more indirect genetic effects, resulting in higher negative correlations between estimated direct sire effects and simulated indirect breeding values for *b* (Table 4.4).

Indirect effects: Correlations between estimated indirect sire effects on variability and simulated indirect breeding values for *b* $\left(A_{I_b}\right)$ were strongly negative, in competition, cooperation, and neutral scenarios (Table 4.5). These correlations suggest that group mates of offspring of sires that have high A_{I_b}, i.e., sires that are cooperative, have lower variability. Similar to the previous model, correlations between estimated indirect sire effects and simulated direct breeding values for *b* (A_{D_b}) were low and negative under competition, and close to zero under cooperation and for neutral *b*. Thus, indirect models of inherited variability capture mostly indirect genetic effects of competition, but not the direct effects (A_{D_b}). In other words, they capture the competitive effects of individuals on the phenotypes of their group mates, but not the sensitivity of individuals to competition.

With higher indirect genetic variation in *b* (σ_{A_I}), the correlation between estimated indirect genetic effects of a sire and indirect breeding values for *b* was more negative. When σ_{A_I} was low or when direct genetic variation in *b* (σ_{A_D}) was high, correlations between estimated indirect genetic effects of a sire and simulated direct breeding values for *b* slightly increased.

4.3.2 Trait models

Correlations between estimated sire and dam effects for growth from both direct and indirect sire-dam models for trait values, and simulated direct and indirect breeding values for *b* were near 0 (results not shown). Trait models, therefore, do not capture genetic effects of competition generated by the model in Equations 1 and 2. This result is not surprising, as the classical sire-dam model does not include IGEs, while the indirect sire-dam model is essentially the variance-component version of an IGE model, which does not make a connection between the level of IGEs and trait variability.

Direct effects: Direct sire and dam effects for growth showed a strongly positive correlation with simulated direct breeding values (~0.83) for all scenarios (results not shown). Correlations were lower than 1 because dam effects were based on only 10 observations; Correlations were near 1 when considering sires only (results not shown).

Indirect effects: Indirect sire and dam effects showed a moderate negative correlation (-0.33) with simulated breeding values for growth under competition, but a moderate and positive correlations (0.26) under cooperation. Thus individuals with

high genetic potential for growth reduce growth of their group mates under competition, but increase growth of their group mates under cooperation.

Changes in values of $\sigma_{A_{GR}}$, σ_{A_I}, and σ_{A_D}, had only minor effect on the estimated correlations.

Table 4.4 Correlations between estimated direct sire effects for variability and simulated breeding values for growth, and direct and indirect breeding values for *b*

Scenario*	$\overline{b}$ effect	A_{GR}	A_{D_b}	A_{I_b}
1	Competition	0.02	-0.96	-0.15
2	Neutral	0.02	-0.96	0.04
3	Cooperation	0.02	-0.91	0.07
4	Competition	0.02	-0.98	-0.05
5	Neutral	0.02	-0.98	-0.02
6	Cooperation	0.02	-0.96	0.02
7	Competition	-0.01	-0.80	-0.33
8	Neutral	0	-0.80	-0.04
9	Cooperation	0	-0.60	0.19
10	Competition	0	-0.80	-0.46
11	Neutral	0	-0.87	-0.22
12	Cooperation	0	-0.85	0.05
13	Competition	-0.01	-0.97	-0.03
14	Neutral	-0.01	-0.96	0.01
15	Cooperation	-0.01	-0.91	0.05
16	Competition	0	-0.96	-0.14
17	Neutral	0	-0.96	-0.02
18	Cooperation	0	-0.91	0.09
19	Competition	0.01	-0.96	-0.16
20	Neutral	0.01	-0.96	-0.04
21	Cooperation	0.01	-0.91	0.07

*Details of the scenario's are summarized in Table 4.2.

Table 4.5 Correlations between estimated indirect sire effects for variability and simulated breeding values for growth, and direct and indirect breeding values for *b*

Scenario*	$\overline{b}$ effect	A_{GR}	A_{D_b}	A_{I_b}
1	Competition	0.01	-0.15	-0.93
2	Neutral	0.01	-0.04	-0.91
3	Cooperation	0.01	0.08	-0.84
4	Competition	0.02	-0.43	-0.81
5	Neutral	0.02	-0.17	-0.87
6	Cooperation	0.01	0.15	-0.83
7	Competition	-0.01	-0.04	-0.94
8	Neutral	-0.01	0	-0.90
9	Cooperation	-0.01	0.03	-0.84
10	Competition	-0.02	-0.04	-0.98
11	Neutral	-0.02	-0.01	-0.98
12	Cooperation	-0.02	0.03	-0.97
13	Competition	0	-0.26	-0.69
14	Neutral	0	0	-0.61
15	Cooperation	0	0.19	-0.47
16	Competition	0	-0.12	-0.93
17	Neutral	0	-0.01	-0.92
18	Cooperation	0	0.11	-0.85
19	Competition	0	-0.15	-0.93
20	Neutral	0	-0.04	-0.91
21	Cooperation	0	0.07	-0.85

*Details of the scenario's are summarized in Table 4.2.

4.3.3 Genetic correlations between breeding values

Above reported results are based on data where genetic correlations between simulated breeding values were 0. We also investigated scenarios with correlations of -0.5 or +0.5 between breeding values (with default values for the other parameters). Results are in Supplementary material, Tables S4.1-S4.4. As expected, estimated correlations between genetic effects from the direct sire model and A_{I_b} and A_{GR} increased, when A_{D_b} had non-zero correlation with A_{I_b} and A_{GR} (Supplementary material, Table S4.1). Similarly, an increase in estimated correlations was observed between genetic effects from the indirect sire model, when A_{I_b} had a non-zero correlation with A_{D_b} and A_{GR} (Supplementary material, Table S4.2), and in trait models when A_{GR} had a non-zero correlation with A_{D_b} and A_{I_b}.

4.4 Discussion

We investigated whether current statistical models for inherited variability and for trait values capture direct and indirect genetic effects of competition on variability. Our results show that a direct model of inherited variability almost entirely captures the direct genetic effect of competition on variability, as illustrated by large correlations between estimated genetic effects and simulated direct breeding values for *b*. Similarly, an indirect model of inherited variability captures indirect genetic effects of competition. Models for trait levels, however, capture only little of the genetic effects of competition.

4.4.1 Capturing *b*

In Marjanovic *et al*. (*in press*) we developed a quantitative genetic model (Equation 1 & 2) in which the regression coefficient *b* comprises both a direct and an indirect genetic effect. Using simulations, we demonstrated that IGEs and variability can co-evolve, because the regression coefficient can respond to selection. Therefore both direct and indirect genetic effects on *b* affect phenotypic variability. In current direct quantitative genetic models for inherited variability, the contribution of the social partner is ignored, which is illustrated by results of this study, where the direct sire model for inherited variability failed to capture indirect genetic effects on *b.* In contrast, the relationship between estimated genetic effects of a sire and simulated direct genetic effects for *b* showed a consistently linear relationship (Figure 4.1). Response to selection for higher uniformity, relying on direct genetic effect only, may be less effective as an entire level of potential genetic variation is not exploited. In addition, presence of IGEs on *b* may cause response in

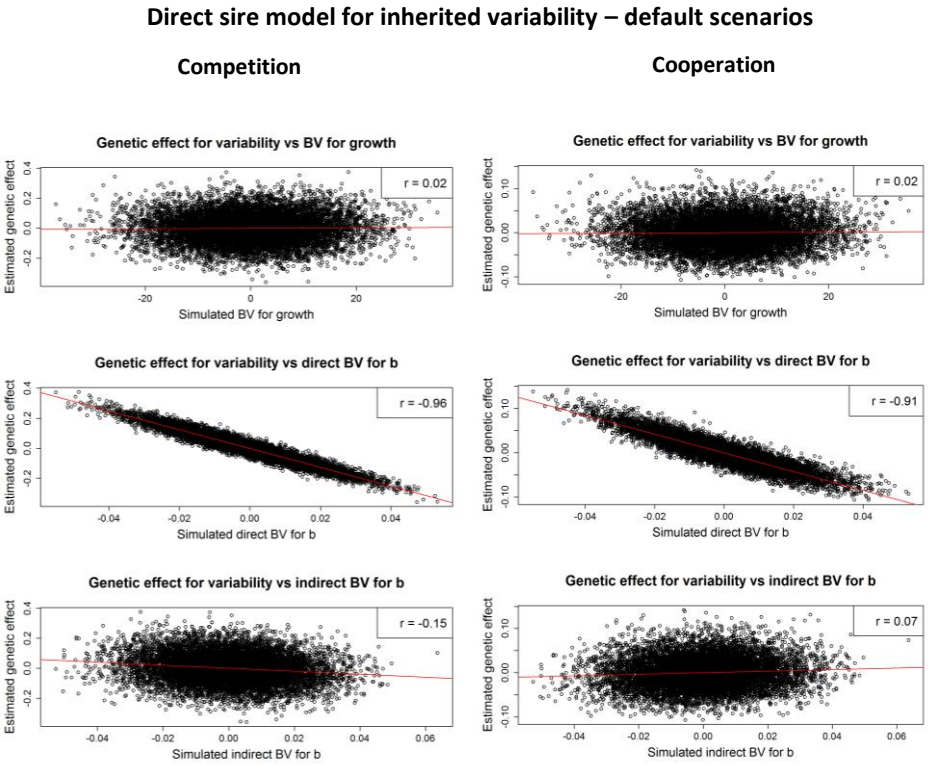

Figure 4.1 Correlations between estimated direct genetic effects of a sire for variability and simulated direct breeding values of a sire for growth (A), simulated direct breeding values of a sire for *b* (B), and indirect breeding values of a sire for *b* (C) under competition and cooperation.

variability to divergence from its expectation, particularly when they are correlated to direct genetic effects on *b* (Ellen *et al.*, 2014).

When traits are affected by social interactions, selection strategies that account for both direct and indirect genetic effects can result in higher response (for example, Griffing, 1976; Muir, 1996; Bijma, Muir, Ellen, et al., 2007). Future breeding programs aiming to reduce variability may need to improve both direct and indirect genetic effects. By using an indirect sire model for inherited variability, we showed that estimated genetic effects of a sire had a high correlation with the simulated indirect breeding values for *b.* Also this relationship is remarkably linear (Figure 4.2).

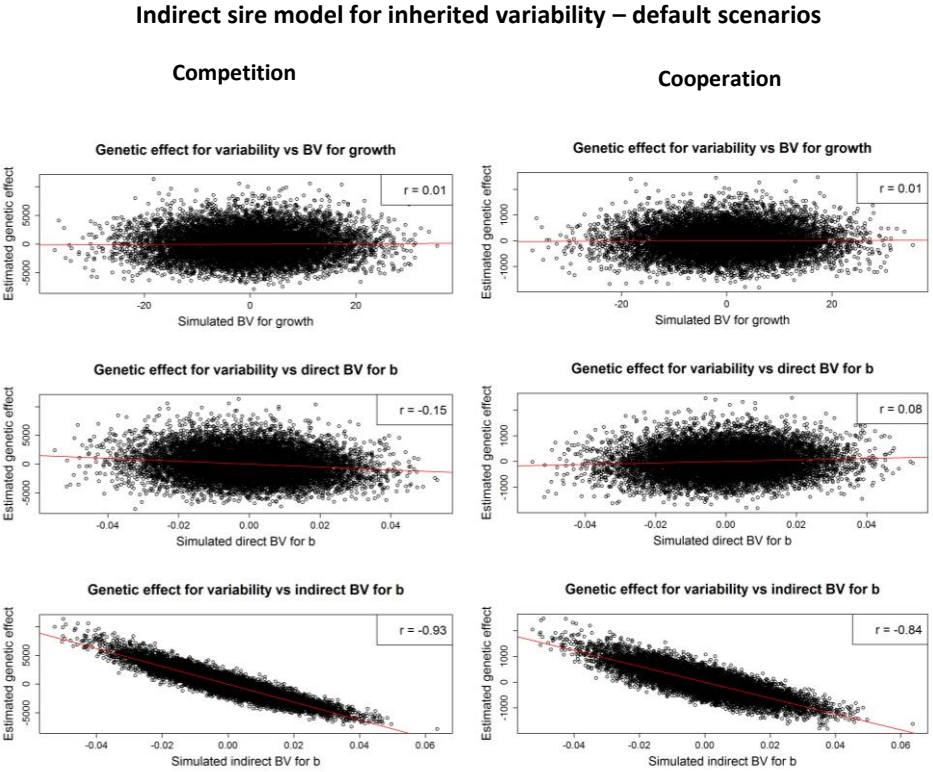

Figure 4.2 Correlations between estimated indirect genetic effects of a sire for variability and simulated direct breeding values of a sire for growth (A), simulated direct breeding values of a sire for b (B), and indirect breeding values of a sire for b (C), under competition and cooperation.

Capturing genetic effect of competition on variability, therefore, is promising with models for inherited variability, but may require a two-step analysis, in which direct and indirect genetic effects are estimated separately, and subsequently combined into a total breeding value for variability, analogous to IGE models for trait values (Bijma *et al*., 2007). The benefit of such an approach is that it only requires group-structured data, but not time-series data, as the analysis is performed on the final phenotype. Using a one-step approach to estimate direct and indirect breeding values for *b* would be challenging with the experimental design used in this study, where groups consisted of two individuals and offspring of a sire were randomly assigned to groups. Since each individual was both a focal individual and a social partner, calculation of the direct and social within-family variance would require using the same individual twice. In other words, the same data would be used to

calculate the variance among the offspring of each sire and to calculate the variance among the social partners of the offspring of each sire. In the present study, we followed the experimental design of Marjanovic *et al*. (*in press*), which has groups of only two individuals. However, the need for a two-step analysis can be avoided by using larger groups consisting of members of two families each. In such a design, the y-variable could be the within-group variance of each family in the group (two records per group), and both a direct effect of the family and an indirect effect of the partner family could be fitted. Alternatively, if multiple observations of body weight of two individuals in a group are available, direct and indirect genetic components of *b* could be estimated using random regression method (Marjanovic *et al*, *in press*).

4.4.2 Validation experiments

To validate results of this study and the previous study by Marjanovic *et al.* (*in press*), the proposed models should be applied to empirical data. Empirical data could give insight into whether the theoretical possibility that IGEs contribute to genetic variation in variability, are also biologically relevant, and in which situations. In addition, it would allow to test the statistical models proposed here and to optimize methods and models for future studies aiming to estimate genetic effects of competition. Selection experiments where one selection strategy involves selection for direct genetic effects on variability only, while the other would select for both direct and indirect genetic effects, would also allow to quantify the contribution of IGEs to response to selection in variability.

The experiments should have a group structure with, e.g., two individuals per group, similar to our simulated data. However, subsequent trials involving larger group sizes may also be conducted to test the single-step analysis suggested above and to quantify the effect of group size on the estimates. For groups of two individuals, data on both individuals in each group should ideally be collected at several time points. Such time-series data would allow to use a random regression approach as suggested by Marjanovic *et al*. (*in press*), but also the direct model and the indirect model for inherited variability presented in this study could be used. The experiment could be performed using zebrafish as a model organism, as this species shows substantial competition and fast growth.

For estimation of direct and indirect breeding values for *b* in a commercial setting, new phenotyping techniques that involve video tracking of individuals in 3D space could be used in the future (see for example idTracker, http://www.idtracker.es/). These techniques would give multiple observations on individual trait values (for

example body weight calculated from the 3D image, i.e., volume of the individual) and information on social interactions between individuals.

4.5 Conclusion

Our results show that a direct model of inherited variability almost entirely captures the direct genetic effect of competition on variability, while an indirect model of inherited variability captures indirect genetic effects of competition. Models for trait levels, however, capture only little of the genetic effects of competition. The estimation of direct and indirect genetic effects of competition therefore is possible with models for inherited variability, but may require a two-step analysis or a different data set-up involving larger groups.

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Supplementary material

Table S4.1 Estimated correlations between simulated breeding values and estimated genetic effects, when simulated breeding values are genetically correlated

Direct sire model for inherited variability

Correlation 0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{D_{V,s}}$	competition	0.0	-0.96	-0.61
	cooperation	0.0	-0.90	-0.31

Correlation -0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{D_{V,s}}$	competition	0.0	-0.95	0.32
	cooperation	0.0	-0.91	0.53

Correlation 0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{D_{V,s}}$	competition	-0.59	-0.95	-0.20
	cooperation	-0.64	-0.90	0.06

Correlation -0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{D_{V,s}}$	competition	0.58	-0.95	-0.18
	cooperation	0.64	-0.90	0.08

Correlation 0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{D_{V,s}}$	competition	0.0	-0.95	-0.11
	cooperation	0.0	-0.91	0.05

Correlation -0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{D_{V,s}}$	competition	0.0	-0.96	-0.16
	cooperation	0.0	-0.91	0.05

Table S4.2 Estimated correlations between simulated breeding values and estimated genetic effects, when simulated breeding values are genetically correlated

Indirect sire model for inherited variability

Correlation 0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.0	-0.56	-0.93
	cooperation	0.0	-0.33	-0.81

Correlation -0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.0	0.38	-0.93
	cooperation	0.0	0.52	-0.86

Correlation 0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.19	-0.01	-0.91
	cooperation	0.34	0.23	-0.78

Correlation -0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.20	0.0	-0.90
	cooperation	-0.36	0.24	-0.78

Correlation 0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.47	-0.13	-0.92
	cooperation	-0.44	0.10	-0.84

Correlation -0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.46	-0.14	-0.93
	cooperation	0.42	0.09	-0.85

Table S4.3 Estimated correlations between simulated breeding values and estimated genetic effects, when simulated breeding values are genetically correlated

Direct sire-dam model for the trait

Correlation 0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.83	0.0	0.0
	cooperation	0.84	0.0	0.0

Correlation -0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.83	0.0	0.0
	cooperation	0.83	0.0	0.0

Correlation 0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.83	0.41	0.0
	cooperation	0.83	0.42	0.0

Correlation -0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.83	-0.41	0.0
	cooperation	0.83	-0.41	0.0

Correlation 0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.83	0.0	0.41
	cooperation	0.83	0.0	0.42

Correlation -0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	0.83	0.0	-0.41
	cooperation	0.83	0.0	-0.41

Table S4.4 Estimated correlations between simulated breeding values and estimated genetic effects, when simulated breeding values are genetically correlated

Indirect sire-dam model for the trait

Correlation 0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.33	0.0	0.0
	cooperation	0.27	0.0	0.0

Correlation -0.5 between simulated A_{D_b} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.33	0.0	0.0
	cooperation	0.28	0.0	0.0

Correlation 0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.33	-0.16	0.0
	cooperation	0.28	0.13	0.0

Correlation -0.5 between simulated A_{GR} and A_{D_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.33	0.15	0.0
	cooperation	0.28	-0.14	0.0

Correlation 0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.33	0.0	-0.17
	cooperation	0.28	0.0	0.13

Correlation -0.5 between simulated A_{GR} and A_{I_b}

		A_{GR}	A_{D_b}	A_{I_b}
$\hat{A}_{I_{V,s}}$	competition	-0.33	0.0	0.16
	cooperation	0.28	0.0	-0.14

5

Effects of relatedness between group mates on body weight and variability of body weight in domestic Nile tilapia

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Abstract

In a wide range of animal species relatives show better social behavior to each other than to unrelated conspecifics, for example, food sharing and reduced aggressiveness. Such behavior has the potential to increase inclusive fitness and it may therefore have evolved through the process known as kin selection. In addition to fitness benefits in natural populations, reduced competition may also lead to increased performance in agricultural populations. In aquaculture populations, competition for feed is a big issue, as it reduces growth, and inflates phenotypic variability among individuals. In domestic Nile tilapia, for example, the coefficient of variation (CV) of body weight is between 40 % - 60 %, which is high. One potential way to reduce competition and increase yield and uniformity of trait values in Nile tilapia is to utilize the consequences of past kin selection, i.e., the evolution of kin discrimination and cooperative behavior among relatives. However, it is almost entirely unknown whether relatedness in Nile tilapia leads to higher growth rates, better uniformity, and possibly higher survival. In this study we compared two experimental treatments: rearing of fish in kin groups vs. rearing in non-kin groups, in order to investigate whether relatedness affects performance traits in domestic Nile tilapia. We analyzed average body weight, standard deviation and CV of body weight, and survival, between the two treatments. Results of our study show that individuals had significantly higher body weight in groups composed of kin (8.6 ± 2.6 g), indicating that domestic Nile tilapia may exhibit kin-biased behavior. The effect of relatedness was higher for males than females, which may be related to a higher level of competition in males. There was no difference in variability of body weight and survival between the two treatments. Aquaculture farming may benefit in yield by rearing individuals in groups composed of relatives.

Key words: Nile tilapia, kin selection, kin discrimination, competition, phenotypic variability, uniformity, body weight

5.1 Introduction

Personal fitness is the ability of an individual to survive and produce offspring, and by that contribute to the gene pool of the next generation. Propagation of own genes, however, is not only achieved through direct descendants. In his influential paper from 1964, Hamilton showed that because relatives tend to share genes, promoting survival and reproduction of relatives may increase the evolutionary success of an individual's own genes (Hamilton, 1964a). This indirect fitness benefit, together with personal or direct fitness, constitutes an inclusive fitness, which is maximized through kin selection. Kin selection describes the process of natural selection which alters the frequencies of genes shared by relatives through actions that increase survival and reproduction of relatives (Fletcher & Michener, 1987; Smith, 1964). The most striking example of kin selection is the evolution of sterile workers in haplodiploid social insects (Hamilton, 1964a, 1964b).

According to kin selection theory, genetic relatedness should influence social behavior, because individuals able to interact differently with kin *vs.* non-kin would have higher inclusive fitness (Hepper, 1986). In a wide range of animal species, it has been noticed that relatives show better social behavior to each other than to unrelated conspecifics, such as food sharing and reduced aggressiveness (Kareem and Barnard, 1982; Wilkinson, 1984; Hepper, 1986; Hiscock and Brown, 2000b; Wahaj *et al*., 2004; Gerlach *et al*., 2007). The ability of an individual to distinguish between kin and non-kin is known as kin recognition (Hepper, 1986).

In aquaculture populations, kin recognition occurs in a sibling-sibling and parent-offspring context (Green *et al.*, 2008). Several species of fish, including Atlantic cod (Herbinger *et al.*, 1997), coho salmon (Quinn and Busack, 1985), common shiners (Ferguson and Noakes, 1981), zebra fish (Gerlach *et al*., 2007), black-chin tilapia (Pouyaud *et al.*, 1999), and Eurasian perch (Gerlach *et al.*, 2001) are known to show kin-biased behavior. Fish mostly use visual and chemosensory cues to recognize kin (Fletcher and Michener, 1987; Hiscock and Brown, 2000a; Olsén *et al.*, 2004).

The majority of studies on kin recognition in fish were focused on the asymmetry in behaviors such as shoaling, aggressiveness, and food sharing of individuals kept in kin versus non-kin groups. In addition, studies on zebra fish, Atlantic salmon, and rainbow trout, showed that rearing in kin groups can significantly increase growth of individuals (Brown and Brown, 1996; Gerlach *et al*., 2007). The observed increase in growth performance has been associated with a lower level of competition and

stress in kin groups, which subsequently allowed individuals to acquire higher amounts of food and spend more time eating (Brown and Brown, 1996; Gerlach *et al.*, 2007).

In aquaculture populations, competition for feed not only reduces performance of individuals, but also inflates phenotypic variability among individuals (Jobling, 1995; Cutts *et al.*, 1998; Hart and Salvanes, 2000). Formation of a social hierarchy, where dominant fish monopolize the majority of the feed, creates large size differences among individuals. Aquaculture breeding programs aim to improve growth and survival of individuals, while uniformity of trait values recently emerged as a commercially important trait (Sae-Lim *et al*, 2012; Khaw, Ponzoni, Yee, Aziz, Mulder, *et al*, 2016; Marjanovic *et al*, 2016; Janssen *et al*, 2017).

The level of competition in aquaculture populations is usually assessed by the coefficient of variation (CV) of body weight, where a high CV indicates strong inter-individual competition and a low CV suggests little competition, i.e., a good social environment (Jobling, 1995). In one of the most cultured fish species around the world, Nile tilapia, the CV of body weight is between 40% and 60%, which is considered as high and undesirable (Ponzoni *et al.*, 2005).

One potential way to reduce competition in Nile tilapia and increase yield and uniformity of trait values, is to utilize the consequences of past kin selection, i.e., the evolution of kin discrimination. If individuals are able to discriminate kin, and as a result show more cooperative behavior to their relatives, then the mean performance, uniformity, and survival of individuals are expected to be higher in groups of kin compared to groups of non-kin (Brown and Brown, 1996). However, to our knowledge, effects of relatedness on growth rate and phenotypic variability have been investigated only in natural and laboratory populations of fish, but not in aquaculture. Also for Nile tilapia it is almost entirely unknown whether relatedness leads to higher growth rates, better uniformity, and higher survival of related individuals.

Here we investigate whether relatedness affects the performance in domestic Nile tilapia. We conducted an experiment in which we compared two treatments: rearing of fish in kin groups *vs.* rearing in non-kin groups. We investigated differences in average body weight, standard deviation, CV of body weight, and survival, between both treatments.

5.2 Material and methods

5.2.1 Environment

The experiment was carried out in two batches between October 2015 and August 2016. Each batch lasted 4 months. The experiment was performed at WorldFish, scientific research organization located in Penang, Malaysia (http://www.worldfishcenter.org). WorldFish complies with the Malaysian laws on animal experiments.

5.2.2 Experimental design

We used brood fish from generations 14 and 15 of the Genetically Improved Farmed Tilapia (GIFT) strain to produce the experimental fish for batch 1 and batch 2, respectively. Families were created by mating each male with a single female, resulting in total of six full-sib families per batch. All families were unrelated to each other. After mating, fertilized eggs of each family were transferred into separate incubators until hatching.

When larvae had reached the fry stage (in 1-2 weeks depending on the temperature), fry of each family were transferred to separate nursery hapas, where they remained until tagging. The nursery hapas for all families in the experiment were installed in the same fiberglass tank to reduce any environmental differences. The water flow in the tank allowed for the exchange of chemical cues between different families. Consequently, all individuals were exposed to both kin and non-kin chemical cues.

When fish reached the tagging size of 5 grams on average, they were individually identified with PIT (Passive Integrated Transponder) tags. At this time, initial weight, size (length, depth, and width), and date of tagging of each individual were recorded. Subsequently, tagged fish were placed in experimental tanks. As experimental tanks, we used 30 units of black round plastic tanks, which were stocked with 50 fish each.

Fish received one of two treatments (Table 5.1):

- Treatment 1: Kin. Tanks contained a single focal family, where all 50 individuals were full-sibs.

- Treatment 2: Mix. Tanks contained a mix of families; Half of the fish (n = 25) were from a single focal family used also in treatment 1, while the other 25 fish were from

a mix of three other families ("partner families") that were unrelated to the focal family from treatment 1.

Table 5.1 Overview of the experimental design for one batch

		Treatment 1 - Kin			Treatment 2 - Mix		
		Focal fish[b]			Focal fish + Partner fish[d]		
Replicate	[e]R1	F[a]	F2	F3	F1 + mix[c]	F2 + mix	F3 + mix
	R2	F1	F2	F3	F1 + mix	F2 + mix	F3 + mix
	R3	F1	F2	F3	F1 + mix	F2 + mix	F3 + mix
	R4	F1	F2	F3	F1 + mix	F2 + mix	F3 + mix
	R5	F1	F2	F3	F1 + mix	F2 + mix	F3 + mix

[a]Focal full-sib family (F1, F2, F3); [b]Fish from the focal full-sib family; [c]Mix of three full-sib families, unrelated to family F1, F2, and F3; [d]Fish from the mix; [e]R1 through R5 indicate replicates 1 through 5. The second batch had the same set-up, but contained 3 different focal families, so that a total of 6 focal families was used in the entire experiment.

Therefore, out of the six families (per batch), three were used as the focal families/fish, while fish from the three other families, which we hereafter refer to as partner fish, were combined together to make a mix. Individuals from the partner families were randomly assigned to each tank. Per batch, fifteen tanks were allocated to each treatment. There were five replicates per full-sib family in both treatments. The second batch had the same set-up, but used three different focal families. Hence, in total there were six focal families, each with five repeats per treatment, and two treatments, giving a total of 60 tanks in the experiment.

All fish were stocked in the experimental tanks on the same day. However, one tank in batch 1 and treatment 2, suffered high mortality just prior to the start of the experiment and was therefore left empty until the first measuring, when it was re-stocked. Fish from this tank, therefore, had a shorter grow-out period of ~27 days compared to individuals from other tanks. From stocking time, the experiment lasted four months, which is close to the ordinary grow-out period in Tilapia kept in ponds. During the growth period, fish were fed twice a day with commercial pellet feed containing 40% of protein. The feed was spread manually across the surface of the tanks. All fish were harvested during a 72h period. The grow-out period of each fish was computed as the time from the stocking date to the harvest date.

5.2.3 Records

During the experiment, body weight and survival of the fish were measured once a month, resulting in five repeated observations, including the initial measurement (Table 5.2). The sex of each fish was also recorded during the harvest.

Each batch started with 1500 fish in total. However, during the experiment some fish died, resulting in a reduced number of live fish at the end of the experiment, being 1152 in batch 1, and 1055 in batch 2. Therefore, the total number of records was 2207 (Table 5.2).

Our main interest was to investigate whether individuals from the same family show differences in body weight, uniformity, and survival, between the two treatments. For this purpose we analyzed body weight both on the individual level and on the group level, while uniformity of body weight and survival were analyzed only on the group level to have measures of variance and survival rate, respectively. Since fish from the partner families were present only in treatment 2, they were excluded from the analysis of the treatment effect. Table 5.3 summarizes the data used in this analysis.

The number of individual observations for the analysis of the treatment effect on the body weight was 1617. Observations on the group level were obtained by pooling all the records of focal individuals in the tank per treatment, for example, average body weight of the focal family in the tank. Uniformity of body weight was expressed as the coefficient of variation (CV) of body weight at the final measuring, and as the standard deviation (SD) of final body weight of the focal family within a tank. The CV was calculated as $\frac{\sigma}{\mu} * 100\%$, where σ is the standard deviation of body weight of the focal family in the tank, and μ is the mean body weight of the focal family in the tank. Survival was calculated as $\frac{n_h}{n_s} * 100\%$, where n_h is number of focal individuals per tank at harvest, and n_s is the number of focal individuals per tank stocked. There were total of 60 observations for CV, SD of body weight, and survival, available for the analysis.

In addition, we wanted to test the effect of the size of the kin group on performance, i.e., whether individuals perform better when they are grouped with more siblings.

Table 5.2 Number of families, groups, and individuals by batch and in total

	Focal families[a]	Partner families[b]	Groups/ Tanks	Total individuals		Individuals in treatment 1		Individuals in treatment 2	
				Stocked	Harvested	Stocked	Harvested	Stocked	Harvested
Batch 1	3	3[c]	30	1500	1152	750	554	750	598
Batch 2	3	3	30	1500	1055	750	521	750	534
Total	6	6	60	3000	2207	1500	1075	1500	1132

[a]Single full-sib families present in both treatments; [b]Full-sib families combined together to make a mix – present only in treatment 2; [c]Three families in each batch were combined together to form a mix for treatment 2.

For this purpose, we used the data from treatment 2, where individuals from the focal families had 24 siblings, whereas individuals from the partner families had only ~7 siblings on average. We compared mean, CV and SD of body weight, and survival, of focal fish versus partner fish. Hence, for this analysis we had two observations per tank, resulting in a total of 60 observations.

5.2.4 Statistical analysis

All statistical analyses were performed using ASReml 4.1 (Gilmour et al., 2015). Summary statistics were obtained using the R programming software (R Development Core Team, 2011). Significance levels were calculated using a Wald-test for fixed effects and a likelihood-ratio test for random effects.

5.2.4.1 Individual level

The model for individual body weight was

$$y_{ijklmnopq} = \mu + days_i + days_i^2 + IW_j + (oxygen \times batch)_{kl} + sex_m + Treatment_n + family_o + Tank_p + (row \times batch)_{ql} + e_{ijklmnopq}, \quad (1)$$

where $y_{ijklmnopq}$ is the individual body weight at harvest, μ is the intercept, $days_i$ is the linear effect of the length of the grow-out period (*i* = 97, 119-125), fitted as a covariate, $days_i^2$ is the corresponding quadratic effect, accommodating a non-linear relationship between body weight at harvest and the grow-out period, IW_j is the effect of individual initial weight, i.e., weight at the start of the experiment, fitted as a covariate, $(oxygen \times batch)_{kl}$ is the fixed effect of interaction between $oxygen$ and $batch$ accounting for differences in oxygen supply to each tank within the batch (*kl* = 1-6), sex_m is the fixed effect of sex of the individual (*m* = Male, Female, Missing), $Treatment_n$ is the fixed effect of the treatment (*n* = 1, 2), $family_o$ is the random effect of full-sib family (*o* = 1-6), $Tank_p$ is the random effect of experimental tank (*o* = 1-60), $(row \times batch)_{ql}$ is the random effect of interaction between row and $batch$ accounting for potential effects of the row within the batch where tank/individual was placed, (*ql* = 1-10), and $e_{ijklmnopq}$ is the residual. All random effects were modeled as independent and normally distributed.

5.2.4.2 Group level

In the analysis of treatment effect on average body weight, CV and SD of body weight we applied the following model:

$$y_{ijkl} = \mu + GIW_i + Treatment_j + family_k + e_{ijkl}, \quad (2)$$

where y_{ijkl} is the average body weight, CV of body, or SD of body weight of a focal family in the tank, GIW_i is the effect of average initial body weight, CV, or SD of initial body weight of the focal family (each corresponding to the given y), fitted as a covariate, $Treatment_j$ is the fixed effect of the treatment (*j* = 1, 2), $family_k$ is the random effect of focal family (*k* = 1-6), and e_{ijkl} is the residual. The effects of $days$, $days^2$, $oxygen \times batch$, and $row \times batch$ were not significant for traits on the group level and were not included in model 2.

For the analysis of survival the model was:

$$y_{ijk} = \mu + Treatment_i + family_j + e_{ijk}, \quad (3)$$

where y_{ijk} is the survival of the focal family in the tank, $Treatment_i$ is the fixed effect of the treatment (*i* = 1, 2), $family_j$ is the random effect of focal family (*j* = 1-6) and e_{ijk} is the residual. The effect of initial weight (GIW) was not significant for survival and thus omitted.

5.2.4.3 Size of kin group

Using the data from treatment 2 we tested whether the number of relatives in a group ("size of kin group") affected the trait values. In treatment 2, individuals of focal families had 24 relatives initially, whereas individuals from partner families initially had ~7 relatives on average. Hence, a difference in trait value between focal and partner families may reflect their number of relatives. We investigated the effect on kin group size on individual and average body weight, CV and SD of body weight, and survival. The model for the individual body weight was:

$$y_{ijklmnop} = \mu + days_i + days_i^2 + IW_j + (oxygen \times batch)_{kl} + sex_m + KGS_n + family_o + Tank_p + (row \times batch)_{ql} + e_{ijklmnopq}, \quad (4)$$

where $y_{ijklmnop}$ is the individual body weight of fish from treatment 2 and KGS_n is the fixed effect of kin group size (*n* = Focal, Partner), $family_o$ is the random effect of the family (*o* = 1-8). Other effects were as described in model 1.

The model for body weight expressed on the group level was:

$$y_{ijklmno} = \mu + days_i + AIW_j + KGS_k + family_l + (row \times batch)_{mn} + e_{ijklmno}, \quad (5)$$

where $y_{ijklmno}$ is the average body weight of a focal or partner family within a tank, AIW_j is the average body weight of that family at the start of the experiment. Other effects were as described in the previous model.

The model for CV of body weight was:

$$y_{ijklm} = \mu + CVIW_j + KGS_k + family_l + e_{ijklm}, \quad (6)$$

where y_{ijkm} is the CV of body weight of a focal or partner family within a tank, $CVIW_j$ is the CV of body weight of that family at the start of the experiment. Other effects were as described in model 4.

For SD of body weight the model was:

$$y_{ijklm} = \mu + days_i + SDIW_j + KGS_k + e_{ijklm}, \quad (7)$$

where y_{ijkm} is the SD of body weight of a focal or partner family within the tank, $SDIW_j$ is the SD of body weight of the family at the start of the experiment. Family effect was not significant and therefore omitted. Other effects were as described in model 4.

For survival the model was:

$$y_{ijklm} = \mu + days_i + KGS_j + family_k + (row \times batch)_l + e_{ijklm} \quad (8)$$

where y_{ijkm} is the survival of a focal or partner family within the tank. See model 4 for details on other effects. Effects included in model 4 but not in models 5-8 were either not significant or not estimable (Tank and Sex).

5.3 Results

5.3.1 Descriptive statistics

Table 5.3 shows the descriptive statistics. Overall survival was 74.5%, which is similar to survival observed in the GIFT population reared in communal ponds (Khaw *et al.*,

2010), also in experimental setting (Khaw *et al*., 2016). Over two experimental batches, we collected a total of 2207 fish with phenotypic records. The average group (tank) size at harvest was 36.8 individuals, indicating an average mortality of 13.2 fish per tank. The overall phenotypic mean of body weight of the whole population was 46.2 g, which is much lower than the ~100-200 g reported in Nile tilapia populations for a similar period of growth in communal ponds (http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en). The lower value can be related to the available space for growth, which was much smaller in the experimental tanks compared to communal ponds. The CV of body weight at harvest of 47.5 %, however, does correspond to values previously reported for the communally reared GIFT strain (48 % by Ponzoni *et al.*, 2005; 59.8 % by Nguyen *et al.*, 2007, 40 % by Khaw *et al.*, 2010). The average CV within groups was slightly lower (38.6 %).

5.3.2 Estimated effects

Table 5.4 gives estimated effects of the treatment and the size of the kin group, and their significance.

5.3.2.1 Effect of the treatment on body weight

The treatment effect on individual body weight was strongly significant (p=0.003), with fish in kin groups (treatment 1) being 8.6 g (se ± 2.6 g) heavier on average compared to individuals in mixed groups (treatment 2), and focal families from kin groups being on average 9.9 g (se ± 2.8 g, p = 0.001) heavier than the same families in mixed groups when analyzing tank averages. Standard deviation (sd) of individual body weight in both treatments was ~20.5 g (22.7 in Treatment 1 and 18.3 in Treatment 2, Table 5.3), meaning that the effect of treatment on both individual body weight and average body weight of focal family is rather large, as it equals almost half a sd.

Males were on average 13.3 g (se ± 1.5 g) heavier than females. To test whether the treatment effect differed for male and female body weight, we divided dataset in two based on sex, and applied model 1. Individuals without recorded sex were excluded (n = 497). Estimated treatment effects (results not shown) indicate that individual body weight in treatment 1 for males was 12.42 g (se ± 3.8 g, p = 0.003) higher compared to treatment 2, and 7 g (se ± 3.4 g, p = 0.04) for females. The larger effect of treatment in males is probably related to their higher phenotypic mean body weight of 53.1 g, compared to 42.1 g for females, but it may also be that a

Table 5.3 Data summary – number of observations (N), mean (μ), minimum and maximum, and standard deviation (σ) of analyzed traits for focal families in two treatments, mix and in overall population

Trait	[a]N	μ	Min	Max	σ
Treatment 1, focal families					
Individual body weight (g)	1074	[a]46.3	3.8	191.2	22.7
Mean body weight (g)	30	50.1	29.8	103.9	17.3
CV of body weight (%)	30	37.1	26.8	58.1	6.9
SD of body weight (g)	30	18.4	9.2	40.9	7.0
Survival (%)	30	71.6	28.0	96.0	16.4
Treatment 2, focal families					
Individual body weight (g)	543	38.1	4.9	116.6	18.3
Mean body weight (g)	30	40.5	23.8	69.6	11.7
CV of body weight (%)	30	37.2	18.7	55.8	9.0
SD of body weight (g)	30	14.8	6.9	29.7	5.6
Survival (%)	30	72.4	36.0	100.0	13.3
Treatment 2, partner families					
Individual body weight (g)	590	52.8	12.3	152.5	22.3
Mean body weight (g)	30	53.7	36.0	82.7	11.3
CV of body weight (%)	30	35.2	24.9	56.5	7.2
SD of body weight (g)	30	19.3	9.9	35.3	7.1
Survival (%)	30	78.5	48.0	96.0	11.1
Overall population					
Individual body weight (g)	2207	46.2	3.8	191.2	22.1
Mean body weight (g)	30	48.5	29.9	103.9	14.3
CV of body weight (%)	2207	47.8	-	-	-
CV of body weight (%)	60	38.6	24.1	58.1	6.6
SD of body weight (g)	60	18.7	9.2	40.1	6.4
Survival (%)	2207	74.5	-	-	-
Survival (%)	60	73.6	28.0	98.0	13.9

[a]Traits with 30 or 60 observations are group-averages. Because of mortality, the number of individuals per group varied. As a consequence, the average of individual trait values may differ from the average of group means.

higher level of relatedness and cooperation in treatment 1 is especially benefiting male performance, as competition is more frequently and more aggressively orchestrated through male-male interactions (Huntingford *et al.*, 2012). Dividing estimated treatment effects for each sex by their average body weight we obtained a relative effect of the treatment of 23.4% for males and 16.6% for females, suggesting that the higher estimated treatment effect in males goes beyond a scaling effect, and may possibly be related to the behavior of male fish.

Table 5.4 Significance and effect of treatment for focal families, and kin group size for focal families versus partner families in treatment 2.

Trait	Model	p-value	Effect (SE)
Focal families, treatment 1&2		Treatment[a]	
Individual body weight (g)	1	0.003	8.6 (2.6)
Mean body weight (g)	2	0.001	9.9 (2.8)
CV of body weight (%)	2	0.863	0.3 (1.8)
SD body weight (g)	2	0.001	4.4 (1.3)
Survival (%)	3	0.722	-1.4 (4.0)
Focal vs partner families in treatment 2		Kin group size[b]	
Individual body weight (g)	4	0.751	-1.2 (3.4)
Mean body weight (g)	5	0.397	-3.4 (3.8)
CV of body weight (%)	6	0.448	3.2 (3.8)
SD body weight (g)	7	0.904	-0.18 (1.5)
Survival (%)	8	0.312	-6.1 (3.1)

[a] Effect of treatment 1 compared to effect of treatment 2 which was set to 0
[b] Effect of large kin group (i.e. focal family compared to smaller kin group (i.e. partner fish) which was set to 0.

5.3.2.2 Effect of the treatment on variability of body weight and survival In the analysis of variability of body weight, the effect of treatment was significant for the SD of body weight ($p = 0.001$). CV of body weight, however was not significantly different between the treatments ($p = 0.863$), suggesting that once standard deviation was adjusted for the mean, the apparent effect of treatment on

variability disappeared. Treatment effect on survival was also non-significant (p=0.722). Grouping traits by sex in the analysis did not affect the significance of estimated treatment effects.

5.3.2.3 Effect of kin group size

While individuals from focal families grew better in treatment 1 compared to treatment 2, within treatment 2 body weight did not differ significantly between focal families and partner families (for individual weight -1.2 ± 3.4, p = 0.751, for mean body weight -3.4 ±3.8, p = 0.397). The difference in variability (for CV 3.2 ± 3.8, p = 0.448, for SD -0.18 ± 1.5, p = 0.904) and survival (-6.1 ± 3.1, p = 0.312) was also not significant. Hence, our results give no evidence for an effect of the size of kin group on body weight, uniformity, or survival.

5.4 Discussion

In this study we conducted an experiment to investigate whether relatedness affects performance, uniformity, and survival in domestic Nile tilapia by comparing two treatments: rearing of fish in kin groups *vs*. rearing in non-kin groups. We found that individuals from focal families grew significantly better in kin groups, which was observed on both individual and average group level. The difference in variability of body weight and survival, however, was not significantly different between the treatments.

5.4.1 Effects of relatedness

Results of our study show that individuals grew significantly better in groups composed of kin, suggesting that domestic Nile tilapia may exhibit kin-biased behavior. This finding is in agreement with previous studies in zebrafish larvae (Gerlach *et al.*, 2007) and juvenile Atlantic salmon and rainbow trout (Brown and Brown, 1996) that both showed improved growth in kin groups. As an indicator of kin-biased behavior, and possible increased cooperation among relatives, we used growth rate on individual and group level in both treatments, rather than fish behavior. In fact, little is known of whether tilapia species modify their behavior in the presence of relatives, except for black-chin tilapia which prefer to aggregate with kin (Pouyaud *et al.*, 1999). In Atlantic salmon and rainbow trout (Brown and Brown, 1996), an increase of body weight in kin groups has been associated with reduced aggressive behavior, while in zebrafish (Gerlach *et al.*, 2007) no such change in behavior was observed. In Nile tilapia we lack such behavioral studies, and therefore the cause of the observed increase in body weight in kin groups is unknown. On the one hand, fish may change their behavior as a reaction to chemosensory cues such

as odor, which does not necessarily require previous exposure to kin. On the other hand, individuals may express less competition towards each other simply due to familiarity. In our study, all fish were placed in the same fiber tank allowing for circulation of chemosensory cues across all families, and therefore, possibly avoiding changes in behavior due to chemosensory familiarity during the nursery period. However, individuals from the same full-sib family were placed in the same nursery hapa, separated from other families, which may have allowed them to recognize their relatives later in life based on visual familiarity. Observational studies on behavior of Nile tilapia in kin versus non-groups may help to unravel the mechanism underlying the effect on body weight found here.

The magnitude of the treatment effect on body effect found in our study may be biased to some degree due to inability to fit the effect of the column in our model, as it was fully confounded with the treatment effect. (Tanks were spatially organized in rows and columns, as illustrated in Table 5.1). The effects of the column in which tanks were placed have partly been accounted for by fitting the oxygen x batch interaction in the model (Model 1). The oxygen supply was the most noticeable difference between columns in the experiment. However it is difficult to say to what extent this effect accounts for all the variation between columns, and whether a part of the potential column effect might have been picked up by the treatment effect.

The relationship between competition and variability of trait values is well-documented in aquaculture (for example, Jobling, 1995; Hart and Salvanes, 2000). However in our study we did not find evidence of increased uniformity of body weight in kin groups *vs.* non-kin groups. The above mentioned studies on juvenile Atlantic salmon and rainbow trout (Brown and Brown, 1996) also assessed the effect of relatedness on variability by comparing coefficients of variation in weight changes between kin and non-kin groups. Their results showed less variability in weight gain in kin groups, which the authors associated with a lower level of aggressive interactions, especially those targeting slower-growing individuals. Beacham (1989) studied variability of growth in juvenile coho salmon, reared under different degrees of relatedness. In contrast to our study, Beacham (1989) found a higher within-family variability in families reared in full-sib groups, compared to families reared in mixed groups of 10 families. The differences in variabilities were attributed to differences in family growth rates and competitive ability, as fish from faster growing families may show lower within-family variance in mixed family groups due to their ability to out-compete slower growing families. In our study, however, we did not observe this effect.

5.4.2 Aquaculture breeding and farming

Aquaculture breeding programs in fish primarily aim to improve growth rate in populations by selective breeding. In GIFT Nile tilapia, substantial genetic gain has been achieved (>100%) through 16 generations of genetic selection for body weight at harvest (Ponzoni *et al.*, 2011; Khaw, 2015). Fish farmers, therefore, can significantly benefit from buying genetically improved fish. However, genetics is only one component of the phenotype; good managing procedures are necessary to express potential response in body weight. The findings of our study suggest that one such managing procedure could be to maintain a high level of relatedness within the rearing group. We found an increase in body weight of individuals growing in kin groups of ~9 grams, which is large compared to an average body weight of ~46 g and a standard deviation of ~22 g (Table 5.3). We only investigated two levels of relatedness – full-sibs and non-kin, i.e., close to 0 relatedness. Despite the large family sizes in aquaculture species, producing full-sib groups sufficiently large to fill an entire pond may be challenging in commercial production. More research is needed to investigate whether the use of a limited number of large families might yield similar benefits as found here for complete full-sib families.

In our experiment males were heavier than females, which is a common observation for GIFT Nile tilapia (for example, Ponzoni *et al.*, 2005). Heavier and faster growing males have drawn the attention of many researchers who advocate the production of 'all male Tilapia' (*e.g.,* Mair *et al.*, 1997). For such practices, grouping related individuals together during the grow-out may be especially beneficial, as we found that the effect of relatedness is much higher for males than for females.

5.5 Conclusions

Results of our study show that individuals grow significantly better in groups composed of kin, suggesting that domestic Nile tilapia exhibits kin-biased behavior. The effect of relatedness was higher for males than for females, beyond a simple scaling effect. This finding may be related to a lower level of competitive interactions among relatives, which are usually more expressed by males. Our results suggest that yields in aquaculture farming may be increased by rearing related individuals together.

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Supplementary material

Table S5.1. Estimated variances of analyzed traits with standard errors within the brackets (Results from ASReml).

Parameter	Individual body weight	Average body weight	SD of body weight	CV of body weight	Survival
Data		*Focal families, treatment 1&2*			
Model	1	2	2	2	3
Family	58.11 (50.1)	59.18 (47.72)	4.84 (4.93)	20.49 (16.39)	17.17 (24.51)
Tank	83.35 (20.38)	-	-	-	-
Row x Batch	3.07 (10.23)	-	-	-	-
Residual	163.92 (5.88)	91.73 (17.95)	20.91 (4.11)	46.58 (9.13)	212.75 (41.31)
Data		*Focal and partner families in treatment 2*			
Model	4	5	6	7	8
Family	16.30 (12.35)	3.05 (5.98)	-	17.06 (15.23)	2.74 (22.86)
Tank	15.50 (7.52)	-	-	-	
Row x Batch	8.42 (8.51)	10.68 (9.05)	-	-	4.39 (15.03)
Residual	171.12 (5.01)	28.25 (6.35)	15.31 (3.060)	48.23 (9.55)	118.18 (26.86)

6

General discussion

6.1 Introduction

Social interactions are common in nature and are an important part of the environment experienced by individuals. When individuals interact, their phenotypes may be affected by genes in their social partners. This heritable effect of a social partner on the trait value of the focal individual is known as an indirect genetic effect (IGE) (Griffing, 1967; Moore *et al.*, 1997). IGEs can also be interpreted as a genetic component in the social environment, i.e., the environment created by social partners. In the terms of classical quantitative genetic model, where the trait value of an individual is a function of genetic and environmental effects, $P = G + E$, the *E*-term is partly heritable when IGEs occur (Wolf *et al.*, 1998; Bleakley and Brodie IV, 2009; Bijma, 2014). However, the classical model assumes that the environmental effects are not heritable. Therefore, there was a need to extend the model to incorporate IGEs, which led to development of two modelling frameworks for IGE, variance component models and trait based models.

IGEs may not only affect the mean trait value, but also variation of the trait around its mean. In fish and some plant populations, competition has been shown to increase variability of trait values. In the past two decades, variability has been studied as a genetic trait in its own right. This trait is often referred to as inherited variability or heritable variation in environmental (residual) variance (SanCristobal-Gaudy *et al.*, 1998; Mulder *et al.*, 2007; Hill and Mulder, 2010). As social interactions are often a source of IGEs, the observed relationship between competition and variability on the phenotypic level (Jobling, 1995; Cutts et al., 1998; Hart and Salvanes, 2000; Denison et al., 2003) strongly suggested an underlying genetic relationship between the two phenomena. Here our knowledge, however, is quite limited, because despite the clear phenotypic relationship between competition and variability, inherited variability has not been connected to competition in quantitative genetic model. On the one hand, variance component and trait-based IGE models cannot fully explain the observed relationship between competition and variability. On the other hand, models for inherited variability treat variability as a property of a single individual.

In this thesis we studied genetics of inherited variability, with specific focus on the relationship between variability and competition, and the contribution of IGEs to genetic variation in variability.

In **Chapter 3** we proposed a quantitative genetic model that allows for indirect genetic effects to lead to differences in variability of trait values, similar to observations in real aquaculture and plant populations. Integrating IGE and inherited variability, and reasons why it was necessary to develop a new model, will be the first topic that I will address in this chapter.

In this thesis we studied genetics of inherited variability. In **Chapter 2** we investigated the genetic basis of variability in body weight and size in a domestic Nile tilapia population. **Chapter 3** & **4** focused on the relationship between variability and competition and how to capture genetic effects of competition on variability. In **Chapter 5** we investigated the effect of relatedness on the level of variability. Understanding the genetic basis of variability is important in animal and plant breeding, both from an economic and an animal welfare point of view. Breeding for uniformity is an analogue of the evolution of canalization in natural populations (Waddington, 1942). In evolutionary biology, canalization is studied for its role in phenotypic evolution (Flatt, 2005). Genetic changes in variability, therefore may have an important impact in both domestic and natural populations. Benefits and downsides of such impact will be next topic I will address.

Finally, I will conclude this chapter by giving perspectives for selection for uniformity, discuss the need for future studies, and possible applications of the model developed in **Chapter 3**.

6.2 Social interactions and inherited variability: bringing two worlds together

As mentioned above, traits affected by social interactions can be modelled using two theoretical frameworks, variance component models and trait based models. Both of these frameworks have been developed from maternal effects theory, which describes a special case of indirect genetic effects, where indirect effects of a mother on the phenotypes of offspring have a heritable component (Dickerson, 1947; Willham, 1963; Falconer, 1965; Cheverud, 1984; Kirkpatrick and Lande, 1989).

In the variance component model, the phenotypic value of the focal individual *i* (P_i), who interacts with a single social partner *j*, is a function of a direct genetic effect of the focal individual ($A_{D,i}$), an indirect genetic effect attributed to the social partner ($A_{I,j}$), and a residual (*e*) (Griffing, 1967):

$$P_i = A_{D,i} + A_{I,j} + e \quad (1)$$

In the trait-based model, the indirect genetic effect of the social partner on the trait value of the focal individual is modelled as a function of the trait value of the social partner. If the trait of interest and the trait causing the IGE are the same, the trait-based model (assuming interaction of two individuals) specifies the phenotypic value of the focal individual *i* as a function of the direct genetic effect of *i* (A_i), non-heritable effects of *i* (e_i), and the phenotype of social partner *j* (P_j) multiplied by an interaction coefficient, ψ (Moore *et al.*, 1997):

$$P_i = A_i + e_i + \psi P_j \quad (2)$$

In the original trait-based IGE-model, the ψ is a population parameter that describes the magnitude of IGEs, i.e., the strength of the social interaction, and is considered constant within a population.

The clear distinction between these models gives them certain advantages and disadvantages in the study of IGEs, depending on the research question and available data. For example, in the variance component model, the traits causing the IGEs do not need to be specified. Instead, the social effect is added to the model as a random genetic effect, and the indirect genetic variance is estimated based on genetic relationships in the data. The variance component model, therefore, gives estimates of direct and indirect genetic effects, but does not disclose the mechanism underlying the IGEs. Trait-based models, in contrast, require knowledge of the traits causing the IGE, but in return quantify the mechanism underlying the social interaction.

To understand the observations from aquaculture and plant populations, where competition for resources increases variability, in this thesis we wanted to integrate IGEs and inherited variability into a single model. Considering available IGE models and models for inherited variability for such study, we encountered the following issues :

1) current IGE-models and models for inherited variability cannot fully explain the observed relationship between competition and variability
2) the interaction coefficient ψ in the trait-based IGE model has the same value for all interacting individuals, i.e., it shows no flexibility

3) IGEs are usually applied to a "final" phenotype, whereas the effect of competition accumulates over time.

6.2.1 Modelling the relationship between competition and variability

In this section I will elaborate on issue number one, by showing the connection between the level of IGEs and variability, or the lack thereof, for each model.

In the variance component model (Equation 1), when pairs of interacting individuals are unrelated, phenotypic variance can be decomposed into the variance of direct genetic effects ($\sigma^2_{A_D}$), the variance of indirect genetic effects ($\sigma^2_{A_I}$), and the residual variance (σ^2_e):

$$\sigma^2_P = \sigma^2_{A_D} + \sigma^2_{A_I} + \sigma^2_e \quad (3)$$

From here it becomes clear that phenotypic variance is only affected by the variance of indirect genetic effects in the population, not by their level. This model, therefore, was not adequate for our research question, as observations from real populations show that competition and cooperation, i.e., sign of average level of IGEs, have a very different effect on variability, whereas variance is always positive and only gives insight in the variation of IGEs in the population around the mean. This was also demonstrated in **Chapter 4**, where indirect models for the trait capture only little of the genetic effects of competition on variability.

In the trait-based model, if we assume that P_i and P_j are the same trait, and that both individuals are both donor and recipient of social interaction, i.e., Equation 2 also applies to individual *j*, then the phenotypic variance on the population level can be derived as follows (Moore *et al.*, 1997):

$$P_i = A_i + e_i + \psi\left(A_j + e_j + \psi\, P_i\right) \quad (4)$$

$$(1 - \psi^2)P_i = A_i + e_i + \psi\left(A_j + e_j\right) \quad (5)$$

Solving the equation gives

$$P_i = \frac{A_i + E_i + \psi\,(A_j + E_j)}{1 - \psi^2}; \qquad P_j = \frac{A_j + E_j + \psi\,(A_i + E_i)}{1 - \psi^2} \quad (6)$$

And phenotypic variance equals

$$\sigma_P^2 = \frac{(1+\psi^2)(\sigma_A^2+\sigma_E^2)}{(1-\psi^2)^2} \quad (7)$$

When $|\psi|$=1, the phenotypic values and the phenotypic variance are undefined (Bijma, 2014). Note that Equation 7 gives the phenotypic variance in a population consisting of many interacting pairs of individuals, not the variance within a pair. Equation 7 shows that the level of ψ affects the phenotypic variance, however, the effect is symmetrical for positive and negative values of ψ, due to ψ^2 term in both the numerator and denominator. Figure 1, Panel A, illustrates how phenotypic variance changes with ψ. This differs from observations from real populations, where competition leads to increase of phenotypic variability, while cooperation decreases variability.

Now let us consider the variance within a pair ("group") of two individuals ($\sigma_{P_{wg}}^2$) **in the trait-based model**

$$\sigma_{P_{wg}}^2 = var\left(P - P_{average}\right) = \frac{1}{4}Var\left(P_i - P_j\right) \quad (8)$$

Using Equation 6, we can express $P_i - P_j$ as

$$P_i - P_j = \frac{(1-\psi)(A_i+E_i) - (1-\psi)\left(A_j+E_j\right)}{1-\psi^2} = \frac{(A_i+E_i) - \left(A_j+E_j\right)}{1+\psi} \quad (9)$$

The variance of $P_i - P_j$ in the trait-based model then becomes

$$Var(P_i - P_j) = \frac{2(\sigma_A^2+\sigma_E^2)}{(1+\psi)^2} \quad (10)$$

and the within-group variance equals

$$\sigma_{P_{wg}}^2 = \frac{1}{2}\frac{(\sigma_A^2+\sigma_E^2)}{(1+\psi)^2} \quad (11)$$

The final equation shows that the within-group variance depends on ψ rather than ψ^2, so that positive and negative values of ψ have different effect on within-group

variance, i.e., negative values lead to higher $\sigma^2_{\bar{P}_{wg}}$, and positive to lower $\sigma^2_{\bar{P}_{wg}}$. This is shown in Figure 1, Panel B, where an increase in ψ causes a drop in variability.

The b_{ij} in our model (**Chapter 3**) measures the effect of a difference in body weight between the social partner and the focal individual on the growth rate of the focal individual. The absolute value of b_{ij} reflects the strength of the social interaction, however *b* can have both positive and negative values. Negative *b* indicates competition, positive *b* cooperation, and an increase in *b* an increase of cooperation. An increase in cooperation in our model leads to a decrease in variability on both population and within-group level, as shown in Figure 2 in **Chapter 3**. Deriving expressions for phenotypic and within-group variance for our model is rather challenging, as the phenotype of the focal individual depends on the phenotypes from the previous time point of both social partner and focal individual. Therefore, in this chapter for our model I present the pattern of change of variability as a function of *b* numerically, by using data simulated in **Chapter 4** and fitting model with mean and random group effect to the final phenotype, i.e., phenotype at the last time point, using ASReml 4.1 (Gilmour *et al.*, 2015). This model gives estimates for within-group, between-group, and phenotypic variance, which were estimated for populations where average *b* is -0.05, 0, or +0.05 (Figure 6.1, Panel D-F).

Comparing our model with the trait-based model, we can see that the main difference occurs for the phenotypic variance. The change in within-group variance shows a similar pattern for both models. Since phenotypic variance includes both within- and between-group variance, the observed difference must be related to the latter.

Starting with the expression from Equation 6, the between-group variance for **trait-based model** is derived as follows:

The group average is given by

$$\bar{P} = \frac{P_i + P_j}{2} = \frac{(A_i + E_i + A_j + E_j)(1 + \psi)}{2(1 - \psi^2)} \qquad (12)$$

The between-group variance equals the variance of the group average,

$$\sigma_P^2 = \sigma_{bg}^2 = \frac{\frac{1}{2}(\sigma_A^2 + \sigma_E^2)(1+\psi)^2}{(1-\psi^2)^2} = \frac{\frac{1}{2}(\sigma_A^2 + \sigma_E^2)}{(1-\psi)^2} \quad (13)$$

Plotting σ_{bg}^2 for different values of ψ using Equation 13 shows an increase in between-group variance with an increase of ψ (Figure 1, Panel C). In our model (Figure 1, Panel F), however, we can see the decrease in the between-group variance. In conclusion, the relationship between competition and variability on the within-group level is modelled in a similar way in our model (**Chapter 3**) and the trait-based model. The main difference between the models can be seen on the population level, where the trait-based model shows symmetrical level of variability for positive and negative values of ψ, while our model shows decrease in variability with positive *b*. My expectation is that competition leads to higher variability on both within-group and population level, which has also been noticed for several species of fish (Mccarthy *et al.*, 1992; Jobling, 1995; Ponzoni *et al.*, 2005, 2011). Therefore our model depicts the co-evolution of competition and variability more realistically compared to ordinary trait-based IGE-models.

Finally, I will show that models for inherited variability fail to connect variability and the level of IGE, using the additive model as an example. The phenotypic value of the focal individual *i* in the classical model is a function of direct genetic effect of *i* on the mean ($A_{m,i}$) and direct environmental effect of *i* on the mean (E_i):

$$P_i = A_{m,i} + E_i \quad \text{or} \quad P_i = A_{m,i} + \chi\sigma_{E,i} \quad (14)$$

where χ is a standard normal deviate, χ~N(0,1) for the environmental effect. With genetic variation in environmental variance:

$$\sigma_{E,i}^2 = \sigma_E^2 + A_{v,i} \quad (15)$$

so that

$$P_i = A_{m,i} + \chi\sqrt{\sigma_E^2 + A_{v,i}} \quad (16)$$

where σ_E^2 is the mean environmental variance and $A_{v,i}$ is the direct genetic effect of *i* for environmental (residual) variance. Models for inherited variability, therefore only consider direct genetic effects of the focal individual on its own variability, ignoring a possible contribution of the social partner. We confirmed this observation

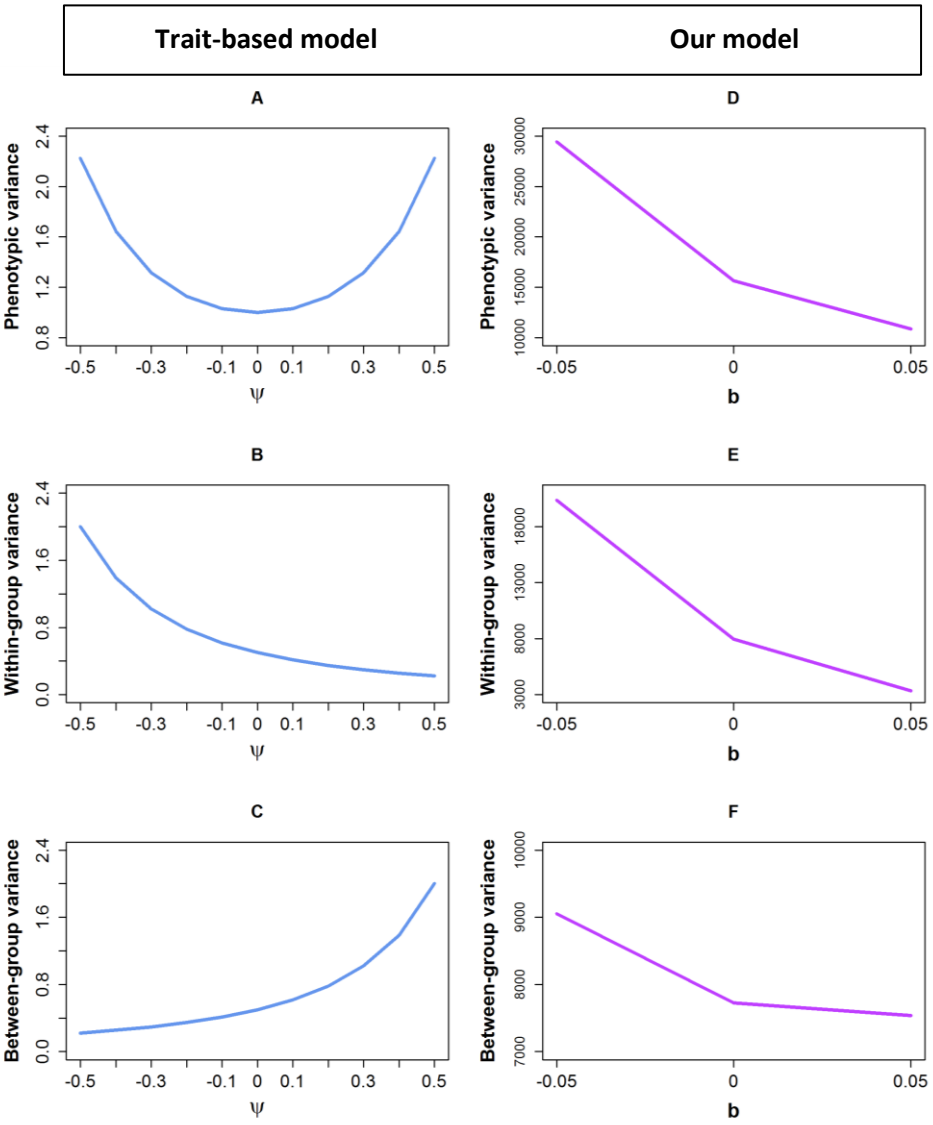

Figure 6.1 Pattern of change in phenotypic, within-group, and between group variance with change in ψ in trait-based model, and change in b in our model. Panels A, B, and C, were made using Equation 7, 11, and 13, receptively, assuming $\sigma_A^2 + \sigma_E^2 = 1$; Panels D, E, and F, were made using estimates from ASReml 4.1, averaged over 10 replicates for each value of b.

in **Chapter 4**, by applying a direct sire model for inherited variability to simulated data. The model captured almost entirely the direct genetic effects of competition (direct breeding values for *b*), but very little of the indirect genetic effect of competition.

6.2.2 Genetic variation in ψ

In the ordinary trait-based model, ψ is assumed to be constant, i.e., to have the same value for all interacting individuals. While done for simplicity, the assumption of constant ψ is rather crude and unrealistic. It is more likely that ψ varies within the population, meaning that ψ itself may respond to selection (Chenoweth *et al.*, 2010). Demonstrating genetic variation in ψ is a challenging task, but can be done, for example, by using multiple discrete genotypes, i.e., inbred lines. Relying on such data, Bleakley and Brodie IV (2009) estimated ψ in guppies and showed that it differs between the focal inbred strains. In addition, the level of ψ in some cases also depended on the social (partner) strain, suggesting that both focal and partner strain contribute to variation in ψ. Similarly, studies on chemical signaling in *D. melanogaster* (Kent *et al.*, 2008) and sexual display traits in *D. serrata* (Chenoweth *et al.*, 2010) also found variation in ψ.

In our study, we wanted to allow variability and competition to co-evolve. For that purpose, the *b* itself needed to be heritable. Inspired by the above-mentioned study on guppies, but also by a study on cannibalistic behavior in laying hens, which shows that such behavior depends on genetic effects of both the social partner (the pecker) and the victim (Ellen *et al.*, 2008), we modelled *b* as a composite quantitative genetic trait. In other words, *b* expresses genetic variation due to direct genetic effects of the focal individual and indirect genetic effects of the social partner. Related to our trait, it means, that the effect of a difference in body weight between the social partner and the focal individual on the growth of the focal individual, depends on genetic competitiveness of the social partner and genetic resistance to competition of the focal individual. Therefore, *b* shows genetic variation and can evolve, which facilitates research on evolution of trait variability due to changes in IGEs.

An additional issue with ψ comes from the feedback effect (Moore *et al.*, 1997; Bijma, 2014). The "feedback" refers to the situation where the "indirect" genetic effects of the focal individual affect its own trait value, indirectly through the social partner. For example, the level of aggression in the focal individual affects the level of aggression in the social partner, which subsequently affects the level of aggression in the focal individual. In those cases, ψ is not a true regression coefficient, because

P and *E* in Equation 2 are correlated (Bijma, 2014). The *b* in our model, however, is a true regression coefficient because the phenotype of the focal individual is affected by phenotype of the social partner from the previous time point, but not vice versa. Therefore, time-series data eliminates the problem of feedback.

6.2.3 Formation of variability

In many species, fitness of an individual depends on its size relative to the size of the other individuals (Smith and Brown, 1986). Fish that are larger often win fights, which allows them to acquire more resources (Huntingford *et al.*, 2012). Because probability of success in a competitive interaction between individuals depends on body size, individuals tend to modify their behavior based on their body size relative to that of social partner. Larger fish, therefore, are usually aggressive, while smaller ones are submissive (Huntingford *et al.*, 2012). In aquaculture, this causes the formation of a social hierarchy, where large fish are at the top of the hierarchy and have priority to feed, while subordinate fish show lower food intake and growth (Vera Cruz and Brown, 2007). As a consequence, dominant individuals show higher and more stable growth, compared to subordinate fish (Mccarthy *et al.*, 1992). Such high discrepancy in growth ultimately leads to increase of variation in body size in time, which has been observed on both group and population level (Jobling, 1995; Ponzoni *et al.*, 2005, 2011).

This brings us to the third issue related to IGE models – as evident from Equation 1 & 2, these models only consider IGEs on the final phenotype. Observations from aquaculture, however, show that variability develops over time. In our model we simulated growth curves in order to incorporate competitive effect of body weight on the growth of focal individuals and mimic the observations from aquaculture population, therefore giving a more realistic impression of how IGEs affect the level of variability. We did, however, for simplicity assume that direct and indirect genetic effects are the same at the different time points, which from biological perspective may not be true, i.e., the level of competition may differ between different stages of fish life.

6.2.4 Other traits

In trait-based models, the indirect effect on the phenotype of the focal individual depends on specific traits of the social partner. Therefore, the traits causing the effect, also known as effector traits, need to be identified. Such information is usually obtained from behavioral studies, and may involve more than one trait. In our model, the effector trait was the difference in body size between the social partner and the

focal individual, which was chosen based on findings in a number of studies on fish behavior (Huntingford *et al.*, 2012). However, other traits may be used as a predictor of variability of body weight instead, or in addition, to the difference in body size. Most likely, these would be traits related to feeding behavior or feed intake and feed efficiency, i.e., traits that affect growth of individual.

In **Chapter 3** & **4** we demonstrated our model using a fish population as an example. However the model may be applicable to other animals, and to plant populations, where a relationship between competition and variability has been observed. In those populations, effector trait(s) may be very different. For example, in domestic pigs variability of body weight can also be related to social hierarchy (Meese and Ewbank, 1973). Several studies suggested initial weight as a key trait for the rank of a certain individual, while higher body weight later in life may not give a competitive advantage (McBride *et al.*, 1964; Meese and Ewbank, 1973). In plants, traits such as height, branching, leaf area, length and branching of the root, determine the competitive ability of an individual (Denison *et al.*, 2003). The difference in level of these traits between social partner and focal individual may be used as an effector traits to investigate relationship between competition and variability. In addition to differences in trait values in plants, distance between interacting individuals would also be needed to take into consideration, as individuals close to each other may exhibit more competitive interactions compared to those that are spaced more distantly.

6.3 Benefits and consequences of selection for uniformity

The main focus of this thesis was on the relationship between competition and variability, which was inspired by observations from aquaculture and plant populations. However, the relationship between these two phenomena may already have existed long before the development of complex organisms and may have played a crucial role in the development of multicellularity.

To understand the evolution of cooperation, scientist often apply game theory, for example a “prisoner’s dilemma” game. According to the prisoner’s dilemma, when two individuals interact, three outcomes are possible: both individuals cooperate; one individual cooperates while other one cheats; both individuals cheat. The scenario where both individuals cooperate brings the highest payoff for both individuals, but that behavior evolves only under certain conditions. Steven A. Frank (2007) gives several examples to demonstrate how mutual cooperation may have

been a key component in the development of multicellular organisms. What I find interesting in these examples is how a high level of cooperation also goes with a high level of uniformity. Slime molds, for instance, live most of the time as single cells, but in certain situations, such as food shortage, they may form aggregations. These aggregates consist of two parts – reproductive cells that form spores, and stalk that raises spores up from the ground. It has been noticed that when these aggregations contain genotypes that are represented more in reproductive part rather than in stalks, i.e., cheating genotypes, the reproductive output of the whole aggregate is decreased because of lower stalk (Frank, 2007). Similarly, if genotypes produce cells in such way that they are equally represented in both parts, success of the whole aggregate is increased. Therefore, in slime molds, mutual cooperation leads to higher uniformity, and vice versa, and higher fitness. These cellular organizations can be considered as predecessors of multicellular organisms (Frank, 2007).

To avoid the possibility of cheating genotypes, multicellular organisms develop from a single-cell, so that all tissue cells are essentially clones. Mutations, however may happen, causing genetic variation and conflict within the tissue. If one of the genotypes has a competitive advantage compared to other, for example, faster cell growth, it may result in severe consequences, such as formation of tumors. Uniformity on the tissue level, therefore, is extremely important. Cell mechanisms such as DNA repair system and apoptotic control evolved to eliminate extreme phenotypes, but in addition genetic and environmental canalization may have had an important role in maintenance of uniformity against small changes in genome and environment (Flatt, 2005). Uniformity, therefore may have relevance for evolution of multicellular organisms and for the stable functioning of such organisms.

In natural populations, uniformity may arise through stabilizing selection for an optimal phenotype (Waddington, 1942; Wagner *et al.*, 1997; Flatt, 2005; Edgell *et al.*, 2009). If the phenotype is at, or near optimum, the variation around optimum is disadvantageous, and an increase in uniformity increases mean fitness of the population. In a study on within-family variance of fledging weight in the great tit, authors found evidence of stabilizing selection on within-family variance (Mulder *et al.*, 2016). In addition, their results show that families with a high or low within-family variance had lower fitness compared to families with an intermediate within-family variance. In some species of fish, uniformity in size, shape, and color, may have evolved through increase of survival of those individuals, as phenotypic similarity between fish that swim together make it difficult for a predator to focus on a single prey, which is known as “confusion effect” (Landeau and Terborgh, 1986). In

conclusion, evolution of uniformity/canalization, is often related to an increase in mean fitness of the population, irrespective of whether such populations consist of single cells or individual organisms.

In domestic populations, uniformity of animal products has a clear economic benefit (Hennessy, 2005). In some cases, an increase in uniformity may also lead to higher survival, for example for litter size in pigs (Sell-Kubiak *et al.*, 2015), and increased welfare, as in aquaculture where uniformity reduces competition and the need for grading (Khaw *et al.*, 2016).

While a reduction of variation may be beneficial, a loss of phenotypic variation may also hinder phenotypic evolution and reduce the ability of a population to adapt to a changing environment (Wagner *et al.*, 1997; Flatt, 2005), which is especially relevant for natural populations. However, while phenotypic variation may be low, the underlying genetic variation may accumulate because it is hidden from the force of natural selection (Wagner *et al.*, 1997; Flatt, 2005). Under extreme environmental conditions, a genotype may become "decanalized", causing more rapid evolution (Flatt, 2005). For example *Drosophila* heat-shock protein Hsp90 buffers genetic variation, unless a stressful environment occurs, such as change in temperature. Buffering ability then becomes compromised and may lead to the expression of new phenotypes (Rutherford and Lindquist, 1998). These results illustrate that phenotypic canalization can go together with the maintenance of heritable variation, so that canalization does not necessarily threaten adaptive potential.

6.4 Future perspectives

Selection for uniformity of body weight in aquaculture could lead to increased profit by producing more fish in the size range that is favored by the consumers, and reducing the need for frequent grading of the fish during the grow-out period, which bares not only financial benefits but also benefits for the welfare of the fish.

Results of theoretical and empirical studies on inherited variability suggest that variability could be reduced by means of genetic selection. However, selection experiments to improve uniformity are scarce, and are mostly limited to laboratory populations (Rendel *et al.*, 1966; Kaufman *et al.*, 1977; Argente *et al.*, 2008; Boldin *et al.*, 2012; Blasco *et al.*, 2017). Findings of **Chapter 2**, together with estimates of genetic variation in variability in several other species of fish (Janhunen *et al.*, 2012; Sonesson *et al.*, 2013; Sae-Lim, Gjerde, *et al.*, 2015; Sae-Lim, Kause, *et al.*, 2015),

suggest that aquaculture populations are suitable to validate the estimated genetic parameters by a selection experiment.

Given the finding of **Chapter 3**, two selection experiments could be performed. A first experiment, where selection is based only on direct genetic effects on variability, and a second experiment where selection involves both direct and indirect genetic effects on variability. These experiments could give us insight into how much of genetic variation in variability could be attributed to variation in IGEs. The experiments should have a group structure with, e.g., two individuals in a group, similar to our simulated data in **Chapter 3** & **4**. However, subsequent trials involving larger group sizes may also be conducted to test whether the magnitude of effects of competition change with an increase of group size. Data on both individuals in each group should be collected at several time points. Time-series data would allow to use random regression approach as suggested in **Chapter 3**, but also the direct model and the indirect model for inherited variability presented in **Chapter 4**. Half sib – full sib designs, similar to that proposed in **Chapter 4**, with multiple observations of within-family variance per sire, and individuals from the same family in both experiments, could be used for estimation of direct and indirect genetic effects of competition. Validation and comparison of the models using real data could make a significant contribution to optimization of methods and models for future studies aiming to estimate genetic effects of competition.

Ideally, these experiments should be performed on aquaculture populations. However, large scale experiments using commercial fish stocks may require considerable investments in finances, facilities, labor, and time. Alternatively, the two proposed selection strategies could be compared by using zebrafish as a model organism. Zebrafish show fast growth and a substantial level of competition, they are small, robust, and easy to maintain. Even though they are not commercial fish, they could elucidate possibilities to improve uniformity in aquaculture, and give an impression of how much IGEs could contribute to the evolution of uniformity. In addition, the genome of the zebrafish has been fully sequenced at high quality, which would facilitate research on genetic and molecular mechanism underlying inherited variability.

One of the main obstacles in incorporating uniformity in aquaculture breeding programs is often high and positive genetic correlation between level and variance of harvest weight, meaning that selection for uniformity will cause decrease in selection response in body weight, which is highly undesirable, especially giving the

low economic value of uniformity (Janssen *et al.*, 2017). It would be interesting to see how indirect genetic effects for *b* correlate with genetic effects for body weight, and whether selection on IGEs only, could be used to improve uniformity, without consequences for growth.

In **Chapter 3** & **4** we suggested approaches to estimate genetic effects of competition, more specifically how direct and indirect genetic effects on *b* could be estimated for each individual. In **Chapter 3** we indicate that random regression could be used to estimate genetic components of *b*, using group-structured population and time series data, while in **Chapter 4** we tested models which are only applied to the final phenotype of individuals within group, therefore avoiding need for multiple observations. Such specific type of data may not be easily available, especially for fish growing in commercial setting. However, with the development of new phenotyping techniques that involve video tracking of individuals in 3D space, generating such data could become common practice (see for example idTracker, http://www.idtracker.es/). These techniques would give multiple observations on individual trait values (for example body weight calculated from the 3D image, i.e., volume of the individual) and information on social interactions between individuals.

In **Chapter 3** & **4** we proposed a model for interaction of two individuals, and discussed how our model could be extended to incorporate IGEs of multiple individuals on the growth of the focal individual. With an increase of group size, IGEs of an individual may show a so-called dilution effect, i.e., decrease in magnitude, due to less time spent in interacting with each of its group mates (Bijma, 2010). Dilution of IGEs does not always happen with increase of group size, for example, alarm signaling in birds will have a similar effect in small and large groups. However, with traits such as growth, where the amount of food is limited, dilution is likely to happen. One main assumption of the dilution effect is that social partner interacts with all group members and in equally intensity, hence IGEs get diluted over a large number of individuals. However for large groups, my expectation is that individuals will interact mostly with small number of same/familiar individuals. This would lead to partitioning of a large group into small sub-groups, so that IGEs might not become heavily diluted. I believe identification of such sub-groups could also be possible with new phenotyping techniques, once they scale up to simultaneously track larger numbers of individuals, which is one of the main future goals of the developers of such technologies.

6.5 Conclusions

To overcome issues of current IGE models and models for inherited variability, integrating social interactions and inherited variability required development of a new model, which was presented in this thesis. The model allows for competition and variability to co-evolve, suggesting that uniformity could be increased through improvement of direct and indirect genetic effects. Estimation of genetic effects of competition requires group-structured data, and also observations from multiple time points in case of estimation with random regression. With development of new phenotyping techniques such data may become commonly available, facilitating application of our model. Ideally, contribution of IGEs to evolution of variability should be quantified in a selection experiment.

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Summary

Summary

Social interactions are common in nature and are an important part of the environment experienced by individuals. In the traditional quantitative genetic model, the phenotype of an individual is determined by the direct effect of its own genes and an environmental effect. With social interactions, however, the phenotype of an individual may also be affected by genes of its social partners. Such effects are known as Indirect Genetic Effects (IGE). IGEs can contribute substantially to heritable variation underlying the trait, and may even reverse the direction of response to selection. A related topic is the inheritance of phenotypic (or residual) variability. The variability of trait values of a genotype, measured either repeatedly on the same individual, or on multiple individuals belonging to the same family, has been studied as a quantitative trait in its own right. This trait is often referred to as inherited variability, heritable variation in environmental variance, or environmental canalization. Results demonstrated substantial genetic variation in variability for many traits. In some species, IGE and inherited variability are related via competition. In aquaculture species and some plants, for example, competition inflates variation of trait values among individuals.

As social interactions are often a source of IGEs, the observed relationship between social interactions and variability on the phenotypic level, strongly suggests an underlying genetic relationship between the two phenomena, of which very little is known. The main objective of this thesis, therefore, was to study the genetics of inherited variability and possibilities for its genetic improvement, focusing primarily on the relationship between competition and variability, and using Nile tilapia as a model species.

In Chapter 2 we investigate the potential for genetic improvement of inherited variability of harvest weight and body size traits in a domestic Nile tilapia population. We analyzed within-family variance of harvest weight, body length, depth, and width, by applying a double hierarchical generalized linear models to individual trait values. Our results showed substantial genetic variation in variability of all analyzed traits, suggesting good prospects for the genetic improvement of uniformity by means of genetic selection. For example, residual variance of harvest weight could be reduced by 58 % with one generation of selection, while proportional change in phenotypic variance would be 36 %. Selection for lower variability of harvest weight in Nile tilapia, however, would come with a consequence on the level of harvest weight, due to high and positive estimated genetic correlation between the two.

Not only direct, but also indirect genetic effects may contribute to genetic variation in variability, as hinted by observations from real populations. In Chapter 3 we make a first step towards understanding the genetic relationship between social interactions and variability, by presenting a quantitative genetic model that integrates both phenomena. In our model, competition between social partners leads to divergence of their phenotypes (e.g., body weight) over their life time. The effects of competition in our model are heritable, and therefore, can evolve. These effects comprise direct genetic effect of the focal individual and indirect genetic effect of its social partner. Simulation results show that our model yields increased variability of body weight with increase of competition, similar to what is observed in real aquaculture populations. Selection for cooperation, i.e., lower competition, will therefore lead to decreased variability. These findings suggest that we may have been overlooking an entire level of genetic variation in variability, the one due to IGEs.

To exploit genetic variation in inherited variability originating from IGEs, we need statistical models to capture this effect. In Chapter 4 we investigate the potential of current statistical models for inherited variability and trait values, to capture the direct and indirect genetic effects of competition on variability. Our results show that a direct model of inherited variability almost entirely captures the direct genetic effect of competition on variability, as illustrated by high correlations between estimated genetic effects and simulated direct breeding values. Similarly, an indirect model of inherited variability captures indirect genetic effects of competition. Models for trait levels, however, capture only little of the genetic effects of competition. Capturing genetic effects of competition, therefore could be possible with direct and indirect models of inherited variability, but may require a two-step analysis.

According to kin selection theory, genetic relatedness should influence social behavior, because individuals able to interact differently with kin *vs.* non-kin would have higher inclusive fitness. In addition to fitness benefits in natural populations, reduced competition may also lead to increased performance in agricultural populations. One potential way to reduce competition and increase yield and uniformity of trait values in Nile tilapia is to utilize the consequences of past kin selection, i.e., the evolution of kin discrimination and cooperative behavior among relatives. In this study we compared two experimental treatments: rearing of fish in kin groups *vs*. rearing in non-kin groups, in order to investigate whether relatedness affects performance traits in domestic Nile tilapia. We analyzed average body

weight, standard deviation and CV of body weight, and survival, between the two treatments. Results of our study show that individuals had significantly higher body weight in groups composed of kin (8.6 ± 2.6 g), indicating that domestic Nile tilapia may exhibit kin-biased behavior. However, there was no difference in variability of body weight and survival between the two treatments.

In Chapter 6, I showed why integrating social interactions and inherited variability required development of a new model, and what are the advantages of the new model, compared to current IGE models and models for inherited variability. The most striking difference between the models comes from modelling of relationship between competition and variability. IGE models and models of inherited variability cannot fully explain this relationship between competition and variability as observed in real population, especially on the population level. Our model, however, allows for indirect genetic effects to lead to differences in variability of trait values, on both group and population level. Furthermore, in this chapter I discussed benefits and consequences of selection for uniformity, and proposed future empirical studies that could give insight into biological relevancy of the theoretical possibility that IGEs contribute to genetic variation in variability.

About the author

About the author

Jovana Marjanović was born on 8th of February 1987 in Bijeljina, Bosnia and Herzegovina. In 2010 she obtained her bachelor degree in Molecular Biology from the University of Novi Sad, Republic of Serbia, with specialization in human physiology. The following year she pursued her master degree in Molecular Biology at the same University, with focus on Molecular Genetics. For her master thesis she studied internal transcribed spacer II (ITSII) and cytochrome oxidase I (COI) as potential barcodes to be used to study phylogenetic relationships among members of the hoverfly genus Merodon (Diptera, Syrphidae), in collaboration with the Finnish Museum of Natural History. After her graduation, in 2013 she was accepted as a PhD candidate in the European Graduate School in Animal Breeding and Genetics program. Her PhD project was a result of a collaboration between Wageningen University & Research and Swedish University of Life Sciences. Jovana had the opportunity to work in both universities, and in addition, in 2016 she has spent one month at WorldFish, in Penang, Malaysia, as a visiting scientist. During her PhD Jovana studied genetics of inherited variability, particularly the relationship between competition and inherited variability. She developed a quantitative genetic model that integrates both phenomena and described possibilities to capture the genetic effects of competition on variability. The results of her research over the course of her PhD are presented in this thesis. In 2017 Jovana started working as a postdoc at Wageningen Livestock Research on the “ReDiverse” project, that aims to increase resilient and competitive use of European Red dairy breeds.

Publication list

Peer reviewed papers

Marjanovic J, Mulder HA, Khaw HL, Bijma P (2016). Genetic parameters for uniformity of harvest weight and body size traits in the GIFT strain of Nile tilapia. *Genet Sel Evol* 48: 41.

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Manuscripts in preparation

Marjanovic J, Mulder HA, Rönnegård L, Koning DJ, Bijma P. Capturing indirect genetic effects on phenotypic variability: Competition meets canalization. *To be submitted.*

Marjanovic J, Mulder HA, Khaw HL, Bijma P. Effects of relatedness between group mates on body weight and variability of body weight in domestic Nile tilapia. *To be submitted.*

Conference proceedings

Marjanovic J, Mulder HA, Khaw HL, Bijma P. Genetic parameters for within-family variance of harvest weight in Nile tilapia (Oreochromis niloticus). 10th WCGALP, Vancouver, Canada, 2014, 273.

Marjanovic J, Mulder HA, Khaw HL, Bijma P. Genetic parameters for uniformity of harvest weight in the GIFT strain of Nile tilapia estimated using double hierarchical generalized linear models. ISGA XII, Santiago de Compostela, Spain, 2015.

Marjanovic J, Mulder HA, Khaw HL, Bijma P. Genetic Heterogeneity of Residual Variance in GIFT Nile tilapia. 66th EAAP, Warsaw, Poland, 2015, 21:217.

Marjanovic J, Mulder HA, Bijma P. Modelling the relationship between social interactions and inherited variability. 5th ICQG, Madison, Wisconsin, 2016.

Marjanovic J, Mulder HA, Rönnegård L, Bijma P. Modelling the co-evolution of indirect genetic effects and inherited variability. Gordon Research Seminar and Gordon Research Conference in Quantitative Genetics & Genomics, Galveston, Texas, 2017.

Training and supervision plan

Training and supervision plan

The basic package (7 ECTS)	
EGS-ABG Introduction course, Addis Ababa, Ethiopia	2013
EGS-ABG Summer Research School - Sustainable animal breeding and food security, Addis Ababa, Ethiopia	2013
Research ethics, Uppsala, Sweden	2016
EGS-ABG Fall Research School - Emerging technologies in animal breeding, Wageningen, The Netherlands	2017

Scientific exposure (16 ECTS)	
International conferences	
10th WCGALP, Vancouver, Canada	2014
ISGA XII, Santiago de Compostela, Spain	2015
66th EAAP, Warsaw, Poland	2015
5th ICQG, Madison, USA	2016
Gordon Research Seminar, Galveston, Texas	2017
Gordon Research Conference, Galveston, Texas	2017
Seminars and workshops	
WIAS Science Day, Wageningen, The Netherlands	2014
Workshop Carousel, Wageningen, The Netherlands	2014
WIAS Science Day, Wageningen, The Netherlands	2015
Societal impact, Wageningen, The Netherlands	2015
Aquaculture workshop, Penang, Malaysia	2016
Presentations	
10th WCGALP, Vancouver, Canada, oral	2014
ISGA XII, Santiago de Compostela, Spain, oral	2015
66th EAAP, Warsaw, Poland, oral	2015
5th ICQG, Madison, USA, poster	2016
Gordon Research Seminar, Galveston, Texas, oral	2017
Gordon Research Conference, Galveston, Texas, poster	2017

In-depth studies (19 ECTS)	
Disciplinary and interdisciplinary courses	
Introduction to theory and implementation of genomic selection, Wageningen, the Netherlands	2014
NOVA course - Linear models in animal breeding, Lofoten, Norway	2015
In depth course genotype by environment interaction, uniformity and stability, Wageningen, The Netherlands	2015
Advanced statistics courses	
Advanced statistical and genetical analysis of complex data using ASReml 4, Wageningen, The Netherlands	2014
Modern statistics for the life sciences, Wageningen, the Netherlands	2014
PhD students' discussion groups	
Quantitative genetics discussion group, Wageningen, The Netherlands	2013-2016, 2017
Quantitative genetics study days, Uppsala, Sweden	2016-2017
MSc level courses	
Genetic improvement of livestock, Wageningen, The Netherlands	

Professional Skills Support Courses (3 ECTS)	
Techniques for writing and presenting scientific paper	2015
Presenting with Impact	2015
Career assessment	2017
Data management planning	2017
Reviewing a scientific paper	2017

Research Skills Training (2 ECTS)	
Getting started with ASReml	2014
External training period - SLU, Sweden & WorldFish, Malaysia	2016-2017 & 2016

Didactic Skills Training (5 ECTS)	
Supervising practicals	
Animal breeding and genetics	2014-2015
Supervising theses	
BSc thesis	
Management Skills Training (1 ECTS)	
Organization of seminars and courses	
Aquaculture round table meeting	2015
Education and Training Total	**53 ECTS**

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Four years have passed. PhD done. I can't believe it! It seems as if just yesterday I walked into WUR campus and started this whole adventure. It was not always easy, but all in all, I had a great time. And I have many of you to thank for that. I will try to do you justice here.

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Colophon

Colophon

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