GENETICS OF SURVIVAL IN CANNIBALISTIC LAYING HENS

THE CONTRIBUTION OF SOCIAL EFFECTS

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Abstract

Mortality due to cannibalism in laying hens is a worldwide economic and welfare problem occurring in all types of commercial poultry housing systems. Due to prohibition of beak-trimming and the traditional battery system in the European Union in the near future, mortality due to cannibalism in laying hens may increase. To reduce mortality in laying hens, it is possible to use genetic selection. Mortality due to cannibalism, however, depends on social interactions between group members. Traditional selection methods neglect these social interactions, meaning that they ignore the genetic effect an individual has on its group members. These methods are, therefore, not very effective. The main aim of this thesis is to investigate the effect of social interactions on the heritable variance in mortality due to cannibalism in laying hens and to develop a selection method that takes into account social interactions.

To investigate the effect of social interactions on the heritable variance in mortality due to cannibalism, genetic parameters for direct and associative effects on survival time in three layer lines were estimated. For all three layer lines it was found that social interactions contribute approximately two-third of the heritable variation in survival time. The heritable variation in survival time is, therefore, substantially larger than suggested by the traditional methods currently used in poultry breeding.

To improve traits affected by social interactions in laying hens, a solution is to select individually housed candidates based on the performance of their full sibs kept in family groups. Theoretical results suggest that this selection method offers good opportunities to improve traits affected by social interactions. A selection experiment was applied aiming to improve mortality due to cannibalism in laying hens using selection based on relatives. After one generation, mortality was 10% lower in the selection line compared to the control. In the second generation, no significant effect was found, which seemed to be related to environmental factors.

Results in this thesis suggest that prospects for reducing mortality due to cannibalism by means of genetic selection are good. Using selection methods that incorporate social interactions may lead to substantial reduction of one of the major welfare problems in egg production. Further research is needed to investigate the effect of group size and kin recognition on social interactions.

Voorwoord

Op het moment dat ik dit voorwoord schrijf, werk ik alweer vier jaar aan het project ”The genetics of robustness in laying hens”. De afgelopen vier jaar zijn voorbij gevlogen en heb ik het erg naar m’n zin gehad. Dit komt mede door een heleboel mensen die ik hieronder graag even in het bijzonder wil bedanken.

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Graag wil ik nog één keer tegen iedereen zeggen

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Chapter 1

General introduction

INTRODUCTION

Mortality due to cannibalism in laying hens is a worldwide economic and welfare problem occurring in all types of commercial poultry housing systems (Blokhuis and Wiepkema, 1998). The term cannibalism is used to denote damage of an individual caused by its group members, sufficiently severe to cause its death (Allen and Perry, 1975). Cannibalism can be either the final phase of feather pecking (Schaible et al., 1947) or it can be the so-called cloacal cannibalism. The last mentioned is quite unrelated to feather pecking (Hughes and Duncan, 1972; Allen and Perry, 1975). Cannibalism and feather pecking are universally recognized as behaviours that are a disadvantage for the well-being of laying hens (Craig and Muir, 1996). Due to prohibition of beak-trimming and the traditional battery system in the European Union in the near future, mortality due to cannibalism may increase if no further actions are taken, and needs to be solved urgently.

There are two strategies to reduce mortality due to cannibalism: management and breeding. Management strategies are beak-trimming, decrease of light intensity, change of feed composition, environmental enrichment, and optimizing group size (Appleby et al., 2004; Hester, 2005; Van Krimpen, 2008). Although much research has focused on improvement of management factors, problems of mortality due to cannibalism are still not solved. Furthermore, management factors used to reduce cannibalism, such as beak-trimming and low light intensity, have been associated with welfare problems (Gentle, 1986; Manser, 1996; Jones and Hocking, 1999). There is, therefore, a need to use selective breeding against mortality due to cannibalism.

The trait mortality due to cannibalism depends on the behaviour of group members, and is an example of a trait affected by social interactions. Social interactions among individuals affect the outcome of domestic breeding programs and evolutionary processes (Frank, 1998; Muir, 2005) and can have profound influences on the expression of performance and welfare traits (Muir, 1996; Brichette et al., 2001; Denison et al., 2003; Muir, 2005; Bijma et al., 2007a). There is clear evidence that social interactions contribute to the heritable variation in traits (Wade, 1976; 1977; Moore, 1990; Muir, 1996; Brichette et al., 2001; Wolf, 2003; Muir, 2005; Bijma et al., 2007b; Bergsma et al., 2008). Using selective breeding, animal breeders have successfully improved many traits of agricultural importance. Traits that are affected by social interactions, such as mortality due to cannibalism, are, however, difficult to improve using traditional selection methods, like mass or individual selection (Muir and Cheng, 2004). In some cases, it has been found that selection for traits affected by social interactions resulted in response in the opposite

direction than expected (Wade, 1976; 1977; Craig and Muir, 1996). These unexpected results occur because traditional selection methods target only the direct effect of an individual's genotype on its phenotype and neglect social interactions among individuals (also known as associative effects). For this reason, traditional selection methods can result in a negative effect on the group mean trait value (Muir and Cheng, 2004). For example, laying hens that have good genes for survival could have aggressive and competitive behaviour, which reduces survival of their group members (Muir and Cheng, 2004). Negative associative effects can be due to a number of factors, including competition for limited resources, high densities (Brichette et al., 2001), social dominance (Craig et al., 1965; Craig and Toth, 1969), and pecking order (McBride, 1960).

To improve animal well-being and performance and to select against mortality due to cannibalism in laying hens, it is critical to understand how to improve social interactions among individuals (Denison et al., 2003; Muir, 2005). Griffing (1967) showed that, with social interactions, the traditional quantitative genetic model must be extended to include not only the direct effect of an individual's own genotype, but also the associative contributions from other genotypes in the group. Selection of groups rather than individuals is a method to capture associative effects of genotypes. Using group selection, Muir (1996) found that mortality of laying hens decreased from 68% in the second generation to 8.8% in the sixth generation. Implementing group selection in commercial poultry breeding, however, is not possible, because selection candidates are housed individually, which is necessary to record data on an individual basis (like egg production). Therefore, a new selection method is needed to improve mortality due to cannibalism in laying hens, while keeping selection candidates individually.

AIM AND OUTLINE OF THIS THESIS

The main aim of this thesis is to investigate the effect of social interactions on the heritable variance in mortality due to cannibalism in laying hens and to develop a selection method that takes into account social interactions.

Chapter 2 and 3 deal with the estimation of genetic parameters for direct and associative effects on survival in laying hens. In Chapter 2, genetic parameters are estimated using a linear animal model including social interactions. In chapter 3, genetic parameters are estimated using a two-step approach and results are compared to those of the linear animal model. For the two-step approach, survival analysis and a linear animal model including social interactions are combined.

Chapter 4 deals with the development of a new selection method to improve traits affected by social interactions, while keeping selection candidates individually. In Chapter 4, expressions for the accuracy of individual and group selection are derived. Furthermore, the opportunity to improve traits affected by social interactions by using information on relatives kept in family groups, while keeping selection candidates individually is investigated. Selection responses obtained with this new method are compared with selection responses obtained with individual and group selection.

Chapter 5 deals with the effect of social interactions in laboratory populations. An experiment with flour beetles (*Tribolium castaneum*) is shown, to investigate the effect of food competition on pupal body weight and duration of development. This was a pilot experiment and results will be used to set up a larger experiment, to estimate genetic parameters for direct and associative effects on pupal body weight in flour beetles.

Chapter 6 deals with ethical issues concerning the implementation of robustness into a breeding goal. In this chapter, the relation between the concept of robustness and the concepts of health, welfare and integrity is discussed. A similar approach can be used when implementing social interactions into a breeding goal.

In the general discussion, two topics are discussed, what can we learn from evolutionary biology? And can we improve traits affected by social interactions in livestock? In the first part of the general discussion, I will explore theoretical work in the field of evolutionary biology and its potential application in the field of animal breeding. In the second part of the general discussion, I argue that it is possible to improve traits affected by social interactions, such as mortality due to cannibalism in laying hens, using the new selection method described in Chapter 4.

THE GENETICS OF ROBUSTNESS IN LAYING HENS

The research presented in this thesis is part of a larger project "the genetics of robustness in laying hens." This project was in cooperation with one of the main breeding companies of laying hens, Hendrix Genetics. The main objective of this project was to identify parameters that are indicative for robustness in laying hens. These parameters could be used in a breeding program to improve robustness of laying hens. A robust laying hen can be defined as 'an animal that has the potential to keep functioning and take short periods to recover under varying environmental circumstances' (Chapter 6).

In the near future, the poultry industry in the European Union will be faced with some changes in environmental circumstances. Laying hens will be kept in larger groups, because

the traditional battery system will be forbidden. Furthermore, beak-trimming will be forbidden. Based on these legislations, the breeding company formulated three priorities for future poultry, which were addressed in three PhD projects:

- Do not peck with intact beak
- Keep functioning/producing at high temperatures
- Keep functioning/producing at high disease challenge

A robust laying hen will be able to cope with these environmental changes. Robustness was assumed to be related to genetic differences between laying hens in behavioural, immunological and physiological responses towards environmental stressors (Uitdehaag, 2008). The three PhD-projects focussed, therefore, on either a genetic (this thesis), behavioural (Uitdehaag, 2008), or immunological (Star, 2008) approach. A summary of the behavioural and immunological approach is given in the next two paragraphs. Both paragraphs have been taken from Uitdehaag et al. (2008).

Behavioural approach

A robust laying hen is considered to show no feather pecking. Laying hens from different purebred lines show differences in feather damage due to severe feather pecking, indicating that it is possible to select against feather pecking (Uitdehaag et al., 2008b). In addition, feather damage due to severe feather pecking and fear related behaviour were found to be affected by group members. Non-fearful birds became more fearful in presence of fearful birds (Uitdehaag et al., 2008a), whereas fearful birds showed more feather damage in presence of non-fearful birds (Uitdehaag et al., 2009). This indicates that fearful behaviour predisposes birds to develop more easily feather pecking and to be more targeted by feather pecking of group members. These results indicate that reducing the expression of feather pecking could probably be achieved by breeding against expression of fearful behaviour.

Immunological approach

Effects of genetic background, environmental conditions, and early-life experiences on immunological parameters were investigated. Birds from different genetic background clearly differ in their immunological and physiological response to high temperatures and disease challenge (Star et al., 2007b). Results further indicated a predictive value for the level of natural antibodies (indicative for the innate immune system) binding to KLH (Keyhole Limpet Hemocyanine; a protein which laying hens will normally never encounter) for survival of laying hens (Star et al., 2007a). Since natural antibodies have a

moderate heritability, it might be possible to select for this trait. Selection on innate immune parameters will probably not be on the expense of hen-day egg production, because these traits are most likely not correlated. The main conclusion of Star (2008) was, therefore, that implementation of selection for natural antibodies into a breeding goal might improve robustness of laying hens.

Ethics of robustness

Societal concern exists about adapting animals to their environment. Indeed, management actions like vaccination are important in order to improve health and welfare of laying hens. The question, however, is to what extent animals can be adapted. Breeding for improved disease resistance or for reduced occurrence of damaging behaviours can enhance the animal's ability to cope with environmental challenges, which, however, could be achieved at the cost of the integrity of the animals (Uitdehaag, 2008). Irrespective of a possible loss of integrity, breeding for robustness can have positive effects on health and welfare. These ethical issues are addressed in Chapter 6, in which it is argued that it is ethically justifiable to incorporate robustness traits into a commercial poultry breeding program.

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Chapter 2

Survival of laying hens: Genetic parameters for direct and associative effects in three purebred layer lines

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ABSTRACT

Mortality due to cannibalism is a major problem in laying hens. Due to prohibition of beak-trimming in the European Union, this problem will increase in the near future. One solution to reduce mortality due to cannibalism is to use genetic selection. Mortality due to cannibalism, however, differs from conventional breeding traits, because it depends on social interactions among individuals. Selection strategies aiming to reduce cannibalism, therefore, should consider both the direct effect of an individual on its own survival and the social effect of the individual on the survival of its group members (the so-called associative effect). Traditional breeding, however, accounts for only the direct effect. Recently, methods have been proposed to estimate variance components and breeding values for both direct and associative effects. This paper presents estimated genetic parameters for direct and associative effects on survival days in three purebred layer lines. For the analysis 16,780 hens with intact beaks were used. When considering only direct effects, heritabilities ranged from 2% through 10%. When considering both direct and associative effects, the total heritable variance, expressed as a proportion of phenotypic variance, ranged from 6% through 19%. These results show that heritable variation in survival days is substantially larger than suggested by traditional linear animal models. This means that prospects for reducing mortality by means of genetic selection are good and may lead to substantial reduction of one of the major welfare problems in egg production.

Keywords: social interactions, variance component estimation, laying hen, survival, indirect genetic effects

INTRODUCTION

Social interactions among individuals can have profound influences on the expression of performance traits like production and welfare traits in domestic livestock populations (Bijma et al., 2007b; Brichette et al., 2001; Denison et al., 2003; Muir, 1996; Muir, 2005). For instance, social interactions can reduce growth due to competition for limited resources or can result in mortality due to cannibalism. The latter is seen in laying hen production systems. Mortality due to cannibalism is an economic and welfare problem occurring in all types of commercial poultry housing systems (Albentosa et al., 2003). Furthermore, due to prohibition of beak-trimming in the European Union in the near future, this problem may increase. One of the possibilities to reduce mortality due to cannibalism is to use genetic selection (Jones and Hocking, 1999; Muir, 1996).

Mortality due to cannibalism is caused by social interactions among group members. Wolf (2003) mentioned that the environment provided by group members is often the most important component of the environment experienced by an individual in that group. Although the interaction between group members may appear to be purely environmental, they differ from other sorts of environmental influences, because they can have a genetic basis (Wolf et al., 1998; Wolf, 2003). Traditional breeding, using mass selection or selection based on information of relatives, has mainly focused on improving the direct effect of the individual's genotype on its phenotype (except for maternal effect models). With the exception of maternally affected traits, traditional breeding has neglected the social effect of an individual on the phenotypes of its group members. This social effect is often referred to as an associative effect (Griffing, 1967). When the objective is to improve traits affected by interactions among individuals, the use of traditional models can result in response to selection in the opposite direction (Griffing, 1967). For instance, Wade (1976) showed that individual selection for increased population size of flour beetle (*Tribolium castaneum*) decreased population size in the next generation. To improve traits affected by interactions among individuals, the usual model for a given genotype must be extended to consider not only the direct effects of its own genes but also the associative effect of the individual on the phenotypes of its group members (Griffing, 1967). One solution is to use group selection (Griffing, 1967). Using group selection, both Muir (1996) and Wade (1976; 1977) found a decrease in mortality due to cannibalism in, respectively, laying hens and flour beetles.

With respect to agriculture, it is important to understand how to improve traits affected by interactions among individuals so as to enhance animal well-being and productivity in

confined high-intensity rearing conditions (Muir, 2005). Determining the relevance of interactions among individuals for breeding programs requires knowledge of the genetic parameters underlying the interactions (Bijma et al., 2007b). Such knowledge would allow one to quantify the potential contribution of associative effects to response to selection, to optimize poultry breeding programs, and to estimate breeding values for both direct and associative effects.

The existence of social interactions among individuals may increase the total heritable variance in a trait (Bijma et al., 2007b; Brichette et al., 2001; Wolf, 2003). Bijma et al. (2007b) found that total heritable variance in survival days expressed as proportion of phenotypic variance increased from 7% through 20% due to social interactions. This indicates that ⅔ of the heritable variation is due to interactions among individuals and is hidden from traditional analysis. These results, however, were based on a relatively small dataset (n = 3,800). Until now, these are the only results that show evidence that heritable variation will increase due to social interactions. Thus, more evidence is needed to confirm the relevance of social interactions for genetic improvement of poultry populations.

In this paper, we present estimated genetic parameters for two models, the traditional linear animal model and a model combining direct and associative effects (associative effects model). For this, we use data on survival days in three purebred layer lines.

MATERIALS AND METHODS

Genetic Stock

Three purebred White Leghorn layer lines from the Institut de Sélection Animale B.V., a Hendrix Genetics company, were used in this study. The three lines were coded: W1, WB, and WF. The lines W1 and WB were expected to have high mortality with intact beaks. The line WF was chosen, because it was characterized as a high feather-pecking line in earlier experiments (Riedstra and Groothuis, 2002; Rodenburg et al., 2003; Van Hierden et al., 2002).

Housing Conditions and Management

For each strain, observations on a single generation were used. Chickens were hatched in two batches, each batch consisted of three lines. Furthermore, each batch consisted of four age groups, differing by two weeks each. After hatching, chickens were sexed, wing-banded in the right wing, and vaccinated for Marek's disease and Infectious Bronchitis.

Chickens had intact beaks. Chickens of the same line and age group were allocated to rearing cages of 60 individuals per cage. Rearing cages were composed at random with respect to family. From week 5 onwards, chickens were housed with 20 individuals per cage.

When the hens were on average 17 weeks old, they were transported to two laying houses with traditional four-bird battery cages. Each batch was placed in another laying house. In both laying houses, the 17-week-old hens were allocated to laying cages, with four birds of the same line and age in a cage. The individuals making up a cage were combined at random. Due to chance, some of the cages contained full or half sibs, but most cages contained unrelated individuals only. Due to lost wing bands, hens were wing-banded in the left wing as well, to avoid loss of data.

In both laying houses, rows were grouped into eight double rows. Individuals could have contact only with the back neighbours, because the back wall of the cages consisted of mesh allowing limited contact between back neighbours, whereas adjacent cages in the same row were separated by a closed wall. In between each double row, there was a corridor through which the employees could access the cages. Each row consisted of three levels (top, close to the light; middle; and bottom). Hens in laying house 2 were placed only in the middle and bottom level. Each level was divided into blocks of ten cages; each block consisted of the same line and age. In general, the same line and age was also housed in the corresponding back cages. A feeding trough was in the front of the cages, and each pair of back-to-back cages shared two drinking nipples. A standard commercial layer diet and water were provided *ad libitum*.

In both laying houses, the hens started with a light period of 9 hours/day. The light period was increased 1hour/week until 16 hours/day was reached when the hens were on average 26 weeks of age. In laying house 1, alongside the first and the last row, there were windows, giving an effect of daylight. In laying house 2, there was no daylight. On average, light intensity was higher in laying house 2 than in laying house 1 (Table 2.1). Light intensity in laying house 1, however, depended predominantly on the weather conditions outside and was therefore highly variable.

Table 2.1. Mean light intensity (lx) and SD in laying house 1 and 2

	Laying house 1		Laying house 2	
Level	L[a]	B[b]	L	B
Top	240±180	63±42	353±7	22±2
Middle	177±150	105±185	282±23	27±2
Bottom	81±99	54±116	122±8	27±3

[a] L is underneath strip light. [b] B is in between two strip lights.

Pedigree

For both laying houses, almost the same sires were used; for laying house 2, a few sires could not be used because of mortality (Table 2.2). The dams used were different for both laying houses. For all three lines, sires and dams were mated at random. Each sire was mated to approximately eight dams, and each dam contributed on average 12.3 female offspring. Five generations of pedigree were included in the calculation of the relationship matrix (**A**). To ensure correct pedigree, hens with unknown identification or double identification were coded as having unknown pedigree (n = 101). The observations on these hens were included in the analysis but did not contribute to estimates of genetic (co)variances.

Table 2.2. Breeding scheme of the three layer lines per laying house

	Laying house 1		Laying house 2	
Line	Sires	Dams	Sires	Dams
W1	36	287	32	250
WB	35	276	33	261
WF	20	159	18	135

Data

All hens were observed daily. Dead hens were removed, and wing band number, cage number, and cause of death were recorded. Determination of cause of death was done subjectively without dissection. Removed hens were not replaced. When the hens were on average 75 weeks old, the study was terminated. For each hen, information was collected on survival and number of survival days. Survival was defined as alive or dead (0/1) at the end of the study. From this data, survival rate was calculated as the percentage of laying hens still alive at the end of the study. Survival days were defined as the number of days

from the start of the study (day of transport to laying houses, when the hens were on average 17 weeks old) till either death or the end of the study, with a maximum of 447 days. For the statistical analysis, 16,780 records were used; 6,276 records of W1; 6,916 records of WB; and 3,588 records of WF (Table 2.3).

Table 2.3. Number of hens (n), survival rate (%) with standard error, and average survival days (days) with standard error of the three layer lines and the fixed effects

	Laying house 1			Laying house 2		
	n	Survival rate[a]	Survival days[b]	n	Survival rate	Survival days
Line						
W1	3,900	53.6±1.3	344±3.6	2,376	64.6±1.5	366±4.3
WB	3,796	50.2±1.3	323±3.6	3,120	56.3±1.5	329±4.1
WF	2,004	74.1±1.1	376±2.9	1,584	75.1±1.2	370±3.3
Laying house	9,700	56.5±0.5	342±1.3	7,080	63.3±0.8	350±2.1
Level						
Top	3,212	52.5±1.2	330±3.2	-	-	-
Middle	3,236	58.0±1.2	348±3.2	3,540	63.8±1.1	353±3.2
Bottom	3,252	59.0±0.9	350±2.3	3,540	62.8±0.8	348±2.3
Row						
1	1,208	61.3±2.0	353±5.2	872	73.5±2.3	364±6.4
2	1,220	64.5±2.0	359±5.2	884	65.1±2.3	341±6.4
3	1,172	66.9±2.0	368±5.3	880	66.7±2.3	355±6.4
4	1,216	68.8±2.0	373±5.2	880	60.6±2.3	343±6.4
5	1,224	50.7±2.0	330±5.2	896	63.4±2.3	358±6.3
6	1,224	50.8±2.0	333±5.2	872	59.3±2.3	351±6.4
7	1,212	43.2±2.0	306±5.2	896	58.2±2.3	346±6.3
8	1,224	46.1±1.4	318±3.7	900	59.9±1.6	346±4.5

[a] Survival rate is percentage of laying hens still alive at the end of the study. [b] Survival days are average number of days from the start of the study (on average 17 weeks old) till either death or the end of the study, with a maximum of 447 days.

Data Analysis

Model. First, the data on survival days were analysed using the General Linear Model (GLM) procedure of the SAS® Statistical program (SAS, 1996). This program was used to decide which fixed effects to include in the model for estimating genetic parameters. The data were analysed separately for each line. The initial model included a fixed effect for each laying house-row-level combination and for average survival days in the back cage to account for a possible effect of the back neighbours. Age was fully confounded with laying house and row and, therefore, not included as a separate fixed effect.

Second, genetic parameters on survival days were estimated using a linear animal model as implemented in the ASReml software package (Gilmour et al., 2002). The traditional linear animal model was used to estimate genetic parameters for the direct effect:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}, \tag{2.1}$$

in which **y** is a vector of observed survival days, **b** is a vector of fixed effects, with incidence matrix **X** linking observations to fixed effects; **a** is a vector of the usual breeding values, with incidence matrix **Z** linking observations on individuals to their breeding value; and **e** is a vector of random residuals. The fixed effects in **b** account for systematic nongenetic differences among observations. Covariance structures of model terms are: $\mathrm{Var}[\mathbf{a}] = \mathbf{A}\sigma_A^2$, where **A** is a matrix of coefficients of relatedness between individuals and σ_A^2 is the genetic variance, and $\mathrm{Var}[\mathbf{e}] = \mathbf{I}\sigma_e^2$, in which **I** is an identity matrix and σ_e^2 is the residual variance.

To estimate genetic parameters for both direct and associative effects, the model of Bijma et al. (2007b) was used, the associative effects model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_D\mathbf{a}_D + \mathbf{Z}_S\mathbf{a}_S + \mathbf{e}, \tag{2.2}$$

in which $\mathbf{a}_D$ is a vector of direct breeding values, with incidence matrix $\mathbf{Z}_D$ linking observations on individuals to their direct breeding value; $\mathbf{a}_S$ is a vector of associative breeding values, with incidence matrix $\mathbf{Z}_S$ linking observations on individuals to the associative breeding values of their group members (*i.e.*, individuals in the same cage); and **e** is a vector of residuals. When there are no social interactions among individuals, the term $\mathbf{Z}_S\mathbf{a}_S$ equals zero, $\mathbf{Z}_D\mathbf{a}_D$ reduces to **Za**, and Equation 2.2 is identical to Equation 2.1.

The covariance structure of genetic terms is $\text{Var}\begin{bmatrix}\mathbf{a}_\text{D}\\ \mathbf{a}_\text{S}\end{bmatrix} = \mathbf{C} \otimes \mathbf{A}$, where $\mathbf{C} = \begin{bmatrix}\sigma^2_{A_\text{D}} & \sigma_{A_\text{DS}}\\ \sigma_{A_\text{DS}} & \sigma^2_{A_\text{S}}\end{bmatrix}$ and where $\sigma^2_{A_\text{D}}$ is the direct genetic variance, $\sigma^2_{A_\text{S}}$ is the associative genetic variance, and σ_{A_DS} is the direct-associative genetic covariance. The residual term in Equation 2.2 is actually the direct environmental effect of the individual plus the sum of environmental effects of its group members: $e_i = E_{\text{D}_i} + \sum_{j \neq i}^{n-1} E_{\text{S}_j}$. The covariance structure of the residual term, **e**, is given by $\text{Var}(\mathbf{e}) = \mathbf{R}\sigma^2_e$, where $R_{ij} = 1$ when $i = j$ and $R_{ij} = \rho$ when i and j are in the same cage ($i \neq j$), but R_{ij} is zero otherwise, with $\sigma^2_e = \sigma^2_{E_\text{D}} + (n-1)\sigma^2_{E_\text{S}}$ (Bijma et al., 2007b). The residuals of the group members may be correlated due to nongenetic interactions among cage members. The correlation equals $\rho = \left[2\sigma_{E_\text{DS}} + (n-2)\sigma^2_{E_\text{S}}\right] / \sigma^2_e$ (Bijma et al., 2007b). The value of ρ is estimated in the analysis.

Heritable Variation. When there are interactions among individuals, each individual interacts with $n - 1$ group members. The total heritable impact of an individual on the population, referred to as its total breeding value (TBV), equals the sum of its direct breeding value and $n - 1$ times its associative breeding value: $\text{TBV}_i = A_{\text{D}_i} + (n-1)A_{\text{S}_i}$. The total heritable variation equals the variance of the TBV among individuals, $\sigma^2_\text{TBV} = \sigma^2_{A_\text{D}} + 2(n-1)\sigma_{A_\text{DS}} + (n-1)^2\sigma^2_{A_\text{S}}$ (Bijma et al., 2007a,b). The σ^2_TBV represents the total heritable variation that can be utilized to generate response to selection (ΔG). Thus, response to selection per generation is given by $\Delta G = \iota\rho\sigma_\text{TBV}$, where ι is the selection intensity; ρ is the accuracy; and σ_TBV is the standard deviation of the total breeding value. When there are no interactions among individuals, σ_TBV reduces to the usual σ_A (Ellen et al., 2007). It follows from equation 2.2 that the total phenotypic variance equals $\sigma^2_P = \sigma^2_{A_\text{D}} + (n-1)\sigma^2_{A_\text{S}} + \sigma^2_e$. The total heritable variance expressed as a proportion of the phenotypic variance (T^2) equals $\frac{\sigma^2_\text{TBV}}{\sigma^2_P}$.

RESULTS

Survival

Line WF showed the highest survival rate of 74.6% and the highest survival days of 373 days, whereas line WB showed the lowest survival rate of 52.9% and the lowest survival days of 326 days (Table 2.3). Both survival days and survival rate differed significantly between lines. Laying house 2 showed significantly higher survival rate over the whole study period (63.3%) and a slightly higher number of survival days (350 days) than laying house 1 (56.5% and 342 days; Table 2.3). A difference in survival rate and survival days was found between the three levels and between the eight corridors. All fixed effects included in the model were significant.

Line WB showed the lowest survival rate in both laying houses (Figure 2.1). At the end of the laying period, ranking of the lines was the same for both laying houses. In laying house 2, however, Line W1 showed until 260 days the highest survival rate, whereas from 260 days onwards, line WF showed the highest survival rate.

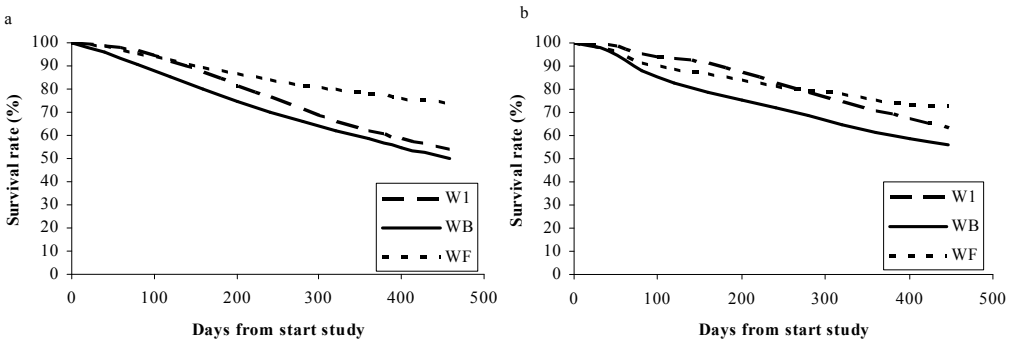

Figure 2.1. Survival curve of the three layer lines W1, WB, and WF of laying house 1(a) and laying house 2 (b)

Genetic Parameters

The estimated genetic parameters on survival days in the three layer lines, using the traditional linear animal model, are given in Table 2.4. The lowest additive genetic standard deviation (σ_A) was found in line WF and the highest in line WB, ranging from 16 through 44 days. Heritabilities ranged from 2% in line WF (not significantly different from zero) through 10% in line WB (significantly different from zero).

Table 2.4. Estimates of genetic parameters[a] with standard error for direct effect on survival days in three layer lines using a traditional linear animal model

	Unit	W1	WB	WF
σ_A	Days	30±4	44±5	16±5
σ_P^2	Days^2	12,814±239	20,066±367	13,936±333
h^2		0.07±0.02	0.10±0.02	0.02±0.01

[a] σ_A is the additive genetic standard deviation. σ_P^2 is the phenotypic variance: $\sigma_P^2 = \sigma_A^2 + \sigma_e^2$. h^2 is the heritability: $h^2 = \sigma_A^2 / \sigma_P^2$.

In table 2.5, results of the associative effects model are given. For the line WF, all results are not significantly different from zero. Both the direct genetic variance ($\sigma^2_{A_D}$) and the associative genetic variance ($\sigma^2_{A_S}$) were highest in line WB and lowest in line WF. The $\sigma^2_{A_D}$ ranged from 246 through 1,917 days^2 and $\sigma^2_{A_S}$ ranged from 60 through 273 days^2. The covariance between direct and associative effect ($\sigma_{A_{DS}}$) was negative in line WB and positive in line W1 and WF; ranging from -228 through 62 days^2 (W1). The standard deviation of the total breeding value (σ_{TBV}) ranged from 30 days (WF) through 55 days (WB). Line WF showed the lowest total heritable variance in survival days expressed as proportion of phenotypic variance (T^2), whereas line W1 showed the highest T^2; ranging from 6 through 19%. The T^2 expresses the total heritable variance relative to the phenotypic variance and is, therefore, a generalization of the conventional h^2 to account for social interactions. The genetic correlation between direct breeding value and associative breeding value (r_A) was positive but not significantly different from zero in line W1 (0.18) and in line WF (0.11) and negative and significantly different from zero in line WB (-0.31). Furthermore, the estimates of the correlation between the residuals of the group members (ρ), ranged from 0.08 in line W1 and WB through 0.10 in line WF and were highly significant.

Table 2.5. Estimates of genetic parameters[a] with standard error for direct and associative effect on survival days in three layer lines using the linear animal model of Bijma et al. (2007b)

	Unit	W1	WB	WF
$\sigma^2_{A_D}$	Days^2	915±218	1,917±394	246±159
$\sigma^2_{A_S}$	Days^2	134±51	273±85	60±61
$\sigma_{A_{DS}}$	Days^2	62±76	-228±132	13±69
σ_{TBV}	Days	50± 8	55± 9	30± 21
σ^2_P	Days^2	12,847±245	20,111±374	13,999±343
T^2		0.19±0.06	0.15±0.05	0.06±0.06
r_A		0.18±0.21	-0.31±0.18	0.11±0.55
ρ		0.08±0.02	0.08±0.02	0.10±0.02

[a] $\sigma^2_{A_D}$, $\sigma^2_{A_S}$, and $\sigma_{A_{DS}}$ are estimates of direct genetic variance, associative genetic variance, and direct-associative genetic covariance. σ_{TBV} is the standard deviation of the total breeding value: $\sigma^2_{TBV} = \sigma^2_{A_D} + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma^2_{A_S}$. σ^2_P is the phenotypic variance: $\sigma^2_P = \sigma^2_{A_D} + (n-1)\sigma^2_{A_S} + \sigma^2_e$. T^2 expresses the total heritable variance relative to the phenotypic variance: $T^2 = \sigma^2_{TBV}/\sigma^2_P$. r_A is the genetic correlation between direct breeding value and associative breeding value. ρ is the correlation between the residuals of the group members.

DISCUSSION

In this paper, we showed that it is possible to estimate genetic parameters for both direct and associative effect on survival days in three purebred layer lines. Furthermore, we showed that including associative effects in the model resulted in a substantially larger heritable variation than was found when using the traditional linear animal models. This result demonstrates the relevance of associative effects for poultry breeders and indicates that prospects for genetic improvement of survival in laying hens are substantially better than suggested by traditional heritabilities.

In our study, survival rate ranged from 52.9% through 74.6% between lines. In other studies, survival rates were found ranging from 69.4% through 94.2% (Craig and Muir, 1989; Kjaer and Vestergaard, 1999). In both studies, however, the hens were beak-trimmed and were kept in larger groups. The fact that we used birds that had intact beaks explains the, on average, lower survival rates in our study.

In our study, survival rate was different between the two laying houses. Survival rate was lowest in laying house 1, which could be due to the effect of daylight. Furthermore, survival rate was lowest in the top level (laying house 1), which could be due to higher light

intensity (Table 2.1). Difference in light intensity, however, did not change the ranking of the lines; it only influenced the level of the survival rate. In other studies, it was also found that high light intensity resulted in a decrease in survival rate (Hughes and Duncan, 1972; Kjaer and Vestergaard, 1999).

In poultry breeding, the trait survival days are more important than the trait survival rate, because survival days show when a laying hen died (*i.e.,* in the beginning or at the end of the laying period). That is why, in this study, the trait survival days were chosen. No literature, however, was found that showed heritabilies for survival days using the traditional linear animal model. Estimated heritabilities were found only for survival as a binary trait. Using the traditional linear animal model, estimated heritabilities for survival days were comparable with heritabilities found for survival as a binary trait, ranging from 3.2% through 9.9% (Craig and Muir, 1989; Mielenz et al., 2005; Robertson and Lerner, 1949). Furthermore, we found that heritabilities, using the traditional linear animal model, for survival as a binary trait ranged also from 3% through 12% (data not shown).

In a simulation study, Van Vleck and Cassady (2005) showed that ignoring a cage effect biases estimates of genetic parameters. In this study, we accounted for nonheritable social effects by fitting a correlation (ρ) between the residuals of cage members (see also Bijma et al., 2007b). Fitting a correlated residual allows cage members to be either similar or dissimilar, corresponding to either a positive or a negative correlation. When cage members are similar due to nonheritable social effects, fitting a random cage effect instead of a correlated residual yields the identical variance. In other words, when cage members are similar, one can fit either a variance between cages or a covariance within cages. The relationship between both models is that $\sigma^2_{cage} = \rho\sigma^2_e$. The equivalence of both models is, however, limited to the situation in which cage members are similar, because σ^2_{cage} cannot be negative. Whether cage members are similar or not is unknown *a priori*. The covariance between residuals of cage members is equal to $2\sigma_{E_{DS}} + (n-2)\sigma^2_{E_S}$ and can be either positive or negative (Bijma et al., 2007b). The general solution to account for nonheritable social effects is, therefore, to fit a correlation between residuals of cage members, not to fit a random cage effect. Moreover, fitting both a correlated residual and a random group effect means that two variables are fitted to account for a single unknown, which over specifies the variance structure and does not yield a unique solution.

Including associative effects in the model, the total heritable variance in survival days expressed as proportion of phenotypic variance (T^2) was 1.5- through 3-fold greater than when using the traditional linear animal models. Line W1 showed the same T^2, of 19%, as

found by Bijma et al. (2007b) on 50% of the data used in the present study. The underlying genetic parameters were, however, slightly different between the two studies. The present results, therefore, confirm the preliminary results of Bijma et al. (2007b).

For growth in mussel cultures, it was found that the genetic correlation between direct and associative effect was negative; individuals getting more food or space would deprive their group members (Brichette et al., 2001). Based on the results of the survival rates, it was expected that correlations between direct and associative effect for survival in hens would be negative because of strong competition. It was expected that dominant animals may kill others and, as a consequence, survive themselves. For line WB, indeed a negative (significantly different from zero) correlation was found between direct and associative effect for survival. However, for line W1 and WF, a positive genetic correlation between direct and associative effect for survival days was found, suggesting that individuals benefit from not harming others (Bijma et al., 2007b). The results of line W1 and WF, however, are not significantly different from zero; it could be that the genetic correlation between direct and associative effect was positive by coincidence. Furthermore, survival rate in line WF is high, which reduced the accuracy of estimated genetic parameters.

Genetic parameters are usually estimated by a linear animal model in which the dependent variables and the random variables are assumed to be normally distributed. In this study, genetic parameters of survival data were also estimated using a linear animal model. Survival data, however, is heavily skewed (Kachman, 1999). Furthermore, for hens still alive at the end of the study, only a lower bound of the exact survival days will be available. These data are called censored data (Kalbfleisch and Prentice, 1980). To analyze survival data, the appropriate method would be survival analysis, which can be done using the survival kit (Ducrocq and Sölkner, 1998). Until now, however, it is not possible to estimate genetic parameters for both direct and associative effect using that software package. Using survival analysis including associative effects, we would, however, expect that the proportion of heritable variation will even be higher than when using a linear animal model including associative effects.

In conclusion, it is possible to estimate genetic parameters for direct and associative effects on survival in laying hens. The results of this study show that including associative effects in the model will give substantially higher heritable variation than when using the traditional linear animal model. When designing a breeding program, estimation of the genetic parameters for all lines is needed. Furthermore, environmental factors, like group size and light intensity, are important, because they can have an effect on the genetic

parameters. Theoretical work shows that prospects for reduction of mortality using the associative effects model are good (Bijma et al., 2007a; Ellen et al., 2007). Genetic selection targeting both direct and associative effects is expected to substantially reduce one of the major welfare problems in egg production.

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Chapter 3

Combining survival analysis and a linear animal model to estimate genetic parameters for direct and associative effects on survival time in three layer lines

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ABSTRACT

Mortality due to cannibalism in laying hens is an economic and welfare problem. Survival of laying hens, however, is a difficult trait to genetically improve, primarily due to three reasons, 1) heritabilities are low; 2) censoring is high (animals still alive at the end of the testing period); and 3) survival of an individual depends on the behaviour of its group members. To improve survival, an appropriate method should both take into account censoring and the effect an individual has on its group members (so-called "associative effects"). To analyse survival data, survival analysis can be used. Until now, however, it is not possible to include associative effects in the current software for survival analysis. To take into account associative effects, a linear animal model including associative effects can be used. This paper presents a two-step approach, combining survival analysis and a linear animal model including associative effects. Genetic parameters for direct and associative effects on survival time in three layer lines are estimated using the two-step approach. For the analysis, survival data on 16,780 hens kept in four-bird cages with intact beaks were used. Using the two-step approach, the total heritable variance in survival time was 1.5 to 6-fold greater than the traditional direct genetic variance. We used cross validation to compare the two-step approach and the linear animal model including associative effects. Results showed that the rank correlation between predicted and observed phenotypes was similar for both methods.

Keywords: survival analysis, social interactions, variance component estimation, laying hen, associative genetic effects

INTRODUCTION

Mortality due to cannibalism in laying hens is a worldwide economic, health, and welfare problem, occurring in all types of commercial poultry housing systems (Blokhuis and Wiepkema, 1998). Due to the likely prohibition of beak-trimming in the European Union in the near future, this problem may increase if no further actions are taken, and, therefore, needs to be solved urgently.

One of the possibilities is to use genetic selection (Muir, 1996; Jones and Hocking, 1999). Selection for lower mortality or increased survival, however, has not been very effective in most cases (Preisinger, 1998). First, heritabilities of mortality are low, ranging between 3% and 10%, leading to low accuracy (Robertson and Lerner, 1949; Craig and Muir, 1989; Ducrocq et al., 2000; Mielenz et al., 2005; Ellen et al., 2008). Second, censoring is high (animals still alive at the end of the testing period) (Ducrocq et al., 2000; Ellen et al., 2008), leading to low accuracy as well. Third, traditional methods for selection against mortality can lead to unfavourable response to selection, because these methods neglect the social effect of the group members on the individual's phenotype (Griffing, 1967; Muir and Liggett, 1995; Muir, 1996; Ellen et al., 2007).

Heritabilities for survival traits are, in most cases, estimated using a linear animal model. One of the disadvantages of a linear animal model is that, for animals still alive at the end of the testing period (censored animals), only a lower bound of the exact survival days will be known. To analyse survival data, survival analysis is a more appropriate method to use (Kalbfleisch and Prentice, 1980). First, survival data is usually heavily skewed (Kachman, 1999), whereas with linear models residuals are assumed to be normally distributed. Survival analysis appropriately accounts for non-normality in the data. Second, survival analysis uses all the information available, from dead animals as well as from animals still alive at the end of the study period (Kalbfleisch and Prentice, 1980).

Mortality due to cannibalism is a trait affected by social interactions. To reduce mortality due to cannibalism, the classical model for a given genotype must be extended to consider not only the individuals' direct effect of its own genes, but also the associative effect of the individual on the phenotypes of its group members (Griffing, 1967). There is clear evidence that interactions among individuals contribute to the total heritable variance in a trait (Brichette et al., 2001; Arango et al., 2005; Bijma et al., 2007b; Van Vleck et al., 2007; Bergsma et al., 2008; Chen et al., 2008; Ellen et al., 2008). When including associative effects in the model, Bijma et al. (2007b) and Ellen et al. (2008) found that total heritable variance in survival days in three lines of commercial laying hens was 1.5 to 3-fold greater

than the traditional genetic variance. These results, however, were estimated using a linear animal model including associative effects. So far, associative effects have not been implemented in existing software for survival analysis. To analyse survival data, a solution might be to combine survival analysis and a linear animal model including associative effects.

Ducrocq et al. (2001) proposed a two-step approach for multiple trait evaluation of functional and production traits. In this paper, we apply a similar two-step approach to estimate genetic parameters for direct and associative effects on survival in laying hens. In the first step, survival analysis will be performed to compute so-called pseudo-records and their associated weights. Pseudo-records can be regarded as the result in the data of a linearization of the model: when analysed with a simple linear animal model, pseudo-records weighted appropriately lead to the same estimated genetic values as the initial survival model used to compute them. In the second step, genetic parameters for direct and associative effects on pseudo-records with their associated weights will be estimated using a linear animal model including associative effects. Finally, results will be compared with those of a linear animal model including associative effects (Bijma et al., 2007b; Ellen et al., 2008) using cross validation.

MATERIALS AND METHODS

For this study, the same data were used as described in Ellen et al. (2008; Chapter 2). The main characteristics are summarized below. For further details reference is made to Ellen et al. (2008; Chapter 2).

Population and housing

Three purebred White Leghorn layer lines from the Institut de Sélection Animale B.V., a Hendrix Genetics company, were used in this study. The three lines were coded: W1, WB, and WF. For each line, observations on a single generation were used. Chickens of each line were hatched in two batches, each batch consisted of four age groups, differing by two weeks each.

When the hens were on average 17 weeks old, they were transported to two laying houses with traditional four-bird-battery cages. Each batch was placed in another laying house. In both laying houses, the 17-week-old hens were allocated to laying cages, with four birds of the same line and age in a cage. The individuals making up a cage were

combined at random. In both laying houses, cages were grouped into eight double rows. Each row consisted of three levels (top, close to the light; middle; and bottom). A feeding trough was in front of the cages, and each pair of back-to-back cages shared two drinking nipples.

Pedigree

Sires used for both laying houses, were largely the same. The dams used were different for both laying houses. For all three lines, sires and dams were mated at random. Each sire was mated to approximately eight dams, and each dam contributed on average 12.3 female offspring. Five generations of pedigree were included in the calculation of the relationship matrix (**A**). To avoid pedigree errors, hens with unknown identification or double identification were coded as having unknown pedigree (n = 101). The observations on these hens were included in the analysis to better estimate fixed effects, but did not contribute to estimates of genetic (co)variances.

Data

All hens were observed daily. Dead hens were removed from the cages, and wing band number and cage number were recorded. Dead hens were not replaced. The study was terminated when hens were on average 75 weeks old. For each hen, information was collected on survival and number of survival days. Survival was defined as alive or dead (0/1) at the end of the study. From theses data, survival rate was calculated as the percentage of laying hens still alive at the end of the study. Survival days were defined as the number of days from the start of the study (day of transport to laying houses) till either death or the end of the study. Hens that died before the end of the study were referred to as a failure (event = 1), whereas hens still alive at the end of the study were referred to as censored (event = 0). In total, 196 hens were removed from the study, due to reasons other than mortality. These hens were referred to as censored (event = 0). For the statistical analysis, 6,276 records were used for line W1; 6,916 records were used for line WB; and 3,588 records were used for line WF.

Data analysis

Data were analysed separately for each line. To estimate genetic parameters for survival days, a two-step approach was used (Ducrocq et al., 2001; Tarrés et al., 2006). First, data were analysed using survival analysis as implemented in the survival kit V5 (Ducrocq and Sölkner, 1998), to produce pseudo-records as defined below. Survival analysis allows the combination of information from hens still alive (censored records) as well as hens that died (uncensored records). Second, genetic parameters for direct and associative effects on pseudo-records were estimated using a linear animal model including associative effects (Bijma et al., 2007b; Ellen et al., 2008), implemented in the ASReml software package (Gilmour et al., 2002).

Step 1: Survival analysis. Data were analysed using the Cox animal model (Cox, 1972). The Cox model can deal with non-linearity, censoring, and non-normal residuals. The model included a fixed effect for each combination of laying house, row, and level, and for average survival days in the back cage to account for a possible effect of the back neighbours (Ellen et al., 2008). Age was fully confounded with laying house and row and, therefore, not included as a separate fixed effect. All the fixed effects were significant.

Using survival analysis results in a pseudo-record (y_i^*) for each hen *i* corrected for all non genetic effects and an associated weight (ω_i). We define pseudo-records as functions of the data and of the effects estimated in the survival model, such that when a straightforward BLUP animal genetic evaluation is applied on these pseudo-records, the same estimated breeding values are obtained as in the initial survival model. Pseudo-records can also be regarded as records pre-corrected for all non-genetic effects of the survival model. The pseudo-record for survival time of animal *i* was (Ducrocq et al., 2003):

$$y_i^* = \frac{\delta_i}{\omega_i} + a_i - 1\,, \tag{3.1}$$

where δ_i is the censoring code of individual *i* ($\delta_i = 1$ if animal *i* is uncensored; $\delta_i = 0$ if animal *i* is censored); a_i is the estimated direct breeding value of individual *i*; and ω_i is the associated weight of individual *i*. It can be shown that ω_i is the estimated cumulative risk of animal *i* from time 0 to censoring time or death and is therefore a function of the (possibly censored) length of life of hen *i*, her censoring code ($\delta_i = 0/1$), and the fixed effects in the model.

To verify the two-step approach, pseudo-records with appropriate weights were analysed to estimate breeding values with an univariate BLUP animal model with a heterogeneous residual variance $1/\hat{\omega}_i$ for animal *i*. The correlation between the estimated breeding values of the two-step approach and the estimated breeding values of the survival analysis was calculated (Ducrocq et al., 2001). As expected, this correlation was one, meaning that the estimated breeding values are the same. Based on this correlation, it can be concluded that the computation of pseudo-records in the two-step approach is correct.

Step 2: Associative effects model. To estimate genetic parameters for the direct and associative effects, using the pseudo-records and associated weights from step 1, the model of Bijma et al. (2007b) was used:

$$\mathbf{y}^* = \mathbf{Z}_\mathrm{D}\mathbf{a}_\mathrm{D} + \mathbf{Z}_\mathrm{S}\mathbf{a}_\mathrm{S} + \mathbf{e}\,, \tag{3.2}$$

where $\mathbf{y}^*$ is a vector of the pseudo-records y_i^*; $\mathbf{a}_\mathrm{D}$ is a vector of direct breeding values, with incidence matrix $\mathbf{Z}_\mathrm{D}$ linking observations on individuals to their direct breeding value; $\mathbf{a}_\mathrm{S}$ is a vector of associative breeding values, with incidence matrix $\mathbf{Z}_\mathrm{S}$ linking observations on individuals to the associative breeding values of their group members (*i.e.*, individuals in the same cage); and $\mathbf{e}$ is a vector of residuals, where $\mathrm{Var}(e_i) = \frac{1}{\omega_i}\sigma_e^2$. A weighted analysis was performed using the associated weight (ω_i) and the !WT statement in ASReml (Gilmour et al., 2002) and fixing σ_e^2 to one (Ducrocq et al., 2001).

The covariance structure of genetic terms is $\mathrm{Var}\begin{bmatrix}\mathbf{a}_\mathrm{D}\\ \mathbf{a}_\mathrm{S}\end{bmatrix} = \mathbf{C} \otimes \mathbf{A}$, where $\mathbf{C} = \begin{bmatrix}\sigma^2_{A_\mathrm{D}} & \sigma_{A_\mathrm{DS}}\\ \sigma_{A_\mathrm{DS}} & \sigma^2_{A_\mathrm{S}}\end{bmatrix}$, in which $\sigma^2_{A_\mathrm{D}}$ is the direct genetic variance, $\sigma^2_{A_\mathrm{S}}$ is the associative genetic variance, and σ_{A_DS} is the direct-associative genetic covariance. Bijma et al. (2007b) showed that residuals of group members are correlated due to non-genetic associative effects. The covariance structure of the residual term, is given by $\mathrm{Var}(\mathbf{e}) = \mathbf{R}\sigma_e^2$, where R_{ij} = 1 when $i = j$, $R_{ij} = \rho$ when *i* and *j* are in the same cage ($i \neq j$), and R_{ij} is zero otherwise. The value of ρ is estimated in the analysis, using a CORU statement in the residual variance structure in ASReml (Gilmour et al., 2002).

Heritable variation. When there are interactions among individuals, each individual interacts with $n - 1$ group members. In this study, $n = 4$. The total heritable impact of an

individual on the population, referred to as its total breeding value (TBV), equals the sum of its direct breeding value and n – 1 times its associative breeding value: $\mathrm{TBV}_i = A_{\mathrm{D}_i} + (n-1)A_{\mathrm{S}_i}$ (Muir, 2005). The total heritable variation equals the variance of the TBV among individuals, $\sigma^2_{\mathrm{TBV}} = \sigma^2_{A_{\mathrm{D}}} + 2(n-1)\sigma_{A_{\mathrm{DS}}} + (n-1)^2\sigma^2_{A_{\mathrm{S}}}$ (Bijma et al., 2007a; Bijma et al., 2007b). With unrelated group members, the total phenotypic variance equals $\sigma^2_P = \sigma^2_{A_{\mathrm{D}}} + (n-1)\sigma^2_{A_{\mathrm{S}}} + \sigma^2_e$. The total heritable variance expressed relative to the phenotypic variance equals $T^2 = \sigma^2_{\mathrm{TBV}} / \sigma^2_p$. The T^2 expresses the total heritable variance relative to the phenotypic variance and is, therefore, a generalisation of the conventional h^2 to account for social interactions.

Cross validation. Comparing two methods can be done using cross validation (Stone, 1974). With cross validation, known phenotypes are set to missing and their value is predicted and compared to their observed value. The correlation between predicted and observed phenotypes is a quality measure of an estimation procedure. The two-step approach and the linear animal model including associative effects (Ellen et al., 2008) were compared using cross validation. Validation focused on line W1 and WF. For this purpose, a number was randomly allocated to each cage, ranging from 1 through n (n corresponds to the number of cages in the total dataset for a particular line). From the total dataset, 20% of the cages were removed, which resulted in five subsets, each containing 80% of the data. In this way, each cage was once removed from the total dataset. For the missing data, the phenotypes set to missing were predicted using either the two-step approach or the linear animal model including associative effects.

Comparing the predicted phenotypes of the two methods is difficult for two reasons. First, a scale difference exist between estimated breeding values of the two-step approach and estimated breeding values of the linear animal model including associative effects. Estimated breeding values of the linear animal model are on the observed scale for survival days, whereas estimated breeding values of the two-step approach are on the hazard scale. Transforming estimated breeding values of the two-step approach into survival days is somewhat difficult, because the transformation is non-linear. Second, in our dataset approximately 50-70% of the data were censored (animals that were still alive at the end of the study). These animals do not have an observed phenotype, but we know that their observed phenotype is larger than the observed phenotypes of animals that are not censored.

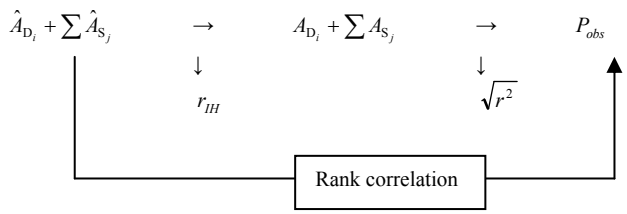
Due to the scale difference and censoring, it is not possible to estimate Pearson correlations between predicted and observed phenotypes for each method and between predicted phenotypes of the two methods. In both methods, however, animals with the highest predicted phenotype (linear animal model) or lowest predicted hazard (two-step approach) have the highest expected value for observed survival days. This means that the rank of the predicted phenotype or hazard can be used for both methods. Therefore, to evaluate quality of both estimation procedures, we compared the rank correlation between observed phenotypes and predicted phenotypes from the linear model to the rank correlation between observed phenotypes and predicted hazard from the two-step approach. This addresses the scale issue. The remaining problem is censored animals, which have unknown rank for the observed phenotype. The fact that animals were censored, however, represents important information because those animals had the highest observed survival days. For example, suppose we have a dataset of 200 individuals with 60% censoring. The phenotype is known of 80 individuals, whereas the phenotype is unknown of 120 individuals. The individuals with known phenotypes have rank 1 through 80, whereas the individuals with unknown phenotypes have rank 81 through 200, but in unknown order. For the censored phenotypes, we assumed that their rank is in random order between 81 and 200. In this case, the rank correlation can be calculated by giving individuals the average rank of 140.5 (in this example, see Appendix 3A for a mathematical proof). In this way, we utilize the information that animals were censored, but make as little assumptions as possible about their order.

Before calculating rank correlations, observed phenotypes were corrected for fixed effects using the linear model: survival_days = laying_house + row + level + survival_days_backcage + *e*. The residual of this model represents the phenotype corrected for fixed effects, ($P_i - \overline{P}$) = *e*. Next, for the 20% missing data, the correlation was calculated between the rank of the observed phenotypes corrected for the fixed effects, *rank*($P_i - \overline{P}$), accounting for censoring as described above, and the rank of the predicted phenotype or hazard: $corr\left[rank\left(P_i - \overline{P}\right)_i, rank(\hat{P}_i)\right]$. In this expression, $\hat{P}_i$ denotes the predicted phenotype in case of the linear model, and the predicted hazard in case of the two-step approach. The $\hat{P}_i$ of individual *i* is the sum of the estimated direct breeding value (or hazard) of hen *i* ($\hat{A}_{\mathrm{D}_i}$) and the estimated associative breeding values (or hazards) of its group members *j* ($\sum_{n-1} \hat{A}_{\mathrm{S}_j}$). Furthermore, to quantify similarity of both methods, the rank

correlation between the $\hat{P}_i$ of the two-step approach and the $\hat{P}_i$ of the linear animal model including associative effects was calculated.

The rank correlation between predictions and observed phenotype depends not only on the accuracy of the estimated breeding values underlying the predictions, but is also affected by non-genetic components of the observed phenotype. When breeding values of predictions would be estimated with full accuracy, the correlation between predictions and observed phenotypes would be equal to the square root of the proportion of phenotypic variance explained by breeding values, $\sqrt{r^2} = \sqrt{[\sigma^2_{A_D} + (n-1)\sigma^2_{A_S}]/\sigma^2_p}$. For any accuracy of predicted breeding values (r_{IH}), the expected rank correlation between predictions and observed phenotypes would be equal to $corr = r_{IH}\sqrt{r^2}$ (Box 3.1). Because animal breeders are interested in prediction of breeding values rather than phenotypes, we calculated an approximate accuracy as $\hat{r}_{IH} = corr\left[rank\left(P_i - \overline{P}\right)_i, rank(\hat{P}_i)\right]/\sqrt{r^2}$. Hence, $\hat{r}_{IH}$ represents the approximate accuracy with which the genetic components underlying the observed phenotype, $A_{D_i} + \sum_{n-1} A_{S_j}$, referring either to the hazard or the to survival days, were predicted. This accuracy is only approximate because it refers to the ranks rather than the phenotypes, and because the prediction from the two-step approach refers to the scale of the hazard rather than the observed phenotype. For line W1, $\sqrt{r^2}$ = 0.32 and for line WF, $\sqrt{r^2}$ = 0.17.

Box 3.1. Approximate accuracy



$\sigma^2_p = \sigma^2_{A_D} + (n-1)\sigma^2_{A_S} + \sigma^2_e$ (Bijma et al., 2007b), which results in $\sqrt{r^2} = \sqrt{\frac{\sigma^2_{A_D} + (n-1)\sigma^2_{A_S}}{\sigma^2_p}}$

RESULTS

Survival

The Kaplan-Meier estimate of the survival function (Kaplan and Meier, 1958) was plotted for the survival of the three layer lines (Figure 3.1). The survival function represents the proportion of laying hens that survived up to time *t*. The survival rate differed significantly between lines in both laying houses ($p < 0.01$). On average, line WF showed the highest survival rate of 74.6%, whereas line WB showed the lowest survival rate of 52.9%.

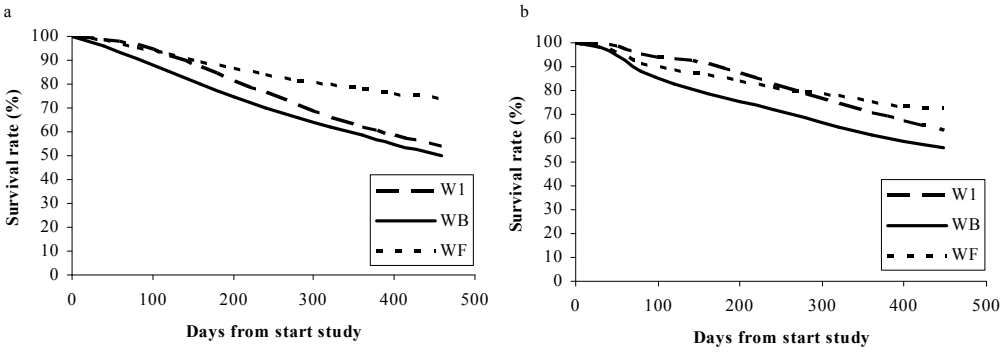


Figure 3.1. Survival curve of the three strains, W1, WB, and WF for laying house 1 (a) and laying house 2 (b)

Genetic parameters

The estimated genetic parameters for direct and associative effects using the two-step approach are given in Table 3.1. For all three lines, both the direct genetic variance ($\sigma^2_{A_D}$) and the associative genetic variance ($\sigma^2_{A_S}$) were significantly different from zero. The $\sigma^2_{A_D}$ was lowest in line WF and highest in line W1, ranging from 0.12 through 0.31, whereas the $\sigma^2_{A_S}$ was lowest in line WB and highest in line WF, ranging from 0.028 through 0.049. The covariance between direct and associative effect ($\sigma_{A_{DS}}$) was negative but not significantly different from zero in line WB and positive in line W1 and WF (but not significantly different from zero); ranging from -0.02 through 0.04. The total heritable variance (σ^2_{TBV}) ranged from 0.44 (WB) through 0.81 (WF) and were significantly different from zero. Line WB showed the lowest total heritable variance in survival days expressed relative to the

phenotypic variance (T^2), whereas line WF showed the highest T^2; ranging from 32% through 64%. The estimated genetic correlation between direct breeding value and associative breeding value (r_A) was positive but not significantly different from zero in line W1 (0.13) and line WF (0.55), and negative and not significantly different from zero in line WB (-0.20). Furthermore, the estimates of the correlation between the residuals of the group members (ρ), were significantly different from zero and ranged from -0.005 in line WB through -0.003 in line W1.

Table 3.1. Estimates of genetic parameters[a] with standard errors for direct and associative effects on survival time in three layer lines using the two-step approach

	W1	WB	WF
$\sigma^2_{A_D}$	0.31±0.05	0.30±0.05	0.12±0.06
$\sigma^2_{A_S}$	0.041±0.011	0.028±0.009	0.049±0.021
$\sigma_{A_{DS}}$	0.01±0.02	-0.02±0.01	0.04±0.02
σ^2_{TBV}	0.77±0.13	0.44±0.09	0.81±0.26
σ^2_P	1.44±0.06	1.38±0.05	1.27±0.08
T^2	0.53±0.08	0.32±0.06	0.64±0.17
r_A	0.13±0.15	-0.20±0.14	0.55±0.28
ρ	-0.003±0.0003	-0.005±0.0001	-0.004±0.0003

[a] $\sigma^2_{A_D}$, $\sigma^2_{A_S}$, and $\sigma_{A_{DS}}$ are estimates of direct genetic variance, associative genetic variance, and direct-associative genetic covariance. σ^2_{TBV} is the total heritable variance: $\sigma^2_{TBV} = \sigma^2_{A_D} + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma^2_{A_S}$. σ^2_P is the phenotypic variance: $\sigma^2_P = \sigma^2_{A_D} + (n-1)\sigma^2_{A_S} + \sigma^2_e$, where $\sigma^2_e = 1$. T^2 expresses the total heritable variance relative to the phenotypic variance: $T^2 = \sigma^2_{TBV} / \sigma^2_P$. r_A is the genetic correlation between direct breeding value and associative breeding value. ρ is the correlation between the residuals of the group members.

Validation

The rank correlations between the observed phenotype, adjusted for fixed effects and censoring, and the prediction, $corr\left[rank\left(P-\overline{P}\right)_i, rank(\hat{P}_i)\right]$, for both the two-step approach and the linear animal model including associative effects, are given in Table 3.2. The rank correlation was approximately the same for both methods. For line W1, the rank correlation was approximately 0.14 and for line WF, the rank correlation was approximately 0.03. The rank correlation between the prediction of the two-step approach and of the linear animal model including associative effects was on average 0.954 for line W1 and 0.648 for line WF. Furthermore, the approximate accuracy for both methods was calculated (Table 3.2).

For both layer lines, the approximate accuracy was marginally higher for the two-step approach.

Table 3.2. Rank correlation[a] with standard error and approximate accuracy between observed phenotype[b] and predicted phenotype[c] using the two methods, two-step approach and linear animal model including associative effects for line W1 and WF

	Rank correlation		Approximate accuracy	
	W1	WF	W1	WF
Corr(2-step[d]; $\hat{P}_i$)	0.141±0.015	0.035±0.019	0.44	0.21
Corr(Animal[e]; $\hat{P}_i$)	0.136±0.013	0.032±0.004	0.42	0.18
Corr(2-step;Animal)	0.954±0.002	0.648±0.010		

[a] Results are averages of five subsets, each containing 20% of the data. [b] Observed phenotype is the phenotype corrected for fixed effects ($P - \overline{P}$). [c] Predicted phenotype is the sum of the estimated direct breeding value of hen i ($\hat{A}_{D_i}$) and the estimated associative breeding values of its group members j ($\sum_{n-1} \hat{A}_{S_j}$), $A_{D_i} + \sum_{n-1} A_{S_j}$. [d] Two-step approach. [e] Linear animal model.

DISCUSSION

In this paper, we showed that it is possible to estimate genetic parameters for direct and associative effects using a two-step approach, combining survival analysis and a linear animal model including associative effects. Using this two-step approach, the total heritable variance was 1.5 to 6-fold greater than the traditional direct genetic variance. For line W1 and WB, this increase in total heritable variance is comparable with results found using the linear animal model including associative effects (Bijma et al., 2007b; Ellen et al., 2008). For line WF, increase is much larger using the two-step approach (6-fold) than using the linear animal model including associative effects (3-fold), which could be due to the fact that censoring is higher in line WF and the two-step approach takes this better into account. Using the two-step approach, the total heritable variance expressed relative to the phenotypic variance (T^2) is substantially larger than using the linear animal model including associative effects. Results of the cross validation, however, do not show any difference.

Comparing genetic parameters of the two-step approach and the linear animal model including associative effects is not possible. For the two-step approach, genetic parameters are given on the hazard scale, the risk of an event at a given time *t*, whereas genetic

parameters of the linear animal model including associative effects are on the observed scale for survival days.

Theoretically, the two-step approach would be a better method to analyse survival data, based on fewer assumptions known to be incorrect. Comparing the two-step approach with the linear animal model including associative effects using the rank correlation between the predicted and observed phenotypes, showed that the correlation was approximately the same (Table 3.2). This applied to both lines, W1 and WF. Note that the rank correlation is low, whereas the accuracy is moderate for line W1 and low to moderate for line WF. Even though the accuracy seems low, it is in accordance with the accuracy for methods that contain only half or full sib information (at least for line W1) (Falconer and Mackay, 1996; Ellen et al., 2007). Furthermore, a moderate (line WF) to high (line W1) rank correlation was found between the predicted phenotypes of the two-step approach and the predicted phenotypes of the linear animal model including associative effects. For line W1, both methods gave nearly the same ranking of predicted phenotypes. For line WF, differences exist in the ranking of the predicted phenotypes, which could be due to the high censoring. Based on these results, it cannot be concluded that the two-step approach is a better method for analysing survival data.

We made a number of assumptions in the cross-validation that may have affected results. First, observed phenotypes were corrected for fixed effects using the linear model, which may have favoured the linear animal model including associative effects compared to the two-step approach. Further investigation is needed, comparing our results to those obtained when using the uncorrected phenotypes, or when using a corrected record on the level of the hazard obtained from survival analysis. Second, when calculating the rank correlation, we assumed that ranks of censored observations were in random order. This will probably not be true when censored individuals would be given the opportunity to actually produce a record. Alternatively, we could have used the ranks of the uncensored records only. In that case, however, we would have ignored the information that the censored records are actually the “best records”. Nevertheless, an investigation of the rank correlation among the uncensored records only would be valuable, since it should at least have the appropriate sign.

For all three layer lines, censoring was at the same time, at the end of the study. It could be that when censoring is at different moments during the study period, differences in accuracy of the two methods could occur. It will be expected that, with different censoring times, the two-step approach will give more accurate breeding values than the linear animal

model including associative effects. Further research is needed to investigate whether different moments of censoring will result in a difference in accuracy of the two methods.

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APPENDIX 3A: RANK CORRELATION WITH CENSORING

Let k denote the ranks of the observed phenotypes, $k = (1, N)$, with $k \leq n$ representing the known records, and $k = (n + 1, N)$ representing the unknown ranks of the censored records. Thus k is known for $k \leq n$, and k is unknown for $k = (n + 1, N)$. Moreover, let $\hat{k}$ denote the ranks of the predicted phenotypes, with $\hat{k} = (1, N)$, and all $\hat{k}$ are known. The problem is to calculate the correlation between the ranks k and $\hat{k}$, taking into account censoring. Both k and $\hat{k}$ range between 1 and N, so we can use $\text{Var}(k) = \text{Var}(\hat{k})$. Thus the rank correlation equals $\rho = \text{Cov}(k,\hat{k}) / \text{Var}(\hat{k})$. Next, $\text{Cov}(k,\hat{k}) = \frac{1}{N-1}\left[\sum_N k\hat{k} - \frac{1}{N}\sum_N k \sum_N \hat{k}\right]$. Since all ranks range from 1 through N, the second term in square brackets is known. The first term can be split into a known component, and a component including the censored records given by $\sum_{j=n+1}^{N} k_j \hat{k}_j$, where k_j is censored. Taking the expectation, assuming that ranks of

censored records are in random order between n + 1 and N, gives $E\left[\sum_{j=n+1}^{N} k_j \hat{k}_j\right]$ = $\sum_{j=n+1}^{N} E(k_j \hat{k}_j)$ = $\sum_{j=n+1}^{N} \hat{k}_j E(k_j)$, meaning that we can use the mean rank of the censored records, $\bar{k}_{censored}$ = $E(k_j \mid j = n+1, N)$ = $(n+1+N)/2$. Therefore, we calculated the rank correlation as $\rho = \mathrm{Cov}(k, \hat{k}) / \mathrm{Var}(\hat{k})$, substituting k = $\bar{k}_{censored}$ in the calculation of the censored elements of $\sum_{N} k\hat{k}$.

Chapter 4

Genetic improvement of traits affected by interactions among individuals: Sib selection schemes

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ABSTRACT

Livestock populations are usually kept in groups. As a consequence, social interactions among individuals affect productivity, health, and welfare. Current selection methods (individual selection), however, ignore those social interactions and yield suboptimal or in some cases even negative responses. In principle, selection between groups instead of individuals offers a solution, but has rarely been adopted in practice for two reasons. First, the relationship between group selection theory and common animal breeding concepts, such as the accuracy of selection, is unclear. Second, application of group selection requires keeping selection candidates in groups, which is often undesirable in practice. This work has two objectives. First, we derive expressions for the accuracy of individual and group selection, which provides a measurement of quality for those methods. Second, we investigate the opportunity to improve traits affected by social interactions by using information on relatives kept in family groups, while keeping selection candidates individually. The accuracy of selection based on relatives is shown to be an analogy of the classical expression for traits not affected by interactions. Our results show that selection based on relatives offers good opportunities for effective genetic improvement of traits affected by social interactions.

Keywords: interactions among individuals, group selection, genetic improvement, indirect genetic effects, response to selection

INTRODUCTION

Nearly all living organisms are affected by social interactions among individuals (Wilson, 1977; Griffing, 1989; Moore, 1990; Moore et al., 1997; Agrawal et al., 2001; Clutton-Brock, 2002; Muir, 2005). Such interactions may be due to competition for limited resources, such as daylight or soil nutrients, or due to social behaviours, such as aggression, social dominance, competitive ability, helping behaviour, or interactions between mothers and their offspring (maternal effects). Those interactions have received a lot of attention in the field of evolutionary biology (*e.g.,* Hamilton, 1964; Frank, 1998; Keller, 1999; Clutton-Brock, 2002), but are also of great importance in domestic populations of animals and plants (Muir, 1996; Denison et al., 2003; Muir, 2005).

There is clear evidence that social interactions may contribute to the heritable variation in traits (Wade, 1976, 1977; Moore, 1990; Muir, 1996, 2005; Brichette et al., 2001; Wolf, 2003; Bijma et al., 2007b). For example, Bijma et al. (2007b) found a total heritable variance for survival time in laying hens equal to 20% of the total phenotypic variance, of which two-third originated from social interactions among individuals. Furthermore, selection experiments to reduce mortality due to cannibalism in domestic chicken (Muir, 1996) and in flour beetle (Wade, 1976; Wade, 1977) and to increase or decrease leaf area in cress (Goodnight, 1985) have demonstrated that heritable interactions can contribute substantially to response to selection.

The inheritance of traits affected by social interactions differs from that of classical traits, because trait values are determined in part by heritable effects that originate from other individuals (Moore et al., 1998). As a consequence, response to selection consists of two components (Willham, 1963; Griffing, 1967). The first component is the usual response in the direct effect of a genotype on the phenotype of the individual itself. The second component is the response in the effect of that genotype on phenotypes of other individuals. Following Griffing (1967), we refer to the effect of a genotype on phenotypes of other individuals as the associative effect of that genotype. With competition among individuals, selection methods that target only the direct effects of genotypes yield a negative correlated response in the associative effects and may yield a negative total response (Griffing, 1967). For example, Wade (1976) showed that individual selection for increased population size of *Tribolium* decreased population size in the next generation. Similar results were also found in other studies (Craig, 1982; Goodnight, 1985). Genetic improvement of traits affected by social interactions, therefore, requires selection methods that aim at both the direct effects of genotypes and at the associative effects of genotypes (Griffing, 1967).

Despite the evidence that social interactions contribute to heritable variation in traits, selection methods currently used in livestock genetic improvement, such as mass selection or selection based on information from relatives, consider only the direct effects of genotypes (with the exception of maternal effects). Those methods are, therefore, inadequate for improving traits affected by social interactions among individuals. Both theoretical and experimental work shows that selection between groups, where the group is the unit of selection, offers a solution, because group selection simultaneously improves direct and associative effects (Griffing, 1967; Griffing, 1976a; Maynard-Smith, 1976; Moore et al., 1997; Wolf et al., 1998, 1999; Agrawal et al., 2001; Muir, 2005; Bijma et al., 2007a). Group selection has, however, rarely been adopted in animal breeding practice, primarily for two reasons. First, the theoretical works on group selection have not been written using the usual expression for response to artificial selection, which is the product of intensity of selection, accuracy of selection, and the genetic standard deviation in the trait. This has caused group selection to be not fully understood and accepted in the field of animal breeding. Second, application of group selection requires that the selection candidates are kept in groups. Keeping selection candidates in groups, however, is often undesirable or difficult to apply in practice; first, because it interferes with recording data on an individual basis for important traits such as feed intake and, second, it may increase loss of selection candidates due to both infectious diseases and aggression.

This article has two objectives. First, we derive expressions for the accuracy of individual and group selection, which provides a measurement of quality for those methods. Second, we investigate the opportunity to improve traits affected by social interactions by using information on relatives kept in family groups, while keeping selection candidates individually. Finally, we compare selection responses obtained with this strategy to responses obtained with existing strategies that are based on individual and group selection.

THEORY

In artificial breeding, the general expression for response to selection is

$$\Delta G = \iota\rho\sigma_A, \tag{4.1}$$

in which ι is selection intensity, ρ is the correlation between the selection criterion and the breeding value for the trait of interest, usually referred to as the accuracy, and σ_A is the additive genetic standard deviation in the trait of interest (Falconer and Mackay, 1996). The

quality of selection methods is commonly measured by their accuracy, which is easy to interpret because it takes values between zero and one (Falconer and Mackay, 1996; Kinghorn et al., 2000). Previous studies have yielded expressions for response to individual and group selection (Griffing, 1967; Griffing, 1976a; Wolf, 2003; Bijma et al., 2007a). Those expressions were based on the covariance between the selection criterion and the breeding value, but did not distinguish between accuracy and genetic variance in the trait. Thus the accuracies of individual and group selection are unclear at present.

In the following, we reformulate existing equations for response to individual and group selection into components of Equation 4.1 and provide expressions for their accuracy. The results show that expressions for response to selection with social interactions among individuals are a generalization of classical expressions for response in the absence of social interactions. First, we briefly summarize the basic quantitative genetic theory of social interactions presented in Griffing (1967), Wolf et al. (1998), Muir (2005) and Bijma et al. (2007a).

Table 4.1. Notation key

Symbol	Meaning
P_i	Observed trait value for individual *i*
ΔG	Selection response in observed trait value per generation
P_{D_i} , P_{S_i}	Phenotypic direct and associative effect
A_{D_i} , A_{S_i}	Direct and associative breeding value (DBV, SBV)
TBV	Total breeding value: $\mathrm{TBV}_i = A_{\mathrm{D}_i} + (n-1)A_{\mathrm{S}_j}$
TPV	Total phenotypic value: $\mathrm{TPV}_i = P_{\mathrm{D}_i} + (n-1)P_{\mathrm{S}_j}$
n, m	Group size, no. of groups per selection candidate
r	Relatedness between selection candidate and its relatives
r_{br}	Relatedness between group members
$\sigma^2_{A_{\mathrm{D}}}$, $\sigma^2_{A_{\mathrm{S}}}$, $\sigma_{A_{\mathrm{DS}}}$	Direct genetic variance, associative genetic variance, covariance between direct genetic variance and associative genetic variance
σ_P , $\sigma_{\bar{P}_{\mathrm{grp}}}$, $\sigma_{\bar{P}_{\mathrm{rel}}}$	Standard deviation among phenotypic values of individuals, among average phenotypic values of groups, and among average phenotypic values of relatives in family groups
τ	Intraclass correlation among relatives adjusted for interactions, $\tau = r_{\mathrm{br}}\eta^2$
η^2	Heritability adjusted for interactions, $\eta^2 = \sigma^2_{\mathrm{TBV}} / \sigma^2_{\mathrm{TPV}}$.
i, ρ, σ^2_{TBV}	Selection intensity, accuracy of selection, variance of TBV

Model

In the classical quantitative genetic model, the phenotype of an individual is the sum of its genetic merit or "breeding value" and a residual nonheritable effect ($P = A + E$). With social interactions, the model needs to be extended to incorporate effects originating from other individuals. When social interactions occur among n (number of animals per group) individuals, the phenotype of an individual can be modelled as the sum of its own direct phenotypic effect and the summed associative phenotypic effects of its $n-1$ associates: $P_i = P_{D_i} + \sum_{j=1,n-1} P_{S_j}$ (Griffing, 1967) (see Table 4.1 for notation). Thus, the phenotype of each individual consist of two terms, a direct effect (P_D) originating from the genes and the physical environment of the individual itself and the sum of associative effects (P_S) originating from each of its $n-1$ group members. Because each individual has both a direct and an associative effect, the model applies to each of the n individuals in a group. Note that P_i is the observed phenotype, whereas P_D and P_S may be unobservable. Models used for maternal genetic effects, in which the phenotype of offspring is the sum of an unobserved direct effect due to the offspring and an unobserved maternal effect due to its dam, can be seen as a specific case of this more general model (Willham, 1963).

Both the direct and the associative effect can be divided into an additive genetic (A) and a residual (E) component,

$$P_i = P_{D_i} + \sum_{j=1,n-1} P_{S_j} = A_{D_i} + E_{D_i} + \sum_{j=1,n-1} (A_{S_j} + E_{S_j}) \quad (4.2)$$

(Muir, 2005; Bijma et al., 2007a), where A_{D_i} is the direct breeding value (DBV) of individual i, E_{D_i} is the nonheritable direct effect of individual i, A_{S_j} is the associative breeding value (SBV) of associate j, and E_{S_j} is the nonheritable associative effect of associate j. Note that DBV and SBV are genetically distinct traits, even though they affect a single phenotype. For example, when interest is in growth rate, the DBV refers to the breeding value of an individual for its own growth rate, whereas the SBV refers to its heritable effect on growth rate of other individuals in the group, which may, for example, be related to aggression or competition for feed. So, the DBV is equivalent to the classical breeding value (Lynch and Walsh, 1998), whereas the SBV is a generalization of a breeding value for a maternal effect (Willham, 1963).

Each individual expresses its DBV once in its own phenotype and its SBV $n-1$ times in the phenotypes of its associates. The heritable contribution of a single individual to total

performance of its group, referred to as its total breeding value (TBV), equals therefore $TBV_i = DBV_i + (n-1)SBV_i$ (Bijma et al., 2007a). Response to selection (ΔG), *i.e.*, the genetic change of the mean trait value per generation, equals the per generation increase of the average TBV of the population. Analogous to Equation 4.1, response to selection can be expressed as

$$\Delta G = \iota\rho\sigma_{TBV}, \tag{4.3}$$

in which ρ is the accuracy and σ_{TBV} the standard deviation of total breeding values among individuals. In Equation 4.3, the accuracy is the correlation between the selection criterion and the TBVs of individuals. It measures the quality of selection methods for traits affected by social interactions. The σ_{TBV} is the square root of the total heritable variation in the trait, which equals

$$\sigma^2_{TBV} = \sigma^2_{A_D} + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma^2_{A_S} \tag{4.4}$$

(Bijma et al., 2007a), where $\sigma^2_{A_D}$ is the direct genetic variance, $\sigma^2_{A_S}$ is the associative genetic variance, and $\sigma_{A_{DS}}$ is the covariance between DBVs and SBVs of individuals.

Accuracies of individual and group selection

In this section we derive the accuracies of individual and group selection, so that response of these selection methods can be expressed as in Equation 4.3. Table 4.2 summarizes the selection methods and the corresponding equations for accuracy of selection.

Table 4.2. Accuracies of selection methods

Method	Accuracy	Range
Individual selection	$\left\{\sigma^2_{A_D} + (n-1)\sigma_{A_{DS}} + r(n-1)\left[\sigma_{A_{DS}} + (n-1)\sigma^2_{A_S}\right]\right\}/(\sigma_{TBV}\sigma_P)$	-1– +1
Group selection	$\left[(n-1)r+1\right]\sigma_{TBV}/n\sigma_{\bar{P}_{grp}}$	0–(0.707–1)[a]
Selection based on relatives	$r\eta/\sqrt{\tau+(1-\tau)/mn}$, $\eta = \sigma_{TBV}/\sigma_{TPV}$, $\tau = r_{br}\eta^2$	
Full sib		0–0.707
Half sib		0–0.5
Half-sib progeny		0–1.0

[a] Depending on group size

Individual selection: With individual selection, selection candidates are kept in groups. Individuals with a phenotypic value greater than a chosen threshold value are selected to become parents of the next generation, irrespective of the performance of their group members (Griffing, 1960). Response to individual selection in a population consisting of groups of size *n* equals

$$\Delta G = \left\{\sigma^2_{A_D} + (n-1)\sigma_{A_{DS}} + r(n-1)\left[\sigma_{A_{DS}} + (n-1)\sigma^2_{A_S}\right]\right\}\frac{\imath}{\sigma_P}, \tag{4.5}$$

(Bijma et al., 2007a), in which r denotes the additive genetic relatedness between group members, and σ_P is the standard deviation among phenotypic trait values of individuals, where $\sigma^2_P = \sigma^2_{A_D} + \sigma^2_{E_D} + (n-1)(\sigma^2_{A_S} + \sigma^2_{E_S}) + r\left[2(n-1)\sigma_{A_{DS}} + (n-1)(n-2)\sigma^2_{A_S}\right]$.

Combining Equations 4.3 and 4.5 shows that the accuracy of individual selection equals

$$\rho_{ind} = \frac{\sigma^2_{A_D} + (n-1)\sigma_{A_{DS}} + r(n-1)\left[\sigma_{A_{DS}} + (n-1)\sigma^2_{A_S}\right]}{\sigma_{TBV}\,\sigma_P}. \tag{4.6}$$

When there are no interactions among individuals, so that $\sigma^2_{A_S} = \sigma_{A_{DS}} = 0$ and $\sigma^2_{TBV} = \sigma^2_{A_D}$, Equation 4.6 reduces to $\rho_{ind} = h$, the square root of heritability, and Equation 4.3 to $\Delta G = \imath h \sigma_{A_D}$, the usual expression for response to mass selection.

When there are no social interactions, the accuracy is between zero and one (Falconer and Mackay, 1996; Kinghorn et al., 2000). Investigation of Equation 4.6, however, shows that with social interactions, the accuracy of individual selection can be negative, which would result in a negative response to selection. With unrelated group members ($r = 0$), for example, the latter occurs when $\sigma_{A_{DS}}$ is negative and greater in absolute magnitude than $\sigma^2_{A_D}/(n-1)$, which depends on the genetic correlation between direct and associative effects (r_A). When this correlation is negative, individuals with high DBVs have on average negative SBVs, *i.e.*, a negative effect on the phenotypes of their group members. As a consequence, the use of individual selection can result in a negative response to selection (Griffing, 1967). With full relatedness ($r = 1$), *i.e.*, when interactions are among clones, the numerator of Equation 4.6 becomes equal to σ^2_{TBV}, which is positive by definition. Thus relatedness among interacting individuals has the effect of making the correlation between phenotypes and TBVs of individuals a positive value.

Group selection: With group selection, individuals in groups with an average phenotypic value greater than a given value are selected to become parents of the next generation. Thus the entire group is either selected or rejected solely on the basis of the mean phenotypic value of the entire group, $\overline{P}_{\text{grp}} = (1/n)\sum_{i=1}^{n} P_i$ (Griffing, 1967). Response to group selection is given by Bijma et al. (2007a),

$$\Delta G = \left[(n-1)r+1\right]\sigma^2_{\text{TBV}}\left(\frac{\imath}{n\sigma_{\overline{P}_{\text{grp}}}}\right), \tag{4.7}$$

in which $\sigma_{\overline{P}_{\text{gpr}}}$ is the standard deviation among group means, $\sigma^2_{\overline{P}_{\text{grp}}} = \left\{\sigma^2_P + 2(n-1)\text{Cov}(P_i,P_j) + (n-1)\left[\sigma^2_P + (n-2)\text{Cov}(P_i,P_j)\right]\right\}/n^2$, with σ^2_P given above Equation 4.6, and $\text{Cov}(P_i,P_j)$ = $2(\sigma_{A_{\text{DS}}} + \sigma_{E_{\text{DS}}})$ + $(n-2)(\sigma^2_{A_{\text{S}}} + \sigma^2_{E_{\text{S}}})$ + $r\left[\sigma^2_{A_{\text{D}}} + 2(n-2)\sigma_{A_{\text{DS}}} + (n^2-3n+3)\sigma^2_{A_{\text{S}}}\right]$ (See example in Bijma et al. 2007a). Combining Equations 4.3 and 4.7 shows that the accuracy of group selection equals

$$\rho_{\text{grp}} = \frac{\left[(n-1)r+1\right]\sigma_{\text{TBV}}}{n\sigma_{\overline{P}_{\text{grp}}}}. \tag{4.8}$$

Because both the numerator and the denominator of Equation 4.8 are positive, the accuracy and response to group selection are always positive. Furthermore, as indicated by the term $\left[(n-1)r+1\right]$, the accuracy of group selection is greater with family groups than with groups of unrelated individuals, which agrees with expressions for response to selection obtained by Griffing (1976a,b).

Selection based on relatives: When selection candidates are housed individually, their phenotypes provide no information on their SBVs. In that case, information for selection methods aiming to improve the population average TBV needs to come from relatives of the candidate, which are kept in groups. These relatives of the selection candidates are assumed to be present at the same time as the selection candidates themselves. The phenotypic value of a relative, say *j*, consists of the direct effect of that relative and the summed associative effects of its group members, denoted by *k*: $P_j = P_{\text{D}_j} + \sum_{n-1} P_{\text{S}_k}$. If the group members of the relative are unrelated to the candidate (r_{ik} = 0), then the phenotype of the relative provides information only on the direct effect of the candidate, not on its

associative effect. This is because $\mathrm{Cov}(\mathrm{TBV}_i, \sum_{n-1} P_{\mathrm{S}_k}) = 0$, so that $\mathrm{Cov}(\mathrm{TBV}_i, P_j) = r_{ij}\sigma^2_{A_\mathrm{D}}$. To capture the entire TBV of the selection candidate, relatedness between the candidate and the group members of its relatives needs to be equal to relatedness between the candidate and its relatives, $r_{ik} = r_{ij}$, so that $\mathrm{Cov}(\mathrm{TBV}_i, P_j) = \mathrm{Cov}(\mathrm{TBV}_i, A_{\mathrm{D}_j} + \sum_{n-1} A_{\mathrm{S}_k}) = r\sigma^2_{\mathrm{TBV}}$. A situation with $r_{ik} = r_{ij}$ is obtained by keeping relatives in family groups. For example, when selection is based on sib information, groups may consist of full sibs of the candidate, so that $r_{ik} = r_{ij} = 0.5$. In the following, therefore, we consider selection based on relatives of the candidate that are kept in family groups.

The accuracy of selection based on the mean phenotypic value of relatives kept in family groups can be expressed analogous to the situation with traits not affected by social interactions (see Appendix 4A for the derivation). In the absence of social interactions, the accuracy of selection based on relatives is commonly formulated in terms of relatedness between the candidate and its relatives, r, the square root of heritability, h, and the intraclass correlation t between the relatives,

$$\rho = \frac{rh}{\sqrt{t + (1-t)/N}}, \tag{4.9}$$

in which $t = r_{\mathrm{br}}h^2$, the product of relatedness between the relatives and heritability, and N is the number of relatives (Falconer and Mackay, 1996; Cameron, 1997; Lynch and Walsh, 1998). Hence, we distinguish between relatedness r between the candidate and its relatives and mutual relatedness r_{br} between the relatives. For example, for half-sib progeny of the candidate we would have $r = \frac{1}{2}$ and $r_{br} = \frac{1}{4}$. The analogy of Equation 4.9 for traits affected by interactions is

$$\rho_{\mathrm{rel}} = \frac{r\eta}{\sqrt{\tau + (1-\tau)/mn}} \tag{4.10}$$

(Appendix 4A). In Equation 4.10, we used Greek symbols to denote heritability and intraclass correlation adapted to account for interactions; $\eta = \sigma_{\mathrm{TBV}}/\sigma_{\mathrm{TPV}}$ is an analogy of the square root of heritability, $h = \sigma_{\mathrm{A}}/\sigma_{\mathrm{P}}$, $\tau = r_{\mathrm{br}}\eta^2$ is an analogy of the intraclass correlation between relatives $t = r_{\mathrm{br}}h^2$, and mn is the number of relatives in m groups consisting of n individuals each. The η and τ account for social interactions among

individuals and, therefore, depend on the TBV and on the total phenotypic value (TPV) contributed by an individual. The TPV is an analogy of the TBV. The TPV of individual *i* represents the phenotypic effect on the population mean that originates from individual *i*, which equals its direct phenotypic value plus $n - 1$ its associative phenotypic value, $\mathrm{TPV}_i = P_{\mathrm{D}_i} + (n-1)P_{\mathrm{S}_i}$, so that $\sigma^2_{\mathrm{TPV}} = \sigma^2_{P_{\mathrm{D}}} + 2(n-1)\sigma_{P_{\mathrm{DS}}} + (n-1)^2\sigma^2_{P_{\mathrm{S}}}$. Note that TPV_i differs from the observed phenotypic value of individual *i*, $P_i = P_{\mathrm{D}_i} + \sum_{j=1,n-1} P_{\mathrm{S}_j}$, which contains associative effects of the group members *j* of *i*, whereas TPV_i contains the associative effect of *i* itself. Thus, the TPV measures the total effect of an individual on performance of its group, the TBV is the heritable component of the TPV, and $\eta^2 = \sigma^2_{\mathrm{TBV}} / \sigma^2_{\mathrm{TPV}}$ is the proportion of the variance of the TPV that is heritable, fully analogous to the classical heritability. The intraclass correlation τ equals the correlation between TPVs of relatives, analogous to the classical intraclass correlation *t*, which equals the correlation between phenotypes of relatives for traits not affected by social interactions. In conclusion, Equation 4.10 shows that response to selection based on relatives kept in family groups can be obtained from the classical expression for response to selection based on relatives, when replacing heritability by $\sigma^2_{\mathrm{TBV}}/\sigma^2_{\mathrm{TPV}}$ and the intraclass correlation between relatives by $r_{\mathrm{br}}\sigma^2_{\mathrm{TBV}}/\sigma^2_{\mathrm{TPV}}$. In the absence of social interactions, η reduces to *h*, τ reduces to *t*, and σ_{TBV} reduces to σ_A, so that Equation 4.10 reduces to Equation 4.9.

Investigation of the accuracy for large numbers of observations, *i.e.*, for $m \to \infty$, shows that the limiting accuracy equals $r/\sqrt{r_{\mathrm{br}}}$, which is 0.5 for half-sib information, 0.707 for full-sib information, and 1 for half-sib progeny of the selection candidate. These results are the same as those for classical selection based on relatives in the absence of interactions. Hence, limiting accuracies for traits affected by social interactions among individuals are the same as those for classical traits. For example, it is possible to obtain an accuracy approaching unity by using information on a large number of half-sib progeny kept in groups consisting of half sibs.

APPLICATION

So far, we have presented expressions for responses to selection and accuracies of individual selection, group selection, and selection based on relatives. In this section we numerically illustrate the accuracy for the three selection methods for a varying degree of competition. The magnitude of social interactions was varied by varying the associative phenotypic variance ($\sigma^2_{P_{\mathrm{S}}}$). Large $\sigma^2_{P_{\mathrm{S}}}$ causes large differences in SBVs of individuals and

thus reflects a situation with large social interactions. The type of interactions was varied by varying the genetic correlation (r_A) between direct and associative effects. Negative r_A corresponds to competition among individuals, zero r_A corresponds to neutral interactions, and positive r_A corresponds to cooperation. For example, large $\sigma^2_{P_S}$ together with a strongly negative r_A represents strong competition, whereas small $\sigma^2_{P_S}$ together with a slightly positive r_A represents mild cooperation. Table 4.3 summarizes the default values of genetic parameters used.

Table 4.3. Default values used to compare selection methods

Parameter	Abbreviation	Default value
Number of animals per group	n	4
Number of groups per selection candidate	m	1
Heritability of direct effect[a]	h^2_D	0.10
Heritability of associative effect[b]	h^2_S	0.10
Direct phenotypic variance	$\sigma^2_{P_D}$	1
Associative phenotypic variance	$\sigma^2_{P_S}$	0.33
Genetic, environmental, and phenotypic correlation between direct and associative effect	r_A, r_E, r_P	0

[a] Obtained as $h^2_D = \sigma^2_{A_D} / \sigma^2_P$. [b] Obtained as $h^2_S = \sigma^2_{A_S} / \sigma^2_P$.

The default value of $\sigma^2_{P_S} = 0.33\,\sigma^2_{P_D}$ together with group size (n) of 4 implies that ~50% of the total phenotypic variance is due to associative effects. Larger values of $\sigma^2_{P_S}$ indicate strong social interactions and *vice versa*. The following selection methods were compared: individual selection with groups consisting of either unrelated individuals ($r = 0$) or full sibs ($r = \frac{1}{2}$), group selection with groups of full sibs ($r = \frac{1}{2}$), and selection based on relatives with groups of half sibs ($r = r_{br} = \frac{1}{4}$), full sibs ($r = r_{br} = \frac{1}{2}$), or half-sib progeny ($r = \frac{1}{2}$, $r_{br} = \frac{1}{4}$).

Figure 4.1 illustrates the impact of the magnitude of social interactions ($\sigma^2_{P_S}$) for the case where interactions are neutral ($r_A = 0$) and for a single group of relatives ($m = 1$). The accuracy of group selection and selection based on relatives was not affected by the magnitude of interactions. This equivalence results from the fact that the heritabilities of

direct and associative effects were equal and that the genetic and environmental correlations between direct and associative effects were equal also (Table 4.3).

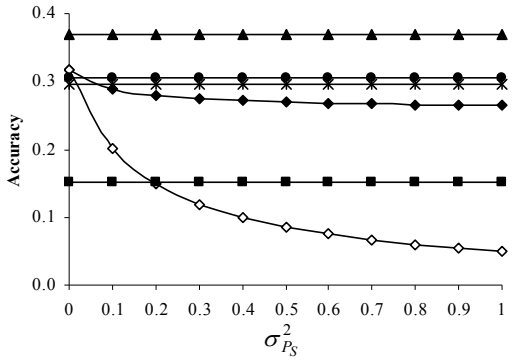

Figure 4.1. Accuracy of selection methods as a function of the associative phenotypic variance ($\sigma^2_{P_S}$) (n = 4; m = 1; $\sigma^2_{P_D}$ = 1; h^2_D = 0.10; h^2_S = 0.10; $r_A = r_E = 0$). The accuracy is shown for individual selection when the animals in a group are full sibs (◆) or unrelated (◇); for group selection with groups of full sibs (▲); and for selection based on relatives where relatives can be half sibs (■), full sibs (∗), or half-sib offspring (●).

In all situations, group selection with groups of full sibs yielded the highest accuracy, even when social interactions were absent. This is because the heritability was low (0.10), so that information on relatives is more important than one's own information. When heritability was high (0.5), individual selection had higher accuracy in the absence of associative effects than selection based on groups of full sibs (results not shown). As expected, selection based on full sibs or progeny yielded higher accuracy than selection based on half sibs. Individual selection with groups of unrelated individuals performed well in the absence of social interactions ($\sigma^2_{P_S}$ = 0), but poorly when the magnitude of social interactions increased. Individual selection with groups of full sibs performed well over the entire range of $\sigma^2_{P_S}$, although somewhat less well than selection based on full sibs or progeny and group selection.

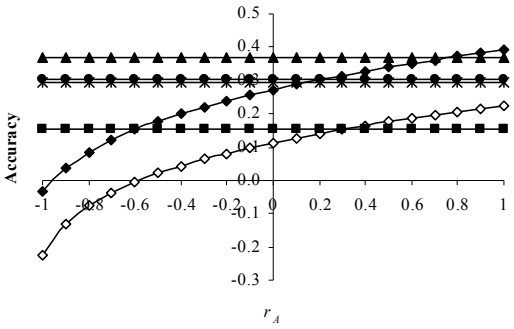

Figure 4.2. Accuracy of selection methods as a function of the genetic correlation (r_A) when $r_A = r_E$ ($n = 4$; $m = 1$; $\sigma^2_{P_D} = 1$; $\sigma^2_{P_S} = 0.33$; $h^2_D = 0.10$; $h^2_S = 0.10$). The accuracy is shown for individual selection when the animals in a group are full sibs (◆) or unrelated (◇); for group selection with groups of full sibs (▲); and for selection based on relatives where relatives can be half sibs (■), full sibs (∗), or half-sib offspring (●).

Figure 4.2 illustrates the impact of the type of interactions (r_A) for the case where interactions contributed 50% of the phenotypic variance ($\sigma^2_{P_S} = 0.33$) and for a single group of relatives ($m = 1$). The accuracy of group selection and selection based on relatives was not affected by r_A and was always positive. The accuracy of individual selection was highest with strong cooperation, but decreased strongly when interactions became more competitive (lower r_A) and became negative with strong competition. Together, Figures 4.1 and 4.2 illustrate that, in contrast to individual selection, group selection and selection based on relatives are robust against variation in the magnitude and type of interactions.

In contrast to group and individual selection, selection based on relatives offers the opportunity to use information on multiple groups of individuals. For example, males may be selected on the mean performance of 10 groups of offspring, instead of on the performance of a single group. Figure 4.3 illustrates the impact of the number of groups of relatives. The accuracy of selection based on relatives was independent of r_A and $\sigma^2_{P_S}$, so that results in Figure 4.3 apply to any magnitude and type of interactions (as long as $r_A = r_E$ and $h^2_D = h^2_S$). The accuracies of selection based on relatives increased substantially with the number of groups of relatives. The relationship between accuracy and the number of groups in Figure 4.3 was similar to that between accuracy and the number of relatives for

classical traits not affected by interactions (Falconer and Mackay, 1996). For comparison, Figure 4.3 also shows accuracies of individual and group selection for neutral interactions (r_A = 0). Those accuracies were substantially smaller than values obtained with selection based on multiple groups of relatives.

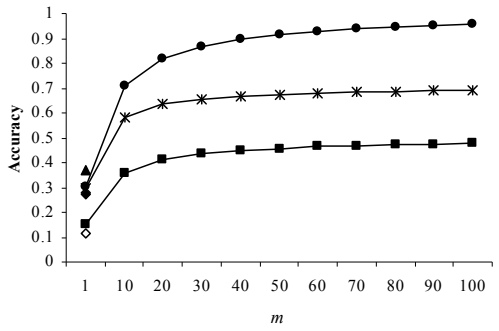

Figure 4.3. Accuracy of selection methods as a function of the number of groups per selection candidate (m) (n = 4; $\sigma^2_{F_D} = 1$; $\sigma^2_{F_S} = 0.33$; $h^2_D = 0.10$; $h^2_S = 0.10$; $r_A = r_E = 0$). The accuracy is shown for individual selection when the animals in a group are full sibs (◆) or unrelated (◇); for group selection with groups of full sibs (▲); and for selection based on relatives, where relatives can be half sibs (■), full sibs (∗) or half-sib offspring (●). When m = 1, selection based on full sibs and selection based on half-sib offspring received the same symbol, because the accuracy is, respectively, 0.29 and 0.31.

DISCUSSION

In this article, we derived expressions for the accuracy of individual and group selection and investigated opportunities for selection based on information from relatives kept in family groups. This work rests primarily on the foundational work of Willham (1963) and Griffing (1967; 1976a). Unfortunately, the work of Griffing (1967; 1976a) has had relatively little impact in the field of livestock genetic improvement, which is mainly due to the difficulty of derivations and the treatment of distinct situations as special cases. In contrast to most previous work on social interactions (*e.g.,* Griffing, 1967, 1976a; Wade, 1978; Wolf et al., 1998), our results are expressed in terms familiar to animal breeders, such as intensity of selection, accuracy of selection, and the standard deviation of (total) breeding values. We expect that this way of expressing results will stimulate the acceptance of quantitative genetic theory of social interactions and its application in livestock genetic improvement. They also provide insight into the prospects for development of better

selection strategies; *i.e.,* low accuracies indicate substantial prospects for improvement of the selection strategy, whereas values near unity indicate little prospects for improvement.

Our results show that selection based on relatives kept in family groups acts directly on the TBVs of selection candidates and always yields a positive response to selection. Selection based on relatives kept in family groups can be interpreted as an analogy of selection based on relatives for classical traits, on the condition that the definition of heritability and intraclass correlation between relatives are extended to account for social interactions. This analogy suggests that, analogous to classical traits, selection on the mean performance of relatives kept in family groups is the optimum way to use information from relatives for traits affected by social interactions. This hypothesis is supported by the fact that asymptotic accuracies obtained with large numbers of relatives are identical to values for classical traits not affected by social interactions.

The added value of selection based on relatives compared to individual or group selection depends on the genetic parameters of the trait, the consequences for parameters of the breeding scheme (intensity of selection and generation interval), and costs involved in keeping different numbers of animals. The advantage is largest with strong competition and low heritability. With cooperation ($r_A > 0$), individual selection yields a positive response, so that group selection or selection based on relatives is not required to ensure positive response (Griffing, 1967). However, when multiple groups of relatives can be used, selection based on relatives yields substantially higher accuracy, particularly when heritability is low. Group selection is robust in the sense that it always yields a positive response (Griffing, 1976a), but requires keeping selection candidates in groups, which is often undesirable. Moreover, group selection cannot be used for traits that require killing the individuals providing the information, such as meat percentage in chickens or pigs, because it would require killing the selection candidate.

At present there is very little information on the genetic parameters underlying traits affected by interactions ($\sigma^2_{A_D}$, $\sigma^2_{A_S}$, r_A; Wolf, 2003; Muir, 2005; Bijma et al., 2007b). The selection method developed here can be implemented without knowledge of the genetic parameters in that population, but the efficiency will depend on the parameters used. Genetic parameters of traits affected by social interactions can be estimated from livestock populations, but the amount of data required to accurately estimate those parameters is substantially larger than that for classical traits (Bijma et al., 2007b). For example, Bijma et al. (2007b) used information on ~3,800 individuals with a full-sib–half-sib pedigree structure and groups composed of unrelated individuals and obtained an estimated genetic

correlation between direct and associative effects of +0.28. This value, however, did not differ significantly from zero. With knowledge of the genetic parameters, individuals could be selected based on the basis of an index of group and individual performance (Griffing, 1969). Furthermore, when information is available on the genetic parameters, the use of best linear unbiased predictions (BLUP) of breeding values with an animal model allows combining information from different types of relatives into a single estimate of the breeding value (Henderson, 1984; Muir, 2005; Bijma et al., 2007b). The accuracy of that breeding value will exceed the accuracy obtained with selection based on relatives. When accurate information on genetic parameters is not available, however, selection based on relatives kept in family groups provides a robust solution that is relatively easy to implement.

An example of a trait affected by social interactions among individuals is survival in laying hens. Survival in laying hens is affected by cannibalistic interactions among cage members, which has made it difficult to improve survival using conventional selection methods (Muir, 1996). Bijma et al. (2007b) estimated variance components for survival time in a commercial line of laying hens with intact beaks. In this line, mean survival till 80 weeks of age was 54%, with a mean survival time of 454 days (SD 122 days). The estimated genetic parameters were $\sigma^2_{A_D}$ = 960 days^2, $\sigma^2_{A_S}$ = 132 days^2, r_A = 0.28, σ^2_E = 12,369 days^2, n = 4, and σ_{TBV} = 52.4 days. With a selection intensity of unity ($\imath$ = 1), predicted responses for this population are 10.7 days for individual selection with groups of unrelated individuals, 16.6 days for individual selection with groups of full sibs, and 22.8 days for group selection with groups of full sibs. With selection based on a single group of relatives, predicted responses are 9.6 days for half sibs, 18.3 days for full sibs and 19.2 days for half-sib progeny. With selection based on 10 groups of relatives, predicted responses are 20.4 days for half sibs, 32.4 days for full sibs and 40.8 days for half-sib progeny. These results show that, even with moderate cooperation, selection based on information from relatives kept in family groups enables substantial response to selection.

The responses to selection mentioned above refer to the total genetic improvement due to the combination of direct and associative effects. There may also be interest, however, in the response to selection for the direct and the associative effect separately. For group selection, responses for direct and associative effects follow from results presented in Bijma et al. (2007a). For selection based on relatives, response in direct effects equals $\Delta G_D = r\left[\sigma^2_{A_D} + (n-1)\sigma_{A_{DS}}\right]\left(\imath/\sigma_{\bar{P}_{rel}}\right)$ and response in associative effects equals $\Delta G_S = r\left[\sigma_{A_{DS}} + (n-1)\sigma^2_{A_S}\right]\left(\imath/\sigma_{\bar{P}_{rel}}\right)$. Using the estimated genetic parameters of Bijma et al.

(2007b), selection based on half sibs yielded a response for the direct effect of 4.4 days and a response for the associative effect of 1.7 days, based on full sibs yielded, respectively, 8.4 days and 3.3 days, and based on half-sib offspring yielded responses of, respectively, 8.8 days and 3.5 days. When judging these results, it is important to realize that the contribution of the associative effects to the total response equals the response in associative effects multiplied by $n - 1$.

Commercial conditions

For all three selection methods we have to keep in mind that the housing conditions of the relatives kept in groups (selection based on relatives) or the selection candidates (individual and group selection) should accurately reflect the commercial conditions under which the animals will be reared by the farmer. Especially group size can have large effects on the impact of social interactions and thus on response to selection. Furthermore, in this article only homogeneous groups of the same type of relatives have been assumed, *e.g.*, only full sibs or only half sibs in one group. Under commercial conditions, however, groups can consist of a mix of full sibs, half sibs, and unrelated individuals. For both group selection and selection based on relatives it is possible to use an average r and r_{br} to estimate the accuracy and response to selection.

Index Selection

When the genetic parameters of direct and associative effects are known, selection of individuals using a selection index is an alternative to selection between groups or based on relatives. With social interactions among individuals, the goal parameter of such an index, usually referred to as the "aggregate genotype", would be the TBV of an individual. In matrix notation, the TBV of individual i is given by $\text{TBV}_i = \mathbf{v}'\mathbf{g}_i$, in which $\mathbf{v}' = (1, n-1)$ and $\mathbf{g}_i' = (A_{\text{D}_i}, A_{\text{S}_i})$. The direct effect of an individual is expressed in its own phenotype, whereas its associative effect is expressed in its group members. Hence, a simple index aiming to maximise response by simultaneous improvement of direct and associative effects can be composed of the phenotype of the individual itself, P_i, and the average phenotype of its $n - 1$ group members, $\overline{P}_{\text{grp}_i}$,

$$I_i = b_1 P_i + b_2 \overline{P}_{\text{grp}_i} = \mathbf{b}'\mathbf{x}_i . \qquad (4.11)$$

It follows from selection index theory that optimum index weights are $\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}\mathbf{v}$, where $\mathbf{P}$ is the 2 × 2 (co)variance matrix of information sources in $\mathbf{x}_i$, and $\mathbf{G}$ is the 2 × 2 matrix of covariances between information sources in $\mathbf{x}_i$ and true breeding values for direct and associative effects in $\mathbf{g}$ (Hazel, 1943). Elements of $\mathbf{P}$ and $\mathbf{G}$ are $P_{11} = \sigma^2_P$, $P_{12} = P_{21} = 2\sigma_{P_{DS}} + (n-2)\sigma^2_{P_S}$, $P_{22} = \{\sigma^2_P + (n-2)[2\sigma_{P_{DS}} + (n-2)\sigma^2_{P_S}]\}/(n-1)$, $G_{11} = \sigma^2_{A_D}$, $G_{12} = G_{21} = \sigma_{A_{DS}}$, $G_{22} = \sigma^2_{A_S}$. From selection index theory, response to selection equals

$$\Delta G = \mathbf{b}'\mathbf{G}\mathbf{v}\frac{\imath}{\sigma_I}, \tag{4.12}$$

in which σ_I is the standard deviation of the index, $\sigma_I = \sqrt{\mathbf{b}'\mathbf{P}\mathbf{b}}$.

To compare response with index selection to other selection methods, we calculated response for the genetic parameters of Bijma et al. (2007b) given above. Predicted response from Equation 4.12 was 11.7 days. This is only a little more than the 10.7 days of response from individual selection with groups of unrelated individuals, but substantially less than the 16.6 days of response for individual selection with groups of full sibs. Thus optimum index selection using groups of unrelated individuals performs worse than individual selection using groups of relatives. This result indicates that using groups composed of relatives contributes more to the accuracy than optimizing index weights. The index calculations can be extended to apply to groups composed of relatives, but the derivations become complex in that case. Selection based on groups composed of relatives, in contrast, is simple and robust. It does not require knowledge of the genetic parameters, and its accuracy does not depend on the real values of the genetic parameters (Figures 4.1 and 4.2).

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APPENDIX 4A: DERIVATION OF EQUATION 4.10

With selection based on relatives kept in family groups, the selection criterion for individual *i* equals the mean phenotypic value of its relatives, $\overline{P}_{\mathrm{rel}_i} = 1/mn \sum_{l=1}^{m} \sum_{j=1}^{n} P_{j,l}$, in which $P_{j,l}$ is the phenotypic trait value of relative *j* in group *l*, and *m* is the number of family groups of selection candidate *i*, each consisting of *n* individuals. The $P_{j,l}$ has components as indicated by Equation 4.2: $\sum_m \sum_n P_{j,l} = \sum_m \sum_n \left(P_{\mathrm{D}_{j,l}} + \sum_{n-1} P_{\mathrm{S}_{k,l}} \right)$, in which *k* denotes group members of *j*. Response to selection is obtained as the regression coefficient of the TBV of the selection candidates on the selection criterion, $b_{\mathrm{TBV},\overline{P}_{\mathrm{rel}}}$, multiplied by the selection differential *S*. Thus, response to selection equals $b_{\mathrm{TBV},\overline{P}_{\mathrm{rel}}} S$, with $b_{\mathrm{TBV},\overline{P}_{\mathrm{rel}}} = \mathrm{Cov}\left(\mathrm{TBV}_i, \overline{P}_{\mathrm{rel}_i}\right) / \mathrm{Var}\left(\overline{P}_{\mathrm{rel}_i}\right)$ and $S = \imath \sigma_{\overline{P}_{\mathrm{rel}}}$, so that $\Delta G = \mathrm{Cov}\left(\mathrm{TBV}_i, \overline{P}_{\mathrm{rel}_i}\right) \imath / \sigma_{\overline{P}_{\mathrm{rel}}}$, in which $\sigma_{\overline{P}_{\mathrm{rel}}}$ is the standard deviation of the selection criterion. The term $\mathrm{Cov}\left(\mathrm{TBV}_i, \overline{P}_{\mathrm{rel}_i}\right)$ can be split into *mn* covariances, giving $(1/mn) \sum_m \sum_n \mathrm{Cov}\left(\mathrm{TBV}_i, P_{j,l}\right) = (1/mn) \sum_m \sum_n \mathrm{Cov}\left(\mathrm{TBV}_i, P_{\mathrm{D}_{j,l}} + \sum_{\mathrm{n-1}} P_{\mathrm{S}_{k,l}}\right) = (1/mn) \sum_m \sum_n \mathrm{Cov}\left(\mathrm{TBV}_i, A_{\mathrm{D}_j} + \sum_{\mathrm{n-1}} A_{\mathrm{S}_i}\right)$. When all relatives have the same relatedness with the selection candidate, *i.e.*, $r_{ij} = r_{ik} = r$ for all *i*, *j* and *k*, then all covariances have the same value, giving $\mathrm{Cov}\left(\mathrm{TBV}_i, A_{\mathrm{D}_j} + \sum_{\mathrm{n-1}} A_{\mathrm{S}_k}\right) = \mathrm{Cov}\left[A_{\mathrm{D}_i} + (n-1) A_{\mathrm{S}_i}, A_{\mathrm{D}_j} + (n-1) A_{\mathrm{S}_k}\right] = r\sigma^2_{A_{\mathrm{D}}} + 2(n-1) r \sigma_{A_{\mathrm{DS}}} + (n-1)^2 r \sigma^2_{A_{\mathrm{S}}} = r\sigma^2_{\mathrm{TBV}}$, in which *r* denotes additive genetic relatedness

between the selection candidate and its relatives kept in family groups. Response to selection based on relatives equals, therefore,

$$\Delta G = r\sigma^2_{\mathrm{TBV}} \frac{\imath}{\sigma_{\bar{P}_{\mathrm{rel}}}} . \qquad (4.A1)$$

Combining Equations 4.1 and 4.A1 show that the accuracy of selection based on relatives kept in family groups equals

$$\rho_{\mathrm{rel}} = \frac{r\sigma_{\mathrm{TBV}}}{\sigma_{\bar{P}_{\mathrm{rel}}}} . \qquad (4.A2)$$

Although Equation 4.A2 provides an expression for the accuracy, it is not easy to use, because it depends on $\sigma_{\bar{P}_{\mathrm{rel}}}$, which is not a common genetic parameter. In the following, therefore, we reformulate Equation 4.A2 in terms of Equation 4.9, starting with the derivation of $\sigma_{\bar{P}_{\mathrm{rel}}}$. To derive $\sigma_{\bar{P}_{\mathrm{rel}}}$, it is convenient to split $\bar{P}_{\mathrm{rel}}$ into a component that is common to all relatives of the selection candidate, plus a remaining term that no longer contains genetic relationships between individuals. For example, when considering half sibs, the common term would be half the TBV of the sire, so that $\bar{P}_{\mathrm{rel}} = \frac{1}{2}A_{\mathrm{D_{sire}}} + \frac{1}{2}(n-1)A_{\mathrm{S_{sire}}} + \bar{\varepsilon}$, in which $\bar{\varepsilon}$ is the remainder of $\bar{P}_{\mathrm{rel}}$ after subtracting $\frac{1}{2}\mathrm{TBV_{sire}}$. The ε is specific to each individual. In general, the common term equals $\sqrt{r_{\mathrm{br}}}\mathrm{TBV}$, in which r_{br} is mutual relatedness between the relatives. In the derivation of $\sigma_{\bar{P}_{\mathrm{rel}}}$, distinguishing between $\sqrt{r_{\mathrm{br}}}\mathrm{TBV}$ and $\bar{\varepsilon}$ is convenient, because we do not have to consider averaging for the $\sqrt{r_{\mathrm{br}}}\mathrm{TBV}$ term, we can ignore relatedness among individuals for $\bar{\varepsilon}$, and both terms are mutually independent (when mating is at random). Thus $\sigma^2_{\bar{P}_{\mathrm{rel}}} = \mathrm{Var}\left(\sqrt{r_{\mathrm{br}}}\mathrm{TBV} + \varepsilon\right) = r_{\mathrm{br}}\sigma^2_{\mathrm{TBV}} + \mathrm{Var}(\bar{\varepsilon})$.

For the derivation of $\mathrm{Var}(\bar{\varepsilon})$, it is convenient to group the direct and associative components in $\bar{\varepsilon} = \sum_{\mathrm{m}}\sum_{\mathrm{n}}\varepsilon_{j,l}$ according to the individual from which they *originate*, instead of according to the individual in whose phenotype they are expressed. Each individual expresses its direct effect once and its associative effect $n - 1$ times. Therefore, we can write that $\sum_m\sum_n\varepsilon_{j,l} = \sum_m\sum_n\left(\varepsilon_{\mathrm{D}_{j,l}} + \sum_{\mathrm{n\text{-}1}}\varepsilon_{\mathrm{S}_{k,l}}\right) = \sum_m\sum_n\left[\varepsilon_{\mathrm{D}_{j,l}} + (n-1)\varepsilon_{\mathrm{S}_{j,l}}\right]$, in

which $\varepsilon_{..} = P_{..} - \sqrt{r}\, A_{..}$, indicating the remainder when subtracting $\sqrt{r_{\text{br}}}\text{TBV}$ from $\bar{P}_{\text{rel}}$. In the last summation, direct and associative effects are grouped according to the individual j from which they originate. Next, $\text{Var}(\bar{\varepsilon}) = \sum_m \sum_n \text{Var}(\varepsilon_{j,l}) / (m^2 n^2) = \text{Var}\{\sum_m \sum_n [\varepsilon_{\text{D}_{j,l}} + (n-1)\varepsilon_{\text{S}_{j,l}}] / (m^2 n^2)\} = \text{Var}[\varepsilon_{\text{D}_{j,l}} + (n-1)\varepsilon_{\text{S}_{j,l}}] / (mn)$, because the $\varepsilon_{\cdot\cdot,j}$ are independent after subtraction of the common part. Finally, using $\sigma^2_{\bar{P}_{\text{rel}}} = r_{\text{br}}\sigma^2_{\text{TBV}} + \text{Var}(\bar{\varepsilon})$ shows that

$$\sigma^2_{\bar{P}_{\text{rel}}} = r_{\text{br}}\sigma^2_{\text{TBV}} + (\sigma^2_{\text{TPV}} - r_{\text{br}}\sigma^2_{\text{TBV}}) / mn\,, \tag{4.A3}$$

in which TPV denotes the total phenotypic value of an individual, $\text{TPV}_i = P_{\text{D}_i} + (n-1)P_{\text{S}_i}$, and $\sigma^2_{\text{TPV}} = \sigma^2_{P_{\text{D}}} + 2(n-1)\sigma_{P_{\text{DS}}} + (n-1)^2\sigma^2_{P_{\text{S}}}$. The TPV is an analogy of the TBV (see main text). In Equation 4.A3, the term $r_{\text{br}}\sigma^2_{\text{TBV}}$ is the variance of the part of $P_{j,l}$ that is common to all relatives, which is not averaged, and the term $(\sigma^2_{\text{TPV}} - r_{\text{br}}\sigma^2_{\text{TBV}})/mn = \text{Var}(\bar{\varepsilon})$ is the variance of the average value of the mn parts of $P_{j,l}$ that are specific to each relative. Combining Equations 4.A2 and 4.A3 gives an expression for the accuracy of selection. First, we rewrite Equation 4.A3 as $\sigma^2_{\text{TPV}}[\tau + (1-\tau)/mn]$, in which $\tau = r_{\text{br}}\sigma^2_{\text{TBV}} / \sigma^2_{\text{TPV}}$, which is the covariance between TPVs of relatives expressed as a proportion of the variance of TPVs, so that τ is a so-called intraclass correlation among relatives adjusted to account for social interactions among individuals. Substituting this result into Equation 4.A2 gives Equation 4.10.

APPENDIX 4B: EXAMPLE OF CALCULATION

This example illustrates the calculation of accuracy and response to selection for selection based on information of a single group of half-sib offspring ($r = \frac{1}{2}$, $r_{\text{br}} = \frac{1}{4}$, and $m = 1$). Estimated genetic parameters for survival time in a commercial line of laying hens are taken from Bijma et al. (2007b) and given in Table 4.A1.

Table 4.A1. Genetic parameters for the example

Parameter	Estimate
σ^2_e	12,369
$\sigma^2_{A_D}$	960
$\sigma^2_{A_S}$	132
$\sigma_{A_{DS}}$	99
ρ	0.09

The accuracy follows from Equation 4.10, $\rho_{rel} = r\eta \big/ \sqrt{\tau + (1-\tau)/mn}$, in which $\eta = \sigma_{TBV}/\sigma_{TPV}$ and $\tau = r_{br}\eta^2$. Thus, calculation of the accuracy requires calculating the variance of total breeding values, $\sigma^2_{TBV} = \sigma^2_{A_D} + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma^2_{A_S} = 960 + 2\times 3\times 99 + 3^2\times 132 = 2742$, and of the variance of total phenotypic values, $\sigma^2_{TPV} = \sigma^2_{P_D} + 2(n-1)\sigma_{P_{DS}} + (n-1)^2\sigma^2_{P_S}$. The statistical data analyses, however, do not provide estimates of the phenotypic variances of direct and associative effects, meaning that we cannot calculate σ^2_{TPV} directly. For this reason, σ^2_{TPV} is calculated as the sum of σ^2_{TBV} and the variance of the total environmental values of individuals, $\sigma^2_{TPV} = \sigma^2_{TBV} + \sigma^2_{TEV}$, in which $\sigma^2_{TEV} = \text{Var}\left[E_{i_D} + (n-1)E_{i_S}\right] = \sigma^2_{E_D} + 2(n-1)\sigma_{E_{DS}} + (n-1)^2\sigma^2_{E_S}$. The data analyses provides an estimate of the residual variance, σ^2_e, and an estimate of the correlation between residuals of group members, denoted ρ in Bijma et al. (2007b). σ^2_e and ρ can be combined into an estimate of σ^2_{TEV}. Bijma et al. (2007b) showed that $\sigma^2_e = \sigma^2_{E_D} + (n-1)\sigma^2_{E_S}$ and $\rho = \left[2\sigma_{E_{DS}} + (n-2)\sigma^2_{E_S}\right] \big/ \sigma^2_e$. Therefore, it follows that $\sigma^2_{TEV} = \sigma^2_e\left[1 + (n-1)\rho\right] = 12{,}369\times\left[1 + (4-1)\times 0.09\right] = 15{,}709$. Next, σ^2_{TPV} = 2742 + 15,709 = 18,451, so that $\eta = \sqrt{2742/18{,}451}$ = 0.39 and $\tau = 0.25\times 0.39^2$ = 0.037. Thus the accuracy of selection based on a single group of half-sib offspring equals $\rho_{rel} = (0.5\times 0.39) \big/ \sqrt{0.037 + (1-0.037)/(1\times 4)}$ = 0.37. When selection is by truncation with a selection intensity of unity (ι = 1) (Falconer and Mackay, 1996), response to selection is $\Delta G = \iota\rho_{rel}\sigma_{TBV}$ = $1\times 0.37\times 52.36 = 19.2$ days.

Chapter 5

Population specific effects on pupae weight and duration of development in the flour beetle, *Tribolium castaneum*, when exposed to food limitation during the larval stage

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ABSTRACT

Food limitation has an effect on ecological and genetic population characteristics. Individuals of the same species can show differences in population characteristics such as body size and duration of development. In this study, we investigate the effect of food limitation during the larval stage on pupal weight and duration of development in seven populations of *Tribolium castaneum* (flour beetle). We limited food by replacing a fixed amount of the standard medium with a non-nutritive component (α-cellulose) to create three environments: (1) Environment 1 with 100% standard medium, abundant resources; (2) Environment 2 with 50% standard medium and 50% α-cellulose, intermediate resources; and, (3) Environment 3 with 25% standard medium and 75% α-cellulose, a deficiency of resources. Population and environment had a large effect on pupal weight and duration of development ($p < 0.001$), as did the interaction between population and environment ($p < 0.015$). Furthermore, sex had a large effect on pupal weight ($p < 0.001$). As expected, larvae reared in Environment 1 yielded highest pupal weight while larvae reared in Environment 3 yielded lowest pupal weight, whereas duration of development increased when deficiency of resources was higher. We found population-level changes in scale of response, resulting in a change in the ranking of the populations within environments. These results suggest that, when exposed to food limitation, populations of flour beetles can differ in the expression of population characteristics. Furthermore, populations show different strategies to enhance local fitness.

Key words: pupal weight, duration of development, genotype-environment interaction, food limitation, *Tribolium castaneum*

INTRODUCTION

In natural populations, temporal and spatial heterogeneity in the environment, due to weather or food limitation, are influential factors in the evolution of life-history strategies (Wilbur, 1980). They can have profound effects on various population characteristics, such as duration of development, body size, reproduction, population size, migration, cannibalism, and inter-specific competition (King, 1967; Sokoloff, 1974; Dawson and Riddle, 1983; Duncan, 1989).

There are several studies that have shown the effect of food limitation on population characteristics. For instance, King (1967) showed that *Euchlanis dilatata* (rotifera) grown in ten times less algal food, yielded a doubling of the juvenile stage and a decrease in the body length of the primipara female. Boggs and Freeman (2005) showed that food limitation during the larval stage in butterflies reduced adult fitness. Besides food limitation's effect on mean population characteristics, individuals within the same population can show different strategies by developing a phenotype that enhances local fitness (DeWitt and Scheiner, 2004). For instance, Moorad (2005) found that individuals of *T. castaneum* developed differently in good versus poor environments: individuals of both sexes were more likely to develop quickly and to small pupal sizes in a nutrient poor environment, but to delay development and emerge at larger sizes in a nutrient rich environment. In both environments, more males tended to adopt the fast-small strategy than females. It was found that this trait was highly heritable ($h^2 = 0.63$).

Genotype-environment interaction is of particular importance in determining the course of evolution in spatially variable environments (Via and Lande, 1985; 1987; Via, 1991), and the *T. castaneum* model system is particularly amenable for the study of evolution in heterogeneous environments. Studies have revealed that flour type affects performance, *e.g.* weight and survival (Sokoloff et al., 1966a,b; Applebaum and Konijn, 1967) which also results in genotype-environment interactions (Hardin et al., 1967; Via and Conner, 1995). These results suggest some degree of specialization for particular resource types. However, it is unknown if there are differences among populations or sex-differences among populations in their response to food limitation. We report our findings from an investigation of the effect of food limitation during the larval stage on pupal weight and duration of development of *T. castaneum*, designed to characterize genotype-environment interaction and sex specific effects of food limitation.

MATERIAL AND METHODS

Populations

The seven populations of *T. castaneum* used in this study included five wild-type populations and two mutant populations (Table 5.1). The mutant *c*SM-black is homozygous for a black body colour marker, which is a naturally occurring mutation found segregating in a laboratory stock population (*c*SM; Wade, 1976; Goodnight and Craig, 1996). The mutant Ring eye is a sex-linked recessive, also derived from the *c*SM population. This phenotype is similar to the autosomal recessive pearl in which the lighter coloured center of the eye is circled by a darker marginal area (Yamada, 1961). The mutant ring eye has both a dark and a white marginal area. Each population has been maintained in large numbers (>200 breeding adults) on standard medium (95% fine-sifted organic whole wheat flour supplemented with 5% brewer's yeast, by weight) under the standard environmental conditions of 29°C, 70% relative humidity, and constant darkness.

Table 5.1. Names and origin of the seven populations of *T. castaneum*

	Population	Origin
Wild populations	*c*SM	Chicago, USA
	DES	Dar-es-salaam, Tanzania
	Bhopal	India
	Purdue	West-Lafayette, USA
	Baños	Ecuador
Mutant populations	Ring eye[a]	Chicago, USA
	*c*SM-black[b]	Chicago, USA

[a] Ring eye (rg) is sex-linked recessive and derived from the *c*SM population of Wade (1976) for which the center of the eye is coloured by a white marginal area. [b] *c*SM-black is homozygous for a black body colour marker. The black mutation is a naturally occurring mutation found segregating in the original stock population (*c*SM; Wade, 1976; Goodnight and Craig, 1996).

Design

For each population, we established cultures consisting of approximately 200 mature adults, and allowed them to lay eggs on standard medium for two days. After two days, we removed the adults and collected larvae a few days after they began hatching. From each population, ten larvae were randomly placed in one cell of a 24-cell tray (a total of 240

larvae per tray per population). Each tray was divided into three nutritional environments: (1) Environment 1 with 100% standard medium; (2) Environment 2 with 50% standard medium and 50% α-cellulose; and, (3) Environment 3 with 25% standard medium and 75% α-cellulose. For each environment, the total volume per cell was 2 gram. The α-cellulose is non-nutritive and addition to the medium is known to result in food limitation. Based on the amount of standard medium, we are confident that larvae in Environment 1 are exposed to abundant resources, whereas larvae in Environment 3 were exposed to a deficiency of resources. There were eight replicates of each environment in each tray and seven trays, one for each population studied. For practical reasons, the study was split into two batches: Batch 1 containing five populations (Baños, Bhopal, *c*SM-black, *c*SM, and DES), and Batch 2 containing two populations (Purdue and Ring eye). This set-up resulted in a total of 1,680 larvae at the start of the study.

After 12 days, cells were checked daily for pupae. Once pupae appeared, we separated larvae and pupae from medium by placing a second empty tray upside down on top of the original tray with a mesh filter between them. All pupae were removed from each cell, date of pupation was recorded, pupae were sexed under a dissecting microscope, and weighed on a Sartorius microbalance to the nearest 0.01 mg. Any remaining larvae were returned to their original cell. After collecting the first pupae, trays with remaining larvae were checked once every three days until all larvae became pupae.

Data analysis

Data on pupal weight and duration of development were analysed using the linear mixed model procedure of the SAS® statistical program (SAS, 1996). Due to some escapes (8.1% of the larvae), there were a total of 1,544 organisms left at the end of the study. Furthermore, some larvae escaped and moved to another cell. These escapes, resulted in a variation in number of pupae per cell, ranging from 1 to 16 with an average of 8.7 individuals per cell. Of the 1,544 organisms, 66 were without observations, because it was not possible to sex them (n = 33), they were already adults when first discovered (n = 11), they were still larvae (n = 2), they were dead (n = 10), or they showed abnormalities (n = 10). For both, pupal weight and duration of development, outliers more than three standard deviations from the mean were deleted (n = 40). Furthermore, cells with only one observation were removed as well. This resulted in 1,434 observations left for the statistical analysis.

Population, environment, and sex were included as fixed effects in the analysis of pupal weight, whereas population and environment were included as fixed effects in the analysis of duration of development. For both dependent variables, cell was included as a random effect, because observations from the same cell may be correlated. Batch was fully confounded with population and, therefore, not included as a separate fixed effect. The initial models included all possible two-way interactions. The non-significant effects ($p > 0.10$) were removed using the backward elimination procedure (Ott and Longnecker, 2001). The final model for pupal weight was:

$$Y_{ijklm} = \mu + P_i + E_j + S_k + (P \times E)_{ij} + c_l + e_{ijklm}$$

where Y is the pupal weight, μ is the overall mean, P_i is the fixed effect of the *i*th population (i = 1 to 7), E_j is the fixed effect of the *j*th nutrient environment (j = 1, 2, 3), S_k is the fixed effect of sex (k = either male or female), $P_i \times E_j$ is the interaction of the *i*th population with the *j*th nutrient environment, c_l is the random cell effect, and e_{ijklm} is the random error term.

The final model for duration of development was:

$$Y_{ijkl} = \mu + P_i + E_j + (P \times E)_{ij} + c_k + e_{ijkl}$$

where Y is the duration of development in days, μ is the overall mean, P_i is the fixed effect of the *i*th population (i = 1 to 7), E_j is the fixed effect of the *j*th nutrient environment (j = 1, 2, 3), $P_i \times E_j$ is the interaction of the *i*th population with the *j*th nutrient environment, c_k is the random cell effect, and e_{ijkl} is the random error term.

To estimate the relation between pupal weight and duration of development, residuals of these two variables were calculated. Pupal weight was the dependent variable and duration of development was the independent variable. The analyses were done among populations and within populations.

RESULTS

Pupal weight

Mean pupal weight of the seven populations ranged from a low of 2.091 mg (Bhopal, India) to a high of 2.791 mg (Purdue, USA; $p < 0.001$; Tables 5.2 and 5.3). Note that the mutant strain Ring eye differed in mean pupal weight from the parent strain (*c*SM). Overall, pupae reared in Environment 3 with a deficiency of resources had the lowest mean weight of 2.310 mg, whereas pupae reared in Environment 1 with standard medium had the highest mean weight of 2.477 mg ($p < 0.001$).

Table 5.2. Number of organisms and mean pupal weight (mg) and mean duration of development (days) with standard error of the fixed effects, and the % of the mean different among environments

	N	Pupal weight (mg)	%	Duration of development (days)	%
Mean[a]	1,434	2.411 ± 0.019		31.32 ± 0.06	
Population					
Baños	206	2.308 ± 0.019		31.44 ± 0.14	
Bhopal	204	2.091 ± 0.019		32.03 ± 0.14	
*c*SM	222	2.505 ± 0.018		29.77 ± 0.13	
DES	186	2.326 ± 0.020		31.00 ± 0.15	
Purdue	209	2.791 ± 0.019		31.51 ± 0.14	
*c*SM-black	223	2.538 ± 0.018		31.97 ± 0.13	
Ring eye	184	2.238 ± 0.020		31.94 ± 0.15	
Environment[b]					
1	520	2.477 ± 0.012		31.05 ± 0.09	
2	495	2.412 ± 0.012	-2.6	31.17 ± 0.09	+0.4
3	419	2.310 ± 0.013	-6.7	31.93 ± 0.10	+2.8
Sex					
Male	752	2.342 ± 0.010		31.43 ± 0.07	
Female	682	2.458 ± 0.010		31.32 ± 0.08	

[a] The standard deviation of mean pupal weight was 0.336 (mg) and of mean duration of development was 2.117 (days). [b] Environment 1 is 100% standard medium (95% fine-shifted organic whole wheat flour supplemented with 5% brewer's yeast, by weight), environment 2 is 50% standard medium and 50% α-cellulose, and environment 3 is 25% standard medium and 75% α-cellulose.

The difference in average weight between the two most extreme environments was 6.3% (Table 5.2). The wild population *c*SM, differed from this overall pattern in that *c*SM pupae reared in Environment 2 were heavier than *c*SM pupae reared in Environment 1, although the difference was not significant (Figure 5.1a).

Figure 5.1b shows the results of transforming the data to a unit normal distribution, to compare the relative performance of a population in one environment with its relative performance in any other environment. For each environment, the standardized observation was obtained as: $x'_{i,j} = \left[x_{i,j} - X_j\right] / S_{x,j}$, where $x_{i,j}$ is the mean of the *i*th population in the *j*th nutrient environment, X_j is the mean across all populations in the *j*th nutrient environment, and $S_{x,j}$ is the standard deviation among populations in the *j*th nutrient environment (Gupta and Lewontin, 1982; Wade, 1990). We found that the relative order differed between environments for some of the populations, demonstrating 'crossing-type' G × E.

For all populations under all conditions, females were heavier than males (2.448 mg and 2.342 mg, respectively; $p < 0.001$). No significant effects of sex-environment or sex-genotype interactions were found.

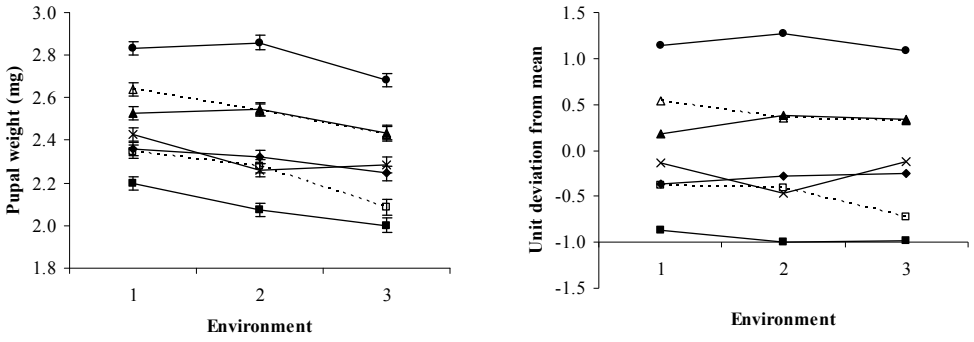

Figure 5.1a. Mean pupal weight across three environments for seven populations of *T. castaneum*. **Figure 5.1b.** A graphical representation of the genotype-environment interaction observed for pupal weight.

Environment 1 is 100% standard medium (95% fine-shifted organic whole wheat flour supplemented with 5% by weight brewer's yeast, by weight), environment 2 is 50% standard medium and 50% α-cellulose, and environment 3 is 25% standard medium and 75% α-cellulose. Pupal weight is shown for the five wild populations (dashed lines), Baños (◆), Bhopal (■), *c*SM (▲), DES (×), and Purdue (●); and for the two mutant populations (dotted lines), *c*SM-black (△) and Ring eye (□).

Duration of development

Mean duration of development of the seven populations ranged from 29.8 days (*c*SM, USA) to 32.0 days (Bhopal, India; $p < 0.001$; Table 5.2 and 5.3). Note that both mutant strains, *c*SM-black and Ring eye, differed in mean duration of development from the parent strain (*c*SM). Overall, pupae reared in Environment 3 with a deficiency of resources developed more slowly (31.9 days), compared to pupae reared in Environment 1 with standard medium (31.0 days; $p < 0.001$). The difference in duration of development between the two environments was almost one day (Table 5.2). The wild population DES differed from this overall pattern in that DES pupae reared in Environment 2 developed significantly faster than DES pupae reared in Environment 1 (Figure 5.2a).

Figure 5.2b shows the results of transforming the data to a unit normal distribution. We found that the relative order differed between environments for some of the populations, demonstrating 'crossing-type' G × E.

For all populations under all conditions, females were faster in development than males (31.3 days and 31.4 days, respectively), although the difference was not significant.

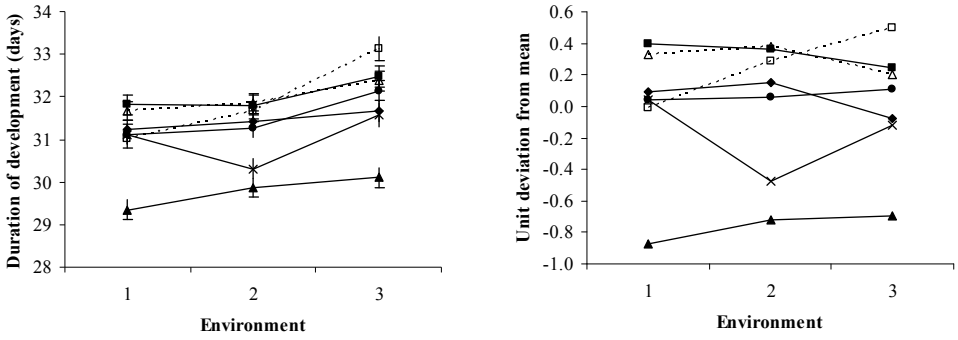

Figure 5.2a. Mean duration of development across three environments for seven populations of *T. castaneum*. **Figure 5.2b.** A graphical representation of the genotype-environment interaction observed for duration of development.

Environment 1 is 100% standard medium (95% fine-shifted organic whole wheat flour supplemented with 5% by weight brewer's yeast, by weight), environment 2 is 50% standard medium and 50% α-cellulose, and environment 3 is 25% standard medium and 75% α-cellulose. Duration of development is shown for the five wild populations (dashed lines), Baños (◆), Bhopal (■), *c*SM (▲), DES (×), and Purdue (●); and for the two mutant populations (dotted lines), *c*SM-black (△) and Ring eye (□).

Table 5.3. Models of pupal weight and duration of development

	DF	F-value	p-value
Pupal weight			
Population (P)	6	152.62	< 0.001
Environment (E)	2	44.74	< 0.001
Sex (S)	1	79.42	< 0.001
P × E	12	2.27	0.008
Cell			0.083
Duration of development			
Population	6	36.05	< 0.001
Environment	2	26.25	< 0.001
P × E	12	2.13	0.013
Cell			0.479

Relation between pupal weight and duration of development

In Figure 5.3, the residuals of pupal weight and duration of development are plotted. Results show that among populations an increase in duration of development goes together with an increase in pupal weight (0.015 mg/day, Table 5.4 [$p < 0.001$]). The same trend was found for the populations Baños, Bhopal, DES, and Purdue. For the other populations no significant effect was found. The correlation between duration of development and pupal weight was 0.12 ($p < 0.001$; data not shown).

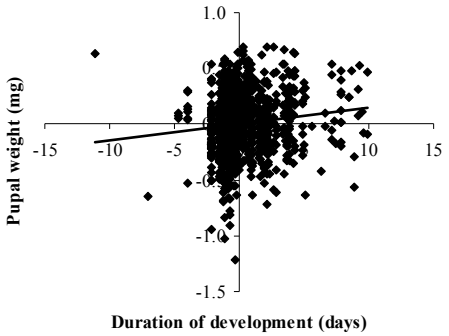

Figure 5.3. The relation between the residuals of pupal weight and the residuals of duration of development.

Table 5.4. Relation between pupal weight and duration of development[a]

	Estimate	Standard error	p-value
All populations	0.015	0.003	< 0.001
Per population			
Baños	0.045	0.015	0.003
Bhopal	0.014	0.007	0.047
*c*SM	-0.003	0.010	0.750
DES	0.031	0.010	0.001
Purdue	0.021	0.009	0.022
*c*SM-black	-0.001	0.007	0.837
Ring eye	0.009	0.007	0.179

[a] Residuals of pupal weight was the dependent variable and residuals of duration of development was the independent variable.

DISCUSSION

Our data show that food limitation during the larval stage of *T. castaneum* affects pupal weight and duration of development. We found, for both pupal weight and duration of development, a genotype-environment interaction. Furthermore, we found that males and females differ in mean pupal weight.

Mean pupal weights, in our study, are comparable with results of other studies, ranging between 2.0 and 3.0 mg (Hardin et al., 1967; Soliman and Hardin, 1971; Sokoloff, 1977; Via and Conner, 1995). Duration of development, in our study, was somewhat longer compared to other studies (± 24 days; Sokoloff, 1974). We found that, overall, female pupae were heavier than males. For almost all populations, pupae reared in the environment with a deficiency of resources yielded a lower mean weight compared to pupae reared in the standard environment for both sexes. These results are similar to those found by Soliman and Hardin (1971).

Moorad and Wade (Submitted) found that the individuals in their study belonged to two distinct phenotypic classes: those that achieved smaller pupal size but developed quickly, and others which developed slowly but achieved larger pupal size. In our study, we did not found that individuals belonged to two distinct phenotypic classes. Overall we found, however, a trend that individuals that developed quickly achieved a lower pupal weight and individuals that developed slowly achieved a higher pupal weight (Figure 5.3). Comparing these results with Table 5.2, it can be seen that larvae reared in environment 3 developed

slower and had a lower pupal weight, which is the opposite result as shown in Figure 5.3. Estimating the correlation between the residuals for pupal weight and duration of development for each genotype-environment combination, we found that for environment 1 and environment 2 all correlations were positive, but in most cases not significant different from zero (data not shown). Whereas for environment 3, for some populations, the correlations were negative, but in most cases not significant different from zero (data not shown). These results suggest that populations can have different strategies when exposed to food limitation.

We found a genotype-environment interaction for pupal weight and duration of development (P × E, Table 5.3 [$p < 0.015$]). Several other studies found genotype-environment interaction in flour beetles as well (Wade, 1990; Via, 1991). The ranking of the populations changed for the different environments. These results suggest that populations show different strategies when exposed to food limitation. The difference between populations when exposed to different levels of food limitation suggests that genetic variation in response to food limitation may occur within laboratory and "natural" populations (Via, 1991).

In this study, larvae were kept with ten individuals per cell. West-Eberhard (1979) mentioned that, when individuals of the same species live in close proximity, they compete directly for essential resources. Furthermore, Park (1962) argued that the more limited the resource, and the larger the population draining it, the greater the intensity of competition. In flour beetles, Park et al. (1964) and Wade (1990) found that the population size in was affected by competition. Jobling and Wandsvik (1983) used the coefficient of variance to estimate the effect of competition within a group. In our study it was not possible to estimate the effect of competition within a group using the coefficient of variance, because the number of replicates per environment was too small and the number of pupae per cell varied too much due to escapes. Further research is needed to investigate the effect of competition for food on pupal weight.

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Chapter 6

A plea to implement robustness into a breeding goal: Poultry as an example

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ABSTRACT

The combination of breeding for increased production and the intensification of housing conditions have resulted in increased occurrence of behavioural, physiological, and immunological disorders. These disorders affect health and welfare of production animals negatively. For future livestock systems, it is important to consider how to manage and breed production animals. In this paper, we will focus on selective breeding of laying hens. Selective breeding should not only be defined in terms of production, but should also include traits related to animal health and welfare. For this we like to introduce the concept of robustness. The concept of robustness includes individual traits of an animal, which are relevant for health and welfare. Improving robustness by selective breeding will increase (or restore) the ability of animals to interact successfully with the environment and thereby to make them more able to adapt to an appropriate husbandry system. Application of robustness into a breeding goal will result in animals with improved health and welfare without affecting the integrity. Therefore, in order to be ethical acceptable, selective breeding in animal production should accept robustness as a breeding goal.

Keywords: health, integrity, laying hen, robustness as a breeding goal, welfare

INTRODUCTION

There is only a limited number of internationally operating poultry breeding companies which have to provide laying hens worldwide. As a consequence, these companies face a wide variety of environmental conditions in which their laying hens have to perform (Knap, 2005). Differences in environmental conditions can be due to climate, housing facilities, disease pressure, exposure to different pathogens, and differences in feed quality and composition. Laying hens are kept from the cold, dry climates in Siberia to the hot, humid climates in Brazil, from battery cages to free range systems that could differ in hygienic circumstances, and are fed corn-based to soy-based diets. Laying hens kept under such different conditions must be able to cope with their environment and, therefore, require sufficient capacities to adapt. Furthermore, these laying hens are expected to produce a maximum number of eggs irrespective of environmental circumstances.

Traditional breeding has resulted in a rapid increase in egg production; in 1930 the average production was 116 eggs per hen per year, whereas nowadays the average production is increased to 300 eggs per hen per year (Preisinger and Flock, 2000). Furthermore, production became even more efficient by intensification; farms increased in size and animals were kept at a higher density (Sandøe et al., 2003).

The combination of breeding for increased production and the intensification of housing conditions have not been without consequences, especially for the animals. Laying hens have become more at risk for behavioural, physiological, and immunological disorders (Rauw et al., 1998) and consequently, for reduced animal welfare. Behavioural disorders include cannibalism[1], feather pecking[2], and absence of broodiness behaviour[3] (Savory, 1995; Price, 1999; Newberry, 2004); physiological disorders include asymmetric growth[4] (Yngvesson and Keeling, 2001; Tuyttens, 2003) and osteoporosis[5] (Bishop et al., 2000;

[1] Cannibalism is the act of consuming tissue of other members of the same species, whether living or dead, and at any stage of the life cycle. Cannibalistic behaviour affects the well-being of attacked laying hens, as evidenced by injuries which, if extensive, result in death (Newberry, 2004).

[2] Feather pecking is characterized as non aggressive pecks towards the plumage of other birds. Generally two forms can be distinguished, *i.e.* gentle and severe feather pecking. Gentle feather pecking can be defined as repeated pecks at the tips and edges of feathers, mostly ignored by the receiver. Severe feather pecking causes feather damage and feather loss. Flocks with high incidence of severe feather pecking suffer from reduced welfare and higher mortality rates due to cannibalism (Savory, 1995).

[3] Broodiness behaviour consists of termination of egg production, the incubation of eggs, and care of the young (Johnson, 2000).

[4] Fluctuating asymmetry is defined as small, randomly directed deviations from perfect symmetrical development in bilateral traits, resulting from the inability of individuals to undergo identical development on both sides of the plane of symmetry. Fluctuating asymmetry provides a useful measure of how well development processes cope with internal genetic and external environmental stressors during morphogenesis (Tuyttens, 2003).

[5] Osteoporosis in laying hens is defined as a decrease in the amount of fully mineralized structural bone, leading to increased fragility and susceptibility to fracture (Whitehead et al., 2003).

Whitehead et al., 2003); and immunological disorders include increased susceptibility against Marek's disease[6] (Dalgaard et al., 2003).

The traditional strategy to reduce these problems is preventive management. Preventive management can be divided in two procedures; physical and non-physical. A physical procedure to reduce feather pecking and cannibalism is beak-trimming (Appleby et al., 2004) and non-physical procedure include decrease of light intensity, change of feed composition, environmental enrichment, and optimizing group size (Hester, 2005). To protect against harmful pathogens, vaccination can be used as a physical procedure, whereas high hygiene systems [specific pathogen free systems (SPF)] are used as a non-physical procedure. Although much research has been focused on improvement of management factors, problems still occur in all types of poultry production systems. Furthermore, management factors used to reduce feather pecking and cannibalism, such as beak-trimming and low light intensity, have been associated with welfare problems (Gentle, 1986; Manser, 1996; Jones and Hocking, 1999). Because of these welfare problems, beak-trimming is, or will be in the near future, prohibited in parts of Western Europe.

Besides the traditional strategy of preventive management, another possibility is to adapt animals by selective breeding or even genetic modification. Selective breeding can be used to improve health and welfare related traits in laying hens (Jones and Hocking, 1999). Health can be enhanced by selective breeding for disease resistance. This may be effective in resistance to a wide range of pathogens and can be used to protect laying hens under different environmental conditions (Lamont, 1998). Welfare can be enhanced by selection against expression of undesirable behaviour. Jones and Hocking (1999) argued that selection against feather pecking and cannibalism might provide powerful, welfare-friendly solutions.

Improving health and welfare by adapting the animal to the housing system, however, can result in violation of the integrity of the animal; for instance, breeding blind laying hens. It is technically possible to breed blind laying hens, which do not show feather pecking or cannibalistic behaviour. Although these laying hens are blind, they are healthy, able to find food and water, and produce a number of eggs according to the expectations (Ali and Cheng, 1985). These hens also seem well adapted to their situation and, assuming that blind hens do not suffer in any other way, they may live a better life than hens that are able to see. Many people, however, intuitively feel that this is a morally wrong approach to improve animal welfare (Sandøe et al., 1999). In this example, integrity of the laying hens

[6] Marek's disease is caused by a highly virulent herpes virus. Marek's disease causes paralysis and mortality in laying hens (Bumstead, 2003).

was violated by selective breeding. By making use of genetic modification, violation of the integrity could even be worse.

In present poultry farming, increased occurrence of behavioural, physiological, and immunological disorders affect health and welfare negatively. Preventive management and selective breeding to reduce disorders, like beak-trimming or breeding blind laying hens, can affect the integrity of laying hens. For future livestock systems it is, therefore, important to consider how to manage and breed laying hens. In this paper, we will focus on selective breeding of laying hens. We argue that in future livestock systems it is necessary that breeding goals[7] should not only be defined in terms of production, but that they should also include traits related to animal health and welfare. For this we introduce robustness as a breeding goal.

Robustness is a term which is rapidly becoming a main interest in animal production (Knap, 2005; Ten Napel et al., 2006). We like to explore the discussion on robustness as a breeding goal for animals kept in future livestock systems. The concept of robustness is related to the concepts of health, welfare, and integrity, but in our opinion, robustness is more comprehensive. We expect that robustness as a breeding goal will result in better health and welfare without affecting the integrity of the laying hen. Based upon this, we argue that it is ethical acceptable to use selective breeding in order to create animals that are able to function better in conventional agricultural systems.

THE CONCEPT OF HEALTH, WELFARE, AND INTEGRITY

Before going into detail about the concept of robustness, the concepts of health, welfare, and integrity will be explored. For the concept of robustness it is important to have a perception about the definitions and considerations behind the realization of the concepts of health, welfare, and integrity. The considerations are important for the implementation of the different concepts into a breeding goal for robustness.

The concept of health

Different approaches towards the concept of health can be found in literature. The very basic definition of health is no more than the absence of disease (Gunnarsson, 2006; Nordenfelt, 2007). Boorse (1997 in Nordenfelt, 2007) defined disease as "a type of internal state that is an impairment of normal functional ability." This definition indicates that disease (and health) are linked to functional ability, *i.e.*, biological functioning (Nordenfelt,

[7] The definition of breeding goal will be elaborated in the section "Introduction to robustness."

2007). For Boorse (1997), biological functioning is tied to the individual's survival and reproduction. This is, however, a very narrow concept of biological functioning. The broader concept of biological functioning, as basis for the concept of health, is related to homeostasis, *i.e.*, regulation of the internal environment of living organisms (Gunnarsson, 2006). In addition, an animal may be in pain and disabled by internal bodily causes (failure in regulating homeostasis) without reducing the probability of the animal's survival. This indicates that there are other possible goals than the one of pure survival (Nordenfelt, 2007). One goal related to health, and commonly used in the debate about animal welfare, is *quality of life*, which includes psychological aspects of health (Fraser et al., 1997). Gunnarsson (2006), however, mentioned that if health is defined as physical and psychological well-being, there will be problems associated with applying the definition to all animals, especially production animals. Gunnarsson (2006) stated that a health definition that puts priority to the physical and psychological well-being of a production animal is misleading in relation to the general purpose of livestock production. In livestock production, economical considerations are involved and can be decisive in the judgment of the animals' health. To achieve good health the animal has to be in harmony with itself and its environment, and has to be in a normal physical condition (free of diseases and other physical disorders) (Rutgers, 1993). Health could than be considered as "the physical condition required to achieve welfare at an acceptable level" (Brom, 1997 derived from Nordenfelt, 1987).

The concept of welfare

Welfare of farm animals is a major concern, in society, in livestock production, as well as in animal science (Kanis et al., 2004). Animal welfare, however, is a complex concept, that is difficult to define operationally, and hence to evaluate empirically (Rowan, 1997). This has led to different welfare definitions.

Fraser et al. (1997) suggested that three main ideas are expressed in public discussion concerning animal welfare: feelings, functioning, and natural living. Fraser et al. (1997) also argued that a scientific approach to animal welfare has to take into account these ideas expressed in public discussion. Animal feelings are related to experiences of animals, *i.e.*, mental harmony, whereas functioning is related to biological functioning, *i.e.*, physical harmony. The concept of experience is based on the presence of positive experiences and the absence of negative experiences, whereas the concept of functioning is based on "doing well," so that the animal is functioning as it should do (Stafleu et al., 1999). The idea that

animals should live natural lives includes considerations of an animal's nature or *telos* (Appleby and Sandøe, 2002), which is related to the concept of integrity, and will be discussed later.

A definition of animal welfare related to the concept of experience is that "animals should feel well by being free from prolonged and intense fear, pain, and other negative states, and by experiencing normal pleasures" (Fraser et al., 1997). Kanis et al. (2004) considered animal welfare as similar to "animal happiness," which can be seen as "the balance between an animal's positive and negative emotions or feelings over a certain time period." It is, however, impossible to ask an animal directly in which situation it feels comfortable and if its preferences are satisfied. Therefore, making use of the concept of experience in scientific studies is rather difficult. To make animal experiences more applicable, the concept of functioning can be used as a tool. The concept of functioning often involves ideas about evolutionary fitness, including successful breeding. When breeding is strongly affected by human intervention, as for production animals, it might be difficult to apply the concept of functioning (Appleby and Sandøe, 2002). The concept of functioning, however, can still be linked to scientific (biological, physiological, social functioning) animal production theories, or models. Definitions of welfare commonly used are often based on the concept of functioning. For instance, welfare definitions given by Broom (1993) "welfare of an animal is reflected by the success of its attempt to cope with its environment" and by Siegel (1995) "welfare depends on physiological ability to respond properly in order to maintain or re-establish homeostatic state or balance."

For scientific models, the concept of functioning is easier to demonstrate than what an animal experiences (Duncan and Fraser, 1997). Although the concept of functioning is more straightforward to quantify, the link between (biological) functioning and the animal's welfare is not always apparent, *e.g.*, there is little consensus on the baseline that should be used in assessing measures and there is less agreement on which levels necessarily denote a better quality of life for the animal. Therefore, assessment of welfare involves a mixture of scientific knowledge and value judgments.

The concept of integrity

Integrity has been described by Rutgers and Heeger (1999) as the "wholeness and intactness of the animal and its species-specific balance, as well as the capacity to sustain itself in an environment suitable to the species." The principle of respect for the integrity of animals leads to considerations and arguments beyond animal health and welfare

(Grommers et al., 1995). The integrity theory of King (2004) proposed that the value of animal life is such that animals should not be harmed or destroyed. The loss of life itself is conceived as the ultimate harm to the animal's integrity, *i.e.*, to its "completeness."

Integrity gives notion to our own moral position, purposes, and perspectives with regard to animals (Vorstenbosch, 1993; De Vries, 2006). Integrity is not a strictly describing term, but it rather refers to the way we think an animal has to be (Brom, 1997). In the former, we already mentioned the possibility to breed blind laying hens and that many people intuitively feel that this is a morally wrong approach to improve animal welfare. The moral notion that gives voice to this intuition is integrity (Bovenkerk et al., 2002). Another example is non-broody behaviour in laying hens. Selection has resulted in strains of chickens that normally do not incubate eggs or brood chicks (Price, 1999). These laying hens seem to be well adapted to their situation and, probably, are still able to brood. However, they do not have the motivation to express their brooding behaviour; it is just not natural to them. These two examples clearly show that it is important to consider the nature and biological needs of animals.

According to Rollin (1989), the nature and biological needs are related to the *telos* of an animal. He defined *telos* as "the unique, evolutionarily determined, genetically encoded, environmentally shaped set of needs and interests which characterize the animal in question." Each animal has a *telos* that is unique to its species, it can be seen as the "chickenness of the chicken" or the "pigness of the pig," which are essential to their well-being as speech is to us (Rollin, 1989). He stated that the animal's well-being is determined by the match between its needs and interest and the treatment it receives (Rollin, 1995). Although, the animal's *telos* is unique to its species, Rollin (1995) argued that changing the *telos* of an animal can be justified. He stated that there is no moral problem in making an animal happier or prevent it from suffering by changing its *telos*, unless changes endanger the animal itself, other animals, humans, or the environment. Verhoog (1992), however, insisted that *telos* is of direct moral relevance in itself and should not be violated or changed. He stated that selective breeding is morally questionable, because it represents interference with the natural species integrity and evolutionary development of animals. In our opinion, selective breeding can violate the animals' integrity in extreme cases like breeding blind laying hens. We can use selective breeding to improve animals, but only if the animals' identity is preserved.

THE CONCEPT OF ROBUSTNESS

Introduction to robustness

In the previous chapter we have explored the concepts of health, welfare, and integrity. All three concepts are related to the quality of life of an animal. To improve the quality of life of an animal in future livestock systems these concepts have to be integrated into a breeding goal. The breeding goal defines which traits have to be improved and how much weight is given to each trait. The breeding goal is the direction in which we want to improve the population (Cameron, 1997). The concepts of health and welfare primarily focus on the state of the animal (mentally and physically) in a specific situation. These concepts do not consider animal related traits and, therefore, could not be implemented into a breeding goal. Integrity considers animal related traits, namely the presence of species specific characters, *e.g.*, it's "completeness." It is, however, not possible to optimize the integrity of an animal, and therefore integrity can not be improved by selective breeding. For this, we like to introduce the concept of robustness. The concept of robustness includes individual traits of an animal that are relevant for health, welfare, and integrity. Because robustness includes individual traits, it can be integrated into a breeding goal.

The concept of robustness is defined in different fields, *e.g.*, ecology, biological systems, statistics, and animal production. A broad definition of the concept of biological robustness is "the maintenance of specific functionalities of the system against perturbations, and it often requires the system to change its mode of operation in a flexible way" (Kitano, 2004). This definition can be used as a starting point for definitions of robustness in other fields, like animal production. Knap (2005) defined robust pigs as "pigs that combine high production potential with resilience to external stressors, allowing for unproblematic expression of high production potential in a wide variety of environmental conditions." Whereas Ten Napel et al. (2006) defined robustness in a broad sense as "the minimal variation in a target feature following a disturbance, regardless of whether it is due to switching between underlying processes, insensitivity or quickly regaining the balance," and in a narrow sense as "the ability to switch between underlying processes to maintain balance." The definitions of Ten Napel et al. (2006) are independent of species.

From these definitions, it can be concluded that the main characteristics informative for robustness of production animals are production and adaptation in a wide variety of environmental conditions. Production is important because it is one of the parameters related to the functioning of an animal. Besides, production is important because of its

economical value. In the concept of robustness, adaptation can be seen as a mechanism of the animal that enables it to cope with internal or external disturbances, or with changes in the environment. Ideally, we would like to breed a strain of laying hens that can adapt to different environmental conditions. In practice, however, strains of laying hens can perform differently in different environments; this is called genotype by environment interaction (Falconer and Mackay, 1996). As mentioned earlier, there is a limited number of internationally operating poultry breeding companies that provide laying hens worldwide. For these companies, it is favourable to have animals that can function under a wide variety of environmental conditions.

Using the main characteristics informative for robustness, *e.g.*, production, adaptation, and a wide variety of environmental conditions, we define a robust laying hen as "an animal under a normal physical condition that has the potential to keep functioning and take short periods to recover under varying environmental conditions." Functioning can be evaluated in terms of physiological, behavioural, and immunological traits. This definition of robustness includes different measurable characters and traits that make the concept of robustness applicable for breeding programs.

Implementation of health in the breeding goal for robustness

In the definition of robustness, "keep functioning" and "take short periods to recover" are referring primarily to health. The definition of Rutgers (1993), "the harmony between an animal itself and its environment, where the animal is free of diseases and other physical disorders," primarily focuses on "functioning." Whereas the definition of Gunnarsson (2006) "regulation of the internal environment of living organisms," primarily focuses on "take short periods to recover." Robust animals will be less sensitive for disease pressure and are expected to recover more quickly than less robust animals. Therefore, by implementing the concept of robustness as a breeding goal, the health of laying hens should improve simultaneously.

Implementation of welfare in the breeding goal for robustness

Together, the welfare definitions given by Broom (1993) and Siegel (1995) "welfare of an animal is reflected by the success of its attempt to cope with its environment" and "welfare depends on physiological ability to respond properly in order to maintain or re-establish homeostatic state or balance," respectively, corresponds with the definition of the concept of robustness. The distinction between animal welfare and robustness is that animal

welfare is often measured by an animals' response to a current stressor, whereas robustness is based on the possibility to respond adequately to a stressor and is aiming at less disturbed functioning by challenge with a stressor. Implementation of robustness into a breeding goal should result in animals with improved coping abilities for conventional housing systems, and, therefore, should result in improved animal welfare.

Implementation of integrity in the breeding goal for robustness

As described earlier, the concept of integrity indicates how an animal has to be. We have to be aware that selective breeding can have either positive or negative side effects on the ability to function. Sometimes a change in genotype would be an advantage to both animals and humans (Sandøe et al., 1999). But in other cases it could have a negative side effect. These negative side effects are not only morally problematic due to undesired consequences for health and welfare. They are also problematic because two core elements in the concept of integrity, as described by Rutgers and Heegers (1999) are at issue, namely "the balance in species specificity" and "to sustain itself in an environment suitable to the species." According to Rollin (1995), changing the animal by selective breeding does not necessary lead to impoverishment of the *telos*. In line with this, notion of integrity is a requirement for robustness. Therefore, improvement of health and welfare by implementation of the breeding goal of robustness should not be achieved by violation of the integrity or impoverishment of the *telos*.

APPLICATION OF ROBUSTNESS AS A BREEDING GOAL

As mentioned earlier, robustness embraces health, welfare, and integrity. Therefore, different traits can be implemented in the breeding goal of robustness. To utilize robustness as a breeding goal, the traits have to be (a) relevant, *i.e.*, they have to say something about robustness, (b) simple, *i.e.*, they have to be understandable for users, (c) sensitive, *i.e.*, they have to react to changes in the system, (d) reliable, *i.e.*, different measurements must lead to the same outcome, (e) it must be possible to establish a target value or trend, and (f) data have to be accessible. Robustness as a breeding goal can be used for different production animals. Each production animal has its species specific characteristics. In this paper, we will focus on traits interesting for improvement of robustness in laying hens. An overview will be given of traits that can be implemented into a breeding goal. These traits cover behavioural, physiological, and immune characters. In practical - commercial - context, selection for these robustness traits must be in balance with selection for production traits.

Traits to breed for

Behavioural traits. To quantify behavioural aspects for robustness in laying hens, parameters like fear, social stress, feather pecking, and cannibalism could be used. The different behavioural parameters are related. For instance, fearful laying hens tend to show more feather pecking behaviour (Jones et al., 1995), and severe feather pecking can lead to cannibalism. Methods used to asses fear in laying hens involve fear towards humans or towards a novel object. Whereas determining plumage and skin condition is a method to asses feather pecking behaviour. Variation in fearful behaviour (novel object test) and incidence of feather pecking exists between genetically different layer lines (Uitdehaag et al., 2008). Rodenburg et al. (2004) estimated heritabilities for fearful behaviour (open-field test) and feather pecking behaviour ranging between 0.35 and 0.60, and 0.10 and 0.24, respectively. The estimated heritabilities were based on individual measurements. More or less fearful and pecking behaviour, however, will also depend on the social behaviour of group members, *e.g.*, plumage condition of a hen does not only depend on her own pecking behaviour, but also depends on the pecking behaviour of her group members. Therefore, it is important to use a breeding method that makes use of information of group members, rather than individual information (Muir, 2003; Ellen et al., 2007).

Immunological traits. Animal health data are rarely straightforward to use. Veterinary treatment records do not give a precise measure for disease (Sørensen et al., 2001), and diagnoses do not normally describe implications useful for robustness. Increasing robustness of animals is important to reduce occurrence of diseases. To reduce occurrence of diseases, animals need a well developed immune system that adequately responds to invading pathogens. The immunological capacity of animals might be enhanced by genetic selection for disease resistance. Variation in immune competence exists between genetically different layer lines (Star et al., 2007a). Siwek et al. (2006) estimated heritabilities for natural antibodies determined in blood ranging between 0.11 and 0.42, whereas Bovenhuis et al. (2002) estimated heritabilities for specific antibodies ranging between 0.16 and 0.19. Furthermore, immune responses towards environmental stressors vary between layer lines (Star et al., 2007b). Therefore, genetic selection for immune traits may improve resistance to a wide range of pathogens and may be an effective strategy to protect laying hens under a wide variety of environmental conditions (Lamont, 1998).

Physiological traits. Genetic selection for production efficiency can have adverse effects on health. In poultry, for instance, this selection has unwittingly produced birds with poor structural bone mass (Bishop et al., 2000; Whitehead et al., 2003). Laying hens selected for

high egg number and a low maintenance requirement (which implies a small body mass) can become prone to osteoporosis towards the end of the laying cycle, because of the high metabolism of calcium for egg shell formation. Such birds have fragile bones and when caught and transported, fractures are common (Hughes and Curtis, 1997). Because selection for egg production has contributed to osteoporosis, this implies that susceptibility to osteoporosis has a genetic component. Bishop et al. (2000) found that traits describing bone strength are moderately to strongly inherited, where heritabilities range between 0.30 and 0.45. Therefore, selection for enhanced bone strength can be used to alleviate the problem of osteoporosis in laying hens.

Potential for a successful result

In our opinion robustness as a breeding goal can be successful to improve health and welfare of production animals in future livestock systems. Before robustness can be implemented into a breeding goal, large scale genetic research on the different traits has to be done. Large scale genetic research is for most traits labour intensive and expensive. For instance, behavioural measurements and collecting blood samples for immunological parameters have to be done at individual level.

After determining the most promising traits, the next step will be the implementation of these traits into the breeding goal. Implementation of the traits is difficult and riskful, but the potential of success for robustness as a breeding goal depends on this implementation. One of the difficulties for the implementation is to decide which trait is more important than another, *e.g.*, how much weight is given to each trait. It is, however, important to implement all traits, because the success of selective breeding for robustness depends on all traits and not on a singular trait.

Genetic research for robustness traits and the implementation of these traits into the breeding goal have to be established by cooperation between science and breeders. Additionally, successful result of robustness as a breeding goal depends on the opinion and motivation of the farmer. The principle aspects of robustness may be different for each individual farmer (or breeder), but also reference values can change. Besides, in the future other traits may arise that have to be implemented into the breeding goal of robustness. By implementation of new traits, it is, however, important that these traits concern the animal itself.

Finally, the potential for a successful result of robustness as a breeding goal depends on the economic value. In his decision-making, a farmer has to consider not just animal robustness, but also how to produce efficiently, at competitive cost.

QUESTIONS RELATED TO ROBUSTNESS

In this paper, we explored the discussion of robustness as a breeding goal for laying hens kept in future livestock systems. Although we think it is possible to implement robustness into a breeding goal, it still raises several ethical questions like: Is it acceptable to adapt animals to the production environment, rather than by changing their environments? Should animals be adapted to all environments, even the worst? And does selection for robustness affect the integrity of the animal?

When looking at the definition of robustness, a robust animal is an animal that has the potential to keep functioning and take short periods to recover under varying environmental conditions. This indicates that the animal has to function under a wide range of circumstances. It is, therefore, preferable to select for robustness traits that are common to different types of production environments. But, are we really aiming at adapting the animal to even the worst environment? No. The aim is to breed animals that can function well in a range of environments and not to breed animals specifically for the worst environments. However, even in the most optimal environments welfare of laying hens can be improved as illustrated by the fact that they show abnormal behaviour. Increasing robustness by selective breeding, therefore, improves welfare by adapting animals to the production environment. This does, however, not take away the need for improvement of housing conditions.

Christiansen and Sandøe (2000) mentioned that breeding for animals that are better suited for intensive farming *instead* of adapting the farming system may be considered violations of animal integrity. This, however, is only the case in those situations where adapting the animal involves diminishing its ability to live a good life or by depriving the animals of natural abilities, such as being able to see. However, improving the ability to cope with stress and improving the ability to recover by using robustness as a breeding goal does not deprive natural abilities, and is, therefore, not a violation of animal integrity. Of course, we have to be aware that when selecting for robust laying hens it is unknown if problems negatively correlated with the genetic make-up underlying robustness will occur.

CONCLUSION

The aim of this paper was to develop the concept of robustness as a breeding goal. Improving robustness by selective breeding will increase (or restore) the animals' ability to interact successfully with the environment and thereby to make the animal better able to adapt to an appropriate husbandry system. This, in turn, is likely to improve both welfare and productivity, although this also depends on management and housing conditions.

The implementation and application of robustness as a breeding goal is desirable. We are convinced that this application will result in animals with improved health and welfare without affecting the integrity. Therefore, improving robustness by introducing this concept as a breeding goal is ethical acceptable.

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Chapter 7

General discussion

INTRODUCTION

Social interactions among individuals can have a large effect on traits important in livestock and in natural populations. In natural populations, many studies have investigated the effect of social interactions on fitness (Wilson, 1974; Wade, 1976; 1977; Wilson, 1977; Griffing, 1989; Higgins et al., 2005). Furthermore, the theory of social interactions has received a lot of attention in the field of evolutionary biology (*e.g.,* Hamilton, 1964; Griffing, 1967; Frank, 1998; Keller, 1999; Clutton-Brock, 2002). Griffing (1967) developed models that take into account social interactions in artificial selection. These models have been implemented in the field of animal breeding to capture the heritable effect of social interactions on trait values (Arango et al., 2005; Muir, 2005; Van Vleck and Cassady, 2005; Bijma et al., 2007a; Bijma et al., 2007b). There is clear evidence that social interactions contribute to heritable variation in traits (Chapter 2 and 3; Wade, 1976; 1977; Moore, 1990; Muir, 1996; Brichette et al., 2001; Wolf, 2003; Arango et al., 2005; Muir, 2005; Van Vleck and Cassady, 2005; Bijma et al., 2007b; Van Vleck et al., 2007; Bergsma et al., 2008; Chen et al., 2008). For instance, Bergsma et al. (2008) found that social interactions contribute two-third of the heritable variance in growth rate and feed intake in domestic pigs. In laying hens, similar results are found for the heritable variance of survival (Chapter 2 and 3).

In this thesis, I have demonstrated that concepts used in evolutionary biology are relevant for animal breeding (Chapter 2 and 4). I have, however, not exploited this knowledge entirely. In the first part of this chapter, I explore work in the field of evolutionary biology and its potential application in the field of animal breeding. The aim is not to give a complete overview, but to discuss two topics (kin recognition and group size) that can be important for animal breeding. However, before going to the field of evolutionary biology it is important to have a little more background on social interactions and on the differences and similarities between models used in evolutionary biology and animal breeding.

Ultimately, genetic improvement of social interactions will be implemented into a breeding program. This can be done using at least two different selection methods, group selection (Muir, 1996) or selection based on information of relatives kept in family groups (Chapter 4). Muir (1996) showed that it is possible to decrease mortality in laying hens using group selection. In commercial laying hen breeding, however, it is not possible to implement group selection, because selection candidates are housed individually to record data on an individual basis. In the second part of this chapter I argue that it is possible to

improve traits affected by social interactions in laying hens using selection based on the information of relatives kept in family groups.

SOCIAL INTERACTIONS

Nearly all living organisms are affected by social interactions (Wilson, 1977; Griffing, 1989; Moore, 1990; Moore et al., 1997; Agrawal et al., 2001; Clutton-Brock, 2002; Muir, 2005). Social interactions can occur due to competition for limited resources, such as daylight or soil nutrients in plants, or due to social behaviours, such as aggression, social dominance, competitive ability, helping behaviour, or interaction between mother and offspring (maternal effects). In many cases, these social interactions affect both trait values and fitness of individuals (Hamilton, 1964; Wade, 1976; 1977; Wolf et al., 1999; Bijma and Wade, 2008). Social interactions can be either negative or positive. Negative social interactions can, for instance, be found in laying hens and flour beetles, where social interactions result in mortality due to cannibalism, or in fish and pigs, where social interactions inflate differences in growth rate and feed intake. Positive social interactions can, for instance, be found in lions, where social interactions result in helping behaviour. Solitary lions join groups, which increases the capacity of the group to defend their territory or young against rival groups (Packer et al., 1990). In the same time, however, positive social interactions can have negative effects, because it can reduce the feeding success or fitness of the lions they join (Packer et al., 1990).

MODELS IN EVOLUTIONARY BIOLOGY VS. ANIMAL BREEDING

In evolutionary biology[8], there are three common approaches for modelling the evolutionary consequences of social interactions; kin selection, multilevel selection, and associative effects models (Keller, 1999). Associative effects models are also known as indirect genetic effect models (IGE; Moore et al., 1997). Kin selection and multilevel selection models focus on the fitness consequences of social interactions, either for an individual or a group of individuals. Associative effects models, in contrast, focus on the consequences of social interactions on trait values of individuals, which may subsequently affect fitness of the individuals.

[8] Different terms are used in the field of evolutionary biology and animal breeding. In this discussion, I will use the terms common for animal breeding. In Box 7.1., terms used in the evolutionary biology are explained.

Box 7.1. Glossary

Actor = Individual that performs a behaviour
Altruism = A behaviour that is costly to the actor and beneficial to the recipient. Cost and benefit are defined on the basis of the lifetime direct fitness consequences of a behaviour
Associative effect = Heritable effect of one individual on the trait value of another individual (also known as indirect genetic effects (IGE))
Co-operation = Behaviour which provides a benefit to another individual (recipient)
Direct fitness effect = The component of fitness gained through the impact of an individual's behaviour on its own fitness
Direct genetic effect = The effect of an individual's genotype on its own trait value
Familiarity based recognition = Recognition based on previous social interactions with conspecifics (also known as association)
Fitness = Capability of an individual of a certain genotype to reproduce the next generation
Group selection = See Multilevel selection
Inclusive fitness = The sum of the direct and indirect fitness effects of an individual's behaviour
Indirect fitness effect = Impact on the fitness of its social partners, weighted by the degree of relatedness between the individual and its social partners
Indirect genetic effect = See Associative effect
Kin = Family related individuals (*e.g.* clones, full sibs, half sibs)
Kin recognition = Individual's capability to distinguish between genetic kin and non-kin (also known as kin discrimination)
Kin selection = Process by which traits are favoured because of their beneficial effects on the fitness of relatives (also known as inclusive-fitness models)
Multilevel selection = Consequences on fitness depend on selection within and among groups (also known as levels-of-selection models or group selection).
Mutual benefit = A behaviour which is beneficial to both the actor and the recipient
Recipient = Individual receiving a behaviour
Relatedness = A measure of genetic similarity
Trait = Any observable or measurable characteristic of an individual (*e.g.* body weight)

Figure 7.1 illustrates the models used in evolutionary biology. Kin selection models centre on inclusive fitness and direct fitness costs (c) and indirect benefits (b) of social interactions among related individuals (Hamilton, 1964; Michod, 1982). They recognize that individuals can increase their inclusive fitness through their behaviour that increases the fitness of related individuals, and *vice versa*. In these models, a social behaviour evolves when the fitness benefit (b) for the recipient weighted by relatedness (r) minus fitness costs (c) for the actor is larger than zero: $rb - c > 0$, this inequality is known as Hamilton's rule (Hamilton, 1964). The $rb - c$ is the inclusive fitness effect of the behaviour.

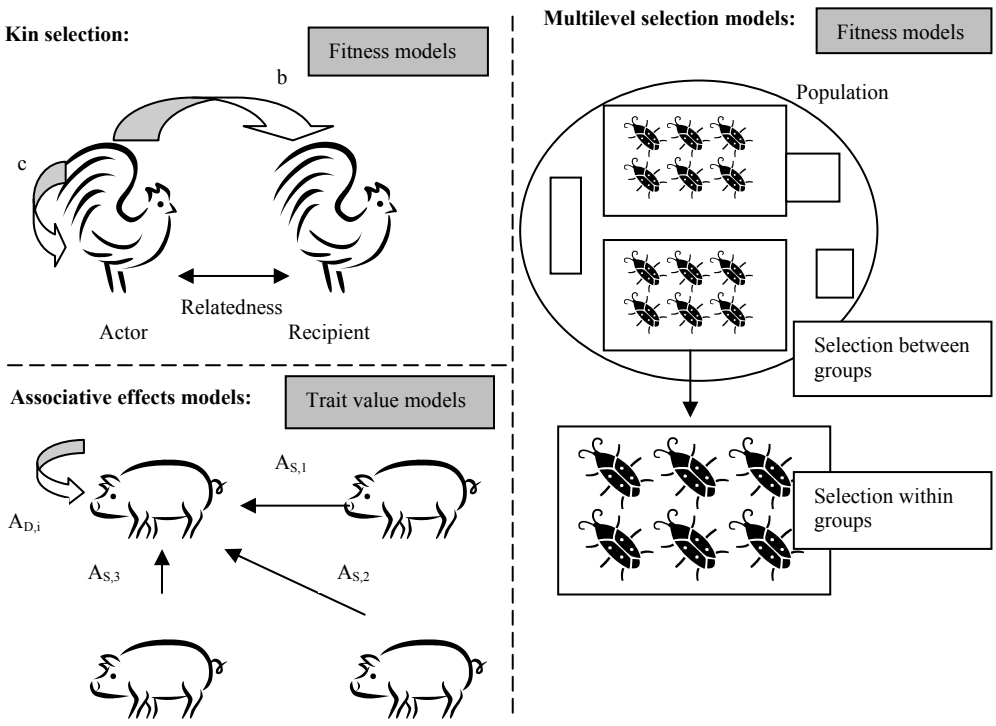

Figure 7.1. Three models of social effects; kin selection, multilevel selection, and associative effects models

Multilevel selection models centre on partitioning total selection pressure into components, one for each of the levels, most commonly, individual and group level (Wilson, 1975; Wade, 1979; 1980; Keller, 1999). In the multilevel selection models, groups with highest mean fitness are selected. In these models, selection within groups opposes the evolution of a social behaviour because of its fitness costs, but selection among groups favours the behaviour because more social groups have higher mean fitness than less social groups (Bijma and Wade, 2008). A behaviour evolves when selection among groups times the "group heritability" exceeds the opposing selection within groups (Goodnight, 2005). Wilson and Wilson (2007) have summarized this as "selfishness beats altruism within groups and altruistic groups beat selfish groups."

Theory shows that kin selection and multilevel selection are the same process (Wade, 1985; Queller, 1992a; b; Lehmann et al., 2007; Wenseleers et al., 2009). For both, there are

two distinct biological factors necessary: 1) relatedness, being the correlation between genes in interacting individuals, *r*; and 2) multilevel selection process, *g*, which can be defined as the degree of multilevel selection (Bijma and Wade, 2008). In artificial breeding, in the absence of social interactions, the general expression for response to selection is

$$\Delta G = h^2 S\,, \tag{7.1}$$

in which h^2 is the heritability and *S* is the selection differential. Equation 7.1 is also known as the "breeders equation" (Lynch and Walsh, 1998). When there is both relatedness and multilevel selection (both *r* and *g* are non-zero), response to selection equals (Bijma and Wade, 2008)

$$\Delta G = h^2 S\left[1 + (n-1)gr\right]. \tag{7.2}$$

Equation 7.2 shows that, if either *r* or *g* is zero, Equation 7.2 equals Equation 7.1. Equation 7.2 shows that both relatedness and multilevel selection are necessary for social interactions to affect response to selection.

The third model used in evolutionary biology is known as the associative effects model (also known as the indirect genetic effect model (IGE)). An associative effect is the heritable effect of one individual on the trait value of another individual (Griffing, 1967). Associative effects models focus on the consequences of social interactions on trait values. For the associative effects model, the traditional model ($P_i = A_i + E_i$) is extended and includes associative effects in the model: $P_i = A_{\mathrm{D}_i} + A_{\mathrm{S}_j} + E_{\mathrm{D}_i} + E_{\mathrm{S}_j}$, where A_{D_i} is the direct breeding value of individual *i*, E_{D_i} is the nonheritable direct effect of individual *i*, A_{S_j} is the associative breeding value of associate *j*, and E_{S_j} is the nonheritable associative effect of individual *j* (*e.g.,* Griffing, 1967; Moore et al., 1997; Wolf et al., 1998; Wolf et al., 1999; Bijma et al., 2007a). Thus the trait value of an individual is modelled as the sum of a direct genetic effect due to the individual itself, and associative genetic effects due to the group members. In animal breeding, associative effects models have been used to model social interactions (Chapter 2; Muir, 2005; Bijma et al., 2007b; Bergsma et al., 2008), and maternal effects (Willham, 1963).

In natural populations, the fitness of an individual is an important parameter. In animal breeding, in contrast, fitness of an individual is decided by the breeder, meaning that fitness is not of main interest in artificial selection. The breeder makes, in most cases, the decision

how long an animal lives and how many offspring it produces. Of course it is important that animals have the potential to survive and reproduce. Models focussing only at the fitness of an individual, like kin and multilevel selection models, are, therefore, not important for animal breeding. Animal breeders are, instead, interested in improving trait values by means of artificial selection. To improve trait values, associative effects models are important, because associative effects models focus on the consequences of social interactions on trait values.

WHAT CAN WE LEARN FROM EVOLUTIONARY BIOLOGY?

In evolutionary biology, a behaviour is social if it has fitness consequences for both the individual that performs the behaviour (actor) and the individual receiving the behaviour (recipient; Hamilton, 1964). Hamilton (1964) classified social behaviours, according to the consequences they have for the actor and recipient. The most common social behaviours distinguished are (Table 7.1), 1) selfishness, beneficial to the actor and costly to the recipient; 2) altruism, costly to the actor and beneficial to the recipient; 3) spite, costly to both the actor and recipient; and 4) mutual benefit, beneficial to both the actor and recipient (Hamilton, 1964; 1970; 1972; West et al., 2007). Both the evolution of altruism and mutual benefit are important issues in evolutionary biology.

Table 7.1. Most common classifications of behaviour (from West et al., 2007)

		Effect on recipient	
		+	-
Effect on actor	+	Mutual Benefit	Selfishness
	-	Altruism	Spite

"Co-operation" includes the "mutual beneficial" and the "altruism" categories. I refer to the term co-operation as a behaviour which provides a benefit to another individual (recipient). There are two general situations where co-operation can evolve; 1) co-operation may provide a direct fitness benefit to the individual, which outweighs the cost of performing the behaviour (mutual benefit; Sachs et al., 2004; Lehmann and Keller, 2006), and 2) co-operation may provide an indirect benefit because it is directed towards other individuals who also carry the co-operative gene (altruism; Hamilton, 1964; Lehmann and Keller, 2006). The second situation is known as kin selection, $rb - c > 0$. For costly behaviour, c is larger than zero, so that r should be larger than zero if a behaviour will

evolve. This implies that for the evolution of costly co-operation positive relatedness is required (Hamilton, 1964; Kokko et al., 2001). High relatedness can be achieved in different ways. One way is limited dispersal, leading to population viscosity. This means that related individuals automatically stay together for extended periods. Another means by which high relatedness can be achieved is kin recognition. In the next paragraph, I discuss the existence of kin recognition and the potential importance for animal breeding and farmers.

Kin recognition

Kin recognition refers to the process in which individuals distinguish between close genetic kin and non-kin. Kin recognition or kin discrimination is the differential treatment of conspecifics as a function of their genetic relatedness (Holmes and Sherman, 1983; Waldman, 1987; Gamboa et al., 1991). Mechanisms that allow individuals to recognize their kin can facilitate the evolution of co-operation by means of kin selection (Waldman, 1988). Interaction with kin differs from interaction with non-kin (West et al., 2002). Kin recognition has been demonstrated in a wide variety of animals, for example in long-tailed tits (Russell and Hatchwell, 2001), rodents (Grau, 1982), and social insects (Greenberg, 1979), but also in plants (Dudley and File, 2007) and livestock (Ligout and Porter, 2003). Several researchers have offered theoretical explanations of potential kin recognition mechanisms (*e.g.* Hamilton, 1964; Holmes and Sherman, 1983; Waldman, 1988). In literature, four possible mechanism of kin recognition are discussed: spatially-based recognition, familiarity based recognition, phenotypic comparisons, and genetic recognition systems. To my opinion, however, spatially-based recognition does not really belong to kin recognition, even though the outcome will be the same. Spatially based recognition occurs when relatives are distributed predictably in space, nepotism may occur as a result of a behaviour specific to a given location (Holmes and Sherman, 1983). In the next paragraph, I discuss the last three mechanisms. For familiarity based recognition, kinship classifications are based on individual recognition, whereas for phenotypic comparisons and genetic recognition systems, kinship classifications are based on group or class recognition (Waldman, 1987; 1988).

Individual recognition: Most frequently, kin recognition appears to be based on previous social interactions with conspecifics (Maynard Smith, 1978; Holmes and Sherman, 1983). These previous social interactions could affect social interactions at a later stage. In many species, rearing environments like nests or burrows provide ideal settings for kin to learn

each other's individual traits (Holmes and Sherman, 1983; Waldman, 1987). Familiarity based recognition is the usual mechanism for recognition between mother and offspring. Recognition of the mother can be due to the odour of the mother (Leon, 1975) or due to vocalization (Trillmich, 1981). However, familiarity based recognition could also exist between siblings. For instance, Russell and Hatchwell (2001) performed a study in long-tailed tits (*Aegithalos caudatus*), to investigate whether helpers prefer to help kin versus non-kin when given the choice. They found that failed breeders became helpers only when kin were present in the same clan, and most helpers assisted at the nests of relatives. When there were no kin present in the same clan, failed breeders offered no help.

In commercial pigs, piglets are reared with their full sibs for several weeks. Bergsma et al. (2008) found that a pen of full sibs shows 15 g/day (~0.2 phenotypic standard deviation, indicating a moderate effect) higher growth than a pen of non-relatives. This difference in growth rate could be due to familiarity based recognition. It could, however, also be that this difference is due to another social component. For instance, it could be that full sibs have genetically similar feed-intake capacity or similar social competition behaviour, which stimulates them to eat all together and avoids fighting. If familiarity based recognition has a positive effect on social interactions in pigs, it could be advised to the farmer to keep sibs in one group, to improve growth rate and welfare.

Group or class recognition: Kin recognition is possible when kin express some traits in common, either because they have been exposed to the same environmental factors or because the traits are genetically determined (Getz, 1981; Lacy and Sherman, 1983; Gamboa et al., 1986; Grafen, 1990). In some species, individuals are not reared with all kin together, for instance when parents have several nests of offspring during their life. Offspring of one nest are not reared with offspring of another nest. Individuals could recognize kin from another nest by their alleles which causes the expression of a unique phenotypic trait (Hamilton, 1964). For instance, it has been found that in bees (*Lasioglossum zephyrum*) recognition of kin is by odour, which permits them to recognize the degree of relatedness even though they have never met (Greenberg, 1979). In other species, individuals are reared with non-kin. Individuals can recognize these non-kin at a later stage, due to the phenotypes they learned during the rearing period. Buckle and Greenberg (1981) performed a study in bees (*Lasioglossum zephyrum*) to investigate recognition when guards were reared in mixed nests with non-kin and kin. They found that these guards admitted both their unfamiliar sisters and the unfamiliar sisters of their unrelated nest mates. Based on these results it appeared that guards discriminate among

unfamiliar conspecifics based on their phenotypic similarity to individuals with whom the guards were reared. Grau (1982) performed a study with white-footed deermice (*Peromyscus leucopus*) and showed that they can distinguish between siblings from parents' subsequent litters and non-kin, despite not having associated with either.

Kin recognition in commercial poultry: In the commercial poultry industry, there is no social contact between mother and offspring. Social contact between sibs will be limited, because eggs are placed at random in large groups in the incubator. It is unknown if sibs are placed together. In some cases, however, sibs will be placed in the same hatching-basket, but after hatching chicks are randomly placed in rearing cages. In this case, there is a possibility that hens could recognize their full sibs, because they were in the same hatching-basket. Overall, it can be concluded that the mechanism of familiarity based recognition will not play a big role in commercial poultry.

In the rearing period, chickens are kept with kin and non-kin in large groups. After rearing, hens are kept in small or large groups, depending on the housing system. It could be that hens recognize their group members of the rearing period, which can result in positive (or negative) social interactions. Recognition could be based on phenotypic comparisons (kin or non-kin) or genetic recognition (kin). If kin recognition exist in commercial poultry, it could be advised to farmers to keep groups the same during the rearing and laying period. This is, in particular, important when laying hens are kept in smaller groups (like the battery system), because contact is more intense. However, in larger group housing systems, like the aviary system (Appleby et al., 1992), it could be important as well, because hens could make sub-groups. D'Eath and Keeling (2003) showed that subject hens were presented to a group with either familiar or unfamiliar birds, hens in that group were more aggressive to unfamiliar subject hens. They only found this result for hens kept in small groups, hens kept in large groups did not discriminate between familiar and unfamiliar subject hens. Their experiment indicates that phenotypic comparisons exist in laying hens. From their experiment, it is, however, unknown if hens in the same group are kin or non-kin.

For poultry breeding, kin recognition could affect the outcome of the associative effects models and selection methods used to improve traits affected by social interactions. So far, genetic parameters for two components, the direct and associative effect, are estimated on traits affected by social interactions (Chapter 2 and 3). If kin recognition exist, we may have to take into account three components, direct effect, associative effect on kin, and

associative effect on non-kin (Bijma et al., 2007a). In that case, the total breeding value (TBV) of individual *i* can be given as,

$$\mathrm{TBV}_i = A_{\mathrm{D}_i} + f_k(n-1)A_{\mathrm{S}_i,k} + f_{nk}(n-1)A_{\mathrm{S}_i,nk} \tag{7.3}$$

where A_{D_i} is the heritable direct effect of individual *i* on its own trait value; f_k is the fraction of kin among group members; $A_{\mathrm{S}_i,k}$ is the heritable social effect of individual *i* on the trait value of its kin; f_{nk} is the fraction non-kin among group members; and $A_{\mathrm{S}_i,nk}$ is the heritable social effect of individual *i* on the trait value of its non-kin.

The equation of the total heritable variation (σ^2_{TBV}) becomes more difficult, because 1) it depends on the number of kin and non-kin in a group; and 2) three genetic correlations are involved, two genetic correlations between the direct and associative effects on kin and non-kin respectively, and the genetic correlation between the associative effect on kin and associative effect on non-kin. When the genetic correlation between the associative effect on kin and associative effect on non-kin is one, σ^2_{TBV} will be similar to Equation 4.4 (Chapter 4). A negative correlation indicates that individuals with high associative effects on kin have lower associative effects on non-kin, resulting in a conflict between co-operation with kin *vs.* co-operation with non-kin (Bijma et al., 2007a). It is, however, not possible to estimate genetic parameters for the associative effect on kin and the associative effect on non-kin, because heritable and nonheritable social effects are confounded with each other and with the physical environment when groups consist of kin. To investigate whether kin recognition exist, a selection experiment may be required.

Selection methods used to improve traits affected by social interactions, like group selection and selection based on relatives (Chapter 4), are based on family groups. When kin recognition does not exist in commercial poultry, still group selection and selection based on relatives should be based on family groups. With family groups there is genetic variation between groups, which allows for between group selection. It is, however, important to investigate whether kin recognition exist, because selection based on relatives focuses on family groups, whereas the commercial product will be kept in random groups. If poultry behave different to kin than non-kin (correlation is negative), results could be different than expected.

Group size

In some natural populations, it has been found that the fitness of all group members commonly increases with the size of their group, because group size increases the capacity of group members to catch, produce, defend food, or raise young successfully (reviewed in Clutton-Brock, 2002). In other species, however, it has been found that the advantage of an individual to join a group may have neutral or negative consequences for the fitness of the group, for instance in lions (Packer et al., 1990; Heinsohn and Packer, 1995; Clutton-Brock, 2002). Furthermore, there could be an optimal group size, an increase in group size will result in a decrease in group fitness when groups are larger than the optimal group size.

Group size affects heritable variance due to social effects and response to selection (Bijma et al., 2007a; Hadfield and Wilson, 2007). For many livestock species, large differences exist among group sizes in breeding and commercial populations. Furthermore, for many livestock species, there is a trend towards larger groups in commercial populations, for reasons pertaining to animal well-being and public acceptance. Due to legislation, the housing system of laying hens in the European Union will change in the near future. In the near future, traditional battery cages will be forbidden and laying hens will be kept in larger groups, like aviary or free range systems. In battery cages, problems like mortality due to cannibalism have smaller impact because cages consist of only a small number of individuals (*e.g.* four or five). Keeping laying hens in large groups may increase the prevalence of mortality due to cannibalism. For instance, Bilčik and Keeling (2000) compared four group sizes and found an increase in frequency of aggressive pecks with increasing group size.

Equation 4.4. suggests that increasing group size increases the heritable variation. Bijma et al. (2007a) mentioned that the relationship is complicated. They suggest that an increase in group size may yield a decrease in the associative effects. Besides, they suggest that the dependence of heritable variance on group size creates genotype-environment interactions (G × E interactions). As a consequence, positive selection in small groups may yield suboptimal and even negative responses in large groups, and *vice versa*. So far, no studies have been reported on the investigation of the correlation between the TBV on survival time in small groups and the TBV on survival time in large groups. If this correlation is significantly lower than one, different results will be expected in small *vs.* large groups, which indicates that different selection methods are needed for survival of laying hens kept in small *vs.* large groups.

Investigation of the correlation between TBV in large groups and TBV in small groups can be done by estimating genetic parameters for the direct and associative effects using different group sizes, for instance n = 4, 8, and 12. Based on the genetic parameters for different group sizes, Equation 4.4. can be adapted. For large groups (n > 20), however, it is very difficult to estimate genetic parameters for the direct and associative effects, because number of groups is small. Furthermore, intensity of interaction between individuals may be different, whereas in our models we assume that the intensity of interaction between group members is the same. I can imagine that contact in large groups is limited or that smaller groups originate within large groups. D'Eath and Keeling (2003), however, suggested that hens living in groups of 120 individuals did not show a pecking order. Furthermore, they found that hens of larger groups did not form territorially distinct sub-groups. I think that we have to investigate this more in detail. To compare small and large groups, family structure of small and large groups should be the same, so that we can investigate if the performance of a sire-dam combination is the same in small and large groups. Furthermore, in large groups behavioural studies should be performed to investigate the intensity of interaction among individuals.

IMPROVEMENT OF TRAITS AFFECTED BY SOCIAL INTERACTIONS IN LAYERS

In Chapter 4, selection based on the information of relatives kept in family groups (selection based on relatives) has been proposed to improve traits affected by social interactions among individuals (Figure 7.2). Based on theoretical results and results in practice (Chapter 2 and 3), selection based on relatives offers good opportunities to genetically improve traits affected by social interactions. In this paragraph, I show some results of a selection experiment applying selection based on relatives to improve survival in laying hens and argue that it is possible to improve traits affected by social interactions. Furthermore, I discuss the consequences for other traits important for the commercial laying hen industry, when applying the new selection method to select for survival in practice.

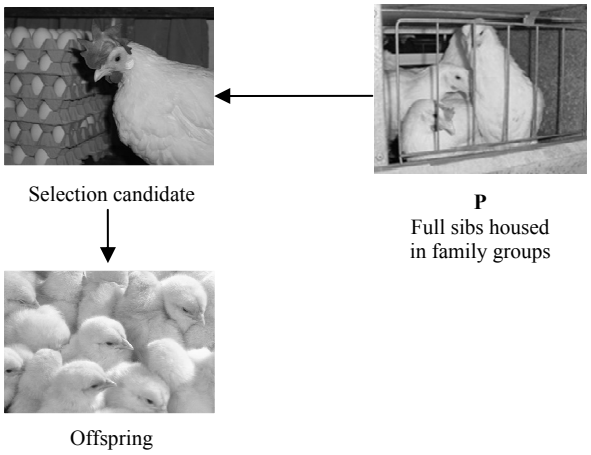

Figure 7.2. Selection based on relatives

Genetic improvement of survival in laying hens

Genetic improvement of survival in laying hens is often difficult or not even possible using the traditional selection methods (Muir, 1996). Muir (1996) showed that it is possible to increase survival when using group selection. In laying hens, he found that survival increased from 32% in generation 2 to 91.2% in generation 6. It is difficult, however, to apply group selection in commercial poultry breeding, because in commercial poultry breeding selection candidates are housed individually as described in Chapter 4. An

alternative was suggested in Chapter 4, selection based on relatives, which showed theoretically evidence that it is possible to improve traits affected by social interactions.

In our project, a selection experiment has been started in 2005 that combined results of chapter 2 and 4, aiming to improve survival in cannibalistic laying hens. For each generation, selection candidates were housed individually and were selected based on the survival of their full sibs kept in four-bird family cages (Figure 7.2).

For the first generation, selection was done in two directions, high and low survival line. Offspring of the selection lines were compared with a control. Figure 7.3a shows the survival rate in the first generation. As expected, the high survival line yielded the highest survival rate of 84.8% for hatch week[9] 1 and 73.9% for hatch week 2, the low survival line yielded the lowest survival rate of 72.2% for hatch week 1 and 51.3% for hatch week 2, and the control was in between. Note the large difference in mean survival between both hatch weeks, which suggests large environmental impacts.

For the second generation, selection was done in one direction, high survival line. Offspring of this selection line were compared with a control. In this generation, small results and even results in undesired direction on survival rate were found. The control yielded the highest survival rate of 74.6%, whereas the high survival line yielded a survival rate ranging between 72.7% and 54.9% (Figure 7.3b). In generation 2, it was only possible to make a comparison for the first hatch week. Due to logistics and low hatchability, it was not possible to have laying hens of the high selection line hatched in one time.

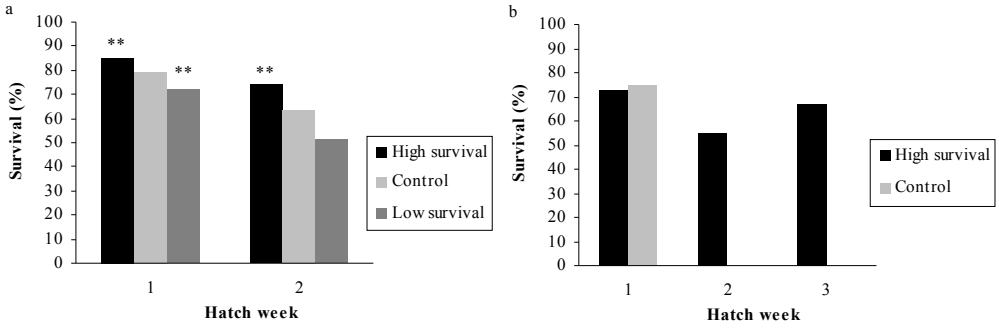

Figure 7.3. Survival rate of the selection lines and control in the (a) first generation and (b) second generation (** = $p < 0.05$ compared to control).

[9] Hatch week can be defined as the week eggs are hatched and chickens are born

For both generations, hatch week had a large effect on survival rate ($p < 0.05$). For the second generation, no significant effect of the direction of selection was found when taking into account hatch week. Differences in survival rate between hatch weeks can be due to age of the dams, preincubation storage conditions, incubation conditions, or rearing conditions, like light intensity (Meijerhof, 1992; Kjaer and Vestergaard, 1999; Yalçin and Siegel, 2003; Yalçin et al., 2008). For generation 1 and 2 it is unknown if differences occurred due to preincubation storage conditions, incubation conditions, or rearing conditions. For the next generations, it is important to keep these conditions the same.

In generation 2, there was an age difference between the parents of the high survival line and the control. Dams used to breed the high survival line were much older (1.5 years old) than dams used to breed the control (one year old). Age of breeders affects hatchability and performance of offspring later in life, because the quality of eggs, such as the egg composition, egg weight, and shell quality, is affected (Wilson, 1991; Vieira and Moran Jr., 1998; Yassin et al., 2008). Yassin et al. (2008) showed that the hatchability decreases when breeders exceed 45 weeks of age. Furthermore, when breeders are older than 65 weeks, hatchability was only 40%. In generation 2, it was found that the hatchability of the high survival line was much lower than the control (on average 43% *vs.* 65%, respectively), which could be due to the old breeders used. Vieira and Moran (1998) showed that egg size of old breeders was larger than those from younger breeders. In general, a larger egg size is considered to be advantageous, because it is often associated with a higher absolute nutrient content (Williams, 1994). Eggs of a larger size, however, hatch earlier than smaller eggs (Crittenden and Bohren, 1962; Shanawany, 1984), and egg temperature could be different, meaning that incubation conditions should be adjusted to the size of the eggs.

Based on the results of generation 1, I am convinced that selection based on relatives has good opportunities to improve survival in laying hens, even though results in generation 2 are disappointing. Based on the results of generation 2, I can conclude that survival in laying hens is a multi-factorial trait, many environmental and management factors affect the survival rate. To reduce or prevent one of the major economic and welfare problems in laying hens, we have to use a combination of selection based on relatives and good management strategies and a constant environment.

Effect on other traits

Selection on mortality due to cannibalism can have an effect on other traits, even though selection was not directed on these other traits. In the worst case, negative correlations could exist between selection for survival and traits important for the poultry industry or traits important for the individual itself. For instance, selection on mortality due to cannibalism could affect egg production, body weight, and fear related behaviour, but it could also affect the integrity of the laying hen (Chapter 6). It is important to investigate the effect of selection against mortality due to cannibalism on other traits. In this paragraph, I show the results of egg production in the first generation of the selection experiment and discuss the outcome of these results.

Egg production: The egg production per cage was recorded for the whole laying period (from 18 through 75 weeks). The egg production traits are precocity from 18 until 24 weeks of age (Figure 7.4a), total egg production per hen housed (Figure 7.4b), and total egg production per cage at the end of the laying period, corrected for mortality (Figure 7.4c).

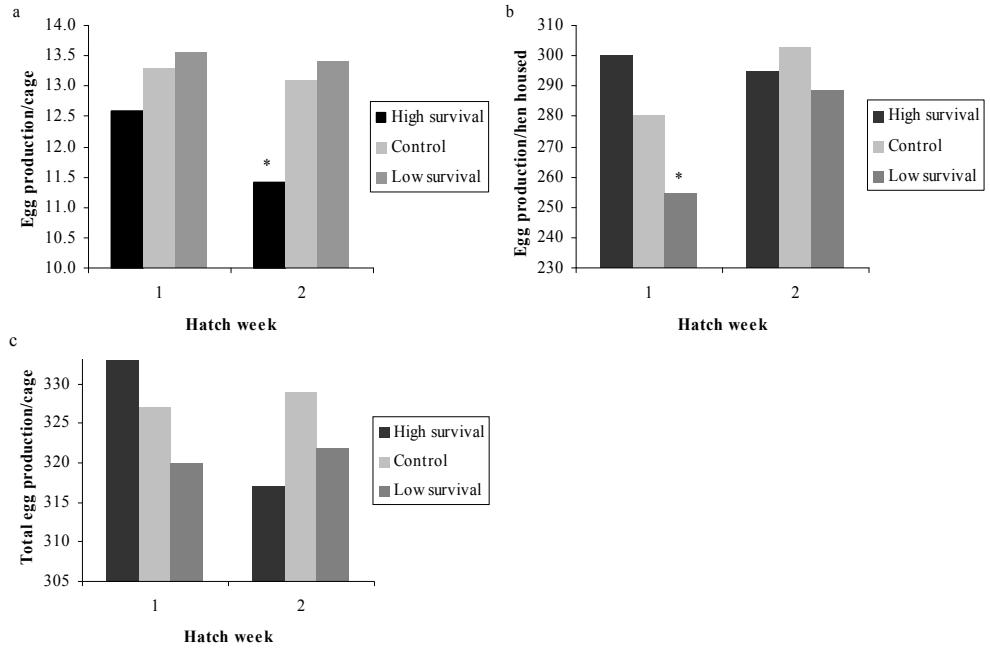

Figure 7.4. a) Precocity per cage, from 18 until 24 weeks of age; b) Total egg production per hen housed; c) Total egg production per cage at the end of the laying period, corrected for mortality (* = $p < 0.10$ compared to control).

The low survival line yielded the highest precocity of approximately 13.5 eggs, whereas the high survival line yielded the lowest precocity of 12.6 eggs in hatch week 1 and 11.4 eggs in hatch week 2. Low precocity means that hens start later with the egg production, so that they are later sexual mature. Jensen et al. (2005) showed that feather pecking is related to early sexual maturation. Based on their and our results, it could be concluded that selection against feather pecking or mortality due to cannibalism, could result in later sexual maturation. Even though the high survival line started later with the egg production, at the end total egg production and egg production per hen housed were highest, at least for hatch week 1. For hatch week 2, the high survival line yielded a lower, but not significant, egg production per hen housed compared to the control (295 *vs.* 303 eggs, respectively; Figure 7.4b) and the lowest total egg production per cage corrected for mortality (Figure 7.4c).

After one generation of selection, a decrease in precocity was found for the high survival line. Furthermore, the high survival line showed an increase in egg production for the first hatch week and a decrease in egg production for the second hatch week. So far, it is difficult to say if selection for survival results in a decrease in total egg production. It can, however, be concluded, that selection for survival results in a decrease in precocity. Based on these results we could say that the correlation between precocity and survival is negative, nevertheless further research is needed to estimate this genetic correlation between precocity and survival. So far, poultry breeding companies select on improvement of precocity, meaning that laying hens start earlier with egg production than a couple of years ago. It could be that animal breeders, without knowing it, indirect selected for lower survival rate and an increase in mortality due to cannibalism.

In the end, farmers are interested in the total egg production per laying house. It is important to investigate whether there is a negative genetic correlation between selection for survival and total egg production. If this correlation is negative, the new selection method will result in a decrease in the total egg production. In that case, selection should focus on both survival and total egg production. This, however, would reduce the selection response for survival.

When poultry breeding companies would like to reduce one of the major welfare and economic problems in the laying hens industry, it is important that their breeding program takes into account social interactions among individuals. It is, however, important that group size and structure are comparable between breeding populations and commercial populations, because it is unknown if differences in group size and structure will result in

unexpected results. Further research is needed to investigate the effect of kin recognition and genotype × environment interaction in poultry. Furthermore, research is needed to investigate trade offs in traits important for poultry, when selecting for survival in laying hens.

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Summary

INTRODUCTION

Mortality due to cannibalism in laying hens is a worldwide economic and welfare problem occurring in all types of commercial poultry housing systems. Due to prohibition of beak-trimming and the traditional battery system in the European Union in the near future, mortality due to cannibalism may increase if no further actions are taken, and needs to be solved urgently. To reduce mortality in laying hens, one of the possibilities is to use genetic selection. Traditional selection methods, however, have not been very effective because they neglect the genetic effect an individual has on its group members (so called "social interactions"). The main aim of this thesis is to investigate the effect of social interactions on the heritable variance in mortality due to cannibalism in laying hens and to develop a selection method that takes into account social interactions.

GENETIC PARAMETERS ON SURVIVAL TIME IN LAYING HENS

In the classical quantitative genetic model, the phenotype of an individual depends on its own direct genotype. With social interactions, the model needs to be extended with a social effect, the effect other group members have on the phenotype of the individual (also known as "associative effects"). The aim of the first two chapters was to estimate genetic parameters for the direct and associative effects on survival time in three layer lines (Chapter 2 and 3). Genetic parameters were estimated using three methods, traditional linear animal model, linear animal model including associative effects (Chapter 2), and two-step approach (Chapter 3). The traditional linear animal model included only the direct effect of an individual's genotype on its phenotype. The linear animal model including associative effects, included both direct and associative effects. The two-step approach is a combination of survival analysis and the linear animal model including associative effects.

Three purebred White Leghorn layer lines were used. Hens of the same line were housed at random in four-bird cages. For each hen, information was collected on survival rate and number of survival days. Survival rate was defined as the percentage of laying hens still alive at the end of the study. Survival days were defined as the number of days from the start of the study till either death or the end of the study.

The three lines showed differences in survival rate, ranging from 53% through 74%. Using the traditional linear animal model, heritabilities of survival time ranged from 2% through 10%. When including associative effects in the model, the total heritable variance in survival time was 1.5 to 3-fold greater than the traditional genetic variance, for both the linear animal model including associative effects and the two-step approach. Both methods

showed that total heritable variation in survival time is substantially larger than suggested by the traditional linear animal model. Results of the two methods suggest that prospects for reducing mortality by means of genetic selection are good and may lead to substantial reduction of one of the major welfare problems in egg production.

GENETIC IMPROVEMENT OF TRAITS AFFECTED BY SOCIAL INTERACTIONS

A second aim was to develop a selection method that can be used to improve traits affected by social interactions (Chapter 4) and apply this in practice (Chapter 7). The inheritance of traits affected by social interactions differs from that of classical traits, because there are two components involved; the direct effect of the individual's genotype on its own phenotype and the associative effect of an individual's genotype on the phenotype of its group members. As a consequence, response to selection depends on these two components. Using individual selection, response to selection can be negative, because the associative effect is neglected. Using group selection, the response to selection is always positive and is greater with family groups than with groups of unrelated individuals. Group selection offers a solution to improve traits affected by social interactions. Using group selection, selection candidates should be housed in family groups. Application of group selection in the poultry breeding industry is, therefore, not possible, because selection candidates are housed individually to record data on an individual basis (such as egg production).

Chapter 4 shows that, to improve traits affected by social interactions in laying hens, a solution is to select individually housed candidates based on the performance of their full sibs kept in family groups. Theoretical results show that the accuracy of selection based on relatives is an analogy of the classical expression for traits not affected by social interactions. Based on the theoretical results, this method offers good opportunities to improve traits affected by social interactions. A selection experiment was applied aiming to improve survival in laying hens using selection based on relatives (Chapter 7). In the first generation, survival improved using selection based on relatives. In the second generation, however, small results and even results in undesired direction were found, which seemed to be related to environmental factors. Based on the results of generation 1, selection based on relatives offers good opportunities to improve survival in cannibalistic laying hens, even though results in generation 2 are disappointing. Based on the results of generation 2, it can be concluded that survival in laying hens is a multi-factorial trait. To reduce mortality due

to cannibalism in laying hens, it is important to combine selection based on relatives and a good management strategy and constant environment.

It is important to investigate whether selection based on relatives affects other traits important for the poultry industry (like egg production traits) or the laying hen itself (integrity of the animal). In Chapter 6, ethical issues related to incorporating robustness traits into a breeding program are addressed. Even though Chapter 6 is based on robustness, ethical issues discussed in this chapter can also be important when incorporating social effects into a breeding program.

SOCIAL INTERACTIONS IN FLOUR BEETLES

So far, genetic parameters for direct and associative effects have been estimated only for livestock. To investigate the mechanism behind social interactions, laboratory populations, such as flour beetles (*Tribolium castaneum*), can be used. Besides, using laboratory populations, research can be performed on a scale not feasible in livestock. Flour beetles have been used extensively as model organism to investigate the effects of interspecies competition and competition due to limited resources.

The aim of Chapter 5 was to investigate the effect of food limitation on body weight and duration of development in seven populations. Results suggest that, when exposed to food limitation, populations of flour beetles can differ in the expression of population characteristics. Based on the results of this pilot experiment, two populations were selected. Currently, a large experiment is carried out to collect data on body weight, to investigate the heritable effect of social interactions on pupal body weight, when larvae are exposed to food limitation. In the near future, we will estimate genetic parameters for the direct and associative effects on pupal body weight. Furthermore, we will use flour beetles as a model organism for laying hens to investigate, for instance, the effect of group size on social interactions.

CONCLUSION

Social interactions substantially increase the heritable variance in mortality due to cannibalism in laying hens. To reduce mortality due to cannibalism in laying hens, it is important to incorporate social effects in the current poultry breeding program. Further research is needed, to investigate whether social interactions affect other traits in laying hens. Furthermore, as discussed in the general discussion, further research is needed to investigate the effect of group size and kin recognition.

Samenvatting

INTRODUCTIE

Sterfte door kannibalisme bij leghennen is een wereldwijd economisch- en welzijns probleem dat voorkomt in alle commerciële huisvestingssystemen, variërend van batterij tot scharrelhuisvesting. Tot nu toe wordt de punt van zowel de boven- als ondersnavel verwijderd om kannibalisme tegen te gaan. Dit vindt plaats als kuikens minder dan 10 dagen oud zijn. Echter, in de nabije toekomst zal deze ingreep worden verboden in de Europese Unie. Ook zal er een verbod komen voor het traditionele batterijsysteem. Deze veranderingen in de pluimveehouderij zullen ervoor zorgen dat sterfte door kannibalisme in de toekomst toe zal nemen. Daarom moeten andere manieren gevonden worden die een stijging in sterfte tegengaan. Fokkerij is één van de mogelijkheden om kannibalisme en sterfte van leghennen tegen te gaan. Echter, selectiemethodes die tot nu toe in de pluimveefokkerij worden gebruikt zijn niet doeltreffend, omdat deze methoden het erfelijke effect dat een individu heeft op zijn groepsgenoten negeren. Dit wordt ook wel sociale interactie genoemd. In dit proefschrift is gekeken naar het effect van sociale interacties op de erfelijke variatie in sterfte door kannibalisme bij leghennen en het ontwikkelen van een selectie methode die gebruikt kan worden om sterfte door kannibalisme bij leghennen tegen te gaan.

ERFELIJKE AANLEG VOOR OVERLEVING VAN LEGHENNEN

Binnen de fokkerij zijn we geïnteresseerd in het verbeteren van de erfelijke aanleg van een ras wat resulteert in een verandering van kenmerken van dat ras (bijvoorbeeld verbetering van eiproductie). Als sociale interacties invloed hebben op de kenmerken van een dier dan moet in het model naast de eigen erfelijke aanleg van het dier zelf ook de sociaal erfelijke aanleg van zijn groepsgenoten worden meegenomen. In hoofdstuk 2 en 3 van dit proefschrift laat ik zien dat sociale interacties bijdragen aan de erfelijke variatie in sterfte door kannibalisme bij leghennen. Hierbij heb ik gekeken naar het percentage van de variatie in een kenmerk dat bepaald wordt door de erfelijke aanleg (=erfelijkheidsgraad). Hiervoor heb ik twee methodes gebruikt; 1) de traditionele methode waarbij alleen eigen erfelijke aanleg wordt meegenomen, en 2) de methode met sociale interacties waarbij zowel eigen erfelijke aanleg als sociaal erfelijke aanleg worden meegenomen. Sociaal erfelijke aanleg is het effect wat een dier heeft op zijn groepsgenoten.

Voor het experiment heb ik drie zuivere leghenlijnen gebruikt (Witte Leghorn). Hennen van deze drie lijnen zijn gehuisvest met vier dieren van dezelfde lijn in één kooi. Het kenmerk waarnaar ik heb gekeken is de sterfte. Voor elke hen die overlijdt wordt de dag

van overlijden genoteerd zodat ik het aantal overlevingsdagen kan berekenen. Op basis van deze gegevens kan ik het overlevingspercentage aan het eind van het experiment berekenen.

Het overlevingspercentage varieert van 53% tot 74%. Overleving is laag omdat de punt van de snavels van deze dieren niet is verwijderd. Daarna heb ik gekeken of er genetische verschillen zijn binnen de lijnen. Het blijkt dat met de traditionele methode de erfelijkheidsgraad varieert van 2% tot 10%. Dit betekent dat maar een klein gedeelte van de variatie in sterfte bepaald wordt door de erfelijke aanleg, terwijl overige effecten (zoals omgeving) een veel groter effect hebben. Hierdoor is het moeilijk om te selecteren tegen sterfte door kannibalisme. De methode met sociale interacties laten zien dat er veel meer bepaald wordt door de erfelijke aanleg dan wat de traditionele methode laat zien. Als we gebruik maken van selectiemethodes met sociale interacties dan kunnen we veel meer vooruitgang boeken bij het tegengaan van sterfte door kannibalisme bij leghennen dan met de tot nu toe beschikbare methodes.

ERFELIJKE VOORUITGANG VAN KENMERKEN DIE BEÏNVLOED WORDEN DOOR SOCIALE INTERACTIES

Tijdens mijn promotieonderzoek heb ik ook gekeken naar het ontwikkelen van een nieuwe selectiemethode die gebruikt kan worden om kenmerken te verbeteren die beïnvloed worden door sociale interacties (Hoofdstuk 4) en om deze selectiemethode toe te passen in de praktijk (Hoofdstuk 7). Als kenmerken beïnvloed worden door sociale interacties dan zijn er twee componenten die een rol spelen; eigen erfelijke aanleg van een individu en sociaal erfelijke aanleg van datzelfde individu op kenmerken van zijn groepsgenoten. Deze twee componenten beïnvloeden de vooruitgang die door fokkerij behaald kan worden. In hoofdstuk 4 heb ik drie verschillende selectiemethodes onderzocht; individuele selectie, groepselectie en selectie op basis van volle zussen gehuisvest in familiegroepen. Zowel groepselectie als selectie op basis van volle zussen kunnen gebruikt worden om kenmerken te verbeteren die beïnvloed worden door sociale interacties. Een nadeel van groepselectie is dat hennen die gebruikt worden om de volgende generatie te maken in een groep met volle zussen gehuisvest moeten worden. In de commerciële pluimveefokkerij zijn ouderdieren doorgaans individueel gehuisvest zodat ook kenmerken aan individueel gehuisveste dieren gemeten kunnen worden (zoals bijvoorbeeld eiproductie). Hierdoor is het moeilijk om groepselectie toe te passen in de commerciële pluimveefokkerij.

Een oplossing om kenmerken te verbeteren die beïnvloed worden door sociale interacties, is selectie op basis van volle zussen gehuisvest in familiegroepen. Ik heb een selectie-experiment opgestart waarbij selectie op basis van volle zussen wordt gebruikt om te selecteren tegen sterfte door kannibalisme bij leghennen. In de eerste generatie is er een afname in sterfte door kannibalisme van 10% ten opzichte van de controle lijn. In de tweede generatie is er geen verschil ten opzichte van de controle lijn. Dit komt waarschijnlijk door verschillen in de omgeving en de leeftijd van de ouderdieren. Op basis van de eerste generatie concludeer ik dat selectie op basis van volle zussen goede perspectieven biedt bij het verminderen van sterfte door kannibalisme. Op basis van de tweede generatie concludeer ik dat omgeving veel effect heeft op sterfte door kannibalisme. Om sterfte door kannibalisme bij leghennen tegen te gaan is het belangrijk om selectie op basis van volle zussen te combineren met een goed management en een constante omgeving.

SOCIALE INTERACTIES BIJ MEELKEVERS

Naast leghennen heb ik tijdens mijn promotieonderzoek ook gekeken naar meelkevers (*Tribolium castaneum*). Meelkevers worden veel gebruikt in onderzoek naar competitie tussen populaties en competitie door een voertekort. Daarnaast is het makkelijker om van meelkevers grotere aantallen nakomelingen te krijgen dan van leghennen, waardoor onderzoeken op grotere schaal kunnen worden uitgevoerd. Het doel van Hoofdstuk 5 is om te onderzoeken of een voertekort een effect heeft op lichaamsgewicht en ontwikkelingstijd van zeven verschillende populaties. Tijdens de gehele larvale periode worden meelkevers blootgesteld aan een voertekort. Daarna heb ik de dag van verpoppen genoteerd en heb ik de poppen gewogen. Uit de resultaten blijkt dat genetisch verschillende populaties anders reageren op een voertekort. Zowel in lichaamsgewicht als in ontwikkelingstijd. Op basis van de resultaten van dit kleine experiment heb ik twee populaties geselecteerd die nu worden gebruikt in een grootschalig experiment, dit om te onderzoeken of sociale interacties die ontstaan door een voertekort een effect hebben op de erfelijke variatie in lichaamsgewicht.

CONCLUSIE

Sociale interacties moeten worden opgenomen in de huidige pluimveefokprogramma's, zodat er efficiënt geselecteerd kan worden tegen sterfte door kannibalisme bij leghennen. Verder onderzoek is nodig om te kijken of sociale interacties een invloed hebben op andere kenmerken die belangrijk zijn in de commerciële pluimveehouderij. Daarnaast is het belangrijk om te onderzoeken of het effect van sociale interacties op de erfelijke variatie in sterfte door kannibalisme verandert als hennen in grotere groepen of met familie worden gehuisvest.

Curriculum vitae

CURRICULUM VITAE (NEDERLANDS)

Op 6 november 1980 werd Esther Dorien Ellen geboren in Wageningen. Esther groeide op in Indoornik (Betuwe). Van 1984 tot 1988 werd Basisschool De Zonnewijzer bezocht en van 1988 tot 1992 werd Basisschool De Lammerts van Bueren school bezocht. In 1998 behaalde zij haar HAVO diploma aan het Hendrik Pierson College te Zetten. Datzelfde jaar begon zij met de opleiding Tropische Landbouw aan Hogeschool Larenstein te Deventer. In het derde jaar (2000) koos zij ervoor om zich te specialiseren in dierlijke productie. Van januari tot juli 2001 werd een stage uitgevoerd bij NGO (non-governmental organisation) Anthra in Pune, India. Het afstudeervak werd uitgevoerd bij Dier en Omgeving van ID-Lelystad. In november 2002 werd de opleiding Tropische Landbouw afgerond. In september 2002 begon zij met de masters opleiding Animal Sciences aan Wageningen Universiteit. Van augustus tot december 2003 werd een klein afstudeervak Fokkerij en Genetica uitgevoerd. Dit afstudeervak werd uitgevoerd met Claudia Kamphuis, waar zij keken naar de genetische achtergrond van klauwproblemen bij melkkoeien en de relatie met exterieurkenmerken. Van januari tot oktober 2004 werd een groot afstudeervak Kwantitatieve Epidemiologie uitgevoerd. Tijdens dit afstudeervak werd gekeken naar de prevalentie van *E. coli* O157 bij kalveren en melkkoeien en de relatie met huisvesting en dierkenmerken. In oktober 2004 werd de opleiding Animal Sciences afgerond. In november 2004 begon zij met promotieonderzoek bij de leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit, waarvan dit proefschrift het resultaat is. Als onderdeel van dit promotieonderzoek verrichte zij van september tot december 2007 onderzoek met meelkevers (*Tribolium castaneum*) in samenwerking met Prof. Dr. M.J. Wade aan de vakgroep Evolutionaire Biologie, Indiana University, USA. Sinds 1 januari 2009 is zij werkzaam als post-doc bij de leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit, waar zij het werk wat in dit proefschrift gepresenteerd is zal voortzetten.

CURRICULUM VITAE (ENGLISH)

On November 6th 1980 Esther Dorien Ellen was born in Wageningen. Esther was raised in Indoornik (Betuwe). From 1984 through 1988 she visited the primary school De Zonnewijzer and from 1988 through 1992 she visited the primary school De Lammerts van Bueren school. In 1998, she graduated from high school Hendrik Pierson college in Zetten. During the same year, she started with the BSc Tropical Agriculture at Hogeschool Larenstein in Deventer. In the third year, she specialised in animal production. From January through July 2001, she performed a practical period at the NGO (non-governmental organisation) Anthra in Pune, India. The thesis was performed at Animal and Surrounding of ID-Lelystad. In November 2002, she completed the BSc Tropical Agriculture. In September 2002, she started with the MSc Animal Sciences at Wageningen University. From August through December 2003 she performed a minor thesis Animal Breeding and Genetics. This thesis was in cooperation with Claudia Kamphuis. The aim of this thesis was to investigate the genetics of claw disorders in dairy cattle and the correlation with conformation traits. From January through October 2004 she performed a major thesis Quantitative Epidemiology. The aim of this thesis was to investigate the prevalence of *E. coli* O157 in veal calves and dairy cattle and the relation with herd and animal characteristics. In October 2004, she completed the MSc Animal Sciences. In November 2004, she started as PhD-student at the Animal Breeding and Genomics Centre of Wageningen University, which resulted in this thesis. As part of her PhD research, she visited from September through December 2007 the USA to perform a study with flour beetles (*Tribolium castaneum*) in cooperation with Professor Dr. M.J. Wade at the Evolutionary Biology group of Indiana University. Since January 2009 she is employed as post-doc at the Animal Breeding and Genomics Centre of Wageningen University, where she continued working on the subject presented in this thesis.

PUBLICATIONS

Papers in refereed scientific journals

Bijma, P., W.M. Muir, E.D. Ellen, J.B. Wolf and J.A.M. van Arendonk. 2007. Multilevel Selection 2: Estimating the genetic parameters determining inheritance and response to selection. Genetics 175: 289-299.

Bolhuis, J.E., E.D. Ellen, C.G. Van Reenen, J. De Groot, J. Ten Napel, R.E. Koopmanschap, G. De Vries-Reilingh, K.A. Uitdehaag, B. Kemp and T.B. Rodenburg. 2008. Effect of group selection against mortality on behaviour and peripheral serotonin in domestic laying hens with trimmed and intact beaks. Submitted to Physiology and Behavior.

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Star, L., E.D. Ellen, K.A. Uitdehaag, and F.W.A. Brom. 2006. Robustness of laying hens: An ethical approach. *In*: Ethics and the politics of food. Preprints of the 6th Congress of the European Society for Agricultural and Food Ethics, Oslo, Norway.

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TRAINING AND SUPERVISION PLAN

The basic package (3 ECTS)

WIAS introduction course	2005
Course on philosophy of science and/or ethics	2006

International conferences and presentations (14 ECTS)

56th Annual meeting of the EAAP, Uppsala (SW)	2005
4th European Poultry Genetics Symposium, Dubrovnik (KR)	2005
12th Benelux Congress of Zoology, Wageningen (NL)	2005
6th Congress of the EurSaFE, Oslo (NW)	2006
8th WCGALP, Belo Horizonte, (BR)	2006
58th Annual meeting of the EAAP, Dublin (IR)	2007
20th International Congress of Genetics, Berlin (GE)	2008

Seminars, workshops, and presentations (9 ECTS)

WIAS science day (4x), Wageningen (NL)	2005-2008
FandG connection days, Vught (2x) (NL)	2004-2006
Symposium “How to participate in dynamic sciences?”, Arnhem (NL)	2006
WIAS seminar “Science meets society”, Wageningen (NL)	2007
Studiedag van Agrivaknet, GGL en WPSA, Wychen (NL)	2007
EADGENE Local ethical matrix workshop, Wageningen (NL)	2008
Workshop verenpikken, Lelystad (NL)	2008

In-depth studies (8 ECTS)

Incorporation of competitive effects in breeding programs, Wageningen (NL)	2004
Genes and environment, Uppsala (SW)	2005
Biological basis for management and selection tools.,Wageningen (NL)	2005
Science meets society: robustness in the context of animal production, Wageningen (NL)	2006
Design of animal experiments, Wageningen (NL)	2007
New insights into mixed model methodology, la-londe-les-Maures (FR)	2007

PhD students' discussion groups (3 ECTS)	
Quantitative genetics discussion group (weekly meeting)	2006-2008
Undergraduate course (6 ECTS)	
Animal breeding and genetics	2004
Professional skills support courses (3 ECTS)	
WIAS course "Techniques for scientific writing"	2006
OWU course "Supervising MSc thesis work"	2007
OWU course "Gespreksvaardigheden één-op-één begeleiding"	2008
Waiver (Writing STW research proposal)	2008
Research skills training (8 ECTS)	
Preparing own PhD research proposal	2004
External training period Bloomington, Indiana, USA (3.5 months)	2007
Didactic skills training: teaching and supervising (10 ECTS)	
Practical inleiding dierwetenschappen	2006
Assisting course population genetics	2008
Supervising students (5 students)	2006-2008
Management skills training (5 ECTS)	
Organisation WIAS science day (2x)	2006-2007
WAPS general member	2006
Total	**69 ECTS**

Colophon

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