Indirect Genetic Effects for Survival in Domestic Chickens (*Gallus gallus*) Are Magnified in Crossbred Genotypes and Show a Parent-of-Origin Effect

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ABSTRACT Through social interactions, individuals can affect one another's phenotype. The heritable effect of an individual on the phenotype of a conspecific is known as an indirect genetic effect (IGE). Although IGEs can have a substantial impact on heritable variation and response to selection, little is known about the genetic architecture of traits affected by IGEs. We studied IGEs for survival in domestic chickens (*Gallus gallus*), using data on two purebred lines and their reciprocal cross. Birds were kept in groups of four. Feather pecking and cannibalism caused mortality, as beaks were kept intact. Survival time was shorter in crossbreds than in purebreds, indicating outbreeding depression and the presence of nonadditive genetic effects. IGEs contributed the majority of heritable variation in crossbreds (87 and 72%) and around half of heritable variation in purebreds (65 and 44%). There was no evidence of dominance variance, neither direct nor indirect. Absence of dominance variance in combination with considerable outbreeding depression suggests that survival is affected by many loci. Direct–indirect genetic correlations were moderately to highly negative in crossbreds (-0.37 ± 0.17 and -0.83 ± 0.10), but low and not significantly different from zero in purebreds (0.20 ± 0.21 and -0.28 ± 0.18). Consequently, unlike purebreds, crossbreds would fail to respond positively to mass selection. The direct genetic correlation between both crosses was high (0.95 ± 0.23), whereas the indirect genetic correlation was moderate (0.41 ± 0.26). Thus, for IGEs, it mattered which parental line provided the sire and which provided the dam. This indirect parent-of-origin effect appeared to be paternally transmitted and is probably Z chromosome linked.

SOcial interactions among individuals are widespread in natural and domestic populations (Frank 2007). When individuals interact, their phenotype can change under the influence of the (behavioral) characteristics of conspecifics. In case these characteristics have a genetic basis, the social environment contains a heritable component (Willham 1963; Griffing 1967; Kirkpatrick and Lande 1989; Moore *et al.* 1997; Muir 2005; Bijma *et al.* 2007). The heritable effect of an individual on the phenotype of a conspecific is known as an indirect genetic effect (IGE) in evolutionary literature, and as an associative, competition, or social effect in animal, plant, and tree breeding literature (Griffing 1967;

Kirkpatrick and Lande 1989; Moore *et al.* 1997; Muir 2005; Bijma *et al.* 2007; Van Vleck *et al.* 2007; Bergsma *et al.* 2008). The most frequently studied IGE is a maternal genetic effect, which is the heritable environmental effect of a mother on the phenotype of her offspring (Willham 1963; Cheverud 1984; Kirkpatrick and Lande 1989; Koerhuis and Thompson 1997; Mousseau and Fox 1998; Eaglen and Bijma 2009; Bouwman *et al.* 2010).

The genetic architecture of traits affected by IGEs can differ substantially from ordinary traits. IGEs influence a trait's inheritance and contribute to heritable variation (Hamilton 1964a,b; Griffing 1967, 1977; Kirkpatrick and Lande 1989; Moore *et al.* 1997; Wolf *et al.* 1998; Bijma and Wade 2008). Early theoretical work shows that IGEs can explain both positive response to negative selection, e.g., evolution of altruism (Hamilton 1964a,b), and negative response to positive selection, e.g., failure of artificial selection for increased or decreased trait values (Griffing 1967). Those theoretical predictions have been substantiated by

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selection experiments in animals, plants, and bacteria (Wade 1977; Craig 1982; Goodnight 1985; Kyriakou and Fasoulas 1985; Griffin *et al.* 2004; Muir 2005). More recent theoretical work shows that IGEs can contribute substantially to heritable variation, even to the extent that heritable variance exceeds phenotypic variance (Bijma 2011a). Estimates of indirect genetic variance in beef cattle, pigs, and laying hens confirm that IGEs can contribute substantially to heritable variation in agricultural populations (Van Vleck *et al.* 2007; Bergsma *et al.* 2008; Chen *et al.* 2008; Ellen *et al.* 2008; Chen *et al.* 2009). These findings are in accordance with predictions of Denison *et al.* (2003), who argued that IGEs are likely to harbor substantial heritable variation, which can be used for genetic improvement. Moreover, the IGE-modeling approach can explain why certain heritable traits, such as success in pairwise contests, will never respond to selection (Wilson *et al.* 2011) and allows the quantitative genetic modeling of traits that cannot be attributed to a single individual, such as the number of prey caught by a hunting pack (Bijma 2011a). The above demonstrates that IGEs can have a big impact on a trait's inheritance and heritable variation. More knowledge of IGEs is needed to predict and understand response to selection in domestic and natural populations.

Traits affected by IGEs can be modeled using either a trait-based approach or a variance component approach. In trait-based models, IGEs are attributed to specific traits and an individual's IGE is the product of its trait values and a coefficient representing the strength of the interaction. These models require knowledge of the social traits that affect the phenotype of a conspecific (Kirkpatrick and Lande 1989; Moore *et al.* 1997). In variance component models, in contrast, direct and indirect genetic (co)variances are estimated without knowledge of the social traits that underlie IGEs (Willham 1963; Griffing 1967; Muir 2005; Bijma *et al.* 2007). A trait-based approach can help us understand the biological mechanism of social interactions. However, when the underlying social traits are unknown or unrecorded, a variance component approach is needed.

At present, knowledge of the magnitude and nature of IGEs is limited (apart from maternal genetic effects; reviewed by Bijma 2011b). In laying hens, large and statistically significant indirect genetic variances were found for survival time in two out of three investigated purebred lines (Ellen *et al.* 2008). Results in beef cattle and pigs are diverse. Some studies report large and statistically significant indirect genetic variances, while others report the opposite (Van Vleck *et al.* 2007; Bergsma *et al.* 2008; Chen *et al.* 2008, 2009; Bouwman *et al.* 2010; Hsu *et al.* 2010). In addition to additive genetic effects, IGEs might depend on dominance and epistasis, affecting the maintenance of genetic variation and the level of heterosis or inbreeding depression (Lynch and Walsh 1998). Moreover, IGEs might depend on maternal effects, sex chromosome linked effects, or imprinting, enforcing differences among reciprocal crosses. Further study on the magnitude and nature of IGEs is needed to understand the inheritance of traits affected by IGEs and to optimize genetic improvement in agriculture and aquaculture.

Here we present estimated genetic parameters for survival time in domestic chickens (*Gallus gallus*), using data on two purebred lines and their reciprocal cross. Survival in livestock populations usually has low heritabilities (Dematawega and Berger 1998; Knol *et al.* 2002; Quinton *et al.* 2011). In laying hens, estimates of genetic parameters for survival during the productive period of group-housed individuals are, to our knowledge, limited to those of Ellen *et al.* (2008). Ellen *et al.* (2008) estimated genetic parameters for survival time in three purebred layer lines. Heritability estimates varied between 0.02 and 0.10. However, Ellen *et al.* (2008) found substantially more heritable variation when accounting for IGEs. To gain knowledge of the nature of IGEs for survival in domestic chickens, we investigated whether there is evidence for dominance, epistasis, maternal effects, sex chromosome linked effects, or imprinting.

Background

This section introduces basic quantitative genetic principles of traits affected by IGEs, using a variance component approach, and introduces the genetic parameters that will be estimated in the next sections. See Table 1 for notation.

Classical quantitative genetic theory defines the phenotype (P) as the sum of a genetic (A) and a nonheritable (E) component; $P = A + E$ (Lynch and Walsh 1998). For traits under the influence of social interactions, the classical model is expanded with IGEs (Willham 1963; Griffing 1967). An individual's phenotype now consists of the direct genetic (A_{Di}) and nonheritable (E_{Di}) effect of the individual itself and the indirect genetic (A_{Ij}) and nonheritable (E_{Ij}) effects of its group mates,

$$P_i = A_{Di} + E_{Di} + \sum_{i \neq j}^{n-1} A_{Ij} + \sum_{i \neq j}^{n-1} E_{Ij},$$

where n is the number of group members (Griffing 1967). With unrelated group members, the phenotypic variance (σ_p^2) equals $\sigma_{A_D}^2 + \sigma_{E_D}^2 + (n-1)\sigma_{A_I}^2 + (n-1)\sigma_{E_I}^2$ (Griffing 1967).

The heritable impact of an individual i on the mean trait value of the population, known as the total breeding value (A_T), consists of its direct breeding value (A_D) and $n-1$ times its indirect breeding value (A_I):

$$A_{T_i} = A_{D_i} + (n-1)A_{I_i}.$$

Consequently, the total heritable variance ($\sigma_{A_T}^2$), determining a population's potential to respond to selection, equals $\sigma_{A_D}^2 + 2(n-1)\sigma_{A_{DI}} + (n-1)^2\sigma_{A_I}^2$ (Bijma *et al.* 2007). By analogy of the classical heritability (h^2), $\sigma_{A_T}^2$ can be expressed relative to σ_p^2 (Bergsma *et al.* 2008):

$$T^2 = \sigma_{A_T}^2 / \sigma_p^2.$$

In conclusion, the relevant genetic parameters for traits affected by IGEs are $\sigma_{A_D}^2$, $\sigma_{A_I}^2$, and $\sigma_{A_{DI}}$. On the basis of these parameters, $\sigma_{A_T}^2$, T^2 , and the direct-indirect genetic correlation (r_{DI}) are calculated.

Table 1 Notation key

Symbol	Meaning
$i - j$	Focal individual, group mates of the focal individual
A_D	Direct genetic effect, direct breeding value
A_I	Indirect genetic effect, indirect breeding value
A_T	Total genetic effect, total breeding value
E_D	Direct nonheritable effect
E_I	Indirect nonheritable effect
σ_{Cage}^2	Cage variance
$\sigma_{A_D}^2$	Direct genetic variance
$\sigma_{A_I}^2$	Indirect genetic variance
$\sigma_{A_T}^2$	Total genetic variance
σ_E^2	Error variance
σ_P^2	Phenotypic variance
$\sigma_{A_{1_DI}}; \sigma_{A_{2_DI}}$	Direct–indirect genetic covariance within a cross
r_{DI}	Genetic correlation between A_D 's and A_I 's within a cross
h^2	Heritability
T^2	Total heritable variance relative to phenotypic variance
$\sigma_{A_{1_D_2_I}}; \sigma_{A_{2_D_1_I}}$	Direct–indirect genetic covariance between crosses
$\sigma_{A_{12_D}}$	Direct genetic covariance between crosses
$\sigma_{A_{12_I}}$	Indirect genetic covariance between crosses
$\sigma_{A_{12_T}}$	Total genetic covariance between crosses
r_{12_D}	Genetic correlation between the A_D 's of both crosses
r_{12_I}	Genetic correlation between the A_I 's of both crosses
r_{12_T}	Genetic correlation between the A_T 's of both crosses

Materials

Data were provided by the Institut de Sélection Animale B.V., the layer breeding division of Hendrix Genetics. Two commercial purebred White Leghorn layer lines, W1 and WB, were used to produce 15,012 crossbred laying hens of which 7668 were W1 $\times$ WB ($\sigma \times \varphi$) and 7344 were WB $\times$ W1 ($\sigma \times \varphi$). Each cross was produced by randomly mating ~ 50 sires to ~ 705 dams, where dams were nested within sires.

Eggs were hatched in two batches. Each batch contained two groups that differed 2 weeks in age. Post-hatching, chicks were wing-banded, sexed, and vaccinated for infectious bronchitis and Marek's disease. Their beaks were kept intact. Chicks of the same cross were housed in cages of 60 individuals. At 5 weeks of age, group size was reduced to 20 individuals. At approximately 17 weeks of age, each batch was placed in a different laying house. The laying houses consisted of four or five double rows. Only eight rows were used per laying house; consequently, the outer two rows were left empty in one of the laying houses. Each row consisted of three levels, *i.e.*, top, middle, and bottom. Four hens of the same cross and age were randomly assigned to a cage. A feeding trough was located in front of the cage. Drinking nipples were located in the back of the cage and were shared with back neighbors. Hence, some interaction with back neighbors was possible, but interaction with side neighbors was prevented.

The trait of interest, "survival time", was defined as "the number of days from the start of the laying period till either death or the end of the experiment", with a maximum of 398 days. Cages were checked daily. Dead hens were removed and the cause of death was determined subjectively. The record was set to missing when the cause of death was

clearly unrelated to feather pecking or cannibalism ($n = 23$, birds with broken wings or legs, and birds that were trapped).

In addition, to investigate the impact of crossbreeding on genetic parameters, data on 6276 W1 and 6916 WB purebred laying hens, previously analyzed by Ellen *et al.* (2008), were reused. More details on the purebred material can be found in Ellen *et al.* (2008).

Methods

A linear mixed model was used to estimate genetic parameters for survival time (motivated in *Results and Discussion*). To determine which fixed effects to include in the model, a general linear model was run in SAS v. 9.1 (SAS Institute 2003). First, an interaction term for each laying_house*row*level was included to correct for infra-structural effects (*e.g.*, differences in light intensity). Second, a fixed effect for the content of the back cage was included, which was either empty or contained hens. Third, a covariate for the average number of survival days in the back cage was included. The model was then extended with random effects in ASReml v. 3.0 (Gilmour *et al.* 2009).

To investigate whether genetic parameters differ between both crosses, a bivariate animal model was used, in which survival time was analyzed as a statistically different trait for each cross.

Direct animal model

The following model was used to estimate direct genetic parameters

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_{1,D} & 0 \\ 0 & Z_{2,D} \end{bmatrix} \begin{bmatrix} a_{1,D} \\ a_{2,D} \end{bmatrix} + \begin{bmatrix} V_1 & 0 \\ 0 & V_2 \end{bmatrix} \begin{bmatrix} \text{cage}_1 \\ \text{cage}_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix},$$

where subscript 1 refers to W1 × WB and subscript 2 refers to WB × W1; y is a vector of observations; X is an incidence matrix linking observations to fixed effects; b is a vector of fixed effects; Z_D is an incidence matrix linking an animal's phenotype to its own A_D ; a_D is a vector of A_D 's; V is an incidence matrix linking observations to random cage effects; cage is a vector of independent random cage effects; and e is a vector of residuals.

The direct genetic covariance structure was

$$\text{Var} \begin{bmatrix} a_{1,D} \\ a_{2,D} \end{bmatrix} = \begin{bmatrix} \sigma_{A_{1,D}}^2 & \sigma_{A_{12,D}} \\ \sigma_{A_{12,D}} & \sigma_{A_{2,D}}^2 \end{bmatrix} \otimes A,$$

where $\sigma_{A_{1,D}}^2$ is the direct genetic variance for W1 × WB; $\sigma_{A_{2,D}}^2$ is the direct genetic variance for WB × W1; $\sigma_{A_{12,D}}$ is the direct genetic covariance between crosses; and A is the matrix of additive genetic relationships between individuals, based on five generations of pedigree.

Direct-indirect animal model

The following model was used to estimate direct and indirect genetic parameters

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_{1,D} & 0 \\ 0 & Z_{2,D} \end{bmatrix} \begin{bmatrix} a_{1,D} \\ a_{2,D} \end{bmatrix} + \begin{bmatrix} Z_{1,I} & 0 \\ 0 & Z_{2,I} \end{bmatrix} \begin{bmatrix} a_{1,I} \\ a_{2,I} \end{bmatrix} + \begin{bmatrix} V_1 & 0 \\ 0 & V_2 \end{bmatrix} \begin{bmatrix} \text{cage}_1 \\ \text{cage}_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix},$$

where the vectors and incidence matrices correspond to those in the direct animal model; Z_I is an incidence matrix linking an animal's phenotype to its cage mates' A_I ; and a_I is a vector of A_I 's.

The direct-indirect genetic covariance structure was

$$\text{Var} \begin{bmatrix} a_{1,D} \\ a_{2,D} \\ a_{1,I} \\ a_{2,I} \end{bmatrix} = \begin{bmatrix} \sigma_{A_{1,D}}^2 & \sigma_{A_{12,D}} & \sigma_{A_{1,D,I}} & \sigma_{A_{1,D,2,I}} \\ \sigma_{A_{12,D}} & \sigma_{A_{2,D}}^2 & \sigma_{A_{2,D,1,I}} & \sigma_{A_{2,D,I}} \\ \sigma_{A_{1,D,I}} & \sigma_{A_{2,D,1,I}} & \sigma_{A_{1,I}}^2 & \sigma_{A_{12,I}} \\ \sigma_{A_{1,D,2,I}} & \sigma_{A_{2,D,I}} & \sigma_{A_{12,I}} & \sigma_{A_{2,I}}^2 \end{bmatrix} \otimes A,$$

where $\sigma_{A_{1,I}}^2$ is the indirect genetic variance for W1 × WB; $\sigma_{A_{2,I}}^2$ is the indirect genetic variance for WB × W1; $\sigma_{A_{12,I}}$ is the indirect genetic covariance between crosses; $\sigma_{A_{1,D,I}}$ is the direct-indirect genetic covariance within W1 × WB; $\sigma_{A_{2,D,I}}$ is the direct-indirect genetic covariance within WB × W1; $\sigma_{A_{1,D,2,I}}$ is the genetic covariance between the direct effect of W1 × WB and the indirect effect of WB × W1; and $\sigma_{A_{2,D,1,I}}$ is the genetic covariance between the direct effect of WB × W1 and the indirect effect of W1 × WB.

The above direct-indirect animal model was also used univariate to estimate genetic parameters in the purebred

parental lines, i.e., W1 and WB. The purebred data were previously analyzed by Ellen *et al.* (2008), but with a different experimental time span and a slightly different model. Therefore, the purebred data were reanalyzed using the same experimental time span (398 instead of 447 days) and the above direct-indirect animal model.

Direct-indirect animal model with nongenetic maternal effects

The following model was used to estimate nongenetic direct and indirect maternal effects

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_{1,D} & 0 \\ 0 & Z_{2,D} \end{bmatrix} \begin{bmatrix} a_{1,D} \\ a_{2,D} \end{bmatrix} + \begin{bmatrix} Z_{1,I} & 0 \\ 0 & Z_{2,I} \end{bmatrix} \begin{bmatrix} a_{1,I} \\ a_{2,I} \end{bmatrix} + \begin{bmatrix} W_{1,D} & 0 \\ 0 & W_{2,D} \end{bmatrix} \begin{bmatrix} \text{dam}_{1,D} \\ \text{dam}_{2,D} \end{bmatrix} + \begin{bmatrix} W_{1,I} & 0 \\ 0 & W_{2,I} \end{bmatrix} \begin{bmatrix} \text{dam}_{1,I} \\ \text{dam}_{2,I} \end{bmatrix} + \begin{bmatrix} V_1 & 0 \\ 0 & V_2 \end{bmatrix} \begin{bmatrix} \text{cage}_1 \\ \text{cage}_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix},$$

where the vectors and incidence matrices correspond to those in the direct-indirect animal model; W_D is an incidence matrix linking an animal's phenotype to its own dam; dam_D is a vector of independent random direct maternal effects; W_I is an incidence matrix linking an animal's phenotype to its cage mates' dam; and dam_I is a vector of independent random indirect maternal effects.

Nongenetic maternal effects account for the covariance between maternal siblings apart from their additive genetic relationship. Such a covariance may arise because of shared maternal environment, causing full sibs to express similar direct or indirect effects. Moreover, because of the nested mating structure, the maternal effect also accounts for the covariance among siblings due to nonadditive effects such as dominance and epistasis, both direct and indirect. Omitting nongenetic maternal effects from the model may cause overestimation of the additive genetic variance.

Results and Discussion

Descriptive statistics

A significant difference in survival was found between both crosses. Up to day 398, 61% of the W1 × WB hens survived, while only 51% of the WB × W1 hens survived (Figure 1). A significant difference in survival was also observed between laying houses. In laying house 1, 50% of the hens survived, while in laying house 2, 62% of the hens survived (Table 2). This is probably related to the higher light intensity in laying house 1 (Ellen *et al.* 2008), which is known to evoke feather pecking and cannibalism (Hughes and Duncan 1972; Savory 1995; Kjaer and Vestergaard 1999).

Model

When analyzing survival time, one should ideally use survival analysis methodology to account for the skewed and censored nature of the data (Kalbfleisch and Prentice 2002). However, survival analysis software does not yet allow the inclusion of

Figure 1 The Kaplan–Meier survival curve. Plotting survival (%) against the number of test days (0–398) for the reciprocal cross of two purebred layer lines.

both direct and indirect genetic effects. To circumvent this problem, Ellen *et al.* (2010) proposed a two-step procedure, which combines survival analysis (step 1) with a linear mixed model (step 2). In the first step, only a direct genetic effect is modeled using survival analysis. The estimate of this effect is then used to create a pseudo-record. In the second step, the pseudo-record is modeled using a linear direct–indirect mixed model. Cross validation results showed that the ordinary linear mixed model had the same predictive ability of breeding values as the two-step procedure (Ellen *et al.* 2010). Ellen *et al.* (2010) did not investigate the variance component estimation with the two-step procedure. When analyzing the current data with the two-step procedure, the estimated genetic parameters showed to be highly dependent on the animal to which the phenotype was allocated in the first step, *i.e.*, the focal animal or one of its group mates.

The decision whether to fit a direct or an indirect genetic effect in the first step should not affect the final outcome. Hence, more research is needed to optimize the two-step procedure for variance component estimations. Therefore, the decision was made to fit a linear mixed model.

Genetic parameters within crosses

Table 3 shows the estimated parameters, for crossbreds, from the direct animal model. The additive direct genetic variance was highly significant in both crosses. The heritability was 0.05 for W1 × WB and 0.06 for WB × W1. When comparing both crosses, all variance components were smaller in W1 × WB. This is a direct consequence of the difference in mean survival between both crosses. Since W1 × WB has a higher survival, more observations were censored and less variation was observed.

Table 3 also shows the estimated parameters from the direct–indirect animal model. The model fitted the data considerably better than the direct animal model (χ -square test; $P < 0.001$). Both $\sigma_{A_D}^2$ and $\sigma_{A_I}^2$ were highly significant in both crosses. For the same reason as for the direct animal model, all variance components were smaller in W1 × WB. Although $\sigma_{A_I}^2$ has a similar magnitude as $\sigma_{A_D}^2$, its contribution to σ_P^2 is three times larger and its contribution to $\sigma_{A_T}^2$ is nine times larger ($n - 1 = 3$ and $(n - 1)^2 = 9$, see *Background*). T^2 was 0.26 for W1 × WB and 0.17 for WB × W1. These values substantially exceed the ordinary (direct) h^2 , indicating that the majority of heritable variation was hidden in the direct animal model. The difference in T^2 between both crosses is primarily due to the difference in $\sigma_{A_{DI}}$, rather than a difference in direct or indirect genetic and nonheritable variance. The direct–indirect genetic correlation was moderately negative for W1 × WB (−0.37), but highly negative for WB × W1 (−0.83). These negative correlations indicate

Table 2 Number of laying hens (*n*), their survival (%) with standard error, and their average number of survival days with standard error for each cross, level, and row within each laying house

	Laying house 1			Laying house 2		
	<i>n</i>	Survival	Survival days	<i>n</i>	Survival	Survival days
Total	8,072	50 ± 1	273 ± 2	6,940	62 ± 1	307 ± 2
Cross						
W1 × WB	4,292	53 ± 1	290 ± 2	3,376	68 ± 1	328 ± 2
WB × W1	3,780	46 ± 1	254 ± 3	3,564	56 ± 1	287 ± 2
Level						
Top	2,444	42 ± 1	244 ± 3	356	72 ± 2	338 ± 6
Middle	2,596	51 ± 1	279 ± 3	3,280	62 ± 1	306 ± 2
Bottom	3,032	55 ± 1	290 ± 3	3,304	62 ± 1	305 ± 2
Row						
1	176	55 ± 4	282 ± 11	632	65 ± 2	314 ± 5
2	1,224	51 ± 1	269 ± 4	884	60 ± 2	305 ± 4
3	1,220	50 ± 1	276 ± 4	888	65 ± 2	314 ± 4
4	1,220	51 ± 1	278 ± 4	888	59 ± 2	297 ± 5
5	1,224	58 ± 1	305 ± 4	888	61 ± 2	305 ± 5
6	1,224	49 ± 1	277 ± 4	884	56 ± 2	285 ± 5
7	1,224	44 ± 1	249 ± 4	884	57 ± 2	297 ± 5
8	560	41 ± 2	231 ± 7	992	75 ± 1	339 ± 4

Table 3 Estimated parameters with standard error, for crossbreds, from a direct and a direct-indirect animal model

	Direct animal model		Direct-indirect animal model	
	W1 × WB	WB × W1	W1 × WB	WB × W1
σ_{Cage}^2	2,764 ± 205	2,914 ± 265	1,984 ± 260	2,379 ± 306
$\sigma_{A_D}^2$	711 ± 179	1,292 ± 281	536 ± 152	997 ± 226
$\sigma_{A_{DI}}^2$			−197 ± 93	−726 ± 140
$\sigma_{A_I}^2$			536 ± 109	767 ± 148
σ_E^2	12,326 ± 270	17,109 ± 393	11,732 ± 298	15,655 ± 460
σ_P^2	15,802 ± 273	21,315 ± 373	15,860 ± 289	21,332 ± 401
h^2, T^2	0.05 ± 0.01	0.06 ± 0.01	0.26 ± 0.06	0.17 ± 0.05
r_{DI}			−0.37 ± 0.17	−0.83 ± 0.10
$\sigma_{A_{12,D}}^2$		770 ± 253		697 ± 209
$\sigma_{A_{12,I}}^2$				261 ± 172
$\sigma_{A_{1,D,2,I}}^2$			−285 ± 185	
$\sigma_{A_{1,I,2,D}}^2$			90 ± 190	
r_D		0.80 ± 0.22	0.95 ± 0.23	
r_I			0.41 ± 0.26	
r_T			0.64 ± 0.31	

All estimated parameters are explained in the notation key (Table 1).

that individuals with a positive (direct) breeding value for their own survival have, on average, a negative (indirect) breeding value for the survival of their cage mates and *vice versa*. This can be interpreted as heritable competition. With heritable competition, selection for direct genetic effects results in a negative indirect genetic response and potentially in a negative net response in the phenotype. When unrelated group members are selected on the basis of their own performance, the realized heritability equals $[\sigma_{A_D}^2 + (n-1)\sigma_{A_{DI}}^2]/\sigma_P^2$ (Griffing 1967). Based on the estimates in Table 3, mass selection would result in a realized heritability of 0.00 for W1 × WB and −0.06 for WB × W1. Hence, despite substantial heritable variance, W1 × WB would fail to respond to mass selection and WB × W1 would respond in the opposite direction. The large difference between the realized heritability for mass selection and T^2 demonstrates that breeders need to adapt their selection criterion to achieve positive response to selection in these crosses.

Estimates of the nongenetic direct and indirect maternal effects were small and statistically nonsignificant. This implies that common environmental effects due to the dam are negligible. Moreover, variance due to dominance and epistasis seems negligible.

Purebred-crossbred comparison

In crossbreds, 61% of the W1 × WB and 51% of the WB × W1 hens survived up to day 398. In the purebred lines, survival up to day 398 was 64% for W1 and 58% for WB. Thus, on average, survival time was shorter in crossbreds than in purebreds. Because test circumstances were similar for pure- and crossbreds (same stables, different year), the decrease in survival is most probably due to nonadditive genetic effects, rather than environmental effects. Nonadditive genetic effects and negligible nonadditive genetic variance seem to contradict each other. However, if many loci influence the trait, dominance variance can be small, despite substantial

(negative) heterosis. Because heterosis is proportional to the dominance effect (d) at a locus ($H \sim \sum_{loci} d$) and dominance variance is proportional to d^2 ($\sigma_D^2 \sim \sum_{loci} d^2$), dominance variance will decrease when the number of loci increases and heterosis is constant (Robertson *et al.* 1983; Falconer and Mackay 1996). Hence, this suggests that survival time is affected by many loci, which is consistent with results from Biscarini *et al.* (2010), who found 11 direct QTL and 81 indirect QTL for feather score, which is a precursor of survival.

Table 4 shows the estimated parameters, for purebreds, from the direct-indirect animal model. T^2 was 0.19 for W1 and 0.16 for WB. These values are slightly lower than in crossbreds. The underlying parameters, however, showed substantial differences. Although $\sigma_{A_D}^2$ was similar in pure- and crossbreds, $\sigma_{A_I}^2$ was two to six times larger in crossbreds than in purebreds. Moreover, r_{DI} was low and not significantly different from zero in purebreds, but moderately to highly negative in crossbreds. Although T^2 was similar in pure- and crossbreds, the contribution of IGEs to $\sigma_{A_T}^2$ differed. The contribution of $2(n-1)\sigma_{A_{DI}}^2 + (n-1)^2\sigma_{A_I}^2$ to $\sigma_{A_T}^2$ was 87% in W1 × WB and 72% in WB × W1, while it was 65% in W1 and 44% in WB. Moreover, the realized heritability in case of mass selection differed. The realized heritability was 0.00 for W1 × WB and −0.06 for WB × W1, while it was 0.08 for W1 and 0.06 for WB. This indicates that IGEs in crossbreds contribute more to heritable variation and have more impact on response to selection than in the parental purebred lines.

Unfortunately, because of a lack of close pedigree links between pure- and crossbreds, purebred-crossbred correlations could not be estimated. The large difference in r_{DI} between pure- and crossbreds implies that the purebred-crossbred correlation must be smaller than one, at least for one of the effects (*i.e.*, direct or indirect). This indicates the presence of nonadditive effects such as dominance or epistasis (Wei *et al.* 1991), or parent-of-origin effects such as sex chromosome linked effects or imprinting.

Table 4 Estimated parameters with standard error, for purebreds, from a direct-indirect animal model

	W1	WB
σ_{Cage}^2	803 $\pm$ 161	1,200 $\pm$ 237
σ_{Ad}^2	656 $\pm$ 161	1,400 $\pm$ 299
σ_{Adi}^2	51 $\pm$ 58	-161 $\pm$ 104
σ_{Ai}^2	100 $\pm$ 39	228 $\pm$ 71
σ_{E}^2	7,976 $\pm$ 205	12,686 $\pm$ 364
σ_{P}^2	9,735 $\pm$ 187	15,971 $\pm$ 297
T^2	0.19 $\pm$ 0.06	0.16 $\pm$ 0.05
r_{Di}	0.20 $\pm$ 0.21	-0.28 $\pm$ 0.18

All estimated parameters are explained in the notation key (Table 1).

Genetic parameters between crosses

The genetic correlation between the A_T 's of both crosses ($r_{12,T}$; see Appendix for derivation) was moderate (0.64) and not significantly different from one (Table 3). Underlying, the genetic correlation between direct genetic effects ($r_{12,D}$) was high (0.95) and not significantly different from one, while the genetic correlation between indirect genetic effects ($r_{12,I}$) was moderate (0.41) and significantly different from one (Table 3). This moderate genetic correlation indicates that, for IGEs, it mattered which parental line provided the sire and which provided the dam, *i.e.*, an indirect parent-of-origin effect.

So far, parent-of-origin effects have been reported for direct effects only. In chickens, direct parent-of-origin effects have been found for feed intake, body weight, sexual maturity, egg-production traits, egg-quality traits, and viability (Fairfull *et al.* 1983; Fairfull and Gowe 1986; Ledur *et al.* 2003; Tuiskula-Haavisto *et al.* 2004). Parent-of-origin effects can have multiple underlying causes, such as (cytoplasmatic) maternal effects, sex chromosome linked effects, or imprinting (Fairfull *et al.* 1983; Fairfull and Gowe 1986; Tuiskula-Haavisto *et al.* 2004). Because maternal variances were small and statistically nonsignificant, they can be excluded as a potential cause of the indirect parent-of-origin effect found here.

Comparing pure- and crossbred data revealed that the cross with the highest survival ($W1 \times WB$) received the paternal chromosome from the pure line with the highest survival (W1) and *vice versa* (Figure 2). This result suggests that part of the genes affecting survival time is located on the paternal sex chromosome (the Z chromosome, which carries more genetic information than the W chromosome) or is maternally imprinted. This result agrees with findings of Rodenburg *et al.* (2003), who reported a higher sire-based than dam-based heritability for feather pecking. Severe feather pecking can kill the recipient (Savory 1995) and has a major impact on survival time.

Sex chromosomes are known to have a substantial impact on sex-specific behavioral characteristics (Xu *et al.* 2002; Gatewood *et al.* 2006). This could also apply for feather pecking and cannibalism, which is more common in females than in males (Hughes 1973; Jensen *et al.* 2005). On the one hand, sex chromosomes contain genes that regulate the

Figure 2 Mean survival (in days) for the purebred lines W1 and WB, and their reciprocal cross.

expression of gonadal steroid hormones. Hughes (1973) observed that the simultaneous admission of estrogen and progesterone resulted in more feather pecking and cannibalism, while the admission of testosterone had the opposite effect. On the other hand, sex chromosomes contain genes that are not involved in male or female determination, but do affect sex-specific characteristics (Gatewood *et al.* 2006). These genes can reinforce differences between males and females as, despite a certain degree of dosage compensation, certain parts of the chromosome remain unequally expressed in males and females (Xu *et al.* 2002; Arnold *et al.* 2008). Biscarini *et al.* (2010) found evidence for Z chromosome linked IGEs in an association study on feather condition score in laying hens. Feather condition score serves as a measure for the severity of feather pecking. Biscarini *et al.* (2010) identified 81 QTL for IGEs, of which six were located on the Z chromosome. Once more, this suggests that IGEs for survival time are Z chromosome linked. On the basis of these observations, the decision was made to perform a sex chromosome linked analysis. However, this model failed to converge.

Alternatively, maternal imprinting, where only paternally inherited alleles are expressed, could explain the observed parent-of-origin effect. Imprinting in animals is assumed to be a phenomenon exclusive to placental-marsupial mammals, fish, and insects, expressed at the embryonic or post-natal stage (Reik and Walter 2001). However, there are indications that imprinting occurs in birds as well (Reik and Walter 2001; Tuiskula-Haavisto *et al.* 2004; Tuiskula-Haavisto and Vilkki 2007; Úbeda and Gardner 2010). Moreover, imprinting is recently linked to social behavior in later stages of life (Garfield *et al.* 2011). But, because of a lack of biological evidence, through expression studies at RNA or protein level, imprinting is an unlikely explanation for the indirect parent-of-origin effect observed here.

If IGEs for survival time are indeed Z chromosome linked, this could cause the sire variance to exceed the dam variance.

This would occur only if the causal genes on the Z chromosome are still segregating within the pure lines. When both pure lines carry different IGEs on the Z chromosome, but those do not segregate within pure lines, then the sire and dam variance would be equal, but r_{12J} may still be smaller than one. To investigate this issue, a_1 in the above direct-indirect animal model was replaced by an indirect sire and dam effect (model not shown). Results showed no consistent or significant difference between sire and dam variance. This suggests that IGE genes on the Z chromosome do not segregate within the pure lines. Alternatively, this issue could be explained by a combination of dominance variance and IGEs segregating on the Z chromosome within pure lines. In theory, dominance could inflate the dam variance, while IGEs on the Z chromosome could inflate the sire variance by approximately the same amount, resulting in a similar sire and dam variance. Genome-wide association studies are needed to further investigate the genetic architecture of survival in chickens.

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Appendix

To quantify the similarity between crosses, the genetic correlation between A_T 's ($r_{12,T}$) needed to be calculated. $r_{12,T}$ is dependent on the total heritable variance within crosses ($\sigma_{A_{1,T}}^2$ and $\sigma_{A_{2,T}}^2$) and the total genetic covariance between crosses ($\sigma_{A_{12,T}}$), as $r_{12,T} = \sigma_{A_{12,T}} / \sqrt{\sigma_{A_{1,T}}^2 \sigma_{A_{2,T}}^2}$. With $\sigma_{A_{1,T}}^2 = \text{Var}(A_{1,D} + (n-1)A_{1,I})$, it follows that $\sigma_{A_{1,T}}^2 = \sigma_{A_{1,D}}^2 +$

$2(n-1)\sigma_{A_{1,D,I}} + (n-1)^2\sigma_{A_{1,I}}^2$. With $\sigma_{A_{2,T}}^2 = \text{Var}(A_{2,D} + (n-1)A_{2,I})$, it follows that $\sigma_{A_{2,T}}^2 = \sigma_{A_{2,D}}^2 + 2(n-1)\sigma_{A_{2,D,I}} + (n-1)^2\sigma_{A_{2,I}}^2$. And with $\sigma_{A_{12,T}} = \text{Cov}(A_{1,D} + (n-1)A_{1,I}, A_{2,D} + (n-1)A_{2,I})$, it follows that $\sigma_{A_{12,T}} = \sigma_{A_{12,D}} + (n-1)\sigma_{A_{1,D,I}} + (n-1)\sigma_{A_{2,D,I}} + (n-1)^2\sigma_{A_{12,I}}$. Therefore,

$$r_{12,T} = \frac{\sigma_{A_{12,D}} + (n-1)\sigma_{A_{1,D,I}} + (n-1)\sigma_{A_{2,D,I}} + (n-1)^2\sigma_{A_{12,I}}}{\sqrt{(\sigma_{A_{1,D}}^2 + 2(n-1)\sigma_{A_{1,D,I}} + (n-1)^2\sigma_{A_{1,I}}^2)(\sigma_{A_{2,D}}^2 + 2(n-1)\sigma_{A_{2,D,I}} + (n-1)^2\sigma_{A_{2,I}}^2)}}$$