Modelling of genetic interactions improves prediction of hybrid patterns – a case study in domestic fowl

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Summary

A major challenge in complex trait genetics is to unravel how multiple loci and environmental factors together cause phenotypic diversity. Both first (F_1) and second (F_2) generation hybrids often display phenotypes that deviate from what is expected under intermediate inheritance. We have here studied two chicken F_2 populations generated by crossing divergent chicken lines to assess how epistatic loci, identified in earlier quantitative trait locus (QTL) studies, contribute to hybrid deviations from the mid-parent phenotype. Empirical evidence suggests that the average phenotypes of the intercross birds tend to be lower than the midpoint between the parental means in both crosses. Our results confirm that epistatic interactions, despite a relatively small contribution to the phenotypic variance, play an important role in the deviation of hybrid phenotypes from the mid-parent values (i.e. multi-locus hybrid genotypes lead to lower rather than higher body weights). To a lesser extent, dominance also appears to contribute to the mid-parent deviation, at least in one of the crosses. This observation coincides with the hypothesis that hybridization tends to break up co-adapted gene complexes, i.e. generate Bateson–Dobzhansky–Muller incompatibilities.

1. Introduction

Geneticists have for many years studied the genetics of hybrid populations (Lynch, 1991; Rieseberg *et al.*, 1999; Burke & Arnold, 2001; Hochholdinger & Hoecker, 2007; Lippman & Zamir, 2007). Hybridization can affect both the mean and the variance of the population. When F_1 and F_2 hybrids are on average different from the mid-parent value (or out of the parental range, depending on the definition), they display either hybrid vigour (heterosis) or hybrid inferiority (negative heterosis) (Hochholdinger & Hoecker, 2007; Lippman & Zamir, 2007). Another outcome of hybridization is transgressive segregation (TS) (Grant, 1975; deVicente & Tanksley, 1993), which occurs when there exist individuals in a hybrid population that do not lie between those of the founder lines or populations.

There are two main genetic mechanisms potentially involved in hybrid deviations: intralocus interactions (dominance) and interlocus interactions (epistasis). When F_1 hybrids from two genetically diverged lines are viable and fertile, it is possible to breed F_2 hybrids. A large number of such intercrosses have been generated between divergent lines of animals (Andersson & Georges, 2004) and plants (Maloof, 2003) for mapping genes underlying the phenotypic differences between those lines. F_2 populations display, in contrast to the F_1 hybrids, a large amount of genetic variation, and their average may differ from the F_1 as more types of genetic interactions can contribute to extreme phenotypes. Studies of segregating hybrids also allow mapping of loci and estimating their genetic effects. Multiple studies have identified loci that contribute to heterosis in agricultural production

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traits in such populations (e.g. Rieseberg *et al.*, 1999; Hua *et al.*, 2002, 2003).

A number of hypotheses have been proposed to explain heterosis including dominance, over-dominance, pseudo-over-dominance and epistasis (Hochholdinger & Hoecker, 2007; Lippman & Zamir, 2007). Empirical investigations that support the dominance-based hypotheses have often used reductionist approaches, either through modelling (Lippman & Zamir, 2007) or experimental design (Semel *et al.*, 2006). These studies focus primarily on detecting the role of individual loci in the genetic architecture underlying inferior or superior hybrid phenotypes. There are, on the other hand, theoretical approaches pointing to the importance of accounting for epistasis when analysing the genetic architectures underlying hybrid phenotypes, particularly when heterosis is involved (e.g. Melchinger *et al.*, 2007*b*). Most statistical models used for quantitative trait locus (QTL) mapping are, however, known to underestimate the significance of epistasis (as pointed out by e.g. Melchinger *et al.*, 2008), whereas several recent empirical studies using multilocus epistatic models to detect QTLs have found epistasis to influence heterosis (Yu *et al.*, 1997; Li *et al.*, 2001; Luo *et al.*, 2001; Hua *et al.*, 2003; Melchinger *et al.*, 2007*a*; Reif *et al.*, 2009; Meyer *et al.*, 2010). The genetics of hybrid phenotypes is thus complex, involves multiple loci and most likely also interactions between loci (Melchinger *et al.*, 2007*b*, 2008).

All the above illustrate the importance of finding ways to simultaneously evaluate the importance of all potential genetic mechanisms determining the properties of the phenotypic distributions in hybrid populations. Here, we address this topic using QTL mapping data for body weight from two independent chicken intercross populations. The first population is a cross between two lines artificially selected for low and high body weight at 56 days of age (BW56) (Dunnington & Siegel, 1996; Marquez *et al.*, 2010). The second population is a cross between the wild ancestor of the domesticated chicken (Red Junglefowl) and a White Leghorn line selected for egg production, where both early (46 days, BW46) and late (200 days, BW200) body weights were available. In both populations, empirical observations suggest that hybrid early (juvenile) body weights are lower than the average of their parents (Liu *et al.*, 1995; Schutz *et al.*, 2002). In this study we aim to (i) determine whether the low-observed hybrid phenotypes can be understood from a multilocus genotype-to-phenotype (GP) map computed from QTLs detected in previous studies on the same populations (Carlborg *et al.*, 2003; Kerje *et al.*, 2003; Jacobsson *et al.*, 2005), (ii) evaluate the type of genetic effects (additive, dominance, epistasis or all) that contribute to low-hybrid phenotypes and TS and (iii) assess how similar

the genetic architectures of a trait (or closely related traits) are when obtained from studies in independent animal populations.

2. Materials and methods

(i) *Animal populations*

(a) *The Virginia (High × Low) body weight-selected line intercross*

An F_2 intercross was generated by reciprocal inter-crossing of birds from two divergently selected lines of chickens obtained by bi-directional selection for body weight at 56 days of age (referred to as the 'high' and 'low' body weight selected lines, Dunnington & Siegel, 1996; Marquez *et al.*, 2010). The lines originate from a common base population, consisting of crosses of seven partially inbred lines of White Plymouth Rock chickens. The sex-averaged 56-day body weight (BW56) for the base population was 793 g with a SD of 120 g. After 40 generations of selection, the high line weighed about eight times (1412 ± 125 g) as much as the low line (170 ± 47 g) at the age of selection. The age at sexual maturity was different in both lines (190 ± 9 days in the high line versus 221 ± 9 days in the low line). The F_1 population was generated by mating 10 males and 19 females from the high line to 8 males and 22 females from the low line. Eight males and 75 females from the F_1 were mated to generate a large segregating F_2. The F_2 that survived to 56 days of age ($n = 795$; BW56 $\pm$ SD: 624 ± 168 g; 18 % mortality) were genotyped for 145 genetic markers covering 2427 cM on 25 linkage groups. All F_2 progeny were from the same hatch and from parents of the same age, and all generations were raised in the same facilities with the same food, in order to limit cross-generation environmental effects. The observed mean values for the F_1 and F_2 progeny were below the arithmetic average for the parental lines (Fig. 1), which is consistent with the previous finding of negative heterosis in F_1 crosses of these lines (Liu *et al.*, 1995). Hybrids from the high- and low-selection lines do, however, not deviate from the parental mean at sexual maturity (Williams *et al.*, 2002). All procedures involving animals used in this experiment were carried out in accordance with the Virginia Tech Animal Care Committee animal use protocols.

(b) *The Red Junglefowl × White Leghorn (Wild × Domestic) intercross*

An F_2 intercross was bred from one Red Junglefowl male raised in captivity and three White Leghorn females (Kerje *et al.*, 2003). The F_1 individuals that were used as parents for the F_2 generation were not phenotyped. The F_2 animals were raised in six

separate batches as previously described (Schutz *et al.*, 2002) and body weights were recorded at 1, 8, 46, 112 and 200 days of age. A total of 827 F_2 animals were first genotyped for 105 genetic markers evenly distributed on 25 linkage groups (Kerje *et al.*, 2003) and later for an additional 384 single nucleotide polymorphism (SNP) markers to cover in total 3214 cM on 32 linkage groups. A total of 756 birds with phenotypic records for all body-weight traits were used in this study (Le Rouzic *et al.*, 2008). The F_2 hybrids had a lower juvenile body weight than the parental lines at 46 days of age (BW46), but a more intermediate phenotype to the parentals at 200 days of age (BW200) (Fig. 1). All procedures involving animals in this experiment were carried out in accordance with protocols approved by the local ethics committee.

(ii) *QTL mapping*

(a) *Mapping methods*

QTLs have earlier been mapped in both F_2 crosses using a simultaneous search for pairs of QTLs using a statistical model including the fixed effects of sex, batch (only in the Wild × Domestic cross) and the additive, dominance and all pair-wise epistatic effects of QTL pairs (Carlborg *et al.*, 2003, 2006). The use of a statistical model including epistasis increases the power to identify loci whose effects are dependent on the genotype at another locus (Carlborg *et al.*, 2000, 2003; Carlborg & Andersson, 2002). QTL pairs that reached the 5% genome-wide significance threshold in a randomization test for the joint effect of the epistatic pair (no QTL or one marginal effect QTL versus two interacting QTLs) and a 1% significance threshold in a model-selection randomization test for the joint effect of the epistatic parameters (two non-interacting QTLs versus two interacting QTLs) were reported as pairs. The search for epistatic QTLs did not include analyses of the Z chromosomes, and QTLs that mapped within 25 cM of each other were assumed to represent the same locus.

(b) *QTLs in the High × Low intercross*

In the High × Low F_2 population, an interacting QTL network involving six significant loci has been reported. Four of these loci explain nearly half of the 8-fold difference in juvenile body weight between the lines (Carlborg *et al.*, 2006), and the interaction pattern suggests a role of selection-driven genetic differentiation involving epistasis (Le Rouzic *et al.*, 2007). The four major loci, labelled *Growth4*, *Growth6*, *Growth9* and *Growth12* in Carlborg *et al.* (2006), were included in the analyses performed in this study (Table 1).

(c) *QTLs in the Wild × Domestic intercross*

Around 20 significant QTLs affecting at least one of the measured body weights have been detected for the Wild × Domestic F_2 intercross (Carlborg *et al.*, 2003; Kerje *et al.*, 2003; Le Rouzic *et al.*, 2008). The four loci having 5% genome-wide significant effects (additive, dominance and interactions) for the largest number of traits (labelled 1A, 1C, 11B and 27A in Le Rouzic *et al.*, 2008) were selected for this study (Table 1). We selected four loci in order to avoid potential problems with over-parameterization from including too many loci and also to use the same number of loci for prediction in both the studied crosses. Two of the loci (1A and 1C) had very strong effects on both early (BW46) and late (BW200) body weight and were therefore obvious loci to be included. Selection of the third and fourth loci was not as straightforward as the number of loci had similar body weight effects in the original QTL analysis. As we were primarily interested in exploring the contribution of loci to hybrid deviations from the mid-parent value, we opted to include the two loci (11B and 27A) that had the most pronounced non-additive effects.

(iii) *Modelling of genetic effects*

Many statistical and functional models of genetic effects have been proposed to capture the essential features of multi-locus GP-maps (Fisher, 1918; Cockerham, 1954; Kempthorne, 1954; Cheverud & Routman, 1995; Hansen & Wagner, 2001; Yang, 2004; Zeng *et al.*, 2005). As a continuation of this work, we recently proposed the natural and orthogonal interactions (NOIA) model (Álvarez-Castro & Carlborg, 2007) as a unifier of different earlier approaches to modelling genetic effects.

(a) *Estimation of genetic effects using the NOIA model*

The genetic effects of the selected QTLs (Table 1) were recomputed using the NOIA framework as implemented in the software package '*noia*' for R (Le Rouzic & Álvarez-Castro, 2008). The use of the statistical formulation of NOIA (Álvarez-Castro & Carlborg, 2007) provides an orthogonal estimation of all included genetic effects, even when the population is a non-ideal F_2 regarding the allelic frequencies at each locus (small departures from orthogonality that may arise from deviations from random association of alleles between loci due to finite sample size). Genetic effects, **E**, are estimated by the linear regression $\mathbf{P} = \mathbf{ZSE} + \boldsymbol{\varepsilon}$, where **P** is the vector of observed phenotypes, **Z** is the incidence matrix coding the genotype of each individual (see e.g. Álvarez-Castro
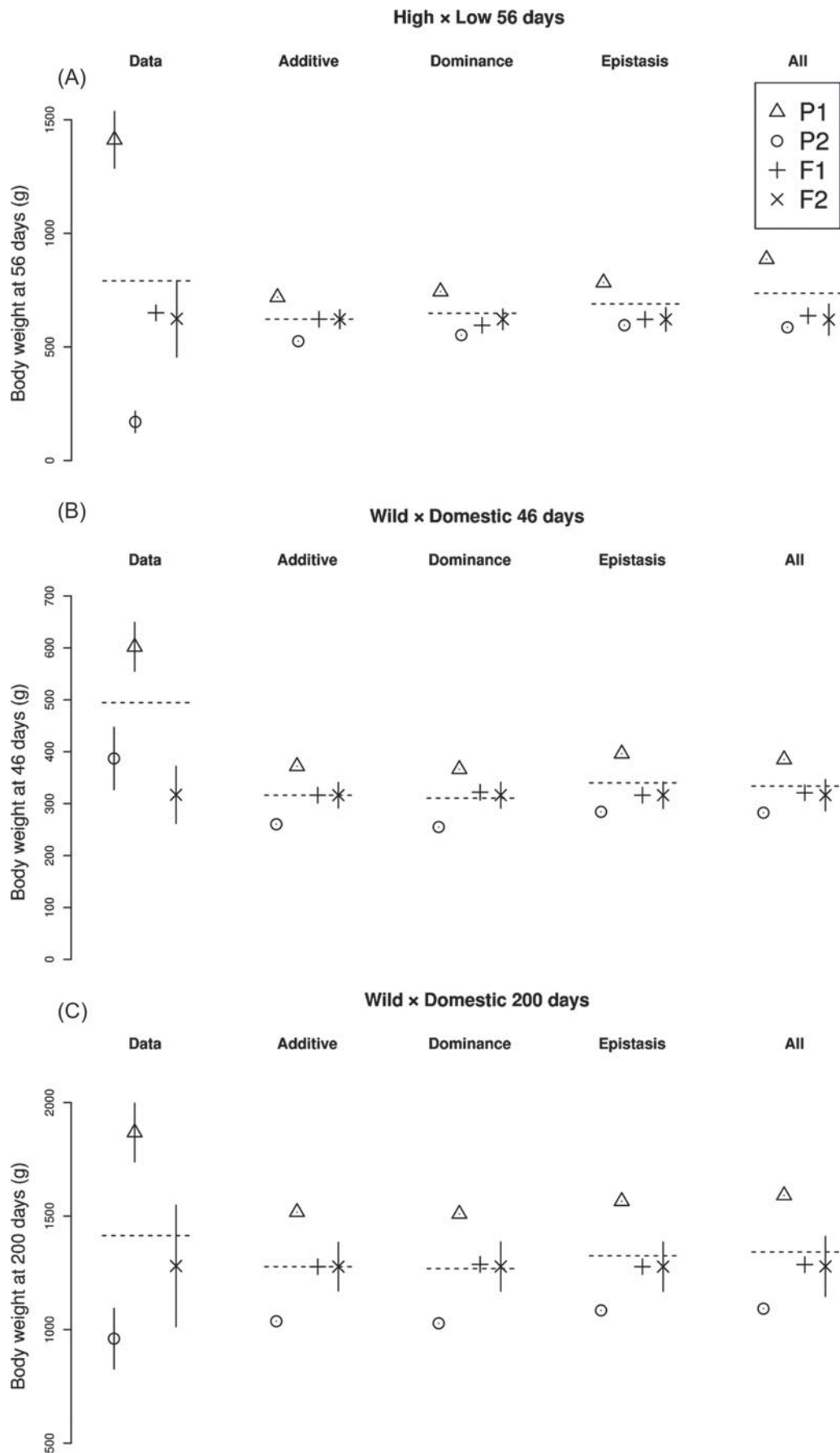
Fig. 1 For legend see opposite page.

Table 1. *QTLs included in this study and their correspondence with the abbreviations in earlier studies*

GGA[a]	Location[b,c] (cM)	Abbreviation[d,e]	ChickenQTLdb ID[f,g,h]
High × Low			
3	0	Growth 4	1957
4	23	Growth 6	1989
7	74	Growth 9	2158
20	55	Growth 12	2371
Wild × Domestic			
1	105	1A	1785
1	484	1C	1847
11	53	11B	2284
27	23	27A	2406

[a] GGA = *Gallus gallus* autosome.
[b] In linkage map by Jacobsson *et al.* (2004).
[c] In linkage map of Le Rouzic *et al.* (2008).
[d] As in Carlborg *et al.* (2006).
[e] As in Le Rouzic *et al.* (2008).
[f] Location of corresponding QTL in Jacobsson *et al.* (2005).
[g] Chicken QTL database Release 13 (31 December 2010) http://www.animalgenome.org/cgi-bin/QTLdb/GG/index.
[h] Corresponding QTL in Kerje *et al.* (2003).

et al., 2008), S is the genetic-effect design matrix implementing the model of genetic effects (Álvarez-Castro & Carlborg, 2007), and ε is the vector of residuals.

Four four-locus models of different complexities were fitted to the data: (i) the 'additive' model with five parameters, including the reference point (i.e. the mean of the population) and one additive effect for each of the four loci; (ii) the 'dominance' model with nine parameters, including the effects of the additive model and also one additional dominance effect per locus; (iii) the 'epistasis' model with 11 parameters, those of the additive model and all additive-by-additive interaction effects; and (iv) the full model (labelled 'all') with 33 parameters, including all effects of the dominance model and all possible pairwise epistatic effects.

(b) *Model-based filtering of the GP map*

As NOIA was used to estimate orthogonal genetic effects (see above), it is only necessary to estimate the genetic effects once using the full genetic model (iv) and then predict the GP map based on the reduced genetic models (i–iii) by simply removing the effects that are not to be used in the respective predictive models without (or only slightly) affecting the GP map (Álvarez-Castro & Carlborg, 2007). This procedure was used for the genetic models described above (i–iv) in the two intercrosses for the traits BW56, BW46 and BW200, using a filtered vector of genetic effects, E_f. For each trait and each model of genetic effects to be used, the vector E_f was built by replacing the effects to be removed from the corresponding vector E by zeros. The four resulting GP maps for each analysed trait – always including four loci and thus consisting of $3^4 = 81$ predicted four-locus genotypes – were then obtained by $G_f = SE_f$, where S is the genetic–effect design matrix as above. In this way, it is possible to use the selected genetic effects obtained from the data to predict the phenotypes of all possible genotypes, including those potentially lacking in the experimental crosses (i.e. in the vector P). The vector G_f entailing the genotypic values of all possible genotypes enabled us, in turn, to obtain phenotype distributions and mean phenotypes of different populations such as the F_1 and F_2 crosses.

(c) *Hypothesis testing using bootstrapping*

In order to evaluate whether the predicted hybrid effects are sufficiently robust for inferring a real

Fig. 1. Empirical and predicted phenotypic means for parental (P_1 and P_2) and hybrid (F_2 and F_1) populations. The panels illustrate these for BW56 (A) in the High × Low body weight selected Virginia lines as well as BW46 (B) and BW200 (C) in the Wild × Domestic intercross. The data column gives the phenotypic means for available populations with SD. The other columns provide predicted phenotypic values for the P_1, P_2 and F_1 populations as the genotype values for the '11 11 11 11', '22 22 22 22' and '12 12 12 12' genotypes, where '1' and '2' represent Domestic/High and Wild/Low parental alleles, respectively, at the four loci (Table 1). The predicted F_2 phenotype is calculated by averaging all 81 genotypes weighted by their expected frequencies in an F_2 population. All predicted values are given together with their genotypic SD (which is 0 for P_1, P_2 and F_1), vertical lines: ± 1 SD. The dotted lines illustrate the arithmetic mean of the parental phenotypes.

Table 2. *Significance testing of the deviation between the predicted F_1 genotype and mid parent values ($D_{F1-mid-parent}$) under the 'epistatic' model (additive and additive-by-additive effects). As a consequence of the epistatic model, the mean F_2 is the same as the predicted F_1 and the tests here are thus identical to a test for $D_{F2-mid-parent}$*

$D_{F1-mid-parent}$	High × Low BW56	Wild × Domestic BW46	Wild × Domestic BW200
Full dataset	−68·3 g	−23·9 g	−47·4 g
Bootstrap average ($n=1000$)			
Means ± SE	−66·8 ± 35·67	−23·80 ± 9·61	−48·79 ± 58·23
$P(\text{obs}|H_0)$	0·08	0·01	0·40

biological effect, we designed a statistical test of whether the difference between our estimates of the four-locus heterozygote genotype (i.e. the F_1 hybrid genotype) and the mean of the two four-locus homozygotes (i.e. the parental phenotypes) was significant. The predictions for the genotypic values used in the test were obtained using the 'epistatic' model (i.e. including additive and additive-by-additive effects). This has the advantage that, as the F_1 and predicted mean F_2 genotype values are equal under this model, we simultaneously test for the significance of the hybrid effects in the F_1 and F_2. Under the null hypothesis, H_0 = no hybrid effects, the F_1 genotype value is identical to the mean of the parental genotype values and the difference is thus zero. The probability of H_0 given the observed distance between the F_1 and the mid-parent value was determined by bootstrapping.

The bootstrapping approach is based on resampling individuals with replacement from the original dataset. In this way, some individuals can be present several times in the analysed bootstrap sample, while others are not included. In each bootstrap resample, the hybrid effect is calculated as described above. The resulting distribution of hybrid effects over $n=1000$ repetitions is centred around the sample mean estimate with a dispersion that approximates the error on the estimate.

3. Results

(i) *The predictive value of the QTL models*

Although a QTL model explains only a fraction of the phenotypic variance, it can predict a substantial portion of the difference between the parental lines (Kerje *et al.*, 2003; Jacobsson *et al.*, 2005; Carlborg *et al.*, 2006). Here, the four QTLs of the High × Low cross predict 24 % of the genetic difference between lines for BW56 (300 g between the two estimates of the parental genotypes versus 1242 g between lines at 56 days of age), and 11·5 % of the phenotypic variance in body weight when considering additive, dominance and epistatic effects. In the Wild × Domestic cross, the QTLs explain 48 % of the genetic divergence (103 g out of 221 g) and 22 % of the phenotypic variance of the body weight at 46 days, with roughly similar

figures for body weight at 200 days (498 g out of 908 g, 54 % of the genetic divergence, 18 % of the phenotypic variance). Most of the genetic variance explained is additive in the Wild × Domestic cross (72·5 % for body weight at 46 days and 72·9 % for body weight at 200 days). In contrast, 65·5 % of the genetic variance in the High × Low cross is epistatic and only 31·4 % is additive.

(a) *Prediction of hybrid patterns from estimated genetic effects*

In both populations, a significant portion of the genetic variance explained by the selected QTL is additive. However, additive effects cannot explain the low-hybrid phenotypes. This is illustrated in Fig. 1, where we present the observed hybrid pattern in the two experimental populations together with the phenotypes predicted from the four genetic models, (i) additive, (ii) additive + dominance, (iii) additive + additive-by-additive epistasis and (iv) the full model, for each of the three datasets.

For BW56 in the High × Low cross (Fig. 1*a*), the dominance model is able to reproduce the low hybrid mean observed in the F_1. It is, however, no better than the additive model in predicting the low F_2 population mean. Both the low F_1 and F_2 means are, however, correctly predicted by the A × A and the full epistatic models, making them better predictors of the empirical observations. In our study, the hybrid patterns predicted from the QTLs mapped in the F_2 population thus appear to forecast the empirical pattern observed when crossing the parental populations. The improved prediction by the dominance and epistatic models is not a result of an increased number of parameters, but rather features with a predictive power outside the studied population. The results from the bootstrapping test presented in Table 2 show that the deviation from the mid-parent explained by the A × A epistatic model is unlikely to be due to sampling effects: the deviation is significant for BW56 in the Wild × Domestic cross, and close to significance for the High × Low cross.

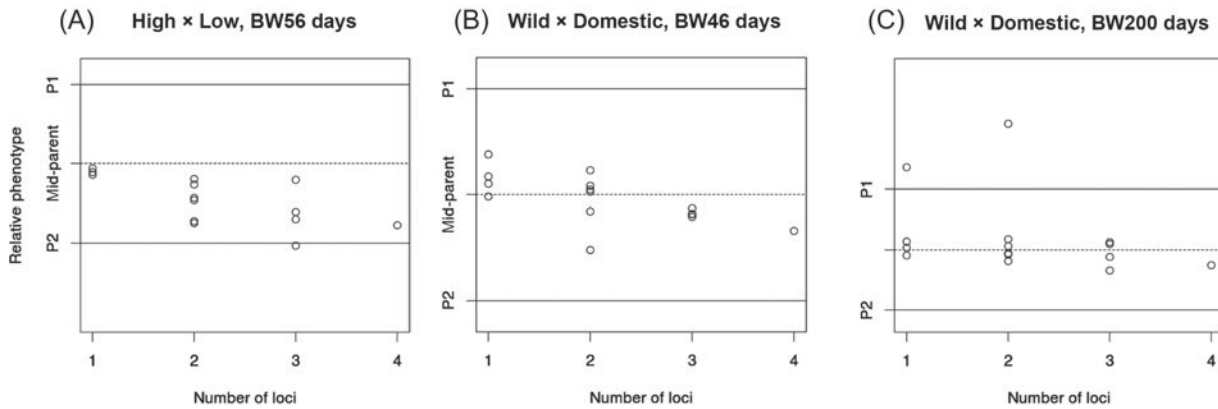For both phenotypes in the Wild × Domestic cross, the dominance model incorrectly predicts a hybrid

Fig. 2. Predictions of phenotypic values for parental and F_2 populations using different subsets of QTLs. Predictions from all combinations of QTLs ranging from single loci with additive and dominance effects to all four loci with additive, dominance and all second-order epistatic interactions are provided for BW56 (A) in the High × Low body weight selected Virginia lines as well as BW46 (B) and BW200 (C) in the Wild × Domestic intercross.

mean higher than the mid-parent value. The models including epistasis correctly reproduce the average F_2 phenotype below the arithmetic mean of the parents, this difference being highly significant for body weight at 46 days, but not at 200 days (Table 2). None of the models are, however, able to predict the empirical observation that BW46 for the hybrid F_2 being below both parents. Thus, in all three studied cases, the models including epistasis provide better predictions of hybrid patterns than additive and dominance models, although not perfect.

(ii) *The genetic basis of hybrid deviations from the mid-parent value*

A difficult challenge when exploring the genetic basis of hybrid effects is to determine how many loci that are involved in causing the deviation from the mid-parent expectation. If the hybrid effect is due to one or very few loci (e.g. through strong over- or under-dominance), the prediction of the deviation from the mid-parent phenotype is highly dependent on the set of genes included in the analysis. On the other hand, if the hybrid effect is a general property of the genetic architecture of the trait, the particular set of loci studied will have a smaller influence on the conclusions.

To investigate the impact of selecting four QTLs in each cross for prediction, we calculated the expected deviation from the mid parent in the F_1 and in the average F_2 for all smaller sets of loci (four single loci individually, all six combinations of two loci, and four combinations of three loci) for all three traits and plot these together with the estimate for the complete four-locus genotypes in Figs. 2a–c. This figure illustrates how epistasis plays a key role in generating the low-hybrid phenotypes in both crosses. The low-hybrid phenotype for the complete four-locus model is not a sum of the predictions from the models including smaller subsets, but rather a result of accounting for

all loci simultaneously. By including more loci together with their interactions in the predictive model, we were able to provide better predictions for the low-hybrid phenotypes.

There are also differences in the results for the two crosses and for the traits within the Wild × Domestic cross. In the High × Low cross (Fig. 2a), virtually all combinations of 2 or 3 loci provide predictions of F_1 and F_2 phenotypes below the mid-parent. This indicates that the low body weight in the hybrids results from the combination of similar epistatic interactions between loci and that the hybrid phenotype is thus an inherent feature in this network of loci rather than the effects of individual loci.

For body weights in the Wild × Domestic cross (Figs. 2b and 2c), the picture is somewhat different. First, for older body weight (200 days), the phenotypic predictions from most individual as well as combinations of loci are in agreement with the original observation in the experimental intercross and the analysis of the four-locus GP map that there is no significant hybrid deviation from the mid-parent. Second, for the younger body weight (46 days), some two- and three-locus combinations provide predictions of low-hybrid phenotypes, but others do not. A detailed analysis showed that lower-than-mid-parent phenotypes were obtained only when the two major loci 1A and 1C were included. Locus 11B was strongly dominant (for BW46) or overdominant (BW200) in the opposite direction and therefore acted to cancel the low-hybrid pattern generated by the other loci. Thus, the inferior hybrid phenotypes in this cross appear to be due to particular combinations of loci rather than a general feature among the evaluated loci.

(iii) *Exploring the genetic basis of TS*

When examining the raw BW56 phenotypes in the High × Low F_2 population, there is a low proportion

(A) **High × Low cross (full model)**
Predicted phenotypic distribution in a F₂ population

(B) **Wild × Domestic cross (full model)**
Predicted phenotypic distribution in a F₂ population

(C) **Wild × Domestic cross (full model)**
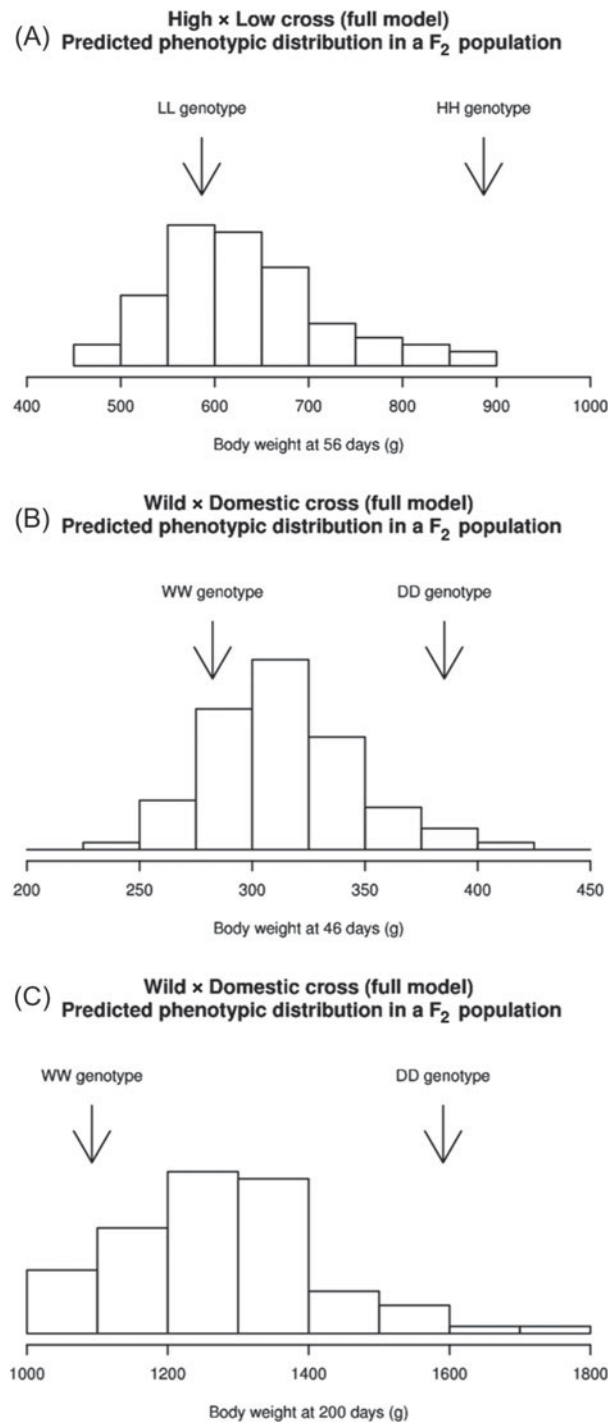**Predicted phenotypic distribution in a F₂ population**

Fig. 3. Distributions of genotypic values predicted using the full model, including four loci with both dominance and pair-wise epistasis. The arrows mark the genotypic values of the parental lines so that the genotype values transgressing the interval between the arrows can be easily identified. Panels A, B and C represent the High × Low body weight selected Virginia lines (BW56) and the Wild × Domestic intercross (BW46 and BW200), respectively.

Fig. 4. An epistatic QTL pair (11B and 27A, Table 1) that through genetic interactions contributes to low-hybrid BW46 phenotypes and TS in the Wild × Domestic intercross.

of transgressive segregants: six out of the 795 chickens have a phenotype below the average of the low line, and none is above the average of the high line. More
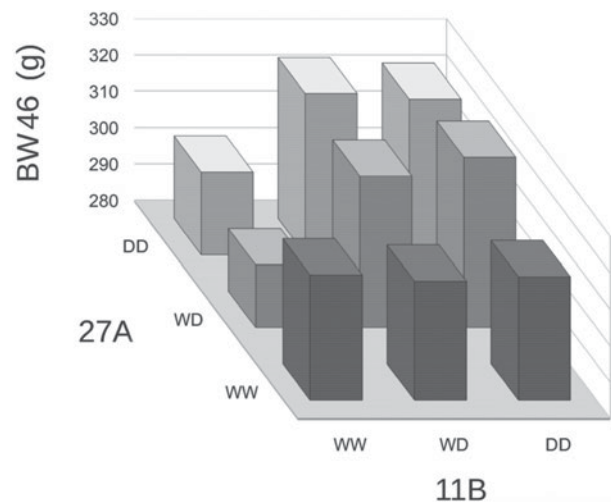
transgressive segregants are observed in the Wild × Domestic cross. For BW200, 76 out of 765 F_2 individuals have phenotypes below the average Red Junglefowl weight, and seven individuals are heavier than the average White Leghorn. When considering BW46 in the same cross, most (90%) of the F_2 individuals are smaller than both parental lines and none is bigger, reflecting a very large hybrid breakdown for this trait. Therefore, most transgressive segregants can be attributed to a lowering of the population mean, rather than to an increase of the genetic or environmental variance (decanalization).

To further explore the genetic basis of TS, we used the predicted genotypic values in the four-locus GP maps, in which all genotypes are present. In the High × Low cross GP map for BW56, 26 out of the 81 genotypes are transgressive; all of them falling below the estimated values for the parental genotypes (Fig. 3). This is in good agreement with the general pattern from the original cross, where only low-parent transgression was observed. In the Wild × Domestic cross GP maps, 18 out of 81 predicted genotypes are transgressive for BW46 (16 below and 2 above the parentals), and 11 out of 81 genotypes for BW200 (8 below, 3 above). Again, the prediction of a larger number of low-parent transgressive genotypes is coherent with the observations in the F_2 cross. Interestingly, the same genotypes of some loci are present in both high- and low-parental transgressive segregants in the Wild × Domestic GP map. This illustrates the importance of epistatic effects for good prediction in this cross and the effects of alleles will be very different depending on the genetic background at other loci. Figure 4 illustrates one such example, where the predicted phenotypes of a pair of loci (11B and 27A) display strong epistatic interactions

contributing to the low-hybrid phenotypes and TS in the Wild × Domestic cross. As already noticed in the previous section, the D allele is highly dominant at locus 11B. Nevertheless, the double heterozygote (WD WD) is intermediate between the parentals (WW WW and DD DD). Thus, this interaction does not lead to lower hybrid phenotypes in the F_1 for these two loci, i.e. heterozygous genotypes alone do not decrease body weight. Recombinant homozygotes (WW DD and DD WW), however, are lighter than both parental lines. The heterozygotes for one locus in the background of the wild homozygote (WW WD and WD WW) in the other locus also display lower phenotypes. The observed transgressive segregant genotypes that result in a lower than expected body weights of the hybrids is thus due to the epistatic effects of loci rather than a property of individual loci.

4. Discussion

Here, GP maps reconstructed from QTL data were used to study the genetic architecture of hybrid phenotypes. Hybrid effects suggested by empirical observations were confirmed by analysing the effects of QTL interactions. We found that (i) despite the limited amount of the phenotypic variance explained by the studied QTLs, they are useful for predicting observed hybrid patterns, (ii) including epistasis in the predictive model was necessary for prediction as neither transgressive additive loci nor dominance nor both together could explain the hybrid effects and (iii) there were both similarities and important discrepancies in the genetic basis that to underlie the hybrid patterns of body weight in the two chicken populations. Our results are consistent with earlier findings in other organisms that multi-locus epistasis is important for low-hybrid phenotypes (e.g. Moyle & Nakazato, 2009). The analytical approach we use here is, however, not restricted to study low-hybrid phenotypes. Its use in genetic modelling and prediction of GP-maps can also make contributions to other long-standing discussions in genetics, of which understanding the mechanisms underlying hybrid vigour and hybrid inferiority are notable examples (Burke & Arnold, 2001; Hochholdinger & Hoecker, 2007; Lippman & Zamir, 2007).

Our study was motivated by the observation that, in both Wild × Domestic and High × Low intercrosses, hybrid populations had a lower body weight than the mid-parent. As experimental and biological constraints associated with such large-scale experiments, parents and hybrids could not be measured at the same generation, and the observed difference might be partly due to generation-specific environmental effects (in particular, they could have amplified the extreme hybrid effect observed for the Wild × Domestic cross). Nevertheless, such cross-generation variability is unlikely to generate large, repeatable spurious hybrid effects. Indeed, populations were raised in standard conditions, which limit the environmental variance, and similar hybrid effects were previously observed among these lines (Liu *et al.*, 1995; Schutz *et al.*, 2002). In any case, the hybrid pattern discovered from the QTL-based GP maps from both crosses arose independently, since only carefully controlled F_2 individuals were used to estimate these maps.

There are, however, some potential challenges to using advanced modelling in moderately sized datasets. By including interaction effects in multilocus loci, there is a risk of overfitting the genetic model. When selecting a model for describing a population, the aim should be to identify and include genetic parameters that allow addressing the biological questions. However, the model should also be as parsimonious as possible statistically: the challenge is to find the appropriate balance between avoiding overfitting and removing effects that will lead to a distortion, or oversimplification, of the GP map. Here, we compared several different parameterizations of the genetic model to find the compromise between biologically useful and statistically parsimonious models that was most useful for prediction. In particular, we considered only models including four QTLs, even if more were detected in the original study. We here assumed that small-effect QTLs would not behave in any radically different way from the major ones concerning dominance or epistasis. To test the validity of this assumption, tests were performed where models with eight, instead of four, loci were fitted in the Wild × Domestic data. These analyses did not affect our conclusions regarding the genetic architecture of the hybrid pattern – epistasis was also necessary here to explain the observed hybrid pattern, but the additional noise in the estimates for the individual genotype-means makes significance tests non-conclusive (results not shown). Therefore, restricting the number of loci improves the estimates of genetic effects, which are more precise and less biased. Indeed, highly significant loci are also less affected by the bias known as the Beavis effect (Xu, 2003), resulting from detecting significant loci and estimating their effect from the same data.

When hybrids display phenotypes that are lower than the mid-parent values for traits related to fitness, this is often referred to as a fitness breakdown resulting from hybrid incompatibilities. The most widely accepted genetic model to explain hybrid incompatibilities is the Bateson–Dobzhansky–Muller (BDM) model (Burke & Arnold, 2001; Palmer & Feldman, 2009). The BDM model proposes that poor hybridization results from poor compatibility between independently co-adapted gene complexes in parental lines. This model is supported by empirical

observations of a relatively widespread role of epistasis in hybrid inferiority (Wu & Palopoli, 1994; Moyle & Nakazato, 2009; Moyle & Nakazato, 2010) and Brideau *et al.* (2006) also reported a mechanistic explanation for the BDM incompatibilities observed in *Drosophila*. However, for many traits (including agricultural production traits such as growth), hybrid deviations are in general relative to productivity traits, and terms such as 'negative heterosis' are used to describe outcomes where the hybrid phenotype is lower than the mid-parent value (Liu *et al.*, 1995). There is thus not necessarily a positive correlation between trait values and fitness, and negative heterosis in one context (e.g. agricultural production) could be positive heterosis in another one (natural selection). Consequently, the sign of heterosis partly relies on arbitrary choices, and it is not unexpected that hybrid breakdown and heterosis may share similar underlying genetic mechanisms.

Other potential contributing factors to the hybrid phenotypes in the non-reciprocal Wild × Domestic F_2 intercross are genes on the W chromosome or in the mitochondria, which only originates from the domestic line. It does, however, seem unlikely that the domestic line would contribute major transgressive alleles decreasing growth on either the sex chromosome or the mitochondria, but further analyses are necessary to exclude this. Neither of these factors is expected to make any noticeable contribution in the High × Low cross as the crossing in that case was reciprocal.

Growth is viewed by many as a multiplicative process and is therefore often modelled on a log-scale. On this scale, the hybrid effect in the High × Low cross becomes a higher body weight (i.e. both the F_1 and the F_2 populations are above the geometric mean of the parental ones), while both Wild × Domestic traits still display a lower hybrid phenotype. Changing the scale for the three traits analysed thus modifies the numerical results, but does not alter our main conclusions that the hybrid deviations from the mid-parent values are better explained by epistatic models than by additive and dominance models (results not shown). In any case, patterns involving qualitative inversions (such as under-dominance, TS, or sign epistasis) are insensitive to scale transformation.

In two independent populations, we observe that epistasis contribute significantly to the phenotypic variation: when epistasis is not taken into account in the genetic model we cannot explain the deviation from the mid-parent value. This outcome provides further support for the impact of epistasis on hybrid effects in accordance with e.g. the BDM model, where epistatic interactions among alleles at different loci having appeared in temporarily isolated subpopulations explain fitness breakdown. The importance of epistasis for the hybrid effects in two independent crosses is particularly interesting given their very different genetic origins. The domestication intercross was based on founder lines divergent by thousands of generations of selection, while in the selected-line intercross, the common base population was merely 44 generations back. This indicates a general role of epistasis in generating hybrid effects, and searching for the corresponding genetic basis could bring important functional insights regarding the influence of genetic interactions on selection response and population divergence.

Declaration of interest

The authors declare no conflict of interest.

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