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Lack of evidence for segregation of a single dominant major gene as the cause of the difference in egg weight between two highly inbred lines of chickens

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Abstract Data on egg weight from experimental crosses with two inbred lines of chickens suggested evidence for segregation of a single dominant major gene. Because the data could not be transformed to satisfy normality and homoscedasticity conditions, the non-parametric test of Elston and the graphical approach used by Stolk et al. were applied. Due to a bad fit of the backcross B2 ($P_2 \times F1$) and the F2 groups, both methods reject the hypothesis of a dominant major gene as the the only cause of the differences in egg weight between the six genetic groups involved.

Key words Inbred lines · Non-parametric test · Major gene · Egg weight · Chicken

Introduction

The summary data on egg weight from two highly inbred lines of chickens (P1 and P2) and their F1, F2 and backcrosses (B1 and B2) in Table 1 suggested that one of the lines might carry a dominant major gene that increases this trait substantially. The table contains both the observed phenotypic means and those expected for the F1, F2 and backcrosses under the hypothesis of (1) complete additivity and (2) a major gene expressing complete dominance, calculated from the observed pure-line means. It is obvious that the dominance hypothesis fits the observed means very closely and much better than does the additive hypothesis. The objective of the present investigation was to validate, through available statistical methods, the hypothesis of a dominant major gene, which substantially increases egg weight, carried by this highly inbred line of chickens.

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Material and methods

Two highly inbred lines of Leghorn chickens with calculated inbreeding coefficients above 0.94 were used in a crossing system for 3 years, beginning in 1986. These lines, named 75 (P1) and 77 (P2), originated from two populations selected during eight generations for large and small eggs, respectively. The two selected populations were derived from a common base population. The development of the inbred lines was done by continous full-sib mating, using artificial insemination. More details on the history of the lines and the crossing experiment are given by Hagger (1985) and Hagger and Stranzinger (1992). The experiment contained contemporaneous birds of the two pure lines, the F1, F2 and both backcrosses (B1, B2) in each of the 3 years. All backcrosses were from matings between inbred males and F1 females. The flock was reproduced by 4–5 weekly hatches every year. Chicks were reared intermingled on deep litter until 16 weeks of age and then transferred to single cages of three-floor batteries. Individual egg production of all hens was recorded for 7 days a week at the beginning of the laying period and for 6 days later on. The trait investigated was the average egg weight (EW) until 40 weeks of age. Hens with less than ten eggs in this period were discarded from the data set. The number of hens finally available in the six genetic groups are given in Table 1. Principles of laboratory animal care (NIH publication No. 85-23, rev. 1985) were followed and the Swiss law on animal protection was applied.

Several methods have been proposed to search for segregation at a major locus in a population. The power of the test statistics used for some of them was recently investigated by LeRoy and Elsen (1992). For the special case of crossings between two homozygous lines, Elston (1984) presented an extended version of the maximum-likelihood method of Elston and Stewart (1973). This method is based on a measure of conformity between the observed distribution of the trait under consideration and the expected distributions under various genetic hypotheses. The degree of conformation is given by the differences among the series of log likelihoods for the genetic models investigated. It was shown by Elston (1984) how, in principle, the hypotheses of one major gene, polygenic inheritance, or a mixture of the two, can be fitted to the data investigated. For the single-locus component, it is possible to have additive or dominant gene action of various kinds. However, the more complicated a model becomes, the more parameters have to be estimated, which leads to a rapid decrease of statistical power for a given data set. In the case of a single locus with two alleles and additive inheritance, three parameters, the means of two genotypic classes and the common variance, have to be fitted.

It was pointed out by Elston (1984) that the likelihoods proposed assume normality of the trait conditional on the genotype. Therefore, it was recommended that the first step in any analysis should be to test for this condition and, if necessary, to transform the observations to satisfy normality and homoscedasticity conditions in order to gain power in the analysis. Thus, the power transformation of Box and Cox (1964) may be applied to the three classes of single genotypes, i.e., the parental strains (P1 and P2) and the F1, to estimate the optimal transformation parameter (Elston 1984). It can be seen in Table 1 that the means of P1 and F1 are very close together, and yet there is a large difference in their standard deviations, a situation that is often observed in crossing systems with highly inbred strains of animals (Falconer 1981, pp. 244). These two circumstances make it impossible to find a transformation that leads to homogeneous variances for the three groups, which means that heteroscedasticity must be taken into account. Whereas it is easy to do this if the genetic variance is solely monogenic or solely polygenic, the number of unknown parameters becomes unwieldy if we wish to detect monogenic segregation in the presence of polygenic variance (Elston 1984).

To circumvent this problem, the graphical approach described by Stolk et al. (1984) and the nonparametric test described by Elston (1981) for one locus with two segregating alleles was adapted to the present data. The first of these methods relies on visual inspection of the cumulative frequency curves of the trait for the different genetic groups plotted against the trait values. For traits whose difference between two inbred lines is due to a single major gene with Mendelian inheritance, the expected cumulative frequency curves show a characteristic shape for each of the various genetic groups. The curves of the groups containing more than one genotype at the major locus (B1, B2, F2) consist of a mixture of the curves found for the corresponding genetic groups containing only one of the genotypes. For completely additive or codominant inheritance, or if a single dominant gene is responsible for the expression of a trait, characteristic pictures for the expected mixtures of the different genetic groups can be constructed and compared with the observed cumulative frequency curves. The fit between these expected curves and the curves observed in the data support or exclude a supposed simple mode of inheritance. For example, under the assumption of an additive major gene segregating with respect to the quantitative trait under consideration, the mean of any offspring population is located halfway between the means of the two parental populations. Cumulatively plotting the parental strain with the smaller mean to the half density interval $[0, \frac{1}{2}]$, the F1 distribution to the half density interval $[\frac{1}{2}, 1]$, and the corresponding backcross distribution cumulatively over the whole frequency range $[0, 1]$, will result in a large degree of overlapping between the backcross curve and the curves of the F1 and parental populations, respectively, if the distributions for the parental lines and F1 are clearly separated. Proceeding analogously with a cumulative plot of the F2 data (with the genotypic proportions $\frac{1}{4}, \frac{1}{2}, \frac{1}{4}$), i.e., plotting the parental distributions and the F1 data constrained to the specific density intervals corresponding to the phenotypic proportions of the underlying genotypes in the F2 population, a corresponding overlap of F2 data with P1, F1 and P2 can be obtained. By assuming dominance at a major locus for the quantitative trait under consideration, and adapting the way the data is plotted under additivity, similar plots can be constructed.

There are many possible mixture curves to which the cumulative plots of the B1, B2 or F2 data may be compared. If one can be found that is clearly different from the picture expected for a specific genetic hypothesis, then this hypothesis will be rejected. Of course, this method does not provide a formal statistical test for the hypothesis, and for this reason a nonparametric test was performed, as described below. However, even if no contradiction to major gene inheritance is present, one can never prove, on the basis of this kind of data alone, that only one or two loci are involved in causing the difference of a quantitative trait between the two inbred lines (Elston 1984).

The nonparametric test of Elston (1981) allows one to test a one-locus hypothesis for the genetic difference of a quantitative trait between two homozygous lines by goodness-of-fit chi-square statistics. The method estimates the probabilities for the phenotypes of the two homozygous lines and their F1 offspring to fall in one of several intervals, using a set of linear equations that contains the observed interval frequencies for the six genetic groups. These estimates then determine the corresponding probabilities for the two backcrosses and the F2 animals in the usual Mendelian manner. The expected frequencies of observations in the given intervals are then calculated for each genetic group. Finally, the observed and expected frequencies are used to calculate the chi-square statistics.

Results and discussion

Because the F1 and P1 distributions of EW show a large degree of overlapping, their means being close together (Table 1), the hypothesis of a single completely additive major gene can be rejected with high probability. The graphical approach of Stolk et al. (1984) was used to further investigate the possibility of non-additive single-gene, segregation whether completely dominant or not (codominant), by examining the backcross (B2) and F2 distributions. The P2, F1 and B2 distributions are cumulatively plotted in Fig. 1. Assuming a single major gene, segregating showing either codominance or complete dominance, the backcross between P2 and F1 is expected to follow approximately the curves of the P2 and F1 group, because the P2 and F1 distributions are well separated from each other. Furthermore, because the P2 and F1 distributions are approximately normal, the best-fitting normal distributions are also plotted in Fig. 1. If there is only monogenic segregation, the backcross distribution should clearly deviate from a normal distribution; it should follow a mixture of two distributions centered around the means of the homozygous recessive genotype (P2) and the heterozygous F1 genotype. In Fig. 1 the cumulative distribution for the backcross B2 deviates only slightly from a cumulative normal curve and its shape differs sharply from a 1:1 mixture of the P2 and F1 distributions. Therefore, the hypothesis of one major gene showing strong codominance or dominance can be rejected. If the slight deviation from normality of the backcross data in Fig. 1 is disregarded, the curves show what would be expected under completely polygenic inheritance, i.e., each of the P2, F1 and B2 groups being approximately normally distributed with a specific phenotypic mean. All phenotypic means in Table 1 indicate complete dominance or a strong codominant effect. Combining these results leads to the hypothesis that more than just one

Table 1 Mean (standard deviation) of observed egg weight (EW) in grams of six genetic groups, and expected means of the crosses under the segregation of a completely additive or completely dominant major gene at a single locus

Group	Number of hens	Means in grams		
		Observed	Additive	Dominant
P1	57	56.5 (5.13)		
P2	128	47.8 (3.61)		
F1	89	56.0 (2.43)	52.2	56.5
B1	56	57.5 (8.13)	54.3	56.5
B2	47	52.7 (5.65)	50.0	52.2
F2	99	54.3 (8.49)	52.2	54.3

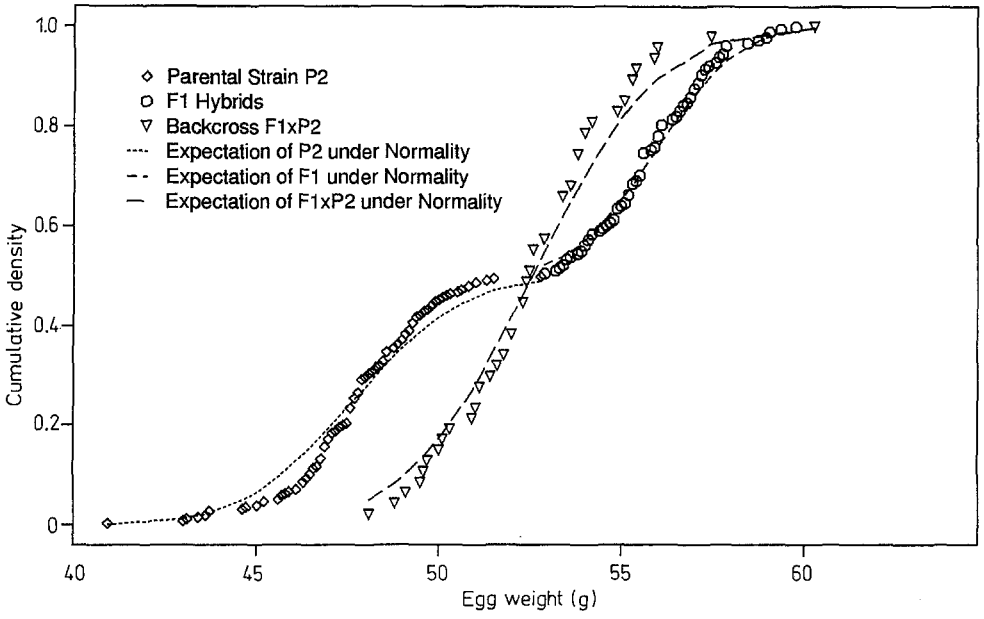

Fig. 1 Cumulative density of the observed distribution of parental line P2, F1 hybrid and corresponding backcross hens. P2 is constrained to the cumulative density interval [0, $\frac{1}{2}$], F1 to [$\frac{1}{2}$, 1] and the backcross (F1 × B2) is cumulatively plotted over the whole density range. The expected cumulative distributions are also shown, as *dotted lines*, assuming normality for the observed data in each genetic group

major gene determines the egg-weight difference between the two lines. It would seen unlikely that genes at a large number of loci would all show dominant inheritance for EW, and so only a limited number of loci may be involved. Data from the F2 population are shown in Fig. 2, compared with the P2 cumulative frequency constrained to the interval [0, $\frac{1}{4}$], and the F1 cumulative frequency constrained to the interval [$\frac{1}{4}$, 1]. Under a major-locus hypothesis with two alleles segregating, the curve of the F2 group would comprise phenotypic proportions of $\frac{1}{4}$ and $\frac{3}{4}$ for the homozygous recessive and the two other genotypes, respectively. Inspection of the F2 curve in Fig. 2 reveals that this hypothesis must also be rejected. Although the curve for the hypothetical dominant genotypes can be seen to be overlapping, the F2 distribution does not show a clear second phenotypic distribution in its lower tail. The deviation from normality could possibly be taken as support for the rejection of pure polygenic inheritance, in addition to the low prior probability of such a hypothesis. Furthermore, it should be noted that the F2 hybrids do not cover the whole data range expected from the P2 and F1 populations. This could be due to a limited number of F2 individuals compared to the large number of possible genotypes under oligogenic (or polygenic) inheritance.

From Table 1 a relative heterosis, i.e., F1 − 0.5 (P1 + P2)/0.5(P1 + P2), of 0.074 is found. If the heterosis is calculated using the expected F1 mean under the

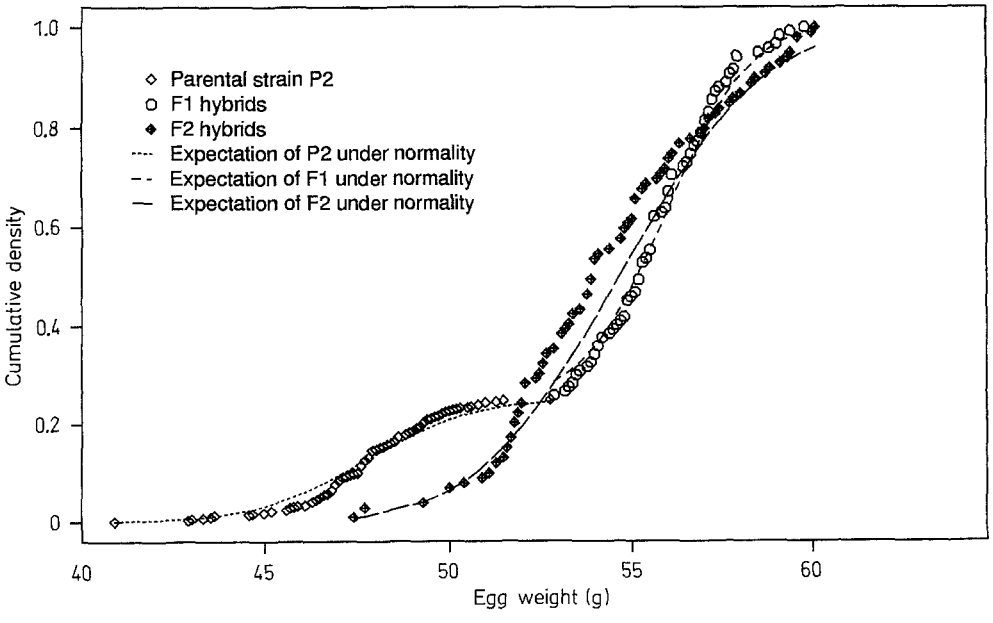

Fig. 2 Cumulative density of the observed distribution of parental line P2, F1 and F2 hybrid hens. P2 is constrained to the cumulative density interval [0, $\frac{1}{4}$], F1 to [$\frac{1}{4}$, 1] and the F2 is cumulatively plotted over the whole density range. The expected cumulative distributions are also shown, as *dotted lines*, assuming normality for the observed data in each genetic group

Table 2 Test statistic for the hypothesis of a single gene segregating for the trait egg weight (EW) using two and three intervals

Number of intervals	2	3
Chi-square values	16.86	34.69
Degrees of freedom	3	6
Tab. chi-square$_{0.001}$	16.27	22.46

hypothesis of a dominant major gene at a single locus (last column in Table 1), a value of 0.083 is found. For pure additive inheritance, a relative heterosis of 0 would be expected. Thus, the observations again point to a type of dominant inheritance.

Table 2 contains the results of the nonparametric test of the hypothesis of a dominant gene at a single locus responsible for the difference in EW between the two inbred lines. This hypothesis cannot be accepted, because the probability of chi-square values of this size is extremely small under the hypothesis, $P \leq 0.0001$. Therefore, the conclusions drawn above, that genes at more than one locus are responsible for the difference in EW between the two lines, is strongly supported. In the case of a significant chi-square value, Elston (1981) suggested inspecting the contributions of each of the genetic groups to the test statistic to see if the significance is due to an overall bad fit or to a particular part of the data. For the present data it was found that with two intervals, the F2 and B2 groups deviated significantly ($P \leq 0.025$) from the expectation under the hypothesis. This has already been observed in the cumulative frequency curves, because in both F2 and B2, the smallest eggs were considerably larger than those in P2, although the two groups should contain one quarter or one half, respectively, of individuals being homozygous for low egg weight under the major-gene hypothesis being considered. The shift in egg weight in F2 and B2 could be explained by assuming that genes with smaller effects at other loci, influencing egg weight in a positive manner, are concentrated in line P1. This could be regarded as a consequence of the selection for large eggs that had been performed for eight generations in the population of origin prior to the development of the inbred line (Hagger 1985). In the F2 and B2, 0.5 and 0.25 of the genome, respectively, are from P1. Considering the joint genotype of the locus carrying the major gene and those genes at other possible loci with smaller effects, hens in the F2 and B2 group with an egg weight in the low range of P2 would occur with a low frequency if the genes tending to increase egg weight were additive or (co-) dominant. Stewart and Elston (1973) have highlighted the possibility that all genes tending to increase a character may be grouped in one parent. The fairly good agreement between the curves for the groups with only one phenotype under the hypothesis, together with the appearance of large eggs in the F2 and B1 groups, nevertheless suggests the existence of a dominant gene with large effect on EW in P1.

Pairwise *t*-tests between the EW means of the three groups P1, F1 and B1, which under a dominant major-gene hypothesis should be identical, were all significant ($P \leq 0.01$). Thus this hypothesis does not seem to be very likely, according to this very simple test. Elston (1981) calculated the nonparametric test statistic for up to ten intervals, to show that a large chi-square value may result from small expected frequencies in some intervals, which occurs as the number of intervals increases. For the present data, it was not possible to calculate a similar sequence of tests, because negative estimates for some probabilities were obtained if more than three intervals were used. This behaviour of the system is due to the data set available: the approximately dominant inheritance leads to a relative large number of empty, or nearly empty, intervals within some of the genetic groups, and this is responsible for the numerical behaviour of the system of equations that is solved to estimate the interval probabilities.

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