Genetics of Broodiness in Poultry - A Review

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ABSTRACT : In poultry, the selection against broodiness took up presumably naturally occurred mutations in the White Leghorn breed and led to an almost complete loss of the avian form of parental behaviour (incubation of eggs). Early studies on the genetics of broodiness demonstrated that the trait is polygenic with a major sex-linked effect. The reassessment of the studies on putative genes located on the Z chromosome, which are implicated in the control of broodiness, has resulted in the denial of this hypothesis. The recent experiments bear witness that incubation behaviour in chickens is not controlled by a major gene (or genes) on Z chromosome and there must, therefore, be major autosomal genes contributing to the expression of the behaviour. If a broody gene does exist on the Z chromosome it is one of at least three genes including two dominant autosomal genes, one causing and other one inhibiting incubation behaviour, with probably equal influence. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 : 1647-1654)*

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INTRODUCTION

Broodiness is a behavioural trait observed in most common breeds of domestic fowl with the exception of the White Leghorn (WL). The red junglefowl (RJF, *Gallus gallus*), which is believed to be a single ancestor of the domestic fowl (Crawford, 1990; Akishinonomiya et al., 1994, 1996) and some "unimproved" chicken breeds show incubation behaviour, whereas commercial breeds, subject to intensive artificial selection, do not go broody.

As a consequence of its fundamental role in avian reproduction, incubation behaviour has been of great interest to poultry scientists, breeders and producers of hatching eggs (El Halawani and Rozenboim, 1993). While some chicken breeds still have strongly a maternal instinct, broodiness has been reduced by selection in some other breeds and strains, like White Leghorns, almost to the vanishing point (Hutt, 1949). Decreased broodiness is also due to a correlated response to selection for increased egg production in turkeys (Emmerson et al., 1991; Nestor et al., 1996). Incubation behaviour in turkey hens continues to be a major hindrance to enhanced reproductive performance in the domestic turkey, leading to ovarian regression, and the termination of ovulation and egg laying (El Halawani et al., 1988; Sharp, 1997) and resulting in substantial loss of potential egg production (El Halawani and Rozenboim, 1993).

The scope of the present review is to survey studies on genes implicated in the control of broodiness.

EARLY STUDIES ON BROODINESS GENETICS

In poultry, the selection against broodiness, that took up presumably naturally occurred mutations in the WL breed, resulted in an almost complete loss of the avian form of parental behaviour, incubation of eggs. There is rather high variability in broodiness manifestation (Hays and Sanborn, 1926; Hays, 1940) that may complicate selection against it. On the other hand, some environmental conditions (high temperature, darkness, removal of the eggs as they are laid, presence of the chicks) are more conducive to broodiness than others (Reaumur, 1750; Punnett, 1923; Hutt, 1949) and may evoke complete broodiness even in White Leghorns (Burrows and Byerly, 1938). In the free range system, 13% of commercial hybrid WL laying hens were reported to become broody in the first year (Fölsch, 1981).

The fact that breeds differ in the degree of broodiness exhibited by them shows that the trait is hereditary (Hays, 1933; Hutt, 1949). The genetics of broodiness has been investigated and has produced conflicting observations. Punnett and Bailey (1920) showed broodiness to be expressed by more than one independent autosomal gene. The hypothesis of Goodale et al. (1920) that complementary genes are involved and that non-broody hens lack one of these or carry an inhibitor of both is supported by the fact that the proportion of broody hens is usually high in the offspring from crossing of two different breeds (Hutt, 1949). The feasibility of reducing broodiness in a strain by selection was demonstrated by Goodale et al. (1920), Hays (1933) and Hays and Sanborn (1939); however, it is very difficult to eliminate the incubation behaviour completely (Hays, 1933). Punnett (1923) suggested that factors for high fecundity may themselves inhibit broody instinct in some cases. Later, Hays (1933) reported a greater egg production in the non-broody line as compared to that in the broody line, and Lippincott and Card (1934) claimed that the broodiness of a breed is in inverse proportion to its fecundity.

Evidence that Z-linked genes may be involved was first

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suggested by Warren (1930) and found later by Roberts and Card (1933), Warren (1942), Kaufman (1946-1947), Mueller (1952), Saeki (1957), and Saeki and Inoue (1979) in various crosses between breeds. In contrast, Hays (1940) could find no evidence of sex-linked genes for broodiness in Rhode Island Reds and proposed a hypothesis of two complementary autosomal dominant genes. In these experiments, expression of the broodiness was shown to be different within families, males and females transmitting the trait equally to their daughters in accordance with the trait incidence in their family. Saeki (1957) still pointed out the controversial evidence of sex-linked inheritance for broodiness, assuming at least one sex-linked gene and the autosomal gene(s), i.e. polygenic inheritance, but later (Saeki and Inoue, 1979) emphasised the sex linkage for this behavioural trait.

Thus, early studies on the genetics of broodiness demonstrated that the trait is likely to be polygenic with a major sex-linked effect, although the latter has been argued.

PHYSIOLOGICAL AND MOLECULAR GENETIC BASES OF BROODINESS

The broody instinct, actually, consists of two phases, incubation of eggs (nesting) and raising the chicks (Saeki and Tanabe, 1955). The neurobiology of incubation behaviour has been extensively studied in birds (Sharp, 1989; Lea et al., 1997), the principal hormones involved being estrogen, progesterone and plasma prolactin (PRL), and brain centres that control incubation behaviour being rich in progesterone and PRL receptors (Askew et al., 1997; Lucas et al., 1998).

As has been long considered, broodiness apparently results from the PRL secretion by the anterior lobe of the pituitary (Riddle et al., 1935; Burrows and Byerly, 1936; Bates et al., 1937; Payne, 1943; Nalbandov, 1945; Hutt, 1949; Saeki and Tanabe, 1954). The onset of incubation in chickens and other birds was thought to be caused by an increase in pituitary PRL (Burrows and Byerly, 1936). However, Burrows and Byerly (1938) stated that the pituitaries of broody hens, as compared with those of laying hens, showed no indication of an increase in PRL-like substance and suggested that PRL is not essential to the broody instinct.

The further investigations demonstrated that plasma PRL secretion appears to act centrally to induce and maintain incubation behaviour (El Halawani et al., 1980, 1986; Sharp et al., 1988; Youngren et al., 1991; March et al., 1994) following PRL transport into the brain through choroid plexus (Buntin et al., 1993).

Wong et al. (1991) isolated cDNA-encoding turkey PRL from a turkey pituitary library and established the increased levels of PRL mRNA and the corresponding increases in plasma PRL levels in photostimulated, laying, and incubating hens relative to that found in nonphotostimulated hens. The transition from incubation to the photorefractory phase resulted in a reduction in PRL mRNA, a decrease in pituitary PRL, and a dramatic decrease in plasma PRL. The changes in the abundance of pituitary PRL mRNA appear to be related to the changes in PRL-releasing activity observed at each of the reproductive stages.

It is unlikely that differences in the expression of this PRL-dependent behaviour in broody and non-broody breeds are due to a breed difference in the structure of PRL (Ohkubo et al., 1998), since the PRL cDNAs from broody Bantams and non-broody White Leghorns have been cloned and the predicted amino acid sequences differ in only three positions (Hanks et al., 1989; Watahiki et al., 1989).

Tanaka et al. (1988) cloned cDNA for the chicken prolactin receptor (PRLR) that is supposed to be involved in the induction of incubation behaviour in the brain (Buntin, 1996). The PRLR cDNA has also been cloned and sequenced in pigeon (Chen and Horseman, 1994) and turkey (Zhou et al., 1994). Intriguingly, the PRLR gene was mapped to the Z chromosome (Dunn et al., 1998) that put it in a number of candidate sex-linked broody genes.

PRL secretion is controlled by the singular avian PRL-releasing factor vasoactive intestinal polypeptide (VIP) (El Halawani et al., 1997). Lately, Ohkubo et al. (1998) demonstrated that the absence of broodiness in White Leghorns is not due to a lack of a PRL response to VIP. These authors discovered PRLR mRNA in the basal and anterior hypothalamus but stated that differences in the expression of broodiness in WL and Bantam hens cannot be explained by differences in the amounts of PRLR mRNA or in the transcription or gross structure of the PRLR gene. They found no evidence for null mutation, as suggested by Lucas et al. (1998), or any other mutation in the WL's PRLR. The identification of the mutation(s) causing the loss of incubation behaviour in White Leghorns remains unknown. The published candidate and related genes for broodiness are listed in table 1.

REANALYSIS OF MAJOR SEX-LINKED EFFECT HYPOTHESIS

To understand the genetic nature of the subject, let us, as a first step, check the early hypothesis that the trait for broodiness is polygenic with a major sex-linked contribution, i.e. the chicken Z chromosome might contain a major gene (or genes) controlling the expression of incubation behaviour.

As mentioned above, the hypothesis that broodiness in birds is a multi-gene trait with a major component located on the Z chromosome was extensively studied in Japan by Saeki (1957) and Saeki and Inoue (1979). For a source of a

Table 1. Candidate, related or possibly involved genes for incubation behaviour in the chicken

Gene	Symbol	GenBank* Accession No.	Chromosome
estrogen receptor	ESR	X03805[1], U60211[2]	GGA3[3]
estrogen receptor beta	not assigned	AB036415[4]	not mapped
progesterone receptor	PGR	Y00092[5], M18813[6], M37518[7], AH002469[8]	GGA1[9, 10]
progesterone receptor binding protein	not assigned	U95088[11]	not mapped
prolactin	PRL	J04614[12], E02259[13], AF288765[14], AJ239131[15]	GGA2[15, 16, 17]
prolactin receptor	PRLR	D13154[18], AJ011128[19], AB030749[20]	GGAZ[16, 19]
vasoactive intestinal peptide	VIP	U09350[21]	GGA3[17, 22]
vasoactive intestinal peptide receptor	VIPR	AB029895[23]	not mapped
luteinizing hormone/choriogonadotropin receptor gene	LHCGR	AB009283[24], AJ289775[25]	GGA3[25]
luteinizing hormone-releasing hormone 1	LHRH1	AB061867[26]	not mapped
dopamine D1D receptor	D1LR	L36877-L36879[27]	not mapped
dopamine D4B receptor	not assigned	AI438108[28]	not mapped
growth hormone	GH1	D10484[29]	GGA1[30]
growth hormone receptor	GHR	M74057[31], AF372659[32]	GGAZ[31, 33, 34, 35, 36]
cytochrome P450 aromatase	not assigned	J04047[37]	not mapped
gonadotrophin releasing hormone I	GNRH	X69491[38]	GGA1[22]

* GenBank®, National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/Genbank/index.html).

References: [1]Krust et al. (1986); [2]Nestor et al. (1994); [3]Van Hest et al. (1994); [4]Suzuki et al. (2000); [5]Gronemeyer et al. (1987); [6]Huckaby et al. (1987); [7]Conneely et al. (1987); [8]Jeltsch et al. (unpublished); [9]Dominguez-Steglich et al. (1992); [10]Toye et al. (1997); [11]Sandhu and Spelsberg (unpublished); [12]Watahiki et al. (1989); [13]Nakajima and Watabiki (1990); [14]Au and Leung (unpublished); [15]Miao et al. (1999); [16]Suzuki et al. (1999b); [17]Smith et al. (2000); [18]Tanaka et al. (1992b); [19]Dunn et al. (1998); [20]Tanaka et al. (2000); [21]McFarlin et al. (1995); [22]Burt et al. (1999); [23]Kansaku (unpublished); [24]Mizutani et al. (1998); [25]Ge et al. (2001); [26]Kansaku et al. (2001); [27]Demchyshyn et al. (1995); [28]Zylka and Reppert (unpublished); [29]Tanaka et al. (1992a); [30]Shaw et al. (1991); [31]Burnside et al. (1991); [32]Leung and Lau (unpublished); [33]Buhr et al. (1991); [34]Burnside et al. (1992); [35]Levin et al. (1993); [36]Suzuki et al. (1999a); [37]McPhaul et al. (1988); [38]Dunn et al. (1993).

prospective broody gene (or genes), Saeki (1957) used the Nagoya (NG) breed chickens that were homogenous for plumage and had 23% inbreeding and 100% broodiness. He reciprocally crossed them to an inbred (31%) WL strain with no broody signs and backcrossed F_1 NG × WL males to Nagoya hens. In another trial, Saeki and Inoue (1979) produced reciprocal crosses between the RJF and WL chickens, the former being descendants of a RJF stock from San Diego Zoo, CA, USA, known genetically to be not pure wild (R. Okimoto, personal communication, 1998), and the latter deriving from the commercial H&N strain, USA.

To reinvestigate the hypothesis of sex-linked inheritance of broodiness, the author has applied the Chi squared (χ^2) test (Mead and Curnow, 1983) to the Japanese data to assess whether or not a difference between a predicted and observed incidence of broodiness was significant.

As seen in table 2, the observed behavioural phenotype segregation in Japanese crosses did significantly differ from the expected one. Therefore, preceding from this analysis, one could hardly single out a major sex-linked factor in the inheritance mode of broodiness.

Recently, a Roslin Institute group (Romanov et al., 1999) reported the failed identification of broody trait loci on the Z chromosome by generating an F_1 cross of a WL male (from an inbred non-broody strain) with two Bantam (B, from a heterogeneous broody strain) hens, which were proven to be true broody before mating. A single male of this F_1 generation was then backcrossed to two WL females. An F_1 reciprocal cross of a B male with two WL hens was also included in the study. The incidence of broodiness was observed in two successive cycles of photoinduced egg laying. If the major dominant sex-linked gene hypothesis is correct, there should be no broodiness in the progeny of a WL male and B hens. Contrary to this prediction, it was observed that 45 of 73 (61.6%) F_1 females from this cross showed the broody behaviour (table 3), which was not significantly different from that in the B stock hens (78.6%).

In the backcross progeny (F_1 male × WL hens), the incidence of broodiness was predicted to be 50%. Contrary to this prediction, the incidence of broodiness was very low with only 5 out of 104 showing the trait (4.8%; table 3). In the reciprocal mating of a B male with two WL females, the incidence of broodiness was predicted to be 100% or at least, the same as that found in the B stock population. Six of the 11 hens became broody (54.5%), which was not significantly different from what was predicted (table 3).

Table 2. Reassessment of data from Saeki (1957) and Saeki and Inoue (1979) based on χ^2 test for sex-linkage of broodiness presuming a single dominant Z-linked gene

Matings [years]	No. of females	Percentage of broodiness observed [expected]	χ^2	P
WL × NG [1951]	152	45.4 [0]	31.3	< 0.001
WL × NG [1952]	46	28.3 [0]	23.7	< 0.001
WL × NG [1954]	32	37.5 [0]	12.5	< 0.001
WL × NG [1951-1954]*	230	40.9 [0]	38.4	< 0.001
WL × RJF [1973-1977]*	18	11.1 [0]	14.2	< 0.001
NG × WL [1952]	161	72.7 [100]	12.0	< 0.001
NG × WL [1954]	27	85.2 [100]	0.6	> 0.05
NG × WL [1952-1954]*	188	74.5 [100]	12.3	< 0.001
RJF × WL [1973-1977]*	34	63.0 [100]	5.0	< 0.01
F_1 (WL × NG) × WL [1952]	55	75.5** [50]	7.6**	< 0.01

*Combined data. **Estimated values.

Table 3. χ^2 test for a major gene controlling broodiness on the Z chromosome in female progeny from crosses between White Leghorns (WL), Bantams (B) and a F_1 backcross (Romanov et al., 1999)

Crosses	Observed phenotypes	Expected phenotypes	χ^2	P
WL × B	45 broody : 28 non-broody	0 broody : 73 non-broody	27.7	< 0.001
F_1 (WL × B) × WL	5 broody : 99 non-broody	52 broody : 52 non-broody	85.0	< 0.001
B × WL	6 broody : 5 non-broody	11 broody : 0 non-broody	2.3	> 0.05

Because of the limited data from this mating, the incidence of incubation behaviour in the progeny was also not significantly different from that in the B stock population (78.6%). However, the occurrence of non-broody progeny in this cross is not consistent with the hypothesis of a single dominant sex-linked gene, controlling broodiness.

It should be noted that the Saeki (1957) experiments done in 1951-1954 were based on the broodiness records of the first laying year only. This would bias the obtained results because of the "deferred broodiness" phenomenon when the trait is not expressed in the first laying cycle but in the second or third one, especially in F_1 progeny from mating WL cocks to broody hens (Goodale et al., 1920; Punnett and Bailey, 1920; Hays, 1933, 1940). It is unknown how incubation behaviour was recorded in another trial of 1973-1977 (Saeki and Inoue, 1979). Moreover, the trait incidence in the "broody" stocks used was not 100%, ranging between 76.9 and 100% in the NG chickens and constituting 87.5% in the RJF hens. On the other hand, there was a considerable variation in percentage of broodiness observed in the test crosses of WL males with "broody" stock females (11.1-45.4%) and reciprocal crosses (63.0-85.2%), the corresponding results produced by Romanov et al. (1999) being 65.3 and 62.5%, respectively. There was a striking difference between Japanese and Romanov et al. (1999) data in the case of backcross (75.5 vs. 5.8%). Taking into account these inconsistent results and controversial evidences about broodiness genetics in early papers, one might conclude that expression of this behavioural trait in pure breeds and crosses is strongly dependant on breed genetic background and probably certain environmental conditions in the experiments.

In the Romanov et al. (1999) study, the F_1 females did not obviously have the hypothetical broody gene on the Z chromosome, yet a significant proportion of them went broody. Taken together, the observations in that study suggested, as a first approximation, that broodiness is controlled by a dominant autosomal gene at one locus in the Bantam and a "non-broody" autosomal gene at another locus in the WL. Assuming *A* be an incompletely dominant gene for broodiness and *B* an incompletely dominant inhibitor of broodiness, the parents and F_1 progeny in the test and reciprocal crosses would have the following genotypes:

P ♂WL × ♀B ♂B × ♀WL
aaBB *AAbb* *AAbb* *aaBB*
↓ ↓
F_1 ♂*AaBb*; ♀*AaBb* F_1 ♂*AaBb*; ♀*AaBb*

In practice, incubation behaviour among crossbred females is rather sensitive to environmental factors and this might cause a decline of broodiness incidence. Assuming an incomplete dominance of both genes and variable environmental effects on the broody trait, the incidence of incubation behaviour in the test and reciprocal crosses is predicted to be about 50%, which is close to the observed values (61.6 and 54.8%, respectively).

In the backcross progeny, the genotype segregation would be as follows:

P ♂F_1 (WL × B) × ♀WL
AaBb *aaBB*
↓
F_2 ♂1/4AaBB, 1/4AaBb, 1/4aaBB, 1/4aaBb;
♀1/4AaBB, 1/4AaBb, 1/4aaBB, 1/4aaBb

In this backcross progeny, incubation behaviour would be expected in female diheterozygotes (*AaBb*) resulting in an incidence of broodiness about 25%. Assuming an incomplete dominance of both genes and variable penetrance of the broody trait, the incidence of incubation behaviour in the test and reciprocal crosses should be much less than 25%. The observed percentage of broodiness was 5.8%.

If more incompletely dominant genes and inhibitors, possibly including sex-linked ones, and/or some other additive genes with smaller effects (both positive and negative) are involved in this complex interaction and there is a varied environmental influence on broodiness expression, the theoretical percentages might fit empirical figures. In this context, we can suggest the existence of behavioural trait loci. For example, in pure RJF males the interrupted manifestation of mating behaviour is associated with so called eclipse plumage when the males in eclipse moult become inactive and sterile; the domestic cocks never show this trait. Some evidences that eclipse plumage is a hereditary character (Kimball, 1958; Morejohn, 1968) might be explained on the base of a similar hypothesis with two dominant genes (R. Okimoto, personal communication, 1998). According to this speculation, the RJF would carry a dominant gene for eclipse plumage at one locus while all domestic breeds would have its inhibitor at another locus. The future investigations of breeding behaviour traits and loci that might be involved in their neurohumoral control are needed to clarify the subject.

The genetic analysis of broodiness is complicated because it is a sex-limited character in the domestic fowl and its expression in the male sex is nil. Besides, there is a certain environmental component in the expression of this trait and two cycles of photoinduced egg production are required to ensure that most birds capable of expressing incubation behaviour do so. The hypothesis proposed by Romanov et al. (1999) explains possible scenarios in genetic control of incubation behaviour in pure broody breeds and their crossbreds with non-broody breeds. In contrast, in White Leghorns, the intensive selection for inhibitors and against broody genes apparently resulted in almost complete absence of incubation behaviour.

These new observations are consistent with the view that incubation behaviour in chickens is not controlled by a major gene (or genes) on the Z chromosome. There must therefore, be major autosomal genes contributing to the expression of the behaviour. If a broody gene exists on the Z chromosome it is one of at least three genes including two dominant autosomal genes, one causing and other one inhibiting incubation behaviour, with probably equal influence.

Using modern sophisticated molecular genetic techniques and resource populations, identification of quantitative trait loci for the expression of incubation behaviour will facilitate in the future the detection of functional genes or anonymous markers and marker-assisted selection against broodiness.

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