

## Sex determination in birds: progeny of nondisjunction canaries of Durham (1926)

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### SUMMARY

The heterogametic sex in birds ( $ZW$ ) is female for presence of the  $W$  or for lack of a second  $Z$  chromosome. These alternatives can be distinguished given  $ZO$  or  $ZZW$  aneuploids and segregation of a  $Z$ -linked marker in their progeny. Having discovered the  $Z$ -linked *cinnamon* locus with two alleles ( $B$ , black eyes;  $b$ , red eyes), Durham observed 21 black-eyed daughters ( $BO$  or  $BbW$ ) of  $bb$  cocks and  $B$  hens. Using Durham's data on sex-colour phenotypes of 14 young of two of the 21 matroclinous females show that the sex chromosome constitution of the two exceptional hens were more likely  $ZO$  than  $ZZW$ . This case plus that of Crew's (1983) trisomic rooster ( $ZZW$ ) shows that sex determination in birds follows the 'genic balance' scheme as in *Drosophila*, not the 'dominant Y' scheme as in mammals.

### 1. INTRODUCTION

The heterogametic sex in birds ( $ZW$ ) is female and the homogametic sex ( $ZZ$ ) is male. This has been known since 1908, when Florence Durham described segregation at the  $Z$ -linked *cinnamon* locus in canaries (Durham & Marryat, 1908; Hutt, 1949: 182). The idea of female heterogamety in birds had originated in January 1908 with William Bateson, Durham's mentor, on the basis of his work with silky fowl (B. Bateson, 1928: 113; Punnett & Bateson, 1908). Existence of the  $W$  sex chromosome was demonstrated only in 1961 (Frederic, 1961; cf. Bloom, 1974), first in chickens and later in canaries (Ohno *et al.* 1964) among many other species of all orders studied barring Ratitae (Bloom, 1974), whose sex chromosomes are homomorphic. Yet the exact mode of sex determination in birds is still unresolved (McCarrey & Abbott, 1979; Austin, Edwards & Mittwoch, 1981). There are two competing switch mechanisms (White, 1973: 586). The  $ZW$  zygote may develop as a female either because a  $W$  is present (analogous to the 'dominant- $Y$ ' scheme in mammals, hypothesis  $H_D$ ) or because only one  $Z$  is present (analogous to the 'genic balance' scheme in *Drosophila*, hypothesis  $H_B$ ).

Many workers seem to favour  $H_B$  (e.g. Ohno *et al.* 1963; Beatty, 1964; White, 1973; Shoffner, 1974; Thorgaard & Gall, 1979) for reasons collated by McCarrey & Abbott (1979). (1) Polyploid intersex chickens; e.g.  $3A-ZZW$  triploids are of female appearance but develop male gonads (Abdel-Hameed & Shoffner, 1972).

(2) Apparent absence of dosage compensation (Cock, 1964; Bloom 1974; Baverstock *et al.* 1982). (3) Crew's (1933) often quoted ZZW rooster. In addition,  $H_B$  is supported by 'half-sider' gynandromorphs (Hutt, 1949: 471; Taber, 1964) where left and right sides of the bird differ not only in Z-linked plumage traits, implying sex chromosome mosaicism (ZZZ/ZO from mitotic nondisjunction or ZZ/ZO from chromatid loss), but also in sex of gonad implying development of functional structures of both sexes in the absence of a W chromosome. Diverse arguments have been held to support  $H_D$ . (1) Since gonadless birds assume a generally male appearance they may be avian counterparts (ZO) of the XO syndrome in man (Taber, 1964). (2) The Y (mammals) or W (birds) chromosome controls sex differentiation by causing early and rapid growth of primitive testicular tissue in the neutral gonad (Mittwoch, 1983). (3) Silvers & Wachtel (1977) attribute a primary sex-determining role to the H-Y antigen that was found to be associated invariably with the heterogametic sex in amphibians, birds and mammals.

Neither Durham (1926) nor her contemporaries (e.g. Duncker, 1928; Crew, 1933) questioned the *Drosophila* scheme of sex determination in birds, as that was universally accepted for birds and mammals until the male-determining role of the mammalian Y was discovered (Stern, 1973: 503). This report re-examines the data of Crew (1933) and analyses for the first time part of the data of Durham (1926). Both sources support the 'balance scheme' of sex determination ( $H_B$ ) in birds.

## 2. MATERIALS AND METHODS

Crew's and Durham's data both concern Z-borne pigment dilution loci: silver (*S*) vs gold (*s*) plumage in chickens and the *cinnamon* locus with black (*B*) vs pink (*b*) eye colour in canaries, which is similar to autosomal pink-eye (*pk*) in fowl (Hutt, 1949: 182).

Crew's (1933) data pertain to one of several exceptional, patroclinous gold roosters produced by a Rhode Island Red male (*ss*, gold) with Light Sussex females (*S*, silver). As may be seen in Table 1, his karyotype was either ZO (by  $H_D$ ) or ZZW (by  $H_B$ ) as stated by Crew and accepted by Abbott & Yee (1975) but not by Hutt (1949: 474). The alternative karyotypes can be distinguished by the segregation at the marker locus in the 67 sons and 50 daughters of this male mated with normal silver hens (*SW*). The proportion of secondary nondisjunction gametes *x* and its standard error were estimated from maximum likelihood formulae based on the expectation  $(1-x)/2$  for each of the disjunction gametes *s* and *sW* and expectation  $x/2$  for each of the nondisjunction games *ss* and *W*.

Unlike Crew's data, those of Durham have been ignored for over 50 years (Sittmann, 1983). They involve 1300 canaries from controlled breeding experiments conducted before 1910 at Cambridge and then till 1915 at the John Innes Horticultural Institution after Bateson had become its first director.

Exceptional progeny appeared in Durham's *bb* ♂B♀ matings: 199 black and 2 pink sons, 21 black and 228 pink daughters. The first four of the 21 matroclinous black daughters (Durham & Marryat, 1908) prompted explanations in terms of partial sex-linkage (Sturtevant, 1912; Bridges, 1916) or primary nondisjunction (Bridges, 1916). Lacking cytological evidence Durham reserved judgement while

Duncker (1928) favoured partial sex-linkage for one part of Durham's data and nondisjunction for another. Exceptional sons appeared also in  $Bb \text{♂} \times B \text{♀}$  matings: 156 black and 3 pink sons, 124 black and 119 pink daughters. Other than primary nondisjunction in meiosis II of spermatogenesis ( $H_B$ ) or in meiosis I of oogenesis ( $H_D$ ), no single plausible mechanism (e.g. misclassification, sex reversal, triploidy, partial sex-linkage, secondary nondisjunction, stock-keeping errors) can simultaneously account for exceptional progeny of both mating types (Sittmann, 1983). (Nondisjunction in male meiosis I or in female meiosis II fails for the pink sons in  $Bb \text{♂} \times B \text{♀}$  matings.) In special cases, trisomy in birds can be attributed directly to misdivision in male meiosis (Bammi, Shoffner & Haiden, 1966).

Table 1. Sex determination in birds according to hypotheses  $H_B$  ('genic balance') and  $H_D$  ('dominant Y')

	$ZZ$	$ZW$	$ZZZ$	$ZZW^*$	$ZWW$	$ZO^*$	$WO, WW$
$H_B$	♂	♀	♂	♂	♀	♀	Lethal
$H_D$	♂	♀	♂	♀	♀	♂	Lethal

\* These karyotypes discriminate between  $H_B$  and  $H_D$ .

For the prediction of progeny phenotypes it is assumed that  $WO$  and  $WW$  zygotes are lethal as apparently no avian embryo without a  $Z$  chromosome has ever been observed at any ploidy level among thousands examined as early as at four days of age or younger. Even in the two 4-day karyotypic mosaics of Bloom (1972) the  $WO$  cells amounted to  $\leq 10\%$ .

### 3. RESULTS

If  $S$  and  $s$  represent  $Z$  chromosomes carrying the dominant silver and the recessive gold alleles, respectively, then by  $H_D$  the parental genotypes in Crew's data are  $sO \text{♂} \times SW \text{♀}$  and expected progeny genotypes are  $1Ss: 1SO: 1sW: 1WO$  or 2 silver sons: 1 gold daughter. But a gold son and silver daughters also appeared which could not arise by  $H_D$ . Thus Crew's observations unequivocally disprove  $H_D$ , unless unexpected phenotypes were due to further primary nondisjunction in the hens or recording errors.

By  $H_B$ , on the other hand, the exceptional male had to be  $ssW$  (Table 1). Expectations for his progeny depend on the rate of disjunction of his two  $Z$  chromosomes. Random disjunction gives  $2Z: 2ZW: 1ZZ: 1W$ , i.e. the rate of secondary nondisjunction ( $ZZ + W$  gametes) is 33%. Complete  $Z-Z$  disjunction gives  $1ZW: 1Z$ , i.e. no  $ZZ$  or  $W$  gametes. Progeny frequencies expected by  $H_B$  with random disjunction are shown in Table 2 and expected numbers in the total progeny of 117 in Table 3. The observed numbers disagree significantly with the expectation for random disjunction ( $\chi^2_3 = 19.4, P < 0.001$ ). The best fit is obtained when the proportion of ( $ZZ + W$ ) gametes is taken to be the maximum likelihood estimate of  $5.45 \pm 3.05\%$  ( $\chi^2_2 = 2.69, P = 0.27$ ). Thus Crew's results agree well with  $H_B$  given preferential disjunction of the  $Z$  chromosomes in the meiosis of  $ZZW$  males. The goodness of fit is not improved by inclusion of a parameter for the

'meiotic drive' observed in *XXX* and *XYY* human and *Drosophila* trisomics who almost invariably transmit a single *X* or *Y* chromosome (Stern, 1973: 515). If *K* equals the proportion of disomic and dysfunctional products of meiosis in Crew's rooster, then the expectation of gold daughters is constant for  $0 \leq K \leq 1$ , while the expectation of silver sons decreases with increasing *K* as a function of *K* and of *x*, the proportion of secondary nondisjunction gametes.

Table 2. *Expectations for the progeny of Crew's patroclinous gold rooster (ssW) in matings with normal silver hens (SW) assuming random disjunction in the male*

		Male gametes and frequencies				
		<i>s</i>	<i>sW</i>	<i>ss</i>	<i>W</i>	
		1/3	1/3	1/6	1/6	
Female gametes	<i>S</i>	1/2	2Ss ♂♂	2SsW ♂♂	1Sss ♂	1SW ♀
	<i>W</i>	1/2	2sW ♀♀	2sWW ♀♀	1ssW ♂	1 lethal
Surviving progeny						
	Silver ♂		Gold ♂	Silver ♀	Gold ♀	Sum
Exp.	5	1	1	4	11	

Table 3. *Tests of observed vs expected numbers in progeny of Crew's gold rooster mated to silver hens assuming, by H<sub>B</sub>, increasing rates of disjunction of Z chromosomes in the male*

	Genotype of rooster	Assumed % of (ZZ + W) gametes	Sons		Daughters		$\chi^2$	d.f.	<i>P</i>
			Silver	Gold	Silver	Gold			
Observed	—	—	66	1	2	48	—	—	—
Expected by <i>H<sub>D</sub></i>	<i>sO</i>	—	78	0	0	39	—	—	—
Expected by <i>H<sub>B</sub></i>	<i>ssW</i>	33.3%	53.2	10.6	10.6	42.6	19.4	3	< 0.001
		5.5	57.7	1.6	1.6	56.1	2.72	2	0.27
		0	58.5	0	0	58.5			

Among Durham's exceptional canaries from *bb* ♂ × *BW* ♀ matings, both pink sons died within days of hatching. But two of the 21 black daughters (*BO* by *H<sub>B</sub>* or *BbW* by *H<sub>D</sub>*; Table 4) reproduced in a total of five matings with three *bb* cocks and two *Bb* cocks, both from *bb* ♂ and *B* ♀ parents, and thus known heterozygotes. Expected progeny numbers were calculated separately for each mating type in the manner shown in Table 2 and then added for comparison with the observed sum (Table 5). The results fit *H<sub>B</sub>* very much better than *H<sub>D</sub>*, which is rejected for secondary nondisjunction rates from zero to as high as half the random rate ( $P \leq 0.04$ ; Table 5). Durham did not comment on the other 19 black females nor on the three pink males (*bbW* by *H<sub>B</sub>* or *bo* by *H<sub>D</sub>*) from *Bb* ♂ × *B* ♀ matings.

## 4. DISCUSSION

In the absence of a clear consensus about the role of  $Z$  and  $W$  chromosomes in sex determination of birds, it seems pertinent to add the observations of Durham (1926) to the widely quoted case of Crew (1933) which, in the judgement of some workers and as re-examined here, disproves  $H_D$ . In canaries,  $H_B$  is acceptable unequivocally ( $P = 0.87$ ) while the strength of rejection of  $H_D$  depends largely on assumed disjunction rates in female meiosis. The rate calculated for male meiosis in Crew's rooster (5.5%; Table 3) suggests that low rates approaching complete, rather than random, disjunction are more likely.

Table 4. *Origin of the exceptional canaries. Possible products of nondisjunction from matings of pink-eyed males (bb) to black-eyed females (BW). B and b represent Z chromosomes carrying respectively the dominant black and the recessive pink alleles*

Type of nondisjunction	Gamete produced	Zygote	Expected phenotype	Sex	
				by $H_B$	by $H_D$
Female					
Division I	$BW$	$BbW$	Black	♂	♀*
	$O$	$bO$	Pink	♀	♂*
Division II	$WW$	$bWW$	Pink	♀	♀
	$BB$	$BBb$	Black	♂	♂
Male					
Division II	$bb$	$Bbb$	Black	♂	♂
		$bbW$	Pink	♂*	♀
	$O$	$BO$	Black	♀*	♂
		$WO$	Lethal		

\* These are the sexes and phenotypes of the observed exceptional progeny.

Table 5. *Tests of observed vs expected numbers in progeny of Durham's two exceptional black hens mated to black (Bb) and pink (bb) males assuming, for  $H_D$ , increasing rates of disjunction of Z chromosomes in the hens*

	Genotype of cocks	Genotype of hens	Assumed % of (ZZ + W) gametes	Sons		Daughters		$\chi^2$	d.f.	P	
				Black	Pink	Black	Pink				
Observed	$Bb$	—	—	2	0	1	1	—	—	—	
	$bb$	—	—	4	0	0	6	—	—	—	
	Total			6	0	1	7	—	—	—	
Expected	by $H_B$	—	$BO$	—	7.0	0	1.0	6.0	1.3	2	0.87
			$BbW$	33.3	5.0	2.0	3.0	4.0	5.8	3	0.13
	by $H_D$	—		16.7	4.5	2.5	3.5	3.5	8.3	3	0.04
				0	4.0	0	4.0	3.0	11.6	3	< 0.01

Genetic evidence in favour of  $H_B$  is supported by cytological observations in chickens. Crew did not identify the analogue to the  $Y$  chromosome of *Drosophila*, but he did find varying numbers of macrochromosomes. The presumed  $Z$  chromosome, largest in the complement according to Crew (and others) but now known

to be fifth in size, was present twice in the gold rooster and his gold son, three times in at least two silver sons and once in both silver daughters.

If  $H_B$  holds, then all of Durham's exceptional canaries as well as Crew's rooster and his gold half- and full-brothers arose from misdivision in male meiosis II.

The sex ratio of exceptional canaries is expected to be equal, yet pink males and black females occurred in  $bb \text{ } \delta \times B \text{ } \text{females}$  matings in the significantly unequal proportions of 2/201 vs 21/249 ( $\chi^2_1 = 12.7$ ,  $P < 0.001$ ). In contrast to the ability of exceptional black females to reproduce, most exceptional pink males that hatched in the stocks of Durham and of contemporary canary breeders failed to mature. It seems reasonable to extend this reduction in post-hatch viability to earlier stages and to regard  $ZZW$  canaries as sublethal (Sittmann, 1983). Furthermore, sex ratios between 39.7% and 44.7% in Durham's three main mating types were interpreted to be due to a reduction in viability of  $ZZZ$  males as well (Sittmann, 1983). For Crew's data (Table 3), the assumption of sublethality of trisomics would reduce the expectation of sons in favour of daughters, thus raising the  $\chi^2$  for 5.5% nondisjunction. Present evidence permits no choice as to whether the sublethality of trisomic canary males or the apparently normal viability of trisomic chicken males (i.e. Crew's rooster and his trisomic brothers and sons) constitute the norm in birds. Normal functioning of  $ZO$  canary females is opposite to the impaired Darwinian fitness of  $XO$  females in humans and other mammals.

Nondisjunction in meiosis II of spermatogenesis in canaries contrasts with the conclusion for chick embryos that all of eight sex-chromosome aneuploids (6  $ZZW + 2 ZO$ ) arose from nondisjunction in meiosis I of oogenesis (Snyder, Fechheimer & Jaap, 1975; also Fechheimer & Jaap, 1980). These chick and canary aneuploids differed not only in origin but also in two aspects of their occurrence. First, the eight chick embryos came from as many dams and were thus clearly not familial, whereas the 23 canary aneuploids in  $bb \text{ } \delta \times B \text{ } \text{females}$  matings were produced by only 17 sires and 17 dams in considerably smaller sibships than the abnormal chicks. Second, the incidence of sex-chromosome aneuploidy was substantially lower in the 16-hour chick embryos (8/2107 = 0.4%) than in the hatched canaries (23/450 = 5.1%;  $\chi^2_1 = 69.8$ ) where, in addition, the incidence of 5.1% is biased down for two reasons. Among trisomic males, at least one class ( $ZZW$ , pink) suffered heavy pre-hatch mortality (~90%; Sittmann, 1983) and the other ( $ZZZ$ , black) was undetectable among normal ( $ZZ$ ) black males. In 4-day chick embryos, furthermore, Bloom (1972) found aneuploidy for any macrochromosome in only 9/4182 = 0.2%, all single or double trisomics. Thus, origin of chicken and canary sex chromosome aneuploids in different meiotic divisions may involve different causes: polygenes or environmental factors or both in chickens and specific genetic causation (Golubovskaya, 1979), possibly a single gene, in canaries.

The structural loci for H-W and H-Y antigens in birds and mammals are similar in being borne by the smaller sex chromosome (Gilmour, 1966; Silvers & Wachtel, 1977; McCarrey, Abplanalp & Abbott, 1981). But genetic evidence presented here suggests an evolutionary discontinuity between  $W$  and  $Y$  chromosomes in sex determination of birds and mammals who last shared a common ancestor 320 million years ago.

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