The evolution of the avian genome as revealed by comparative molecular cytogenetics

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Abstract

Birds are characterised by feathers, flight, a small genome and a very distinctive karyotype. Despite the large numbers of chromosomes, the diploid count of 2n = 80 has remained remarkably constant with 63% of birds where 2n = 74–86, 24% with 2n = 66–74 and extremes of 2n = 40 and 2n = 142. Of these, the most studied is the chicken (2n = 78), and molecular cytogenetic probes generated from this species have been used to further understand the evolution of the avian genome. The ancestral karyotype is, it appears, very similar to that of the chicken, with chicken chromosomes 1, 2, 3, 4q, 5, 6, 7, 8, 9, 4p and Z representing the ancestral avian chromosomes 1–10 + Z; chromosome 4 being the most ancient. Avian evolution occurred primarily in three stages: the divergence of the group represented by extant ratites (emu, ostrich etc.) from the rest; divergence of the Galloanserae (chicken, turkey, duck, goose etc.) – the most studied group; and divergence of the ‘land’ and ‘water’ higher birds. Other than sex chromosome differentiation in the first divergence there are no specific changes associated with any of these evolutionary milestones although certain families and orders have undergone multiple fusions (and some fissions), which has reduced their chromosome number; the Falconiformes are the best described. Most changes, overall, seem to involve chromosomes 1, 2, 4, 10 and Z where the Z changes are intrachromosomal; there are also some recurring (convergent) events. Of these, the most puzzling involves chromosomes 4 and 10, which appear to have undergone multiple fissions and/or fusions throughout evolution – three possible hypotheses are presented to explain the findings. We conclude by speculating as to the reasons for the strange behaviour of these chromosomes as well as the role of telomeres and nuclear organisation in avian evolution.
The unique avian karyotype

Birds have a number of peculiarities: They are the only extant phylogenetic class to possess feathers; flight is near ubiquitous (despite being extremely rare in other vertebrates) with all representatives having, or having lost, the ability to fly. Moreover, they have a characteristically small genome (one third the size of mammals i.e. 0.97–2.16 pg with a mean of 1.45 pg ± 0.1 pg, Burt et al., 1999, www.genomesize.com), which has, it is suggested, evolved in response to the energy conservation requirements associated with the evolution of flight (Hughes and Piontkivska, 2005). A further distinguishable avian feature is the gross organisation of the genome (i.e. karyotype), which is readily identifiable to a relatively trained eye. The ancient and consistent haploid number of around 40, in combination with the large number of microchromosomes is a pattern that is quite distinct in nature. That is, although many reptiles (including lizards, snakes and crocodiles) are known to have microchromosomes, and karyotypes of particular species of turtle (where 2n = 66) are quite similar to avian ones, the ‘so many, so small’ pattern (where 2n ; 80) is a uniquely avian feature. The relatively unchanged nature of the diploid chromosome number among the majority of avian species further implies that such a karyotype was, and is, a highly successful means of genome organisation. Like flight, feathers and a small genome, this characteristic karyotype, once it had appeared in birds, remained relatively constant (with few exceptions) to the present day.

The complete chicken karyotype – a baseline for comparative genomics

Chicken (Gallus gallus domesticus) is the domesticated descendent of the Red Junglefowl (Gallus gallus), on which most genomic and hence cytogenetic studies have been performed. Its pivotal role in agriculture and as a classical embryology model – as well as a model for human disease – made it the primary species from which nearly all comparative vertebrate genome analysis in birds have ensued. Our group recently published a complete chicken karyotype (2n = 78) (Masabanda et al., 2004), which was the first avian karyotype to be completely described. The purpose of this review is to describe our findings and, more importantly, to indicate how they have, through collaborations with colleagues worldwide, led to a greater understanding of avian genome evolution

Perhaps a convenient starting point is in the terminology. The terms macro- and micro-chromosome have been common parlance for many years when describing chicken chromosomes. In many ways though, these terms are somewhat of a misnomer. Closer inspection reveals that there is no clear dividing line between the smaller and the larger chromosomes, which may explain why different authors give different accounts of the relative numbers of macro- and micro-chromosomes. In an attempt to reconcile the confusion (but admittedly running the risk of adding to it), we suggested a different classification system (Masabanda et al., 2004). This was related to the ability to resolve the chromosomes in a flow karyotype and to the newly emerging genome sequence. Thus we assigned group A as the chromosomes that could be resolved in a flow karyotype (which included chromosome 10 which, although slightly smaller that 11 and 12, was resolved alone where 11 and 12 sorted together). Groups B and C comprised the remainder of the chromosomes (11–32) that had known markers from the genome project assigned to them at the time of writing. The groups were separated by the NOR chromosome which, despite its relatively small size, had previously been assigned number 16. Even two years after the original publication, the smallest ‘group D’ chromosomes (33–38) have yet to be anchored to the genome assembly and this raises the question of whether this is simply due to their small size and thus ‘bad luck’ or a more fundamental biochemical reason, e.g. a large proportion of repeats.

The classical way of studying any karyotype is, of course, by G-banding. In general terms however, G-band information is limited in birds firstly because bands on the group A chromosomes are less distinct than in mammals (presumably due to less distinct differences between the molecular correlates of G-banding along the genome) and secondly because the group B–D chromosomes are too small to visualise any banding pattern (Ladjali-Mohammedi et al., 1999). In defining the full karyotype, our group thus developed chromosome-specific FISH probes for each chicken chromosome (Griffin et al., 1999), and we subsequently set up a resource centre (www.farmachrom.net) for, among other things, comparative genomics. Using these probes (both chromosome paints and BACs) it has become possible, through zoo-FISH experiments, to establish chromosome orthologies among birds. This review focuses on the molecular cytogenetic studies and makes reference to the G-banding studies only when a general point is made or
when the zoo-FISH studies have added extra information. We might add therefore that conclusions drawn from banding information alone, which is at best limited, should be viewed with caution until confirmed by molecular methods.

Chromosome numbers in birds – an overview

The most complete account of the chromosome number in birds is given by Christidis (1990), including 723 species with relatively accurate chromosome numbers and partial karyotypes. Rodionov (1997) suggested that there are nearly 800 published avian karyotypes in existence and cites several not quoted by Christidis. As mentioned the diploid number is very consistent with around 63% of birds where \(2n = 74–86\) and 24% with \(2n = 66–74\) (Christidis, 1990). Considering the more rapid rate of change in mammals (Wienberg, 2004), the relative lack of variation in birds is remarkable. That is, chromosomal changes are commonplace in the genome evolution of mammals and the best known examples include comparisons of the Chinese and Indian Muntjacs, *Muntiacus reevesi* (\(2n = 46\)) and *Muntiacus muntjak* (\(2n = 6\) in females and 7 in males, Yang et al., 1997), and gibbons where gross rearrangements are commonplace (Wienberg, 2004). Examples of birds with significantly less than the norm include the Laridae (gulls and terns, where \(2n = 66–70\)), the Pelecaniformes (pelicans etc. where \(2n = 66–70\) with only one known exception, the Little Cormorant, *Phalacrocorax niger* (Christidis, 1990) and the Psittacidae (parrot family) where \(2n = 60–72\). Interestingly the Psittacidae are rare examples of where clear differentiation between macro- and micro-chromosomes can be seen; for instance *Platycerus elegans* (the Crimson Rosella) has seven pairs of macrochromosomes (including Z and W) with the rest at least ten times smaller than that the smallest pair of macroautosomes (Christidis, 1990). In this instance the most likely mechanism therefore is a fusion of several pairs of group B–D chromosomes to form larger chromosomes and/or fusion on to group A chromosomes. In the absence of FISH studies the precise nature of these changes remains to be determined. In the Falconiformes chromosome number varies from \(2n = 50\) to \(2n = 72\) in all but the Cathartidae (new world vultures), Sagittariidae (Secretary bird) and selected Falconidae (falcons and caracaras). Indeed, it is the Falconidae that shows the most variation among the Falconiformes with *Falco jugger*, *peregrinus* and *subbuteo* (Laggar, Peregrine and Hobby Falcons respectively) with \(2n = 50\) and *Polyborus plancus* (the Crested Caracara) with \(2n = 84–86\) (Christidis, 1990). Unlike in parrots however, rather than a tendency to form large chromosomes, chromosome fusion seems to have been more uniform across the karyotype. Recent molecular cytogenetic studies have shed light on the nature of these changes and this is dealt with in detail in a subsequent section

For the sake of completeness it is appropriate to mention the extremes at both ends of the spectrum. Smallest among known diploid chromosome numbers are the stone curlew *Burhinus oedicemus* (\(2n = 40\)), the trumpeter hornbill *Ceratogymnus bucinator* (\(2n = 40\)), the beach thick knee *Burhinus magnirostris* (\(2n = 42\)), and the black and white casqued hornbill *Ceratogymna subcilindrica* (\(2n = 42\)) (Christidis, 1990). On the other end of the scale the hoopoe *Upupa epops* has a diploid number of 126. The greatest number of reported chromosomes in a bird, however, is either (appropriately for a review of molecular cytogenetics) the common kingfisher *Alcedo atthis* where \(2n = 132\) or 138 depending on which paper you read (the Azure kingfisher *Ceyx azurea* has a mere \(2n = 122\)) and the strangely named Gray or Southern Go-away-bird *Corythaixoides concolor* (\(2n = 136–142\)) (Christidis, 1990). These birds are rare examples indeed however, since the next highest number is cited as 108–110 in several unrelated species (Christidis, 1990).

Early evolution of birds and the ancestral karyotype

It has been thought that avian species existed in the Triassic approximately 200 million years ago since discovery of two nearly complete fossil skeletons of *Protoavis* (Rodionov, 1997) which pre-date the Jurassic Archaeopteryx by some 50 million years. Mitochondrial evidence suggests the common ancestor of birds (synapsids) and mammals (diapsids) diverged 310 million years ago (Kumar and Hedges, 1998; Burt et al., 1999), while the common ancestors of birds and crocodilians may have diverged 210–250 million years ago (Muller and Reisz, 2005). The presence of turtles in the scheme of things appears less certain, however they too are thought to have diverged from birds over 210 million years ago and recent molecular evidence from both mitochondrial and nuclear sources places birds, crocodilians and turtles in the same group (Archosaurs) with lizards and snakes (Lepidosauria) separate (Hedges and Poling, 1999; Kumazawa and Nishida, 1999). Matsuda et al. (2005), through the isolation of cDNA libraries from soft-
shelled turtles and comparison with chicken sequences provided compelling evidence that there was highly conserved linkage homology between birds and turtles (specifically chickens and softshelled turtles); moreover bird and turtle chromosomes 1–5 (as well as turtle 6 and avian Z) appear to be precise counterparts of one another. Of these chromosomes the Z chromosome is thought to be an ancient sex chromosome (Marshall Graves and Shetty, 2001) and sequence comparisons from the human and chicken genome projects reveal a remarkable degree of synteny of chicken chromosome 4q and (coincidentally) human chromosome 4 (Chowdhary and Raudsepp, 2000). That is, although unsurprisingly, there is extensive inter-chromosomal rearrangement between all other chromosomes but none between human chromosome 4 and chicken chromosome 4q other than a tiny segment of another chicken chromosome in the p-terminus of human chromosome 4 (Chowdhary and Raudsepp, 2000). Taken together then, the ancestral avian chromosomes 1, 2, 3, 5 and Z will have appeared at least 210 million years ago with the ancestral chromosome 4 appearing at least 310 million years ago.

Attempts to depict the ancestral karyotype of birds by examining banding patterns date back to at least 1982 (Stock and Bunch). The ancestral karyotype for the Galliformes as predicted by Shibusawa et al. (2004a) by comparative chromosome painting appears to be conserved throughout the avian lineages. Hence, for the purposes of this paper we will make reference to chromosome rearrangements in relation to the putative avian ancestor, rather than the chicken. The chicken chromosome 4p has been shown to be, most likely, a fusion of the ancient ancestral chromosome 4 to another ancestral chromosome (ICGSC, 2004). Our own banding comparisons of hybridised paints suggest that this chromosome is chromosome 9 in turkey and thus a group A chromosome. For the purposes of this study therefore we will refer to the orthologue of chicken chromosome 4p as ‘ancestral chromosome 10’. Taking the studies as a whole it seems clear that the pattern of the chicken orthologues of chromosomes 1, 2, 3, 4q, 5, 6, 7, 8, 9, 4p and Z represent the ancestral chromosomes 1–10 + Z for all birds, illustrated in Fig. 1. The timing of appearance of extant chromosomes 6–9 remains to be determined (e.g. by means similar to that employed by Matsuda et al. (2005); it is not unfeasible however to suggest that they appeared at a similar point to their larger counterparts. Moreover, we have not yet seen any direct evidence of the chicken W chromosome orthologues in other birds (indeed we have been unsuccessful in hybridising chicken W paint to turkey metaphases), yet it seems reasonable to assume that this evolved by previously described mechanisms of sex chromosome divergence from a Z chromosome ancestor (Marshall Graves and Shetty, 2001). As will become clear in subsequent sections, it seems to be chromosomes 1, 2, 4 and 10 that are more prone to interchromosomal rearrangements (fissions and/or fusions) and the Z more prone to intra-chromosomal rearrangements. Moreover selected orders and families have multiple rearrangements (mostly microchromosomal fusions) and these are also reviewed in detail presently.

Figure 1: Schematic depicting the ancestral avian karyotype and its chicken orthologues. The only difference is chicken chromosome 4, which is represented by ancestral chromosomes 4 and 10.
The appearance of the microchromosomes (groups B–D) seems to have been a gradual rather than sudden event. Molecular and fossil data suggest that the divergence of all the major amniotes (reptiles, birds and mammals) occurred around 300–310 million years ago (Kumar and Hedges, 1998; Burt et al., 1999). The presence of microchromosomes in birds, lizards, snakes, crocodiles and turtles but not in mammals or amphibians suggests the first appearance of microchromosomes was after this time (Fig. 2). The presence of a 2n = 66 karyotype (including a large number of microchromosomes) in turtles (Stock and Mengden, 1975) might suggest that it is turtles rather than crocodilians that are the closest avian relative although this thesis may be challenged by other evidence. That is, if, as mitochondrial DNA data would suggest, the crocodilians are more closely related to birds than turtles, then it seems likely that the crocodilians underwent a series of microchromosomal fusions that was a feature of their own evolution. It is likely also that the genomes of the reptilian/avian ancestors continued to fragment over a period of 100 million years or so, reaching at least 2n = 66 by the time the lineages that led to turtles and birds diverged and became fixed in the classic 2n ; 80 pattern around the emergence of the first birds around 200 million years ago. An alternative explanation is that the Lepidosauria (lizards, snakes etc.) and birds evolved microchromosomes separately and the crocodilians retained a more ancestral karyotype however this seems very unlikely particularly as bird and turtle macrochromosomes are precise orthologues of one another and the crocodilians are a monophyletic group.

There are thought to be three major events involved in bird evolution: 1) the divergence of Palaeognathae (surviving members include the Struthioniformes or ratites such as emu, ostrich, rhea, kiwi etc.) and Neognathae (others) approximately 100–120 million years ago (van Tuinen and Hedges, 2001); 2) the divergence from the Neognathae of the Galloanserae (e.g. chicken, turkey, goose, duck etc.) approximately 100 million years ago (van Tuinen and Hedges, 2001); and 3) the divergence of the remainder of the Neognathae into ‘higher land’ and ‘higher water’ birds approximately 70–80 million years ago (van Tuinen and Hedges, 2001). These divergences can be seen in Fig. 2.

Figure 2: (overleaf) Phylogenetic tree for which comparative genomic data (zooFISH) exists. The tree has been collated from consensus studies of DNA hybridisation studies, mitochondrial DNA sequencing and comparative protein sequencing (Edwards et al., 2005; Schmid et al., 2005). Only interchromosomal changes are shown, fissions are represented in red, fusions are represented in blue. All numbers correspond to the ancestral avian karyotype (Fig. 1). Chromosome 4 is omitted since it appears in Fig. 3. To the right is a representation of which ancestral chromosomes have split, fused or remained unchanged compared to the ancestral one in all the species thus far studied. Data from Takagi and Sasaki, 1974; Burt et al., 1999; Hedges and Poling, 1999; Shetty et al., 1999; Chowdhary and Raudsepp, 2000; van Tuinen et al., 2000; Zardoya and Meyer, 2001; Dimcheff et al., 2002; Donne-Gousse et al., 2002; Shibusawa et al., 2004a; Matsuda et al., 2005; Summers, 2005; Kohn et al., 2006. Known divergence dates have been taken from the following sources: Birds/mammals: 300–310 Myr (Kumar and Hedges, 1998; Burt et al., 1999); Birds/crocodilians: 210–250 Myr (Muller and Reisz, 2005); Birds/turtles: 210 Myr (Hedges and Poling, 1999; Kumazawa and Nishida, 1999, Matsuda et al., 2005); Palaeognathae and Neognathae: 100–120 Myr (van Tuinen and Hedges, 2001); Neognathae/other Galloanserae 100 Myr (van Tuinen and Hedges, 2001); Neognathae ‘higher land’/‘higher water’: 70–80 Myr (van Tuinen and Hedges, 2001); Anseriformes and Galliformes 90–96 Myr, Ciconiiformes (New world vultures) and Falconiformes: 75 Myr (van Tuinen and Hedges, 2001). Other divergence dates can be regarded as speculative only.
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Legend:
- Green: Ancestral chromosome
- Yellow: Ancestral chromosome, with rearrangements
- Blue: Fusion of ancestral chromosomes
- Orange: Rission of ancestral chromosome
- Brown: No Data

A - Pericentric inversion
B - Centromere repositioning
C - Unknown fusion
D - Fusion of ancestral 4 and 9

NB: Rearrangements of Z chromosome not shown as ancestral form as yet unclear.
The first divergence

Modern day representatives of the divergence of Palaeognathae comprise only two extant orders; the flightless Struthioniformes (ratites; e.g. emu, ostrich, rhea, cassowary and kiwi) and the Tinamiformes (tinamous). One of the first successes of our avian chromosome resource centre (and for avian comparative genomics in general) was the confirmation of what had been long expected by classical studies; i.e. at least for the group A chromosomes, synteny is remarkably conserved. This was revealed by experiments using chicken whole chromosome paints from chromosomes 1–9 + Z on to emu (Dromaius novaehollandiae) metaphases (Shetty et al., 1999). All but one chromosome appeared not to display interchromosomal rearrangements; the exception being the extant chicken chromosome 4 represented by the aforementioned ancestral chromosomes 4 and 10. A similar pattern was more recently noted by Guttenbach et al. (2003) in the American Rhea (Rhea americana). Given that chickens share a very similar karyotype (at least for the Group A chromosomes) to that of ratites, it seems clear that the first divergence was either not accompanied by a major autosomal change or was accompanied by a change in the smaller chromosomes that has yet to be discovered. The diploid number of 2n = 80–82 in all extant ratites suggest the former to be the case. A unique feature of the ratites is that they have homomorphic sex chromosomes, indicative of an ancestral autosomal origin and sex chromosome differentiation after the divergence of this group (Guttenbach et al., 2003).

The second divergence – Galloanserae (Galliformes and Anseriformes)

Galliformes are an order comprising the turkey, grouse, pheasants and quails and they contain the species in which the most genomic sequencing information is available i.e. chicken. Moreover, due to their status as agricultural birds, they are among the most studied avian orders in many other areas of science and, since the availability of chicken chromosome paints (Griffin et al., 1999; Masabanda et al., 2004), have been natural targets for comparative studies. Indeed the largest body of comparative genomic studies has been performed with reference to this order. As mentioned, all birds examined other than the ratites have heteromorphic sex chromosomes, however there is no evidence that the divergence of the Galloanserae was accompanied by any other major chromosomal change (although isolated individual changes are apparent), due to the relatively stable chromosome number in the majority of species and the clearly established orthology of the group A chromosomes.

Chromosome number is conserved and ranges from 2n = 78 (chicken) to 2n = 82 (golden pheasant, Chrysolophus pictus, Guttenbach et al., 2003), and zoo-FISH with chicken whole chromosome paints reveals the interchromosomal rearrangements that have occurred in the Galliform lineage. The majority of the changes from the ancestral form are found in chromosomes 2 and 4. Chromosome 2 is represented as two separate telocentric chromosomes (3 and 6) in the five species of pheasant, turkey (Meleagris gallopavo) and California quail (Callipepla californica) (Shibusawa et al., 2004b). It is also represented as two telocentric chromosomes (3 and 7) in the capercaillie (Tetrao urogallus) (Shibusawa et al., 2004a).

The ancestral chromosome 4 (chicken chromosome 4q) is conserved intact in all the Galliformes and indeed most birds, albeit fused to smaller chromosomes on certain occasions. In the guinea fowl (Numidea meleagris) a fusion has occurred between it and ancestral chromosome 9 (Shibusawa et al., 2004a). However the most common fusion is between ancestral chromosome 4 and ancestral chromosome 10; this is seen in chicken, the partridges; peafowl and two quail species (Blue breasted and Japanese) (Shibusawa et al., 2004b). The pheasants, capercaillie, turkey, California quail and chachalaca (Ortalis vetula) all show the chicken 4p arm hybridising to ancestral chromosome 10 although it is usually described as an unassigned microchromosome (Shibusawa et al., 2004a). Interestingly, molecular evidence (ICGSC, 2004) has suggested that, despite the fusion in chicken, ancestral chromosome 10, when it appears as chicken chromosome 4p still retains the properties (e.g. gene density, recombination rate, CpG island distribution) of the smaller chromosome it once was.

In addition to the rearrangements of chromosomes 2 and 4, there are only four other interchromosomal changes detected to date for the remainder of the karyotype. In Guinea fowl (Numidea meleagris), a fusion has occurred between ancestral chromosomes 6 and 7 (Shibusawa et al., 2002); the capercaillie shows a
fusion of ancestral chromosomes 6 and 8 (Shibusawa et al., 2004a); and the common peafowl (Pavo cristatus) has both a fusion of ancestral chromosomes 8 and 9 and fusion of ancestral chromosome 7 to a microchromosome (Shibusawa et al., 2004a).

With regard to intrachromosomal rearrangements, in the Japanese quail (Coturnix japonica), pericentric inversions have occurred between it and the ancestral type (Shibusawa et al., 2001) for chromosomes 1 and 2 (Schmid et al., 2000). An apparent pericentric inversion in Red-Legged Partridge (Alectoris rufa) has been revealed by Kasai et al. (2003) using comparative BAC mapping to in fact be the repositioning of the centromere to the p terminus. That is, the gene order has not changed along the length of the chromosome though the position of the centromere has. The reported ‘pericentric inversion’ seen in the Blue Breasted and Japanese Quails (Shibusawa et al., 2004b) should be viewed with caution therefore until further BAC studies are performed. One rearrangement that is probably a pericentric inversion however is the ancestral chromosome 8 which is metacentric in chicken and Chinese bamboo partridge but telocentric in other birds. BAC mapping experiments with turkey chromosomes (Robertson et al., unpublished results) have all but confirmed this.

On a related theme, recent studies of lampbrush chromosomes (Galkina et al., 2006) suggest that, while all chicken microchromosomes are telocentric, Japanese quail microchromosomes are all metacentric. The mechanism by which this occurred remains a mystery, however we are currently investigating whether this phenomenon occurred by pericentric inversion or centromere relocation. The reasons why it occurred are as yet unclear.

The Anseriformes are the nearest extant relatives to the Galliformes, diverging 90–96 million years ago, and the only other surviving order from the second divergence. Among them the Greylag goose (Anser anser) studied by Guttenbach et al. (2003) shows a fusion of the ancestral 4 and 10, an identical pattern to that seen in chicken. The swan goose (Anser cygnoides) studied by Jaszcak et al. (2002) shows evidence of rearrangements on chromosome 4, having a metacentric chromosome pair. Though painting data is not yet available to confirm that this is the ancestral 4, the accepted diploid number of 80, equal to that of the Greylag, plus the ease of hybridisation suggest conservation of the ancestral form as well. The Mallard duck (Anas platyrhynchos) shows the ancestral form (with chicken chromosome 4 paint hybridising to two chromosomes (Schmid et al., 2000, 2005), though mapping of chicken BAC clones reveals intrachromosomal rearrangement in the smaller macrochromosomes. The Muscovy duck (Cairina moschata) has not yet been painted with chicken chromosome paints, however banding studies by Denjean et al. (1997) showed few rearrangements in the macrochromosomes, and the diploid number is believed to be the same (2n = 80).

**The final divergence**

During the final divergence (into ‘higher land’ and ‘higher water’ birds) there is no evidence to suggest a change characteristic of either clade. Following this event, however, many birds on both sides of the divide clearly underwent a series of microchromosomal fusions and, to a smaller extent, macrochromosomal fissions with a net result of less chromosomes in the karyotype. In other words, a tendency to reduce the chromosome number has been an independent, convergent event happening in several unrelated families and orders significantly after the last major divergence of the birds. As mentioned, 24% of all birds have an average of 2n = 66–74: the Laridae (gulls and terns), the Pelecaniformes (pelicans etc.) and the Psittacidae (parrot family). Perhaps the most striking example however is seen in the Falconiformes (e.g. vultures, falcons, hawks, eagles etc.) on which the most zoo-FISH studies have been performed.

**Evolution in Falconiformes and Ciconiiformes**

Falconiformes have a low chromosome number ranging from 2n = 50 (American Kestrel, Falco sparverius) to 2n = 68 (Red tailed Hawk, Buteo jamaicensis) (Shields, 1982) and an atypical chromosome morphology suggestive of several fissions and fusions among both the macro- and microchromosomes. The Ciconiiformes (New World vultures) were formerly classed as part of the Falconiformes, but are now separated following a divergence approximately 75 million years ago (van Tuinen and Hedges, 2001). They have 2n = 80, and are thus closer to the ancestral avian karyotype (Nanda et al., 2006). Cytogenetic
findings therefore along with other lines of evidence would suggest that members of the Accipitridae family including old world vultures, eagles, hawks and kites are more closely related to one another than they are to the new world vultures, and that the major chromosomal changes are characteristic of the Accipitridae rather than the Falconiformes as a whole. Accipitridae that have been studied by comparative painting are the Griffon vulture (\textit{Gyps fulvus}), Ruppells vulture (\textit{Gyps rueppelli}) and the Bearded Vulture (\textit{Gypaetus barbatus}) by Nanda et al. (2006) and the Harpy Eagle (\textit{Harpia harpyja}) by de Oliveira et al. (2005). There are no large macrochromosomes in these birds, rather \(\sim\)25 pairs of medium sized chromosomes and 4–6 pairs of microchromosomes suggesting frequent and whole scale microchromosomal fusion; the black-winged kite (\textit{Elanus caeruleus}) has only a single microchromosome pair (2n = 68; Bed’Hom et al., 2003). There are also several fissions of the larger chromosomes apparent when chicken whole chromosome paints are applied to these species; chicken chromosome 1–5 paints show extensive rearrangements; for example chicken chromosome 1 hybridises to six separate chromosomes ranging in size from 7 to 22 in \textit{G. fulvus} and \textit{G. rueppelli}; to four chromosomes in \textit{G. barbatus} ranging from 7 to 12 and five chromosomes ranging from 5 to 24 in the Harpy Eagle (for all rearrangements from chicken chromosomes 1–10 see Table 1). In contrast, where data is available, chromosomes 6–10 hybridise only to a single chromosome or a larger, fused chromosome. An apparent exception is chromosome 4. The chicken chromosome 4 paint hybridises to only two chromosomes in all four species, a larger (~1–4) and a smaller (~13–16) chromosome suggesting conservation of the ancestral karyotype. Among the Ciconiiformes the best studied example is the California Condor (\textit{Gymnogyps californianus}), which also has the distinction of being the largest flying bird. Raudsepp et al. (2002) found few rearrangements with chicken. The chicken 2 paint hybridises to chromosome 2 of this species with weak cross hybridisation to chromosome 3. Moreover, the chicken chromosome 3 paint hybridises to California condor chromosome 3 with a weak signal on chromosome 2. Chromosome 4 has a p arm and is therefore sub-metacentric, however the chicken chromosome 4 paint detects two chromosomes (4 and 9 – presumably the ancestral 4 and 10) in the California condor suggestive of a pericentric inversion or centromere relocation on the California condor chromosome 4.

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<tr>
<th>Paint</th>
<th>Chromosome</th>
<th>Bearded vulture (\textit{Gypaetus barbatus} 2n = 60)</th>
<th>Harpy Eagle (\textit{Harpia harpyja} 2n = 58)</th>
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<td>Chicken (\textit{Gallus gallus} 2n=78) Griffon vulture (\textit{Gyps fulvus})/Ruppells vulture (\textit{Gyps rueppelli} 2n = 66)</td>
<td>7, 12, 15, 19, 20, 22 7, 8p, 11, 12q 5, 6, 19, 21, 24</td>
<td>2, 3, 23 8, 16q, 21, 24 1q, 2, 14q, 23q 2p, 18, 23</td>
<td>14q, 17 1q, 13 15q, 20 8q, 12, 21q, 22q 3, 16 4, 14 2p, 18, 23</td>
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<td>1</td>
<td>7, 12, 15, 19, 20, 22</td>
<td>7, 8p, 11, 12q</td>
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<td>2, 3, 23</td>
<td>1q, 2, 14q, 23q</td>
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<td>8, 16q, 21, 24</td>
<td>8q, 12, 21q, 22q</td>
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\(\textsuperscript{a}\) Nanda et al. (2006).  
\(\textsuperscript{b}\) de Oliveira et al. (2005).

\textbf{Table 1}: Summary of comparative genomics between chicken and three Falconiformes established by painting of chromosome paints 1–10 + Z of chickento metaphases of these species. Note that comparisons in this case are with chicken and not with the ancestral karyotype and thus the orthologies with chicken chromosome 4 are probably ancestral in the Falconiformes.
**Evolution in the Passeriformes**

The Passeriformes are the largest avian order, comprising about half of all known bird species. The most studied is the zebra finch (*Taeniopygia guttata*), an emerging model organism for study of many issues relevant to human health and disease mainly because of its ability to communicate via complex learned vocalisations (Arnold, 2004). Zebra finch has been used as a model species for sex differences in neural structure and function, influences of steroid hormones on neural networks, adult neurogenesis, steroid hormone synthesis in the brain, the neural basis for learning and complex auditory processing and auditory-motor integration (Arnold, 2004). It seems likely for these reasons that zebra finch will be the second bird (after chicken) to have a complete genome sequencing effort. Comparative genomics (zoo-FISH) has also been carried out on the chaffinch, redwing (Derjusheva et al., 2004) and blackbird (Guttenbach et al., 2003). Chicken chromosome paints 1–10 and Z reveal a few distinct rearrangements. The redwing (*Turdus iliacus*) and blackbird (*Turdus merula*) belong to the family Turdidae, and both display a fission of the ancestral chromosome 1 near or at the centromere. The chaffinch (*Fringilla coelebs*) is in the family Fringillidae; it and the zebra finch (*Estrildidae*) show a similar fission (Itoh and Arnold, 2005). Ancestral chromosome 4 is conserved in all four birds; however, due to the chromosome 1 fission it is referred to as chromosome 5 in the chaffinch and zebra finch.

**Strigiformes and Columbiformes (Owls and Doves)**

The Great Grey Owl (*Strix nebulosa*) and the Eagle Owl (*Bubo bubo*) from the family Strigidae were studied by Schmid et al. (2000) and Guttenbach et al. (2003) respectively. There are no interchromosomal changes from the ancestral form in the Great Grey Owl. The Eagle Owl shows a similar fission to that seen in the Turdidae i.e. that of ancestral chromosome 1. The chicken (ancestral) chromosome 2 paint hybridises to the long arm of the largest chromosome in the Eagle Owl with the short arm of the same chromosome orthologous to ancestral chromosome 4. Finally, the ancestral 5 has undergone a fission event in Eagle Owl and split to a macro- and microchromosome.

Among the Columbiformes, the Pigeon (*Columbia livia*) retains the ancestral karyotype (Derjusheva et al., 2004), but the African collared dove (*Streptopelia roseogrisea*) has a fusion of ancestral 4 and 10 (the same as chicken and goose), as well as two fusions of ancestral chromosomes 6, 7, 8 and 9 forming two larger macrochromosomes (Itoh and Arnold, 2005).

**The sex chromosomes**

Despite being an ancient conserved chromosome, the Z chromosome is subject to some of the most extensive intrachromosomal rearrangements within the lineages; the ancestral monomorphic pair seen in emu and rhea have been mentioned already. Thereafter, however, not only has a W chromosome evolved but the Z seems to have undergone numerous intrachromosomal changes. The metacentric chromosome of the chicken appears as a submetacentric chromosome in many of the other Galliformes, as well as in the Anseriformes, and there are indications of additions of heterochromatin accumulation in the q arms of chicken, blue-breasted and Japanese quails (Shibusawa et al., 2004b). Chicken Z paints hybridise to a single turtle chromosome (Marshall-Graves, personal communication), suggesting that chicken (apart from the heterochromatin on the q arm) represents the ancestral form. This form is also found in the ratites, where Z and W are homomorphic (Shetty et al., 1999). Detailed comparisons of BAC order in other birds are hampered by the paucity of markers in the sex chromosomes on the chicken assembly and it seems clear that the overall role of inversions, centromere relocation and heterochromatin accumulation is yet to be determined.
Chromosome 4

Independent convergent changes are not entirely unusual in avian karyotype evolution; for instance both chromosomes 1 and 2 have displayed individual fission events around the centromere. This has occurred in both the Turdidae and the Eagle owl for chromosome 1 and in the Californian Quail and the turkey/panet group for chromosome 2. These pale into insignificance however when compared to the story of chromosome 4. The ancestral chromosome 4 (chicken chromosome 4q) is the most ancient of all the avian chromosomes – appearing intact even in humans (Chowdhary and Raudsepp, 2000). It is somewhat ironic then that, when coupled with its counterpart as it appears on chicken chromosome 4 (i.e. as a submetacentric chromosome from fusion with ancestral chromosome 10) it is subject to the most puzzling of conundrums in avian evolution.

In the majority of species the ancestral pattern (separate chromosomes 4 and 10) is maintained, however the exceptions to this rule include chicken, goose and African collared dove. The most parsimonious explanation of these findings (Fig. 3) is three independent fusion events (in goose, dove and a recent ancestor of turkey, pheasants and some quail; nodes 5, 22 and 10) and one fission in the turkey/panet ancestor (node 15) (hypothesis 1, Fig. 3). Perhaps the least parsimonious involves a fusion before the second major divergence (node 2) and then at least eight different independent fission events (nodes 6, 9, 15, post 7, 20, 21, 22; hypothesis 3, Fig. 3). While ordinarily such a scenario might be disregarded as highly unlikely, it might be argued that it is equally unlikely that three independent fusion events involving the same chromosomes have occurred in the background of very few rearrangements occurring overall – a recurring fission event might at least be explained by the region being particularly fragile and prone to breakage. There is also an interim explanation (hypothesis 2, Fig. 3), for instance if we assume that there was not a fusion before the second major divergence, then a fusion in the Galliform/Anseriform ancestor could have been followed by four independent fission events a) in the turkey/panet group, b) in the group leading to the ducks, c) in the group leading to the Guinea fowl/California quail and d) leading to the chacha-laca (nodes 6, 9, 16, post 7). Of course this still requires an explanation for the pattern seen in the higher birds of which the most parsimonious is an independent fusion event (African Collared dove) – leading to a total of two fusions (nodes 3, post 22) and four fissions (nodes 6, 9, 15, post 17; hypothesis 2, Fig. 3). We have no evidence to suggest that these fissions and fusions are any other than centric although this requires further testing using BAC mapping and/or chromosome paints from non-chicken species on tiling path microarrays (which have recently become available for the chicken) and/or lambrush chromosomes. In a class where there are so few changes overall, having two chromosomes that are constantly splitting and joining is a mystery. A particularly fragile region of the genome (in this case perhaps the centromere) might explain multiple fissions, however genomic reasons as to why two chromosomes might be prone to fusion are more difficult to explain. In mammals, evolutionary breakpoints are more common at sites of segmental duplications, though to the best of our knowledge no such duplications exist in birds (Hillier et al., 2004). Similar sequences at the centromeres of the two chromosome might provide one explanation (c.f. the acrocentric chromosomes in humans are more prone to fusion because of their association with the nucleolus) and/or proximity of the two chromosomes in the interphase nucleus might provide another.

Figure 3: (overleaf) Representation of the same tree but with three interpretations of the likely scenarios to explain the fission and fusion involved in the evolution of ancestral chromosomes 4 and 10. Fissions (of chromosomes 4 and 10) are in red, fusions are in blue and assumed to be fusions of chromosome 4 and 10 unless otherwise stated, i.e. A = fusion of 4 + 9 and B = fusion of 4 + 2. The numbers on the nodes of divergence are referred to in the text for the purposes of easier reading. (For Hypothesis 2 and 3 see next pages.)
Hypothesis 2

- Ratites
  - Greylag goose
  - Swan goose
  - Mallard duck
  - Muscovy duck
  - Chacalaca

- Fission
  - Guinea fowl
  - Californian Quail
  - Chinese Bamboo Partridge
  - Chicken
  - Red Legged Partridge

- Fission
  - Blue-breasted Quail
  - Japanese Quail
  - Peafowl
  - Turkey
  - Pheasants
  - Capercaillie

  - Great Grey owl
  - Eagle owl

- Fusion
  - Pigeon
  - African collared dove

- Fusion
  - Falconiformes
Telomeres and avian evolution

As in mammals, avian telomeres are composed of a repeat sequence, 5'-(TTAGGG)n-3', a pattern conserved throughout vertebrate evolution over 400 million years (Meyne et al., 1989). While the avian genome is only one third the size of the average mammalian genome, the telomeric sequences comprise 4% of it, making them ten times more prevalent than in mammals, (c.f. the prevalence in humans is 0.3%) and with a length range of 0.5–2 Mb (at least in chicken; Delany, 2000). Telomere array organisation studies by Delany (2000) in chicken divided them into three classes based on telomere size, chromosome location and stability. Class I telomeres are interstitial, 0.5–10 kb, and show no evidence of telomere shortening. Class II are terminal, 10–40 kb, and show age related shortening. Class III are terminal, 40 kb–2 Mb, and do not show shortening.

Nanda et al. (2002) used FISH to study the distribution of telomeric sequences in 16 different bird species, and showed an enrichment of telomeric DNA on microchromosomes compared with the macrochromosomes. This pattern of centric and interstitial sequence in addition to chromosome ends has been found in chicken and turkey (Galliformes), Bell’s vireo (Vireo bellii; Passeriformes), red tailed hawk (Buteo jamaicensis; Falconiformes) and Inca dove (Columbina inca; Columbiformes) (Meyne et al., 1990; Nanda and Schmid, 1994). The Californian condor, studied by Raudsepp et al. (2002), in contrast, showed no interstitial hybridisation sites, similar to the house sparrow (Passer domesticus; Passeriformes), and lesser adjutant stork (Leptoptilos javanicus; Ciconiiformes) (Meyne et al., 1990); signals were confined to chromosome ends. This is also found in two vultures studied by Nanda et al. (2006), Gyps fulvus and Gyps barbatrus, as well as in the black-winged kite (Elanus caeruleus) studied by Bed’Hom et al. (2003). When compared to the macrochromosomes, telomere signals were stronger on the microchromosomes in all of the studied bird species, with the strongest signals on the smallest chromosomes. This signifies higher numbers of telomeric repeats, and one suggestion is that these serve as caps to protect the gene dense microchromosomes from telomere erosion (Delany, 2000).

Among the outstanding questions in telomere research is the paradox that, although there is a reduced proportion of repeat sequences in the avian genome overall, the abundance of telomeric sequences does not follow that rule (quite the opposite in fact). A second question is the determination of whether the interstitial arrays on the larger chromosomes reflect an ancient fusion point of smaller microchromosomes during evolution. Nanda et al. (2006) did not find any interstitial telomeres in the Old World Vultures (G. fulvus, G. rueppelli, G. barbatus) and we have further examined whether there is any evidence to suggest that interstitial telomeres represent ancient fusion points; we can find none.

Genome organisation from a different perspective (nuclear positioning of chromosomes)

Assessment of the spatial and temporal arrangement of chromosomes in the interphase nucleus is the best known assay for levels of genome organisation in interphase nuclei. Perturbations in either the gene density arrangement and/or the size-related arrangement have been associated with different cell types, states and with disease (Foster and Bridger, 2005). Habermann et al. (2001) conducted the first detailed two dimensional study and three dimensional reconstruction of chromosome territories in chicken fibroblasts and neurons. They used whole chromosome paints for chicken group A 1–10 and Z, and 19 pairs of smaller chromosomes (from 14 to 4 Mb). In both cell types, the largest chromosomes 1–5 and Z, plus the smaller group A chromosomes 6–10 were predominantly found at the periphery of the nucleus, while the microchromosomes formed clusters, mainly towards the centre of the nucleus (though some microchromosomes formed small clusters at the surface of the nucleus, between the macrochromosome territories). Of course, given that the smaller chromosomes are also the more gene-rich, this arrangement fits both the size-related and the gene density related models. In total 21 neuronal nuclei and 28 fibroblast nuclei were analysed. In neurons, chromosomes 1–5 and Z were peripheral, while 6–10 shifted slightly towards the centre. The microchromosome territories were central in both cell types, although they were more peripheral in the neurons than in the fibroblasts. From this it was suggested that this radial arrangement may be a common motif in all chicken cell types.

Zoo-FISH offers a method of using whole chromosome paints as a means of investigating the genome organisation of other avian species, for example the turkey, in which studies are in progress on the position...
of ancestral chromosomes 4 and 10 (turkey 4 and 9), and may help to explain why these two chromosomes are particularly prone to fusion.

Concluding remarks

The avian genome is fascinating and, as with many studies, the more it is examined, the more complex it seems. Many questions remain that are fundamental to our understanding of not only the avian genome but to our understanding of genome dynamics in general. Of course, performing more zoo-FISH experiments on more species is an obvious way to go. To this end we have available a set of chromosome paints or BACs for all/most of the avian karyotype including a set of ancestral chromosome paints (where 1–3, 5–9 + Z were derived from the chicken and 4 + 10 (chicken 4p + 4q) were derived from turkey chromosomes 4 and 9 (www.farmachrom.net). At present, for obvious reasons, the Galloanserae are over-represented compared to the rest of the orders and this is a situation that will, no doubt, change in the future.

More fundamental questions lie ahead of us however. For instance why, when this unusual genome organisation appeared, did it remain relatively ‘untouched’ for millions of years? It might be speculated that having so many chromosomes was sufficient, through both random segregation and crossing over (at least one chiasma per bivalent) to drive evolutionary change without the need for extensive chromosome rearrangement as a means of speciation. That is, while, in mammals, chromosome rearrangement is a consistent feature of species divergence, it is clearly less so in birds despite them having many more chromosomes. Put simply, birds may have, on the whole, not facilitated their evolution through chromosomal changes because there was less pressure for them to do so. What is the reason behind the very strange behaviour of chromosome 4? Examination of a rapidly evolving chromosome in a background of a genome where change is rare may shed light on the reasons as to why, and under what circumstances, chromosome change occurs. How did the required structures (e.g. telomeres and centromeres) arise in newly formed microchromosomes? The formation of de-novo centromeres is not unknown and perhaps arose through endoreduplication of pre-existing non-coding DNA. What, if anything, is the role of telomeres in avian evolution and why are telomeres so big in birds? Why are ancestral telomeres not detectable following fusion e.g. in the Accipitridae (are they eliminated completely or simply reduced to sub-detectable levels?) What is the role of nuclear organisation in genome evolution? Finally, what can we deduce about the genome organisation of species that are long since extinct (e.g. the dinosaurs); detailed comparative analysis of extant species may now make this possible.

References

De Oliveira EHC, Habermann FA, Lacerda O, Sbalqueiro IJ, Wienberg J, Müller S: Chromosome reshuffling in birds of prey: the karyotype of the world’s largest eagle (Harp eagle, Harpia harpyja) compared to that of the chicken (Gallus gallus). Chromosoma 2005 Nov;114:338–43.


