

Characterization of chromosome structures of Falconinae (Falconidae, Falconiformes, Aves) by chromosome painting and delineation of chromosome rearrangements during their differentiation

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Abstract

Karyotypes of most bird species are characterized by around $2n = 80$ chromosomes, comprising 7–10 pairs of large- and medium-sized macrochromosomes including sex chromosomes and numerous morphologically indistinguishable microchromosomes. The Falconinae of the Falconiformes has a different karyotype from the typical avian karyotype in low chromosome numbers, little size difference between macrochromosomes and a smaller number of microchromosomes. To characterize chromosome structures of Falconinae and to delineate the chromosome rearrangements that occurred in this subfamily, we conducted comparative chromosome painting with chicken chromosomes 1–9 and Z probes and microchromosome-specific probes, and chromosome mapping of the 18S–28S rRNA genes and telomeric (TTAGGG)_n sequences for common kestrel (*Falco tinnunculus*) ($2n = 52$), peregrine falcon (*Falco peregrinus*) ($2n = 50$) and merlin (*Falco columbarius*) ($2n = 40$). *F. tinnunculus* had the highest number of chromosomes and was considered to retain the ancestral karyotype of Falconinae; one and six centric fusions might have occurred in macrochromosomes of *F. peregrinus* and *F. columbarius*, respectively. Tandem fusions of microchromosomes to macrochromosomes and between microchromosomes were also frequently observed, and chromosomal locations of the rRNA genes ranged from two to seven pairs of chromosomes. These karyotypic features of Falconinae were relatively different from those of Accipitridae, indicating that the drastic chromosome rearrangements occurred independently in the lineages of Accipitridae and Falconinae.

Abbreviations

BrdU 5-bromodeoxyuridine
CCD charge-coupled device
FCO *Falco columbarius*
FISH fluorescence *in-situ* hybridization

FPE *Falco peregrinus*
FTI *Falco tinnunculus*
GGA *Gallus gallus domesticus*
rRNA ribosomal RNA
UV ultraviolet

Introduction

Avian karyotypes are usually classified into two major groups. One is the typical avian karyotype characterized by a large diploid chromosome number (around $2n = 80$), which shows a typical chromosome organization with 7–10 pairs of large- and medium-sized macrochromosomes and 30–33 pairs of morphologically indistinguishable microchromosomes or with groups A–D (Masabanda *et al.* 2004). This karyotype has been widely conserved in all palaeognathous birds and most of neognathous bird species as the ancestral state of avian karyotypes (Takagi & Sasaki 1974, Belterman & de Boer 1984, Shetty *et al.* 1999, Nishida-Umehara *et al.* 2007). The other one is a strikingly different karyotype with lower chromosome numbers, which is widely found in Accipitridae of the Falconiformes. Their karyotypes usually have a moderate diploid number around 66 chromosomes, exhibiting many medium- and small-sized, mostly bi-armed macrochromosomes and only few microchromosomes. This atypical organization of chromosomes in Accipitridae has been confirmed in many species including hawks, harriers, buzzards, kites, eagles and Old World vultures (de Boer 1976, de Boer & Sinoo 1984, Padilla *et al.* 1999, Bed'Hom *et al.* 2003). The large number of medium- and small-sized macrochromosomes that gradually decrease in size and the extreme reduction of microchromosomes suggest that chromosome rearrangements in Accipitridae favour the formation of macrochromosomes and the disappearance of microchromosomes.

These morphological similarities or differences of avian karyotypes between different species have been investigated by conventional Giemsa-staining and chromosome banding. Cross-species chromosome painting (termed Zoo-FISH) provides a direct, genome-wide view of chromosome homology between phylogenetically distant species and of chromosome rearrangements that have occurred in each lineage of species since they diverged from a common ancestor (Scherthan *et al.* 1994, Wienberg & Stanyon 1995, Wienberg 2004). Lately, chromosome-specific DNA painting probes for chromosomes 1–9 and Z and fractions of microchromosomes have been developed in chicken (*Gallus gallus domesticus*) (Griffin *et al.* 1999, Habermann *et al.* 2001, Masabanda *et al.* 2004).

To date, comparative chromosome painting with the chicken probes has been performed for a total of 40 species from 10 orders (Shetty *et al.* 1999, Schmid *et al.* 2000, Raudsepp *et al.* 2002, Guttenbach *et al.* 2003, Kasai *et al.* 2003, Derjusheva *et al.* 2004, Shibusawa *et al.* 2004a,b, de Oliveria *et al.* 2005, Itoh & Arnold 2005, Nanda *et al.* 2006, 2007, Griffin *et al.* 2007, Nishida-Umehara *et al.* 2007). These results revealed that the avian karyotypes are highly conserved at the molecular level, and that the typical avian karyotype with a small number of macrochromosomes and a large number of microchromosomes is mostly conserved and hence representative of the ancestral state. In contrast to the slow rate of chromosome rearrangements in most of bird species (Burt *et al.* 1999), chromosomes have been drastically reconstructed in Accipitridae of the Falconiformes. Chromosome painting with chicken probes for four Accipitridae species, the Harpy eagle (*Harpia harpia*) (de Oliveria *et al.* 2005) and three Old World vultures (*Gyps ruppelli*, *Gyps fulvus* and *Gypaetus barbatus*) (Nanda *et al.* 2006) confirmed the dramatic reduction of microchromosomes by fusions and translocations of microchromosomes and the increase of bi-armed medium- and small-sized macrochromosomes by repeated fission and fusion events and many other types of rearrangements in macrochromosomes.

DNA–DNA hybridization and the nucleotide sequences of the mitochondrial cytochrome *b* gene suggest that Falconidae and Accipitridae are clustered as sister groups (Sibley & Ahlquist 1990, Seibold & Helbig 1995), which are classified in a different clade from American vultures (Cathartidae) and storks and ibises (Ciconiidae) that are now recognized as the Ciconiiformes (Sibley & Ahlquist 1990, Avise *et al.* 1994, Lerner & Mindell 2005). This phylogenetic relationship is not contradicted from the cytogenetic data that the karyotypes of Falconidae are more similar to those of Old World vultures (Accipitridae) than New World vultures (Cathartidae) (Takagi & Sasaki 1974, de Boer 1976, Belterman & de Boer 1984, Schmutz & Oliphant 1987, Padilla *et al.* 1999, Raudsepp *et al.* 2002). However, Falconidae species exhibit a variant karyotype that comprises all or mostly acrocentric chromosomes, relatively different from the features of Accipitridae karyotypes. There is karyological

heterogeneity in Falconidae; the karyotypes of which are subdivided into two groups by chromosome number. One is the karyotype with lower diploid chromosome numbers ranging from 40 to 54, comprising 7–11 pairs of large- and medium-sized, mostly acrocentric chromosomes and around 13–16 pairs of microchromosomes for *Falco* species. The other is the karyotype with higher diploid chromosome numbers ranging from 84 to 90, comprising 12–15 pairs of large- and medium-sized acrocentric chromosomes and around 30 pairs of microchromosomes for *Polyborus*, *Milvago* and *Phalcoboenus* species (de Boer 1975, 1976, Belterman & de Boer 1984, 1990, Sasaki *et al.* 1984). The family Falconidae is composed of 61 species in 10 genera, which are divided into two subfamilies: Polyborinae comprising 16 species of six genera, and Falconinae comprising 45 species of four genera (del Hoyo *et al.* 1994). On the basis of the nucleotide sequences of mitochondrial and nuclear genes and the morphological characters, Griffiths (1999) and Griffiths *et al.* (2004) classified the family as follows: (1) Falconinae comprising two tribes, Falconini including *Falco*, and Caracarini including *Phalcoboenus*, *Milvago* and *Polyborus* (*Caracara*), and (2) Herpetotherinae comprising *Herpetotheres* and *Micrastur*. Classification of the Falconidae remains unclear, and no karyotypes of Herpetotherinae have been reported; this difference in the karyotypes, however, parallels the phylogenetic relationship of the two groups. However, no molecular cytogenetic characterization has been performed for Falconidae species; there is therefore little knowledge about the features of their chromosome structures at the molecular level and the process of chromosome rearrangements that have occurred during the evolution of this family.

Here we performed molecular cytogenetic characterization of the chromosome components of Falconinae employing chromosome painting with chicken DNA probes and chromosome mapping of the 18S–28S ribosomal RNA genes and telomeric (TTAGGG)_n sequences for three *Falco* species, common kestrel, peregrine falcon and merlin. We delineate chromosome homology between chicken and the three *Falco* species and discuss the process of chromosomal rearrangements that have occurred in the lineage of Falconinae.

Materials and methods

Specimens, cell culture and chromosome preparation

Three Falconinae species were used in this study: common kestrel (*Falco tinnunculus*), peregrine falcon (*Falco peregrinus*) and merlin (*Falco columbarius*). Small pieces of skin tissue were taken by biopsy, and the fibroblasts were cultured in 199 medium supplemented with 15% fetal bovine serum at 39°C in 5% CO₂. Replication R-banded chromosomes were prepared as described previously (Matsuda & Chapman 1995). 5-Bromodeoxyuridine (BrdU) (25 µg/ml) was added to the culture medium at log phase, and the cell culturing was continued for an additional 5 h including 30 min of colcemid (0.025 µg/ml) treatment before harvesting. The chromosome slides were stained with Hoechst 33258 (1 µg/ml) for 5 min, and then were heated to 70°C for 3 min on a hotplate and subsequently exposed to UV light at 70°C for an additional 5 min.

DNA probes

Chicken (*Gallus gallus domesticus*, GGA) chromosome-specific DNA probes of chromosomes 1–9 and Z (GGA1–9 and GGAZ) (Griffin *et al.* 1999) and a mixture of microchromosome-specific paints, which can detect 19 different pairs of chicken microchromosomes (Habermann *et al.* 2001, de Oliveira *et al.* 2005), were used for chromosome painting. For chromosome mapping of the 18S–28S ribosomal RNA genes, the 5.8 kb pHr21Ab and 7.3 kb pHr14E3 fragments of the human ribosomal RNA genes provided by the Japanese Cancer Research Resource Bank (JCRRB), Tokyo, were used. A commercial biotin-labelled 42 bp oligonucleotide probe complementary to telomeric (TTAGGG)_n sequences was used for chromosomal mapping of telomeres.

Fluorescence in-situ hybridization (FISH)

Comparative chromosome painting with chicken probes was performed as described previously (Nishida-Umehara *et al.* 2007). Chromosomal localization of the 18S–28S rRNA genes and telomeric repeats was performed as described in Matsuda & Chapman (1995). The FISH images were captured

using a cooled CCD camera (MicroMAX 782Y, Princeton Instruments) mounted on a Leica DMRA microscope, and analysed with the 550CW-QFISH application program of Leica Microsystems Imaging Solutions Ltd (Cambridge, UK).

Results

Interspecific chromosome homology

Chromosome-specific paints of chicken chromosomes 1–9 and Z (GGA1–9 and GGAZ) and a microchromosome-specific paint pool of 19 chicken microchromosomes all efficiently cross-hybridized to

metaphase chromosomes of the three species. The chromosome painting patterns of the three species are shown in Figure 1. Hoechst-stained bands obtained by the replication R-banding method, which correspond to G-bands, made it possible to identify each chromosome for the three species. The homology with chicken chromosomes was depicted on Hoechst-stained karyotypes, of which chromosomes were arranged according to our previous report (Sasaki *et al.* 1984) for *F. tinnunculus* (FTI) and *F. peregrinus* (FPE) and Longmire *et al.* (1988) for *F. columbarius* (FCO) (Figures 2, 3, 4). Homologous chromosomes and chromosome arms between chicken and the three *Falco* species are summarized in Table 1. The large-sized microchromosomes

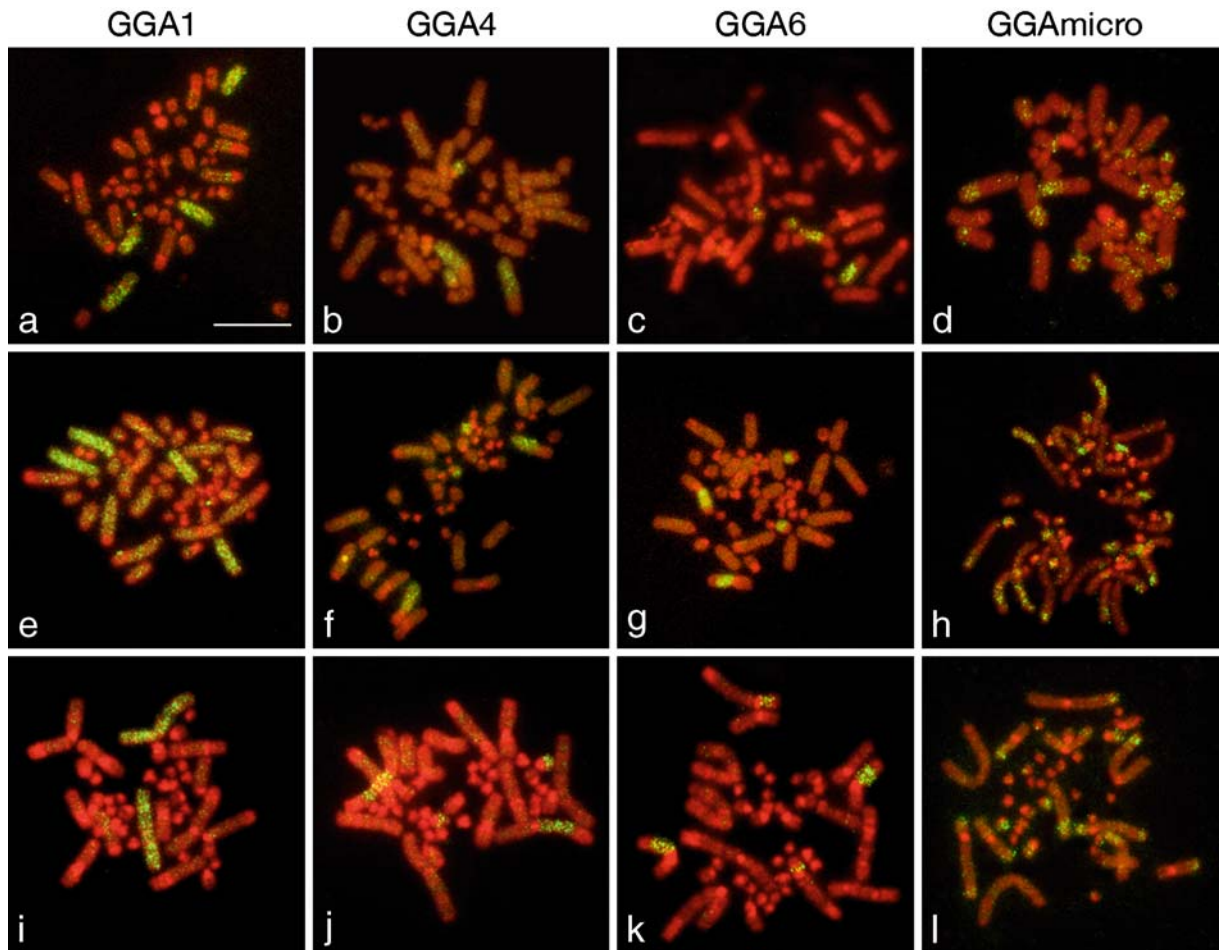


Figure 1. Chromosome hybridization patterns with chicken chromosome 1, 4 and 6 paints (GGA1, GGA4, GGA6) and a paint pool of 19 microchromosomes (GGAmicro) to PI-stained metaphase chromosome spreads of *F. tinnunculus* (a–d), *F. peregrinus* (e–h) and *F. columbarius* (i–l). Scale bar represents 10 μ m. All the images of each species are shown at the same magnification.

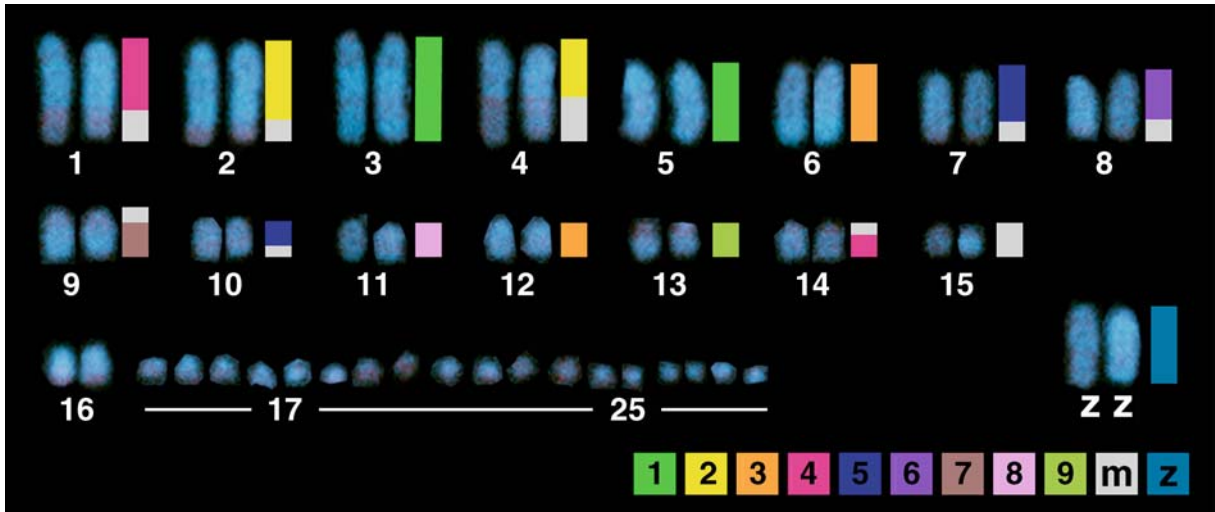


Figure 2. Hoechst-banded karyotypes of *F. tinnunculus* with the assignment of homology with chicken chromosomes delineated by chromosome painting with chicken probes. The conserved chromosome segments to chicken chromosomes 1–9 and Z and a pool of 19 microchromosomes are represented by 11 different colours.

(FTI10–15, FPE9–14, FCO7–9) were numbered according to physical size, although the size difference between the chromosomes was not distinct.

The karyotype of *F. tinnunculus* ($2n = 52$) was composed of all acrocentric chromosomes except for the submetacentric W chromosome (Giemsa-stained karyotype is not shown). GGA1–9 and Z probes detected 15 conserved segments between chicken chromosomes and *F. tinnunculus* chromosomes (Figure 2). GGA1 and GGA2 each hybridized to

two macrochromosomes (FTI3 and FTI5, and FTI2 and FTI4, respectively). GGA3, GGA4 and GGA5 each hybridized to one macrochromosome (FTI6, FTI1 and FTI7, respectively) and one large-sized microchromosome (FTI12, FTI14 and FTI10, respectively). GGA6–9 paints each hybridized to a single chromosome (FTI8, 9, 11 and 13, respectively), and GGAZ corresponded to FTIZ. The microchromosome-specific paint pool hybridized to nine chromosome segments: one large-sized microchromosome (FTI15),

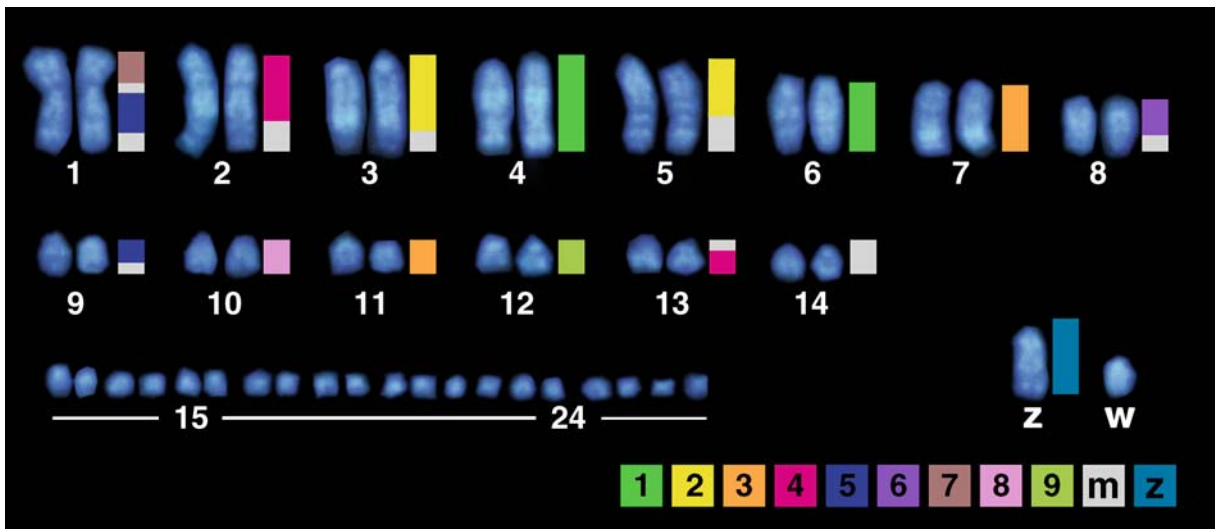


Figure 3. Hoechst-banded karyotypes of *F. peregrinus* with the assignment of homology with chicken chromosomes delineated by chromosome painting with chicken probes.

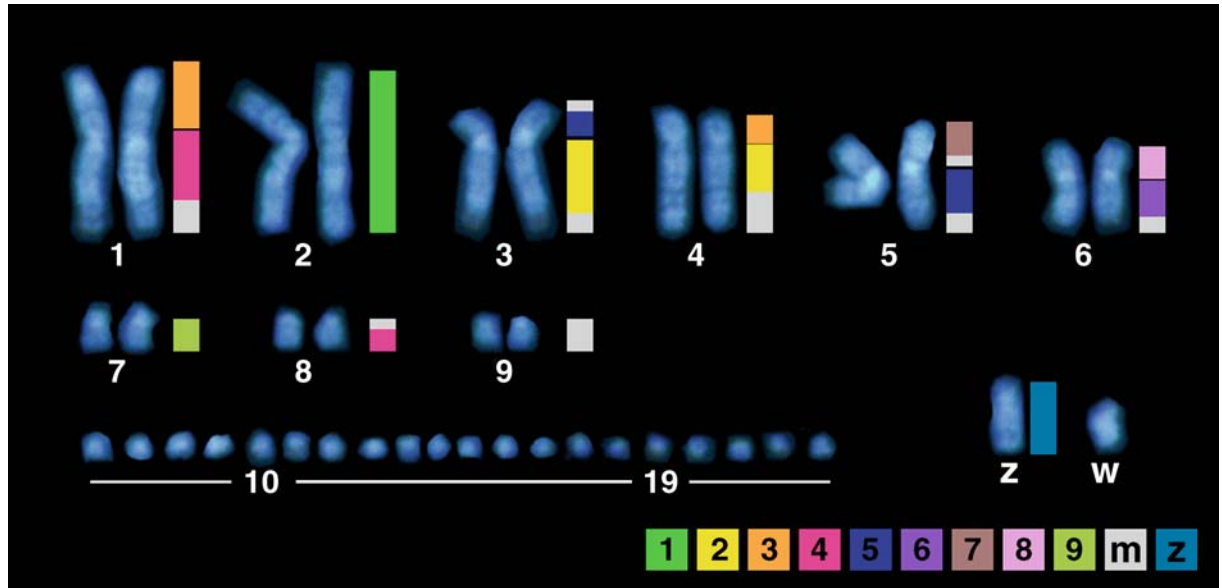


Figure 4. Hoechst-banded karyotypes of *F. columbarius* with the assignment of homology with chicken chromosomes delineated by chromosome painting with chicken probes.

the distal ends of FTI1, 2, 4, 7, 8 and 10, and the proximal ends of FTI9 and FTI14. No hybridization signal was detected on FTI16 with large centromeric heterochromatin block (C-banded karyotype is not shown), which should be categorized with small-sized microchromosomes. The other nine pairs of small-sized microchromosomes also showed no hybridization signals with GGA probes used in this study.

In *F. peregrinus* female ($2n = 50$) all chromosomes except for one pair of large metacentric macrochromosomes (chromosome 1) were acrocentric. The hybridization pattern of *F. peregrinus* chromosomes with GGA macrochromosome paints was the same as that of *F. tinnunculus* except for acrocentric chromosomes 7 and 9 of *F. tinnunculus* and metacentric chromosome 1 of *F. peregrinus* (Figure 3). The homology with chicken chromosomes revealed that FPE1p and FPE1q were homologous to FTI9 and FTI7, respectively, and, therefore,

FPE2, 3, 4, 5, 6, 7 and 8 corresponded to FTI1, 2, 3, 4, 5, 6 and 8, respectively. The pericentromeric region on the p arm of FPE1 was painted with the microchromosome-specific probe, indicating that FPE1 was derived from a centric fusion between FTI7 and FTI9, not a tandem fusion. The hybridization patterns of microchromosomes in FPE were also the same as those in FTI: FPE9, 10, 11, 12, 13 and 14 were homologous to FTI10, 11, 12, 13, 14 and 15, respectively.

The karyotype of *F. columbarius* ($2n = 40$) differed from those of the other two species in possessing the fewest chromosome number and six pairs of large bi-armed macrochromosomes (Figure 4). The seventh largest Z chromosome was acrocentric, and the medium-sized W chromosome was subtelocentric. The largest microchromosome (chromosome 7) was subtelocentric with heterochromatic short arm (C-banded karyotype is not shown). The p and q arms

Table 1. Homologous chromosomes and chromosome segments between chicken and three *Falco* species as detected by chromosome painting using chicken macrochromosome probes

Species	2n	Chromosome									
<i>Gallus gallus domesticus</i>	78	1	2	3	4	5	6	7	8	9	Z
<i>Falco tinnunculus</i>	52	3 + 5	2 + 4	6 + 12	1 + 14	7 + 10	8	9	11	13	Z
<i>Falco peregrinus</i>	50	4 + 6	3 + 5	7 + 11	2 + 13	1q + 9	8	1p	10	12	Z
<i>Falco columbarius</i>	40	2	3q + 4q	1p + 4p	1q + 8	3p + 5q	6q	5p	6p	7q	Z

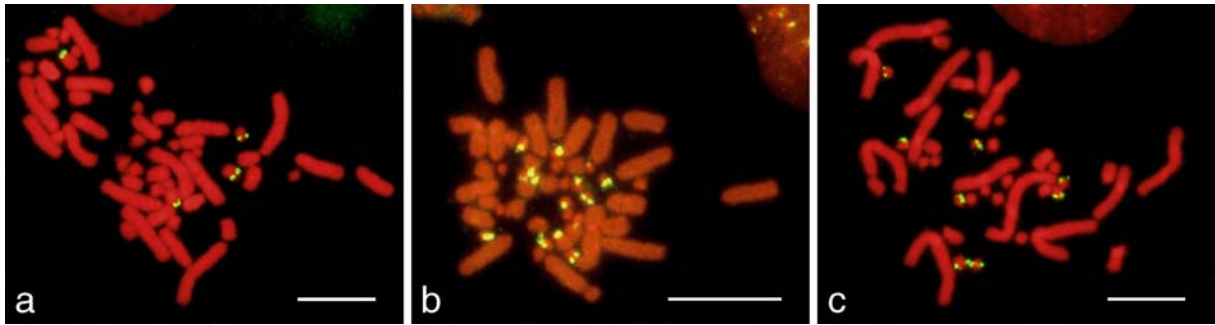


Figure 5. Chromosomal distribution of the 18S–28S ribosomal RNA genes. (a) *F. tinnunculus*; (b) *F. peregrinus*; (c) *F. columbarius*. Scale bars represent 10 μm .

of the largest chromosome (FCO1) corresponded to FTI6 and FTI1, respectively, and FCO3p and FCO3q were homologous to FTI10 and FTI2, FCO4p and FCO4q to FTI12 and FTI4, FCO5p and FCO5q to FTI9 and FTI7, and FCO6p and FCO6q to FTI11 and FTI8, respectively. The p and q arms of the second largest pair (FCO2) were both painted with GGA1 probe, which hybridized to two pairs of acrocentric macrochromosomes in *F. tinnunculus* and *F. peregrinus* (FTI5 and FTI3, and FPE6 and FPE4, respectively). The other large-sized microchromosomes FCO7, FCO8 and FCO9 were homologous to FTI13, FTI14 and FTI15, respectively.

Chromosomal location of the 18S–28S rRNA genes and (TTAGGG)_n sequences

The 18S–28S rRNA genes were localized to two pairs of microchromosomes for one *F. tinnunculus*

male, 12–14 microchromosomes for one *F. peregrinus* male and female, and nine microchromosomes for one *F. columbarius* female (Figure 5).

The signals of the (TTAGGG)_n sequences were observed on both telomeric ends of all chromosomes in the three species (Figure 6). Interstitial signals were only found around the centromere on the long arm of chromosome 3 of *F. columbarius* (Figure 6c). The location of the interstitial signals corresponded to the fusion point between FTI2 and FTI10. A large number of copies of the (TTAGGG)_n sequences were accumulated on 10 pairs of microchromosomes in *F. peregrinus* and on the centromeric regions of the Z and W chromosomes in *F. columbarius*.

Discussion

Molecular cytogenetic characterization of three *Falco* species by chromosome painting with chicken

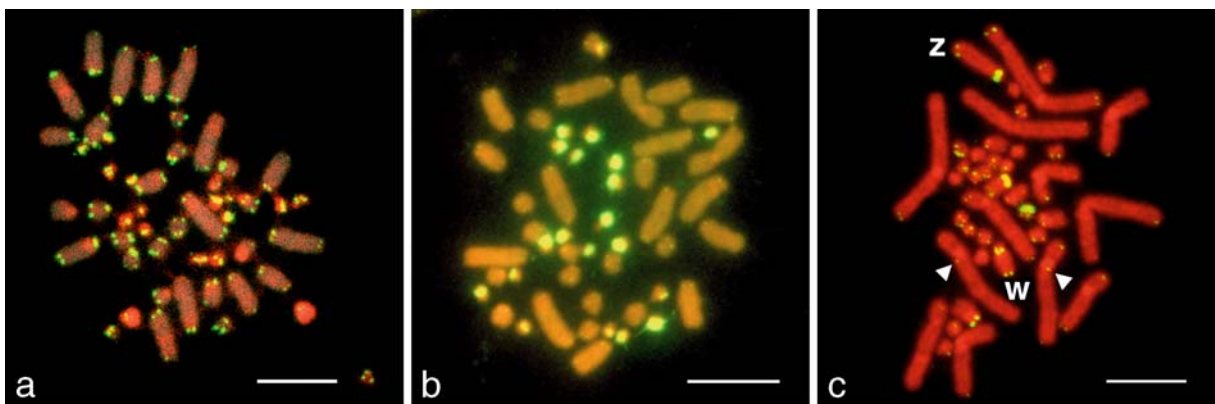


Figure 6. FISH pattern of telomeric (TTAGGG)_n sequences. (a) *F. tinnunculus*; (b) *F. peregrinus*; (c) *F. columbarius*. Arrowheads indicate interstitial signals of the repeated sequences. Scale bars represent 10 μm .

probes demonstrated the difference in the features of karyotypic evolution between Falconinae and Accipitridae, suggesting that drastic chromosome rearrangements such as found in Accipitridae have not occurred in this family. The karyotypes of *Falco* species have been reported for 10 species out of 37 or 39 species (Sibley & Monroe 1990, del Hoyo *et al.* 1994): *Falco columbarius* ($2n = 40$), *F. mexicanus* ($2n = 48$), *F. chicquera*, *F. jugger*, *F. sparverius*, *F. subbuteo* and *F. peregrinus* ($2n = 50$), *F. rusticolus* and *F. tinnunculus* ($2n = 52$), and *F. biarmicus*

($2n = 52$ or 54) (de Boer 1976, Belterman & de Boer 1984, Sasaki *et al.* 1984, Schmutz & Oliphant 1987, Longmire *et al.* 1988, Nishida *et al.* unpublished data, present study). On the basis of our Zoo-FISH data of three *Falco* species and the published data of Giemsa-stained karyotypes of the other seven species, we deduced the process of karyotypic evolution in the genus *Falco* by the most parsimonious events of chromosome rearrangements. Although reciprocal painting data are necessary to identify the true homology of chromosome segments with chicken

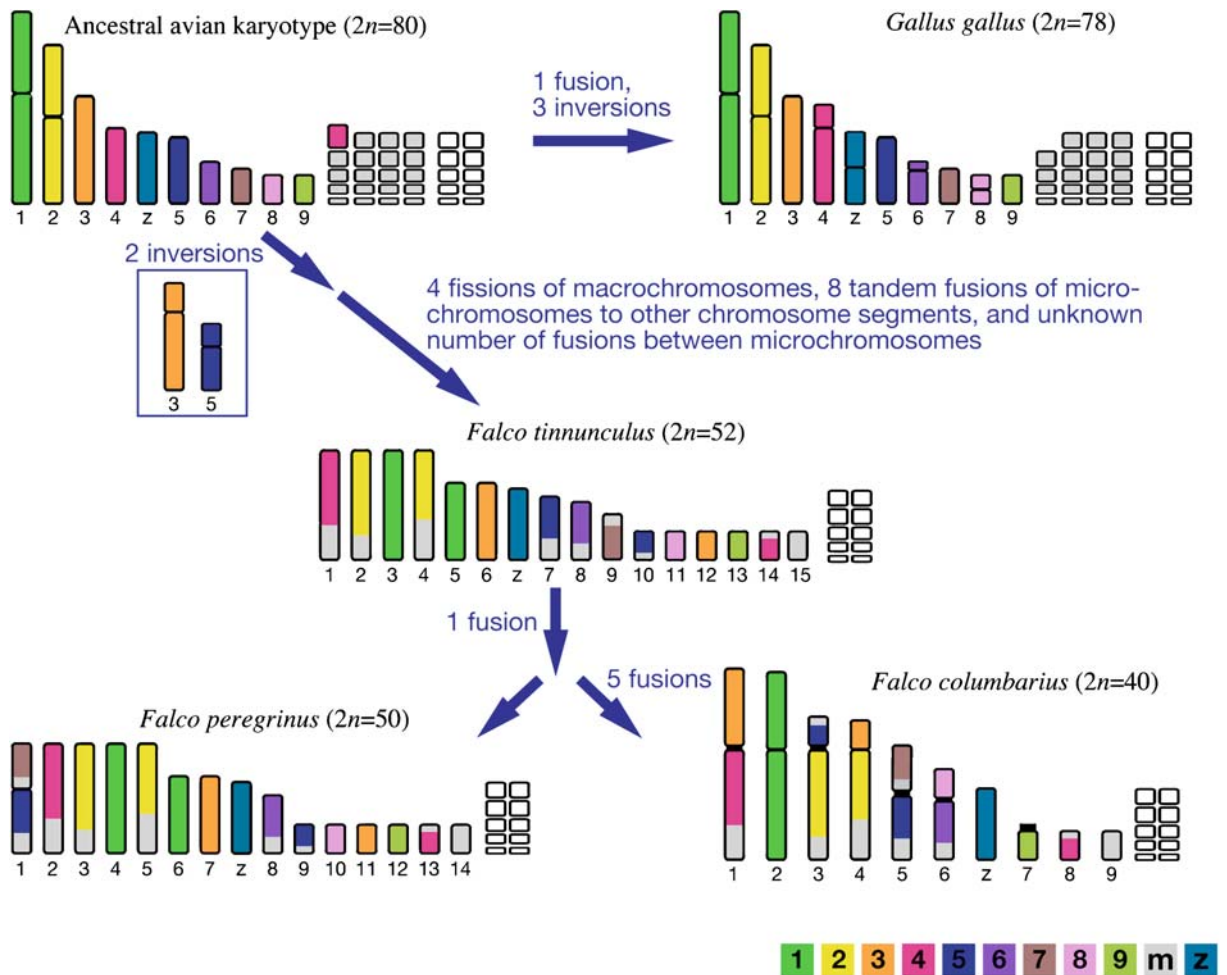


Figure 7. Schematic representation of the putative ancestral avian karyotype and the process of karyotypic evolution in three *Falco* species, *F. tinnunculus*, *F. peregrinus* and *F. columbarius*, after divergence from the common ancestor. The comparative cytogenetic maps showing chromosome homology between chicken and three *Falco* species were constructed by comparative chromosome painting with chicken probes. The homologous chromosome segments with chicken chromosomes 1–9 and Z and a pool of 19 microchromosomes are represented by 11 different colours. Ten pairs of microchromosomes, which were not painted with the probes used in this study, are shown as white-painted boxes. The direction of karyotypic evolution is shown by arrows with the events of chromosome rearrangements that occurred in the species during the evolutionary process from the ancestral avian karyotype.

chromosomes, we concluded that the ancestral karyotype of the genus *Falco* was probably $2n = 52$ or 54 , comprising all acrocentric macrochromosomes including Z and W chromosomes and acrocentric microchromosomes. According to our scheme shown in Figure 7, the karyotype of *F. tinnunculus*, which consists of all acrocentric chromosomes except for the submetacentric W chromosome, was considered to retain the ancestral state of Falconinae karyotypes. Six centric fusions of acrocentric chromosomes are required to transform the *F. tinnunculus* karyotype to the *F. columbarius* karyotype with six pairs of bi-armed macrochromosomes. *F. columbarius* chromosome 5 (FCO5) probably corresponded to *F. peregrinus* chromosome 1 (FPE1), which might have resulted from a centric fusion between *F. tinnunculus* chromosome 7 (FTI7) and chromosome 9 (FTI9). Both the p and q arms of FCO2 were painted with GGA1. The probability is that the bi-armed FCO2 was the result of a centric fusion between FTI3 and FTI5 rather than that it was originally contained in the ancestral karyotype of Falconinae, because the acrocentric FTI3 and FTI5 was considered to have been derived from a centric fission of the bi-armed chromosome 1 of the ancestral avian karyotype as described below.

Our previous study of five Struthioniformes species (emu, double-wattled cassowary, ostrich, greater rhea and lesser rhea) and one Tinamiformes species (elegant crested tinamou) showed that each chicken probe hybridized to a single pair of chromosomes for all six species with the exception that GGA4 hybridized to the fourth largest chromosome and a single pair of microchromosomes (Nishida-Umehara *et al.* 2007). The GGA4 probe consistently hybridizes to a single macrochromosome and a pair of smaller chromosomes (homologous to turkey chromosome 9) in many diverged bird karyotypes (Griffin *et al.* 2007), indicating that the submetacentric chicken chromosome 4 resulted from a centric fusion between an ancestral acrocentric chromosome 4 (GGA4q) and an ancestral smaller chromosome (GGA4p) (Schmid *et al.* 2000, Raudsepp *et al.* 2002, Shibusawa *et al.* 2002, 2004a,b). Except for a centric fusion in chromosome 4 and pericentric inversions in chromosomes 6, 8 and Z, the chicken appears to retain the ancestral karyotype of many other avian orders with diploid chromosome numbers of around 80 (Schmid *et al.* 2000, Guttenbach *et al.* 2003, Shibusawa *et al.* 2004b, Griffin *et al.* 2007). In

Falco species also the homologous region of GGA4 was observed on a single macrochromosome or chromosome arm (FTI1, FPE2, FCO1q) and a single large-sized microchromosome (FTI14, FPE13, FCO8). The establishment of the *F. tinnunculus* karyotype from the ancestral avian karyotype can probably be explained by four fissions of macrochromosomes, which occurred in the ancestral chromosomes 1, 2, 3 and 5, and at least eight tandem fusions of microchromosomes to chromosome segments painted with GGA macrochromosomes. For the occurrence of centric fissions of the ancestral chromosomes 3 and 5, pericentric inversion events should have occurred in the two chromosomes before the centric fission events, because they were probably acrocentric in the ancestral avian karyotype. After the fission of four macrochromosomes, tandem fusions with microchromosomes might have subsequently occurred in the distal ends of chromosomes 1, 2, 4, 7, 8 and 10 and the proximal ends of chromosomes 9 and 14 in the ancestral karyotype of Falconinae. The microchromosome-specific paint pool, which was composed of 19 pairs of microchromosomes, hybridized only to eight chromosome segments and a single large-sized microchromosome. Apparently, the homologues of chicken microchromosomes were frequently fused to other microchromosomes as found in FTI14, which resulted from a tandem fusion between a microchromosome homologous to GGA4p and another microchromosome.

Gain of telomeric repeated sequences can be expected in the fusion points; however, the potential retention of telomeric (TTAGGG)_n sequences at the interstitial chromosome sites was hardly found in the three *Falco* species. Interstitial TTAGGG repeats are frequently observed in chicken and palaeognathous bird species (Nanda *et al.* 2002, Nishida *et al.* unpublished data) but they have not been found in Accipitridae (Bed'Hom *et al.* 2003, de Oliveira *et al.* 2005). Gradual shortening and degradation of non-functional repeated sequences may lead to loss of non-telomeric TTAGGG arrays. Centromere sequences must also have been gained at the interstitial sites by tandem fusion between telomeric ends and centromeres, but the presence of centromere sequences in the fusion points has not been examined. Molecular cloning of centromeric repetitive sequences from Falconinae species and their characterization are needed to investigate this possibility.

In conclusion, our Zoo-FISH data of three *Falco* species suggest that the karyotypes of Falconinae are relatively conserved and much closer to the ancestral avian karyotype than those of Accipitridae. It is conceivable that more extensive chromosome rearrangements have occurred in the lineage of Accipitridae than that of Falconinae after they diverged from the common ancestor. The other subfamily of Falconidae, Polyborinae including *Polyborus plancus* ($2n = 82-86$), *Milvago chimachima* ($2n = 86$) and *Phalcoboenus megalopterus* ($2n = 90$), exhibits higher diploid chromosome numbers with much larger numbers of small-sized and dot-shaped microchromosomes (de Boer 1975, 1976, Belterman & de Boer 1984, 1990, Sasaki et al. 1984). These results provide a possibility that the fusion events between macro- and microchromosomes and between microchromosomes have hardly occurred in this group, suggesting that Polyborinae probably retain the ancestral state of Falconidae karyotypes. An extension of chromosome painting studies to more species of Falconidae including Polyborinae and comparison of the molecular cytogenetic data associated with their molecular phylogenetic analysis would be desirable to accurately delineate the process of karyotypic evolution in the Falconiformes with atypical karyotypes.

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