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ORIGINAL ARTICLE

# Segregation distortion in chicken and the evolutionary consequences of female meiotic drive in birds

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As all four meiotic products give rise to sperm in males, female meiosis result in a single egg in most eukaryotes. Any genetic element with the potential to influence chromosome segregation, so that it is preferentially included in the egg, should therefore gain a transmission advantage; a process termed female meiotic drive. We are aware of two chromosomal components, centromeres and telomeres, which share the potential to influence chromosome movement during meioses and make the following predictions based on the presence of female meiotic drive: (1) centromere-binding proteins should experience rapid evolution as a result of a conflict between driving centromeres and the rest of the genome; and (2) segregation patterns should be skewed near centromeres and telomeres. To test these predictions, we first analyze the molecular evolution of seven centromere-binding proteins in nine divergent bird species. We find strong evidence for positive selection in two genes, lending support to the genomic conflict hypothesis. Then, to directly test for the presence of segregation distortion, we also investigate the transmission of ~9000 single-nucleotide polymorphisms in 197 chicken families. By simulating fair Mendelian meioses, we locate chromosomal regions with statistically significant transmission ratio distortion. One region is located near the centromere on chromosome 1 and a second region is located near the telomere on the p-arm of chromosome 1. Although these observations do not provide conclusive evidence in favour of the meiotic drive/genome conflict hypothesis, they do lend support to the hypothesis that centromeres and telomeres drive during female meioses in chicken.
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## Introduction

Random Mendelian segregation of alleles from parents to offspring is a fundamental principle in genetics. However, numerous processes such as true meiotic drive, gametic selection, postzygotic viability selection and maternal–fetal incompatibilities have the potential to distort segregation ratios away from the Mendelian expectation. Most scientific attention has so far been directed towards post-meiotic processes, although the frequency and biological significance of true segregation distortion (SD) during meiosis remains poorly characterized (Pardo-Manuel de Villena and Sapienza, 2001b). In this study, we first discuss the potential for SD during avian meiosis, and then use two independent strategies to look for signs of SD in birds.

Pardo-Manuel de Villena and Sapienza (2001b) identified three conditions required for non-random segregation of chromosomes during meiosis. The first condition implies that the meiotic division needs to be asymmetric with regard to cell fate. In birds, as in many other eukaryotes, female meiosis proceeds through two rounds of asymmetrical cell divisions, each of which produce an oocyte and a polar body, in the end resulting in the production of only one functional gamete (Pardo-Manuel de Villena and Sapienza, 2001b; Rutkowska and Badyaev, 2008). This is in contrast to the symmetrical male meiosis, in which all four chromatids present before cell division will be represented in the final gametes. If chromosomes are partitioned non-randomly among oocyte and polar body, this asymmetry will lead to SD during female meiosis (Pardo-Manuel de Villena and Sapienza, 2001b).

The second prerequisite for SD is the presence of functional asymmetry of the spindle poles, that is, so that one pole leads to the oocyte, whereas the other leads to the polar body. Although experimental evidence for spindle pole asymmetry has been presented from insects and plants (Pardo-Manuel de Villena and Sapienza, 2001b), so far, no such evidence, nor apparent physical differences in comparisons of oocyte spindle poles have been detected in birds (Rutkowska and Badyaev, 2008). However, non-random segregation of chromosome rearrangements in chicken do indicate that spindle pools may be asymmetric in birds as well (Dinkel *et al.*, 1979).

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Third, populations should harbour genetic variation at loci implemented in the interaction between chromosome and spindle, so that alleles are transmitted to the oocyte with different probability (Pardo-Manuel de Villena and Sapienza, 2001b). There are several potential mechanisms (and hence loci) for influencing the interaction between chromosome and spindle during avian meiosis:

(1) Before spindle attachment (during meiotic prophase), telomeres move along the nuclear envelope and cluster to form the 'bouquet', in a manner that is likely to be conserved in a wide variety of organisms (Chikashige *et al*., 2007; Rutkowska and Badyaev, 2008). It is thus possible that telomere-associated chromosome movement could affect the orientation of chromosomes, so as to facilitate attachment to the preferential spindle pole (Zwick *et al*., 1999). Interestingly, the chicken genome harbours considerably more telomeric DNA compared with the human genome, and bird genomes in general contain repeats belonging to the longest class of telomere repeats (mega-telomeres) known in any vertebrate genome (200 Kb to 3 Mb) (Delany *et al*., 2007). Avian telomeres may thus be good candidates for SD.

(2) Also before spindle attachment, chromosomes congress to the spindle equator; a process that is guided by actin networks and probably conserved in all vertebrates (Lenart *et al*., 2005). Again, the spatial orientation of chromosomes after delivery by the actin network may be important for the outcome of the subsequent chromosome segregation (Rutkowska and Badyaev, 2008). If specific chromosomal elements are responsible for the interaction with actin filaments, it is possible that they too may have the potential to affect the orientation of chromosomes at delivery. Little is, however, known about the interaction between chromosome and the actin network.

(3) Centromeres represent the most obvious genetic elements with ability to influence the interaction between chromosome and spindle. Via their binding to kinetochore proteins, centromeres interact directly with the spindle microtubuli (Henikoff *et al*., 2001) and it is possible that different centromere repeat alleles may vary in ability to attract microtubuli from the oocyte side of an asymmetric spindle pole (Pardo-Manuel de Villena and Sapienza, 2001b).

(4) Finally, in birds, cohesion proteins aggregate into protein bodies that assemble adjacent to centromeres (Krasikova *et al*., 2005). These protein bodies vary in size among and within bird species (Krasikova *et al*., 2006) and it has been suggested that they may interfere with spindle attachment to the centromere (Rutkowska and Badyaev, 2008). The interaction between protein bodies and centromeres may thus represent an additional, but so far poorly characterized, route to SD at centromeres.

In summary, two chromosomal elements, centromeres and telomeres, emerge as good candidates for SD during female meiosis in birds. For centromeres, it has been hypothesized that drive is achieved through expansions in the centromere repeat array, whereby more kinetochore proteins are allowed to bind to the centromere (Sandler and Novitski, 1957). This should then attract the preferential spindle pole and lead to the inclusion of the driving centromere in the egg. Centromeric drive may, however, lead to deleterious effects such as non-disjunction and sterility in males (Pardo-Manuel de Villena and Sapienza, 2001b). Mutations that alter the binding specificity of kinetochore proteins should then be selected for to restore Mendelian segregation among centromere alleles, thus preventing further negative fitness effects. A continued evolutionary dynamics then arises, in which evolutionary changes in centromeres are followed by changes in kinetochore proteins and *vice versa*. Such dynamics would lead to a high rate of evolution in kinetochore proteins, despite their strong functional importance. This theory also predicts the existence of intermittent SD around centromeres. We call this theory the Genomic Conflict Theory.

Several observations provide indirect support for the hypothesis of centromeric drive in female meioses. First, in several known non-random segregation systems there is a difference in the number of centromeres between paired chromosomes; this is the case for B chromosomes, Robertsionian translocations, chromosome fissions and X0 females. Also, in maize, 'knobs' act as neocentromeres that are able to direct the preferential movement of the chromatid carrying it (Henikoff *et al*., 2001). Second, even though centromeres are universally conserved in function, the centromeric repeats themselves undergo rapid evolution and differ between closely related species (Malik and Henikoff, 2002). The centromere of the human X chromosome has a core of homogeneous satellite repeats outside which it gradually becomes less conserved in a pattern that probably reflects the continuous renewal of the centromere sequence at the centre (Malik and Henikoff, 2002). Third, selective sweeps seem to be common in centromeric regions in human (Williamson *et al*., 2007; Hellmann *et al*., 2008), and finally, kinetchore proteins have been shown to evolve rapidly in plants, flies and mammals (Talbert *et al*., 2004).

Direct evidence for meiotic drive at centromeres has, however, been difficult to document. This could in part be due to the transient nature of polymorphisms responsible for drive, as the preferred alleles will rapidly go to fixation due to the drive itself. In addition, the repetitive nature of centromere sequence makes centromere evolution inherently difficult to study directly. Recently though, Fishman and Saunders (2008), in a hybrid cross of two species of the plant genus Mimulus, found the first direct evidence for SD at loci linked to a centromere during female meiosis.

Despite progress in our understanding of telomere-associated chromosome movement during meiosis (Dresser, 2008), it is not clear how telomeres would achieve drive mechanistically. Overall, the potential for telomere drive has been explored relatively little, despite early observations in *Drosophila* showing that distally located chromosomal elements have the ability to alter a chromosome's chances of inclusion in the egg (Novitski, 1951). Indirectly, observations of high rates of subtelomeric sequence turnover in both *Drosophila* and primates (Anderson *et al*., 2008) and frequent selective sweeps in human subtelomeric regions (Hellmann *et al*., 2008) may reflect the action of telomere drive. In addition, if drive at telomeres and centromeres confer similar, negative, fitness consequences, an evolutionary dynamics between telomere sequence and telomere binding proteins, analogous to that described in the Genomic Conflict Theory for centromers, might develop. In line with this expectation, telomere-binding proteins show signs of

elevated rates of evolution in *Drosophila* (Anderson *et al*., 2009).

In this study, we examine if there is support for the Genomic Conflict hypothesis in birds. In line with the arguments presented above, we hypothesize that at least some of the proteins known to interact with centromeres during meiosis should display rapid sequence evolution during avian evolution. To test this we sequence 7 kinetochore proteins in nine divergent bird species covering most of the evolutionary history of birds.

We also analyze how alleles at heterozygous loci in a domestic chicken population are inherited from parent to offspring, and hypothesize that segregation patterns should be skewed near centromeres and/or telomeres in the presence of female meiotic drive. To this end, we used data of 9330 single-nucleotide polymorphisms typed in 197 chicken families with, in total, 1047 offspring. This data allows us to examine segregation patterns across most chicken autosomes. By simulating fair Mendelian meioses in replicate chicken populations identical to that studied, we define critical values for, and locate chromosomal regions with significantly distorted segregation ratios.

## Materials and methods

### Evolutionary analysis of centromere-binding proteins

RNA extraction: Gonad and/or liver tissue from turkey (*Meleagris gallopavo*), pheasant (*Phasianus colchius*), quail (*Coturnix coturnix*), guinea fowl (*Numida meleagris*), mallard (*Anas platyrhynchos*), pigeon (*Columba livia*) and budgerigar (*Melopsittacus undulates*) was dissected immediately post-mortem and stored in RNAlater (Qiagen, Hilden, Germany). We used the RNeasy Mini Kit (Qiagen) to extract RNA from the tissue samples and then reverse transcribed RNA to cDNA using Oligo$(dT)_{20}$ Primer (Invitrogen, Carlsbad, CA, USA) and SuperScript III Reverse Transcriptase (Invitrogen) as described by Invitrogen.

The genes: Genes targeted for sequencing in this study have been identified as members of the kinetochore in chicken (*CENP-U, CENP-R, CENP-Q, CENP-M, CENP-P, CENP-I* (Okada *et al*., 2006) and *NUF2* (DeLuca *et al*., 2005)). The kinetochore is a multiprotein structure, which assembles on centromeric DNA to mediate binding of spindle microtubules to chromosomes and chromosome movement. The CENP genes sequenced in this study are all part of the inner core of the kinetochore, but can be further divided into three groups on the basis of both function and position: *CENP-Q, CENP-U* and *CENP-P* belong to a group of linked proteins, which have been demonstrated to cause slow growth of cell lines when knocked out. *CENP-I* and *CENP-M* represent the two remaining groups, in which both lead to cell death if knocked out (Okada *et al*., 2006). The *NUF2*, in contrast, is a member of the Ncd80 complex, which is required for the anchoring of kinetochore microtubules to the outer plate of the kinetochore. All genes analyzed in this study show a 1-to-1 orthologous relationship between chicken and human.

PCR and sequencing: PCR primers were designed to bind exonic sequence with a high degree of sequence conservation between chicken and a second bird species (if available) or humans. Selected genes were then amplified using PCR conditions and primers as given in Supplementary Table S1 and S2. Before sequencing, PCR products were cleaned using ExoSAP-IT (Amersham Biosciences, Buckinghamshire, UK). Cleaned products were then either sequenced inhouse on a MegaBACE 1000 sequencing instrument or sent to Macrogen for sequencing (Seoul, South Korea). The resultant partial coding sequences of the selected genes were deposited in GenBank under accession numbers GQ281320–GQ281352. Chicken and Zebrafinch orthologous were collected from the assembled genome sequences of chicken (2.1 release of the chicken genome at Ensemble; http://www.ensembl.org/Gallus_gallus) and zebrafinch (first release of the zebra finch genome sequence at Ensemble; http://www.ensembl.org/Taeniopygia_guttata).

Sequence analysis: We aligned the orthologues using ClustalW and checked alignments by eye. Evolutionary analyses were carried out using codeml in the PAML 4.2 package (Yang, 1997, 2007). We tested for positive selection using likelihood ratio tests based on comparisons of models M1a and M2a (Nielsen and Yang, 1998) and models M7 and M8 (Yang and Swanson, 2002). While model M1a assumes two site classes; one for which $\omega$ ($d_N/d_S$) is allowed to take a value between 0 and 1 (negative selection) and a second in which $\omega=1$ (no constraint), model M2a adds a third class of sites with $\omega>1$ (positive selection). Model M7 allows $\omega$ to take values following a $\beta$ distribution between 0 and 1; on top of this model, M8 adds a class of sites under positive selection ($\omega>1$). Phylogenies (Figure 1) for these tests (((((turkey, pheasent), quail), chicken), guinea fowl), mallard), ((budgerigar, zebrafinch), pigeon)) were as described by Kaiser *et al*. (2007) and Ericson *et al*. (2006).

### Segregation analysis of genetic markers in chicken pedigrees

Family data: Detailed information of the generation of families and genotype data is described by Elferink *et al*.

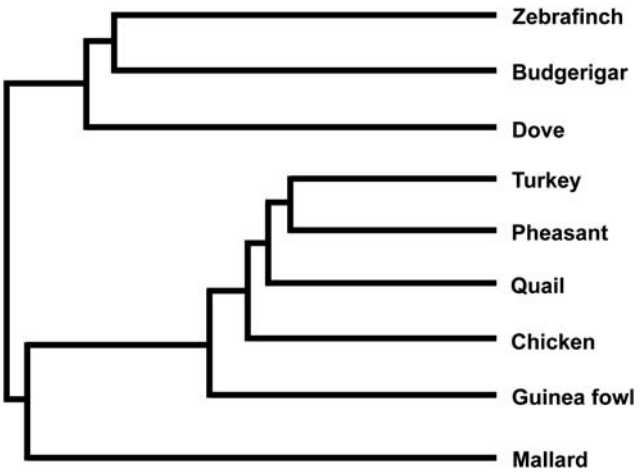


**Figure 1** Phylogenetic tree showing the evolutionary history of bird species included in the analysis of kinetochore protein evolution. The most basal split occurred around 95 MYA, and branch lengths are scaled to roughly represent previously estimated divergence times (van Tuinen and Hedges, 2001; Ericson *et al*., 2006).

(submitted). Briefly, chicken individuals come from a broiler population but are not inbred. Data from 197 families (except for chromosome 27 where data from 196 families were available) with an average of 5.3 offspring (family size ranged from 1 to 12 offspring) were included in the study. In total, 1313 individuals, 1047 of which were offspring, were genotyped at 9330 single-nucleotide polymorphisms using the Infinium assay of Ilumina (CA, USA) (Steemers *et al*., 2006).

Before analysis, the data were processed, family-by-family, to remove loci that contained genotype combinations that resulted in Mendelian incompatibilities. In total, ~0.25% of all family-locus combinations were removed from the original data in this way. Similarly, on the basis of frequency of Mendelian errors, the genotyping error rate was estimated to be 0.14%. Error rates were uniformly distributed across the chicken genome with no regions showing a systematically elevated rate. Furthermore, loci with less than 20 informative meioses were discarded from the analyses.

Segregation analysis: The single-nucleotide polymorphisms are diallelic, with two alleles in each locus, arbitrarily labelled $A$ and $a$. There are two types of informative families: families in which both parents are heterozygous, and families in which only one parent is heterozygous. In families with two heterozygous parents, we expect alleles $A$ and $a$ to appear with equal probability in the offspring. In families with only one heterozygous parent, we expect in average half of the offspring to carry both alleles. Let the total number of offspring of families with two heterozygous parents be $n_2$, and let the number of offspring of the second kind be $n_{1AA}$ and $n_{1aa}$, when the homozygous parent is of genotype $AA$ and $aa$, respectively. Let $n_{A,total}$ be the number of $A$ alleles in all offspring of informative families. Then, in the absence of SD, $n_A = n_{Atotal} - n_{1AA}$ is binomially distributed with parameters $(1/2, n)$, where $n = 2n_2 + n_{1aa} + n_{1AA}$. The test statistic we use to determine deviations from a 50/50 segregating ratio is then the tail probability of this distribution, that is,

$$P = 1 - \sum_{i=n_A+1}^{n-n_A-1} \binom{n}{i} 0.5^n$$

This assumes an absence of genotyping errors. However, genotyping errors will be incorporated into the simulations determining critical values.

We have combined information from families in this way, because each family is relatively small. Our power to detect SD then depends on the existence of linkage disequilibrium (LD) between the markers and the distorter locus. Otherwise, we would have little or no power to detect SD. In a study of LD decay in commercial broiler populations, 24% of marker pairs within 0.5 cM had $r^2$ values $>0.5$ (Andreescu *et al*., 2007), suggesting that LD decays relatively quickly in the population studied here. Therefore, we expect evidence for SD to also erode quickly at relatively short genomic distances away from the distorter locus.

The statistic $P$ was averaged across a sliding window of size 50 or 10 markers depending on the number of markers typed for each chromosome (window size was 50 markers for chromosomes 1–12, and 10 markers for chromosomes 13–28). The sliding window average was plotted against the physical position of the intermediate marker moving the window one marker at a time along the chromosomes. Chromosomes 16 and 25 were excluded from the analysis due to very limited amounts of data from these chromosomes.

As the centromere drive hypothesis predicts SD in females, but not males, we focused our analysis of segregation patterns on female chickens. At a particular locus, this was achieved by including families where only the mother, or both the mother and the father are heterozygous when summing transmitted alleles. This analysis will thus describe a composite of female and male transmission patterns, although females contribute more than males to the result. To be able to further partition the effects of sex on segregation ratios, we also separately analyzed families in which the father is heterozygous and the mother is homozygous, and families in which the mother is heterozygous and the father is homozygous. The results of these analyses represent male only, and female only, meiotic events, respectively.

Simulations: In order to compare the results of the segregation analysis to the expectations from a system with a fair segregation, we simulated 1000 data sets with Mendelian segregation. First (to model LD), we used fastPHASE (Scheet and Stephens, 2006) to infer the phase of chromosomes in the studied chicken population. We then generated the same number of offspring per family as observed in the real data set by allowing recombination between homologous chromosomes. We used a recently developed linkage map (Elferink *et al*., submitted), built from the same data set as that used in this study, to randomly determine the number (Poisson distributed) and position (uniformly distributed) of crossing-overs. Each pair of homologous chromosomes was allowed to segregate randomly (Mendelian segregation) so that each homologue had an equal probability of ending up in the offspring. Genotyping errors were randomly assigned to the genotypes with the same probability as observed in the real data. To this end, we estimated the rate of Mendelian inconsistencies in the real data set among doubly homozygous parents. We used this rate to introduce a binomially distributed number of genotyping errors in both parents and offspring. As in the real data, offspring genotypes inconsistent with the parent genotypes were removed from the data. Each simulated data set was analyzed as described for the real data set. In order to correct for multiple tests, we retrieved the lowest and highest value of $P$ observed in any window (of 50 or 10 markers), from each simulation. Critical values for (the sliding window average of) $P$ were then obtained as tail probabilities of this simulated distribution.

### Test for non-random location of SD regions

To formally test whether observed transmission distortion regions in the chicken genome have a non-random location with regard to telomeres and centromeres, we measure the average genetic distance (in cM) from SD regions to their closest centromere or telomere (depending on which is closest), and apply a permutation procedure. The DNA sequence of centromeric regions are unknown and represented as gaps in the chicken genome assembly. We used the location of these gaps to determine the position of chicken centromeres. Many of

these locations have been confirmed using immunostaining techniques and fluorescence *in situ* hybridization (Krasikova *et al*., 2006). However, for several of the acrocentric microchromosomes the centromere location remains unknown. In testing for non-randomness with regard to centromeres and telomeres, this will, however, have a minor effect, as the centromere in these cases will be tightly liked to one of the telomeres. The start and end point of assembled chromosomes were taken to represent telomere locations. On the basis of the physical map of the chromosomes used in this study, we then randomly select new positions for the transmission distortion regions uniformly along the length of the chromosomes, and again measure the average genetic distance to telomeres and centromeres. We repeat this procedure 1000 times and count how often the average distance to centromeres and telomeres are closer in the simulated data, as compared with the real data.

## Results

### Evolution of avian centromere-binding proteins

The average $d_N/d_S$ value of the seven analyzed centromere-binding genes in this study is 0.53, which is substantially higher than the average $d_N/d_S$ (0.085) from a comparison of ~5000 chicken–zebra finch orthologous (Axelsson *et al*., 2008). The individual $d_N/d_S$ values of all CENP genes studied here merit a rank among the top 130 fastest evolving genes in the study by Axelsson *et al*. (2008). The two most rapidly evolving genes, CENP-U and CENP-Q, have $d_N/d_S$ values placing them among the top 30 most rapidly evolving avian genes from the same study. Tests for positive selection, based on comparisons of the M1a to M2a and M7 to M8 models in codeml (Table 1), furthermore reveal that the molecular evolution of the same two genes, CENP-U and CENP-Q, have been affected by positive selection repeatedly. The exact function of the two rapidly evolving genes remains unknown; however, they have been classified as belonging to the same group of kinetochore proteins on the basis of both physical interaction and function in chicken (Okada *et al*., 2006). In addition, a third member of the same group of proteins, CENP-P, shows weak evidence for positive selection in one of our model comparisons (M7 vs M8; $P = 0.065$).

### SD analysis

**Distribution of SD regions:** We identify four regions ($P < 0.05$) (Table 2) that display SD when all loci in which mothers are heterozygous are analyzed. Among these, we note one region that is located near the centromere on chromosome 1 (Figure 2). Interestingly, chromosome 1 also harbours a distorted region in close proximity to the p-arm telomere (Figure 2). These observations may suggest that there is SD at centromeres and telomeres in chicken. The two additional SD regions show locations that are neither centromeric nor telomeric (Table 2). We tested whether the distribution of SD regions in our analysis is random with regard to centromere and telomere location, by randomly assigning new locations for the observed SD regions. We then measured the average genetic distance from distorted regions to the closest centromere or telomere and compared the results with the real data. This procedure was repeated 1000 times to determine how often the randomly assigned locations were located nearer to centromeres and telomeres than the observed SD regions. This test shows that the detected SD regions are in significantly tighter linkage to centromeres and telomeres than would be expected by chance ($P = 0.015$).

**Female vs male meiosis:** The female meiotic drive hypothesis predicts SD at centromeres and telomeres

**Table 2** Chicken genome regions displaying segregation distortion (SD), or unexpectedly fair segregation, when transmission patterns in families with heterozygous mothers were compared with results from 1000 simulations with fair chromosome segregation

| *Chr* | *SD region start* | *SD region end* |
|---|---|---|
| *SD* | | |
| 1 | 6118633 | 6118633 |
| 1 | 73974022 | 78785092 |
| 9 | 18452293 | 20792207 |
| 12 | 7413419 | 7618810 |
| *Unexpectedly fair segregation* | | |
| 8 | 8620795 | 13504946 |
| 17 | 10696570 | 10844198 |

Start and end positions refer to the centre of the first and last windows in a row of consecutive windows that deviate from simulated segregation patterns.

**Table 1** Results of the evolutionary analysis of avian centromere-binding proteins

| *Gene* | *Models* | *M1a* | *M1a–M2a* | *M1a–M2a* | *M7–M8* | *M7–M8* |
|---|---|---|---|---|---|---|
| | *Species included* | $d_N/d_s$ | *lr* | *lrt* | *lr* | *lrt* |
| NUF2 | C, T, Q, Ph, G, D, Z | 0.26 | 0 | NS | 0 | NS |
| CENP_U | C, T, Q, G, D, Pi, Z | 0.70 | 12.02 | <0.01 | 12.54 | <0.01 |
| CENP_R | C, T, Q, Ph, G, Z | 0.53 | 0 | NS | 0.22 | NS |
| CENP_Q | C, T, Q, Ph, G, D, B, Z | 0.76 | 21.56 | <0.001 | 21.7 | <0.001 |
| CENP_P | C, T, Ph, G, Pi, Z | 0.54 | 3.86 | NS | 5.48 | NS |
| CENP_M | C, Q, T, G, Ph, Z | 0.55 | 0.56 | NS | 0.8 | NS |
| CENP_I | C, Q, T, G, Ph, Pi, Z | 0.39 | 0 | NS | 0 | NS |

$d_N/d_S$ values were estimated using the M1a model. Likelihood ratio values (lr) and significance values from likelihood ratio tests (lrt) refer to comparisons of models M1a and M2a, and models M7 and M8, respectively (see Materials and methods section for brief model descriptions). A significant likelihood ratio test in any of the two model comparisons above provides strong support for positive selection during the molecular evolution of a particular gene. Abbreviations for species included in each analysis are as follows: C, chicken, T, turkey, Q, quail, Ph, pheasant, G, guinea fowl, D, duck, B, budgerigar and Z, zebra finch.

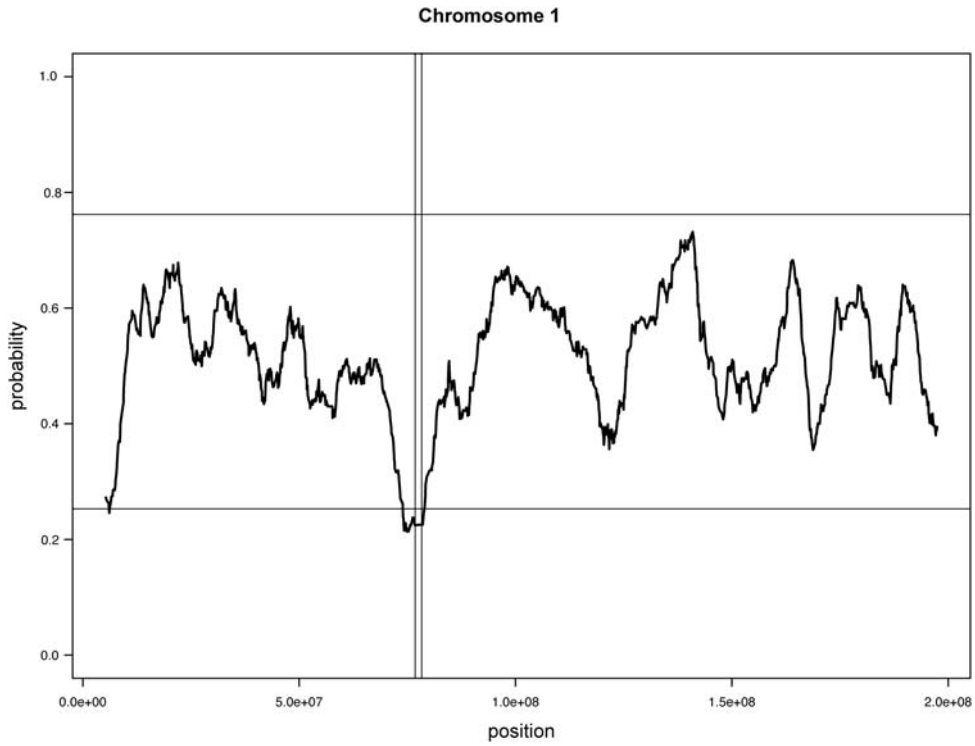


**Figure 2** Transmission analysis at loci heterozygous in mothers for chromosomes 1. Average two-tailed binomial probabilities for observed transmission patterns are plotted for a sliding window (window size is 50 markers) along the chromosomes. Horizontal lines represent the critical values ($P<0.05$ and $P>0.95$) for transmission probabilities obtained through simulations. Vertical bars show the location of the centromere.

during female meioses, but not during male meioses. So far our analyses have considered the transmission of alleles from parent to offspring at all loci, in which mothers are heterozygous (fathers may either be homozygous or heterozygous at these loci), which in turn means the observed transmission patterns will be influenced by both female and male effects, although mainly by transmission in mothers. Our observation of transmission distortion near a centromere and a telo mere, in this analysis, is thus in line with expectations from the female meiotic drive hypothesis. In an attempt to further partition the effect of sex, we also analyzed transmission at loci, in which only fathers and mothers are heterozygous, respectively. Such an analysis should in theory be able to distinguish completely between male and female affects. This analysis did however not detect any significant SD in either of the two sexes for the previously identified regions. This lack of SD may reflect a loss of power when completely separating the effects of sex (there are on average 197 and 213 informative meioses per locus, respectively, when analyzing male and female transmission separately, whereas the initial analysis of all loci where females are heterozygous contain an average of 323 informative meioses per locus), but could also indicate that any differences in male and female transmission patterns are small.

## Discussion

### Rapid evolution of centromere-binding proteins

In this study, we document overall fast rates of molecular evolution among avian kinetochore proteins, and specifically demonstrate that two ($\sim$22%) of the nine kinetochore proteins analyzed in this study have evolved under positive selection. As a comparison Kosiol *et al*. (2008) found evidence for positive selection in 2.4% (400) of $\sim$16 500 mammalian genes, using a similar test to that used here. We attempted, but failed, to generate sequence data from additional kinetochore genes, and suggest that this problem potentially may be due to difficulties in identifying conserved DNA sequence, suitable for primer binding, across divergent bird species. As this problem is likely augmented in rapidly evolving genes, it is possible that our sample of kinetochore genes excludes some of the most rapidly evolving ones. Adaptive evolution could thus have played a more significant role during avian kinetochore evolution than indicated here.

According to the Genomic Conflict hypothesis, centromeres exploit the asymmetry of the female meiosis to gain a transmission advantage. The hypothesis further states that driving centromeres generate negative fitness consequences in males, which in turn causes mutations that alter the binding specificity of centromere-binding proteins to be selected for, to restore random chromosome segregation (Malik and Henikoff, 2002). This develops into a continuous process where changes in centromeres are followed by changes in kinetochore proteins and *vice versa*. Our observations of rapid evolution of kinetochore proteins in birds is thus in line with expectations from the Genomic Conflict Theory.

Could other processes be responsible for the high rate of molecular evolution in kinetochore proteins? First, a scenario where protein–protein interactions at the outer core of the kinetochore drive the rapid kinetochore evolution is unlikely given previous observations of

accelerated evolution, exclusively at the inner, centromere binding (Okada *et al*., 2006), core of the kinetochore (Brar and Amon, 2008). In support of this, we note that the two adaptively evolving kinetochore proteins detected in this study (*CENP-Q* and *CENP-U*) belong to the inner core of the kinetochore (Okada *et al*., 2006). In addition, although we only sequenced one representative (*NUF2*) of the outer kinetochore plate (which interacts with the spindle microtubuli (DeLuca *et al*., 2005)), it is interesting that this gene evolves slower than all representatives of the inner kinetochore analyzed here.

Second, to our knowledge, there are no reports describing interactions between inner core kinetochore proteins and chromosomal elements other than centromeres. This suggests that it is indeed the kinetochore–centromere interaction that drives the rapid kinetochore evolution. This leaves us with the possibility that centromeres evolve rapidly due to some other process than female meiotic drive. Although we are not aware of such a process, we acknowledge that it is impossible to rule out such alternative hypotheses based on our evolutionary analysis. However, on the basis of many observations, from a wide variety of organisms, that indirectly (and directly) support the presence of centromere drive (see introduction), female meiotic drive remains a strong candidate for explaining the rapid divergence of centromere arrays. As a striking example, the complete lack of centromere complexity in *Saccharomyces cerevisiae* (Malik and Henikoff, 2002), where meiosis always is symmetric, suggests that rapid centromere divergence indeed may be caused by drive during asymmetric female meiosis. In short, we argue that our new evidence for rapid evolution of avian kinetochore proteins support, but do not prove, the Genomic Conflict hypothesis, and a view of molecular evolution in which repeated molecular adaptation of parts of the kinetochore is commonplace in eukaryotes.

**Drive at centromeres?** Direct evidence for drive at centromeres is very scarce. Fishman and Saunders (2008) only recently presented what has been described as the first direct evidence for female meiotic drive at a centromere in any organism. Our observation of SD in a region near the centromere on chromosome 1 may represent an additional and new example of this phenomenon. There are, however, caveats to our results that refrain us from concluding this is the case.

First, in addition to identifying four SD regions in the chicken genome, our analysis also indicates that there are two regions (one near the centromere on chromosome 8 and one at the end of chromsome 17) that exhibit unexpectedly fair segregation patterns (Table 2). That is, for these regions, alleles are transmitted at equal, or nearly equal, proportions across several linked loci. We can think of several hypotheses that explain this surprising observation. For meiotic segregation events to produce unexpectedly equal proportions of transmitted alleles, it is necessary to evoke the existence of a 'meiotic memory', such that the result of a previous chromosome segregation is known to the subsequent segregation event. As we are unaware of the existence of such a memory, we regard this as an unlikely explanation. Alternatively, the unexpectedly fair segregation could result from post-zygotic selection for heterozygous genotypes at a locus embedded within the observed region. Finally, although our method for defining critical values takes local recombination rates and errors into account, we may have failed to correct for some unknown genotype calling biases, which may act to systematically increase *P*-values in some regions. At present we see no simple way to discern between these possible explanations, but consider a methodological artefact a plausible explanation. Although it is not likely that factors causing an increase in *P*-values in some regions, also are responsible for the reduced *P*-values in other regions, the observation of regions with significantly increased *P*-values is our main motivation for interpreting the observed SD regions with some caution. Identification of SD based on multiple linked loci requires knowledge of error rates and local recombination rates, both of which are associated with significant statistical uncertainty.

Second, given that the observed centromere proximal SD region truly reflects a skewed transmission pattern, there are several alternative hypotheses, in addition to female meiotic drive, that may explain this observation. The female meiotic drive hypothesis predicts that SD should be of exclusively female origin. We note that our result is based mainly on the analysis of female transmission patterns and thus in line with expectations from this hypothesis. However, a complete partitioning of the effect of sex failed to provide conclusive evidence for a female origin of this observation. Hence, it is not possible to rule out alternative explanations for the observed SD, such as post-meiotic viability selection. We do however argue that gamete selection in males is unlikely to be responsible given the effect of female meioses observed in this analysis. We also believe that gamete selection in females is unlikely given the enormous fitness cost such a process implies.

Although we fail to provide conclusive evidence for SD at centromeres here, it is interesting that centromere drive in chicken has been suggested before based on indirect evidence. Dinkel *et al*. (1979) showed that chromosome fissions in chicken display biased segregation when heterozygous. Pardo-Manuel de Villena and Sapienza (2001a) argued that this phenomenon was due to an imbalanced centromere number (one centromere at the original chromosome and two at the split chromosome) and that the chromosome with the greater number of centromeres preferentially move to the polar body side of the spindle in chicken.

**Drive at telomeres?**
In addition to a possible example of centromere drive, our analysis also suggests that there is SD near a telomere on chromosome 1. Again, given that our observation represents true SD (see Discussion section above), the failure to completely separate male and female effects means we are unable to conclude that the observed bias is caused by female meiotic drive. Observing drive at telomeres would not be unexpected; on the basis of their ability to affect the orientation of chromatids, telomeres have previously been hypothesized to drive during female meiosis (Zwick *et al*., 1999). In support of this it is now becoming increasingly clear that telomeres are heavily involved in chromosome movement during meiosis (Dresser, 2008). Early on, it was also demonstrated that distally located chromoso-

mal elements have the ability to influence chromosome segregation in *Drosophila* during female meiosis (Novitski, 1951). However, apart from this observation, there is no direct evidence for SD at telomeres in any organism. Indirectly, several observations might be attributable to competition among driving telomeres, such as a rapid diversification between human and chimpanzee telomeric regions (Trask *et al*., 1998), a high degree of human subtelomere sequence diversity (Mefford and Trask, 2002) and recurrent selective sweeps in human subtelomeric regions (Hellmann *et al*., 2008). Also, given that telomere drive would cause a similar genetic conflict to that caused by driving centromeres, it is interesting that there is evidence for rapid evolution of telomere-binding proteins in *Drosophila* (Anderson *et al*., 2009).

There are several reasons why it might be difficult to detect drive in studies such as ours. First, centromeres or telomeres with new and driving sequence mutations are likely only transiently polymorphic in a population, as they should drive to fixation rapidly. Second, drive at centromeres could potentially be neutralized by mutations in kinetochore proteins so that Mendelian segregation is restored. In the light of this, it is encouraging that our analyses suggests that both centromeres and telomeres may experience SD, especially as the coverage of single-nucleotide polymorphisms in our data tends to be low near these chromosome features on many chromosomes. At the same time, it is clear that our inability to explain the unexpectedly fair segregation in two regions of the chicken genome makes it difficult to draw any conclusions of the segregation analysis, other than that there may be SD near a centromere and a telomere in chicken. Further analyses of data sets, both from chicken and other organisms, are needed to understand the importance of female meiotic drive in molecular evolution. Such data sets should ideally contain large numbers of informative meioses and high levels of LD across markers; with regard to the latter criterion, data from crosses of inbred lines should be most useful. Our new method for detecting SD should provide a valuable resource for such future analyses.

To conclude, we have used a combination of two independent analyses to search for both indirect and direct evidence of female meiotic drive in birds. While the evolutionary analysis provide the first evidence for positive selection at kinetochore proteins during large parts of avian evolutionary history, as is predicted by the Genomic Conflict hypothesis, we only find weak evidence supporting SD near a centromere and a telomere in a chicken population.

## Conflict of interest

The authors declare no conflict of interest.

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