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# NEWS AND COMMENTARY

Long-range linkage disequilibrium in natural populations ............................................

# Stripes, sex and sparrows: what processes underlie heteromorphic chromosome evolution?

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Are we witnessing the evolution of a new or accessory set of sex chromosomes in the white-throated sparrow (Zonotrichia albicollis)? This common North-American bird is well known for its plumage polymorphism, most strikingly for the white or tan colour of the stripe above the eye. Early on, karyotype studies revealed that the white/tan polymorphism was perfectly associated with a major chromosomal polymorphism on chromosome 2, which comes in two forms distinguished by multiple nested inversions: ZAL2 and ZAL2$^m$ (Thorneycroft, 1966). ZAL2/ZAL2$^m$ birds have the white stripe, while ZAL2/ZAL2 birds have the tan stripe, with a 1:1 frequency in natural populations. In fact, the plumage polymorphism is merely the most conspicuous difference between the ZAL2 homozygotes and ZAL2/ZAL2$^m$ heterozygotes: behavioural differences, particularly in reproductive strategies, as well as hormonal differences have also been noted, with white-striped males and females being more 'male'-like than their sex-matched tan-striped counterparts (Falls and Kopachena, 1994; Tuttle, 2003; Maney et al., 2005; Lake et al., 2008). ZAL2$^m$/ZAL2$^m$ homozygotes are vanishingly rare (only a few birds have ever been recorded), so ZAL2$^m$ chromosomes occur almost exclusively in the heterozygous state with the ZAL2 chromosome. The karyotype frequencies appear to be balanced by strong negative assortative mating, in which almost all breeding pairs are between birds with different plumage colours (and their associated different chromosome complements) (Thomas et al., 2008). The chromosome dimorphism, nearly obligate cross-mating and the rarity of ZAL2$^m$ homozygotes, coupled with the complex inversion patterns and a suggested absence of recombination, are reminiscent of balancing selection associated with heterogametic sex chromosomes (Thomas et al., 2008).

So, are white-throated sparrows in the process of evolving another set of sex

chromosomes? In the current issue, Huynh et al. (2011) report an extensive study of genetic variation and recombination along the polymorphic chromosomes, shedding light on the population processes shaping these chromosomal forms and evaluating their merit as models for the initial stages of sex chromosome evolution. Huynh et al. sequenced 62 loci (totalling 36.7 kb) distributed across the entire chromosome, thoroughly sampling across the inversions and the small flanking non-rearranged terminal portions. Remarkably, extreme linkage disequilibrium (LD) was found over the entire rearranged region, which spans over 100 Mb and contains about 1000 genes, showing that most of the chromosome length behaves like two non-recombining genomic blocks. The two chromosome types differ by ∼1% at the nucleotide level, with fixed differences accounting for two-thirds of the genotyped single-nucleotide polymorphisms. Scaling up from this sample, one arrives at an amazing estimate of half a million fixed differences in total between the two chromosomes. In contrast, the non-rearranged portion of the chromosome shows higher levels of shared diversity, and much lower levels of haplotype structure and LD, suggesting that the chromosome does recombine more freely outside the complex inversions. This arrangement is reminiscent of the recombining pseudoautosomal regions at the termini of X and Y chromosomes, which enable correct pairing and segregation at meiosis.

The long-term evolutionary trajectory of the non-recombining sex chromosome, that is, the Y chromosome in male mammals and Drosophila, or the W chromosome in female birds and butterflies, is one of extensive genomic decay. This process leaves a signature of accumulated deleterious mutations and gene loss, acquired repetitive sequences and heterochromatinization. This characteristic genomic erosion can be driven by various genetic forces: in

non-recombining regions the rate of adaptive evolution is reduced and deleterious mutations can accumulate via Muller's ratchet, background selection and by hitch-hiking effects (Charlesworth and Charlesworth, 2000). However, the initial stages of sex chromosome evolution remain unclear, and the age of many established sex chromosome systems limits their utility to provide insight into this. One particular question concerns the relative roles and timing of events in the evolution of sex chromosomes, which include obligate disassortative mating, the acquisition of sex-specific functions, the degeneration of genes on the non-recombining chromosome and the development of dosage compensation.

There are systems in which incipient or neo-sex chromosomes are known and genomic features of these, particularly where the cessation of recombination appears to have occurred suddenly and recently, offer an interesting comparison to the Z. albicollis chromosomal polymorphism. In Drosophila miranda, a fusion of the Y chromosome to an autosome approximately 1 million years ago created a neo-Y that never recombines, along with a neo-X that can recombine freely in females (Bachtrog and Charlesworth, 2001). Comparative analysis of the neo-X and neo-Y sequences revealed an already marked level of degeneration of the neo-Y, while genes on the neo-X remained intact (Bachtrog, 2003; Bachtrog et al., 2008). Similarly, a chromosomal inversion and fusion event in the black muntjac within the past 0.5 million years also bears an imprint of accumulated mutation on the neo-Y chromosome (Zhou et al., 2008). In marked contrast to these studies, the ZAL2$^m$ chromosome, which is estimated to have split from ZAL2 two million years ago, does not show significant signs of genetic degeneration (Thomas et al., 2008; Romanov et al., 2009; Huynh et al., 2011). What might underlie the differences between these cases? Huynh et al. applied the four-gamete test to detect historical recombination events within the ZAL2 and ZAL2$^m$ haplotypes. At least two events within ZAL2$^m$ were detected, compared with ⩾12 events within ZAL2, a surprising result given the near-constant heterozygosity of ZAL2$^m$ observed in extant populations. The authors argue that this level of recombination may be sufficient to combat the forces driving genomic degeneration (Haddrill et al., 2007). Alternatively, one might hypothesize that the ZAL2$^m$ chromosome is

poised on the cusp of a transition into decay, considering that the rate of early degeneration may be more gradual in *de novo* evolution of sex chromosomes compared with cases involving fusion between an extant sex chromosome and an autosome (Charlesworth and Charlesworth, 2000). However, in one example of *de novo* evolution of an incipient Y chromosome in papaya, signs of genetic degeneration are already seen around the sex-determining region, despite most of the rest of the chromosome being functional and recombining (Liu *et al.*, 2004). This further highlights that the ZAL2 heteromorphism may be following a different evolutionary trajectory from sex chromosomes.

Indeed, the extent of sequence divergence and LD between the ZAL2 forms, revealing the suppression of recombination and their long-term coexistence, suggests that both functional haplotypes are actively maintained, and it was suggested that they might encode coadapted combinations of multiple traits (Falls and Kopachena, 1994; Romanov *et al.*, 2009)—a form of giant supergene combining behavioural as well as plumage variation (Mather, 1950; Clarke *et al.*, 1968; Joron *et al.*, 2006). One remarkable finding is indeed the sheer number of genes locked into very large non-recombining units maintained by balancing selection. Interestingly, despite powerful balancing selection on the two forms, ZAL2$^m$ homozygotes are avoided through strong disassortative mating, which implies that some level of self-incompatibility of ZAL2$^m$ haplotypes is balanced by strong beneficial effects.

Therefore, a deeper understanding of the selective forces and their fitness tradeoffs acting on alternative versions of this chromosome will be central to our understanding of the evolution of their genomic organization. For instance, it was suggested that the multiple 'super-male' behavioural alleles associated with ZAL2$^m$, such as territory defence and aggressiveness, could be maintained through the balancing effects of 'good parent' combinations of alleles at the same or other linked genes associated with ZAL2. Epistatic interactions are likely involved, both within the haplotypes and with other genomic regions. For example, adult pairs are not symmetrically distributed in the wild, such that two-thirds of

the breeding pairs are white-striped male × tan-striped female (Falls and Kopachena, 1994), revealing probable fitness interactions of the ZAL2 polymorphism with the sex chromosome.

Several lines of evidence also suggest that the divergence between the chromosome forms occurred before the radiation of the *Zonotrichia* clade (Thomas *et al*, 2008; Romanov *et al.*, 2009; Huynh *et al.*, 2011), which raises the question of the processes favouring long-term maintenance of the haplotypes in the clade, and the possibility of similar cryptic behavioural polymorphisms occurring in other species in the absence of plumage dimorphism. It is then tempting to speculate that the stripe pattern might represent a male signalling display (Tuttle, 2003) more recently recruited onto the polymorphic chromosome, with optimal effects in combination with the other male behaviours correlated with the ZAL2$^m$ haplotype. Our understanding of the evolution of adaptive genomic architectures, such as supergenes or divergent chromosomal forms, and how they seem to maintain or accumulate the control of coadapted fitness-related traits, therefore requires unravelling the fitness advantages of the different trait combinations, and the evolutionary history of their involvement in integrated phenotypes. As Huyhn *et al.* demonstrate, the white-throated sparrow, with its fascinating blend of morphology and behaviour, is likely to provide a fruitful setting for such studies.

## Conflict of interest

The authors declare no conflict of interest.
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