Disproportionate Roles for the X Chromosome and Proteins in Adaptive Evolution

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Adaptation requires genetic variation. A complete understanding of this key evolutionary process includes identification of the mutations that confer adaptive change. High-resolution genetic dissection of particular adaptive phenotypes can pinpoint these mutations, but this is a challenging task and the resulting conclusions are restricted to the traits under consideration.

An alternative approach is to examine characteristics of adaptive mutations through the powerful lens of population genetics. By measuring intraspecific polymorphism and interspecific divergence across genomes, evolutionary properties of those mutations targeted by natural selection can be discovered. Under this framework as well, generalities are widely sought and difficult to identify. A useful way to make progress is to compare patterns of adaptive evolution among different categories of loci. Two contrasts are based on the genomic location of mutations: X linked *vs.* autosomal and protein coding *vs. cis*-regulatory (*i.e.*, noncoding sequences that affect local gene expression). These contrasts have generated substantial interest because they are tied directly to basic questions about adaptive evolution.

The fate of a beneficial mutation should depend on its mode of inheritance. This idea motivates comparisons between rates of adaptive evolution on the X chromosome and the autosomes. Recent, low-frequency recessive mutations are immediately exposed to selection in males when X-linked and are mostly hidden from selection (in heterozygotes) when autosomal. If beneficial mutations are at least partially recessive on average, they will fix faster on the X chromosome (Avery 1984), elevating the rate of evolution relative to autosomal loci. In an influential theoretical article, Charlesworth *et al.* (1987) quantified this connection between dominance and "faster X" evolution and identified the biological conditions under which it applies. With several assumptions, the relative rates of molecular evolution at X-linked and autosomal loci can even be used to estimate the average dominance of new advantageous mutations, an important quantity in evolutionary biology.

The study of faster X evolution is also motivated by a desire to explain the outsized role of the X chromosome in speciation. In a wide variety of species pairs, the sterility and/or inviability of hybrids created by interspecific crosses maps differentially to the X chromosome (Coyne and Orr 2004). This bias seems to reflect a higher density of hybrid incompatibilities involving the X chromosome (Masly and Presgraves 2007).

The prediction of faster X evolution has been tested repeatedly by comparing between-species divergence at X-linked and autosomal genes (Meisel and Connallon 2013). The most popular approach calculates the relative rate of substitution at nonsynonymous and synonymous sites, reasoning that nonsynonymous changes will often be targeted by selection. A flurry of studies featuring *Drosophila* revealed a range of support for faster X evolution (Betancourt *et al.* 2002; Thornton and Long 2002; Counterman *et al.* 2004; Musters *et al.* 2006; Thornton *et al.* 2006; Begun *et al.* 2007; Presgraves 2008; Singh *et al.* 2008; Vicoso *et al.* 2008; Grath and Parsch 2012; Zhou and Bachtrog 2012). A recent comparison between high-quality genomes sequences of *Drosophila simulans* and *D. melanogaster* found faster X evolution at nonsynonymous sites, UTRs, and long introns (Hu *et al.* 2013). This pattern is expected if beneficial substitutions are recessive on average, though the authors suggest it is more likely due to differences in gene content between the X

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chromosome and the autosomes. Human–chimpanzee divergence (Lu and Wu 2005; Nielsen *et al.* 2005; Chimpanzee Sequencing and Analysis Consortium 2005; Hvilsom *et al.* 2012), human–mouse divergence (Torgerson and Singh 2003), and mouse–rat divergence (Baines and Harr 2007) support faster X, mostly for genes biased toward male expression. The bird Z chromosome (the analog of the X) also shows elevated rates of protein evolution (Borge *et al.* 2005; Mank *et al.* 2007; Ellegren 2009) but this elevation might not be caused by selection (Mank *et al.* 2010).

The divergence-based tests described above cannot discriminate between adaptive, neutral, and deleterious substitutions. But the faster X prediction is strongest for adaptive substitutions (mildly deleterious mutations can also fix faster due to the smaller effective population size of the X chromosome). All substitutions pass through a polymorphic stage, and combining divergence with intraspecific polymorphism generates a much richer portrait of selection. The fraction of substitutions fixed by positive selection can be estimated by comparing polymorphism and divergence at nonsynonymous and synonymous sites, allowing an improved test of faster X. This approach has so far yielded strong evidence for faster X in *Drosophila* (Langley *et al.* 2012; Mackay *et al.* 2012; Campos *et al.* 2014) and chimpanzees (Hvilsom *et al.* 2012) with mixed results for rabbits (Carneiro *et al.* 2012).

Another salient question about the genetics of adaptation concerns the location of causative mutations within genes: Do they primarily fall in protein-coding regions or *cis*-regulatory elements? If *cis*-regulatory mutations more easily allow functional fine tuning (Jacob and Monod 1961; Stern 2000; Wilkins 2002; Carroll *et al.* 2004) and/or are less pleiotropic than protein-coding changes (Stern 2000; Wilkins 2002; Wray *et al.* 2003), the former might disproportionately drive adaptation (Carroll 2005; Wray 2007). Mutations responsible for adaptive phenotypic differences have been identified in both protein-coding and *cis*-regulatory sequences, but the issue of whether these two classes of changes differ generally in their evolutionary properties [as famously proposed by King and Wilson (1975)] remains unresolved (Hoekstra and Coyne 2007; Wray 2007; Stern and Orgogozo 2008).

As in the case of faster X, patterns of molecular evolution can help address this question. Many studies have documented adaptive divergence in proteins; there is also good evidence for adaptive evolution of putative *cis*-regulatory sequences in *Drosophila* (Andolfatto 2005), humans (Torgerson *et al.* 2009), and house mice (Halligan *et al.* 2011; Kousathanas *et al.* 2011). But direct contrasts between signatures of selection at *cis*-regulatory and protein sequences have been sparse.

Results from Peter Keightley's group now provide key insights into both of these topical problems in molecular evolution. New genome sequences from a population sample of *Mus musculus castaneus*, one of the three subspecies of house mice, and *Mus famulus*, are combined with the existing rat genome sequence to describe patterns of polymorphism and divergence genome-wide. In this issue of *Genetics*, Kousathanas *et al.* translate these patterns into conclusions about faster X evolution, whereas Halligan *et al.* (2013) use them to examine the relative roles of protein-coding and *cis*-regulatory variation in adaptation.

Kousathanas *et al.* (2014) analyze variation at 700 X-linked genes and 18,110 autosomal genes. The authors combine two approaches to measure adaptive evolution (Eyre-Walker and Keightley 2009). First, they analyze the site frequency spectrum, fitting a demographic model to synonymous variants (which are presumed neutral) and a selection model to nonsynonymous variants (Keightley and Eyre-Walker 2007). The resulting distribution of fitness effects is used to calculate the average fixation probability of deleterious and neutral nonsynonymous mutations relative to neutral synonymous changes. Second, the authors count the numbers of divergent nonsynonymous and synonymous sites between *M. musculus castaneus* and the outgroup species. They combine divergence counts with fixation probabilities to estimate the proportion of substitutions that are adaptive (α; Fay *et al.* 2001) and the relative rate of adaptive substitution (w; Gossmann *et al.* 2010). This mode of inference accounts for effects of mildly deleterious mutations and demography. Kousathanas *et al.* (2014) categorize genes as male-specific (expressed only in testis or prostate), female-specific (expressed only in ovary or uterus), or non-sex-specific, based on published expression datasets for mice.

X-linked genes exhibit higher values of α and w than autosomal genes, providing clear evidence for more adaptive evolution on the X chromosome. The signal is driven by male-specific genes—neither female-specific nor non-sex-specific genes differ based on whether they are X-linked or autosomal—as expected if exposure of recessive mutations to selection in males is responsible (Charlesworth *et al.* 1987).

Using the relative rates of adaptive evolution on the X chromosome and the autosomes, Kousanthanas *et al.* (2014) estimate the average dominance of a new beneficial mutation to be ≤ 0.2. The authors emphasize that the calculation requires many assumptions (Charlesworth *et al.* 1987; Connallon *et al.* 2012). One assumption unlikely to be met in house mice is that equal numbers of males and females breed in nature.

Kousathanas *et al.* (2014) raise another wrinkle with the dominance explanation for faster X. Before the first meiotic division in spermatogenesis, cells are diploid: recessive X-linked mutations are expressed but recessive autosomal mutations are hidden in heterozygotes. After the first meiosis, cells are haploid and recessive mutations on the autosomes are expressed as well. Therefore, if recessivity is the principal cause of faster X, the elevated adaptive substitution rate on the X chromosome should be seen mostly in genes expressed early in spermatogenesis. To test this prediction, the authors partition genes by timing of expression.

Genes whose expression is restricted to early spermatogenesis (in premeiotic spermatogonia) show similar rates of adaptive evolution on the X chromosome and the autosomes, contrary to the prediction of the dominance model. X-linked and autosomal genes with expression restricted to late spermatogenesis (in postmeiotic spermatids) also show similar rates.

Interestingly, genes expressed in both premeiotic and postmeiotic cells display the signature of faster X. The expression of most X-linked genes is suppressed during the first meiosis of spermatogenesis in a process called meiotic sex chromosome inactivation (MSCI), but some genes escape. Kousathanas *et al.* (2014) suggest that a struggle between selfish elements and host genes for control of expression during spermatogenesis could drive adaptive evolution at the genes that escape MSCI (Presgraves 2008).

The observation of faster X leads to the prediction that the X chromosome will contribute disproportionately to speciation in house mice. Genetic studies of hybrid male sterility—the primary reproductive barrier between house mouse subspecies—support this prediction. Loci that cause F_2 sterility in crosses between *M. musculus castaneus* (the subspecies studied by Kousathanas *et al.* (2014)) and *M. musculus domesticus* map differentially to the X chromosome (White *et al.* 2012) and multiple X-linked loci shape F_1 and F_2 sterility in crosses between *M. musculus domesticus* and *M. musculus musculus* (Storchova *et al.* 2004; Good *et al.* 2008; White *et al.* 2011). Interestingly, disruptions in MSCI are also connected to hybrid male sterility in house mice (Campbell *et al.* 2013).

The Kousathanas *et al.* (2014) report provides some of the strongest evidence for adaptively driven faster X to date. Answering two empirical questions would accelerate progress toward understanding the causes of this interesting pattern. First, are adaptive mutations usually recessive? Newly arisen deleterious mutations appear to be recessive on average (Simmons and Crow 1977; Lynch and Walsh 1998), but the general properties of adaptive mutations remain elusive. Second, how common is faster X? Only a handful of species have been examined—mostly from one genus (*Drosophila*)—preventing general conclusions. The expected signature of faster X depends on relative effective population sizes and relative mutation rates of the X chromosome and autosomes (Kirkpatrick and Hall 2004; Vicoso and Charlesworth 2009; Connallon *et al.* 2012), factors that differ among species. Placing the study of faster X in a comparative framework could help identify the underlying mechanisms.

Halligan *et al.* (2013) use the genome sequences from *M. musculus castaneus*, *M. famulus*, and rat to compare adaptive evolution at protein-coding and *cis*-regulatory sequences. Noncoding elements conserved across placental mammals are treated as putative *cis*-regulatory sequences (and abbreviated as CNEs, for "conserved noncoding elements"). The authors use the same analytical procedures as Kousathanas *et al.* (2014) to measure adaptive evolution.

The percentage of substitutions inferred to be driven by positive selection is substantial for both nonsynonymous sites (28–42%) and CNEs (18–21%). These estimates exceed those for humans (Boyko *et al.* 2008; Torgerson *et al.* 2009; Eyre-Walker and Keightley 2009), presumably because the higher effective population size of house mice makes selection more efficient. Though proteins bear a higher proportion of adaptive substitutions, CNEs cover more sequence real estate. The authors propose that only 20% of adaptive substitutions happen in proteins, with noncoding sequences (mostly CNEs) contributing the remaining 80%. By this metric, *cis*-regulatory mutations are disproportionately responsible for adaptation.

However, the relative significance of mutation classes in adaptive change depends on both the number of substitutions and their effects on fitness. Measuring fitness properties is more difficult than estimating substitution numbers, but the authors take advantage of an indirect approach. When a new adaptive mutation spreads through the population, it brings linked neutral variants along with it. The result is a local reduction in sequence diversity that surrounds the adaptive substitution (Maynard Smith and Haigh 1974). The expected width of this dip in linked variation is related to s/r (where s = strength of selection and r = recombination rate; Kaplan *et al.* 1989), providing a way to roughly gauge the fitness effects of recent adaptive substitutions. The selective purging of deleterious mutations also reduces linked diversity, through a process called background selection (Charlesworth *et al.* 1993).

Halligan *et al.* (2013) measure diversity in narrow windows around exons and CNEs, using neutral divergence to account for local variation in the mutation rate. Whereas diversity around CNEs fits predictions from background selection models, variation near exons is lower than expected. The authors suggest that this discrepancy reflects larger fitness effects for adaptive mutations in proteins. This conclusion is affected by a large number of modeling assumptions, and Halligan *et al.* (2013) appropriately attach caveats. But given the dearth of empirical studies comparing the adaptive significance of proteins and *cis*-regulatory sequences (especially on a genomic scale), these results deserve careful consideration.

Perhaps the X chromosome and proteins play disproportionate roles in adaptation. In addition to providing support for this interesting idea, the work of Kousathanas *et al.* 2014) and Halligan *et al.* (2013) reveal general issues with using genomic data to dissect adaptive evolution. First, combining polymorphism with divergence dramatically improves power over the more popular approach of considering divergence in isolation because beneficial, neutral, and deleterious variants can be separated. Second, both studies rely on synonymous diversity as a neutral benchmark. Although the authors argue that their inferences are robust to this choice, it underscores an issue in the field. As the fraction of the genome believed to be affected by positive and negative selection grows, it becomes increasingly difficult to

characterize selection by comparing diversity among site classes. Third, the predictions tested in both papers assume that new beneficial mutations drive adaptation. If instead selection targets standing variation, autosomal loci can evolve faster than X-linked loci (Charlesworth *et al.* 1987; Orr and Betancourt 2001; Connallon *et al.* 2012) and selective sweeps are not expected to generate local reductions in diversity (Przeworski *et al.* 2005). Debates about faster X and the relative adaptive contributions of *cis*-regulatory *vs.* protein sequences should embrace the reality that a mixture of new mutations and standing variation contributes to adaptation.

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