Reduced Nucleotide Variability at an Androgen-Binding Protein Locus (Abpa) in House Mice: Evidence for Positive Natural Selection

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Previous work has shown that the gene for the alpha subunit of androgen-binding protein, Abpa, may be involved in premating isolation between different subspecies of the house mouse, Mus musculus. We investigated patterns of DNA sequence variation at Abpa within and between species of mice to test several predictions of a model of neutral molecular evolution. Intraspecific variation among 10 Mus musculus domesticus alleles was compared with divergence between M. m. domesticus and M. caroli for Abpa and two X-linked genes, Glra2 and Amg. No variation was observed at Abpa within M. m. domesticus. The ratio of polymorphism to divergence was significantly lower at Abpa than at Glra2 and Amg, despite the fact that all three genes experience similar rates of recombination. Interspecific comparisons among M. m. domesticus, Mus musculus musculus, Mus musculus castaneus, Mus spretus, Mus spicilegus, and Mus caroli revealed that the ratio of nonsynonymous substitutions to synonymous substitutions on a per-site basis (Ks/Ka) was generally greater than one. The combined observations of no variation at Abpa within M. m. domesticus and uniformly high Ks/Ka values between species suggest that positive directional selection has acted recently at this locus.

Introduction

One approach to studying the genetic basis of speciation is to identify genes that may be involved in reproductive isolation and then to describe the evolutionary forces that have acted on those genes within and between closely related species. This approach has been used to document elevated rates of nonsynonymous substitutions for genes involved in a variety of sex-related functions in diverse taxa (e.g., Lee, Ota, and Vacquier 1995; Metz and Palumbi 1996; Ferris et al. 1997; Tsaur and Wu 1997; Civetta and Singh 1998). Sexual selection may be a driving force for changes at genes involved in prezygotic isolation (e.g., Lee, Ota, and Vacquier 1995; Metz and Palumbi 1996; Tsaur and Wu 1997).

Mouse androgen-binding protein (Abp) is an excellent mammalian candidate locus for testing ideas about the role of selection in shaping patterns of genetic variation at genes involved in reproduction. Abp is a small dimeric protein with an alpha subunit that is linked by disulfide bonds to either a beta or a gamma subunit (Dlouhy, Taylor, and Karn 1987). Abp is secreted in the saliva and binds male sex hormones. Male mice coat both their pelage and their territories with Abp, and females show the ability to discriminate among different Abp variants (Laukaitis, Critser, and Karn 1997). Several lines of evidence suggest that selection has driven the evolution of Abp among the three subspecies of Mus musculus. First, the Abp alpha subunit gene (Abpa) displays three major alleles that are fixed in each of the three subspecies: Abpa in M. m. domesticus, Abpa in M. m. musculus, and Abpa in M. m. castaneus.

Abbreviation: Abpa, androgen-binding protein alpha subunit.

Key words: androgen-binding protein, Abpa, Darwinian selection, neutral theory, Mus musculus, sexual selection, pheromone.

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(Karn and Dlouhy 1991; Hwang et al. 1997). This is an unusual pattern for these subspecies; most electrophoretic variants show differences in frequency, rather than fixed differences, among these three groups (e.g., Bournot et al. 1993; Sage, Atchley, and Capanna 1993). Second, Laukaitis, Critser, and Karn (1997) created a congenic strain for the Abpa allele by backcrossing through 16 generations to C3H mice, a strain derived from M. m. domesticus. This produced a strain that was primarily of domesticus origin (>99%) yet contained the musculus Abpa allele. In several laboratory experiments, Laukaitis, Critser, and Karn (1997) demonstrated that female mice prefer to associate with and mate with males of their own Abpa type significantly more frequently than with males of a foreign type. This work suggested that Abpa mediates sexual isolation in mice and may be involved in limiting introgression where M. m. domesticus and M. m. musculus have established a hybrid zone in Europe. Third, comparison of evolutionary rates among Abp variants in different species of mice reveals a large excess of nonsynonymous substitutions over synonymous substitutions, consistent with strong directional selection (Hwang et al. 1997).

Here, we further investigate genetic variation at Abpa in mice by looking at levels of polymorphism within M. m. domesticus in both coding and noncoding regions. Neutral theory predicts that levels of intraspecific polymorphism and interspecific divergence will be correlated for different genes (Kimura 1983), while selection may uncouple levels of polymorphism and divergence (Hudson, Kreitman, and Aguade 1987). We wish to know if we can detect the footprint of recent directional selection in the form of reduced polymorphism at both coding and noncoding sites of Abpa within M. m. domesticus. A survey of 10 M. m. domesticus Abpa sequences revealed no variation within this subspecies, despite high levels of divergence in comparison with Mus caroli. We also investigate rates of nonsynonymous (Ks) and synonymous (Ka) substitution on a per-site basis between closely related species of Mus. Pair-
wise comparisons of $K_s$ and $K_a$ among *Mus* species revealed uniformly high values of $K_s/K_a$. These observations provide further evidence that positive directional selection has acted recently at *Abpa* in mice.

**Materials and Methods**

**Samples**

We obtained genomic DNAs for five *M. m. domesticus* (BNC, DFS, DJO, DSD, and DBV) from outbred stocks maintained by Dr. François Bonhomme at the University of Montpellier, France. The collecting localities for these stocks are as follows: BNC (Cairo, Egypt); DBV (Vlas, Bulgaria); DFS (Alsace, France); DJO (Or cetto, Italy), and DSD (Dakar, Senegal). DNA from *M. caroli* was obtained from the Jackson Laboratory in Bar Harbor, Maine.

**PCR and DNA Sequencing**

We amplified and sequenced 998 bp of *Abpa* spanning portions of two exons (213 bp) and the intervening intron (785 bp) for 10 *M. m. domesticus* and 2 *M. caroli* alleles. PCR was performed on genomic DNA using Taq polymerase (Promega and BRL) as previously described (Hwang et al. 1997). PCR products were purified using the QIAquick PCR cleanup kit (Qiagen). DNA sequencing was accomplished by a combination of automated sequencing (ACGT, Inc.) and manual sequencing using the CircumVent DNA sequencing kit (New England Biolabs). Both strands were sequenced. Sequences have been submitted to GenBank (accession numbers AF144714, AF144715). To check for potential heterozygous sites, a few sites that were ambiguous with automated sequencing were also sequenced manually. Initial sequence data were generated in the laboratory of R.C.K. in Indiana and revealed a surprisingly high level of intron divergence between *M. caroli* and *M. m. domesticus* (see Results). To confirm this observation, introns of one additional *M. caroli* and two additional *M. m. domesticus* were independently sequenced in the laboratory of M.W.N. in Arizona. The coding-region sequences of *Abpa* for *M. musculus* and *M. caroli* have previously been reported (Hwang et al. 1997).

**Analysis**

Sequences were aligned with the DNASIS program (Hitachi). The HKA test (Hudson, Kreitman, and Aguadé 1987) was used to test the neutral expectation of equal ratios of polymorphism to divergence for different genes. Polymorphism data for these tests came from *M. m. domesticus* and *M. caroli*. *Abpa*, which is located on chromosome 7 (Dlouhy, Taylor, and Karn 1987), was compared with two X-linked loci (*Amg* and *Glra2*) experiencing similar rates of recombination. Two measures of nucleotide variability, $\pi$ (Nei and Li 1979) and $\theta$ (Waterson 1975), were calculated for each locus. The HKA test was modified to take into account the difference in effective population size for X-linked and autosomal genes. DNA sequence data for *Amg* and *Glra2* are reported in Nachman (1997). Recombination rates for these loci were estimated as described in Nachman and Churchill (1996). $K_s$ and $K_a$ values were calculated as in Li (1993) for the *Abpa* coding region in pairwise comparisons among *M. caroli*, *Mus spretus*, *Mus spicilegus*, *M. m. domesticus*, *M. m. musculus*, and *M. m. castaneus* using sequences from Hwang et al. (1997).

**Results**

Aligned *Abpa* sequences for *M. m. domesticus* and *M. caroli* are shown in figure 1. No differences were observed between the coding regions shown here and those reported by Hwang et al. (1997). Between *M. m. domesticus* and *M. caroli*, a total of 73 nucleotide substitutions were observed. The overall divergence corrected for multiple hits (Kimura 1980) was 7.7%, more than twice the value (3.2%) observed for 6 kb of intron sequence for X-linked genes between these same taxa (Nachman 1997). The divergence observed for the two exons (7.4%) was very similar to the value observed for the single intron (7.8%). No insertion-deletion differences were observed between *M. m. domesticus* and *M. caroli*, although in several instances, adjacent nucleotide substitutions were observed. These could be interpreted equally parsimoniously as either two point mutations or one insertion and one deletion event. Since several of these occur in the coding region, it is unlikely that they reflect multiple insertion-deletion events, because these would have disrupted the reading frame. We conducted all HKA tests in two manners: (1) we calculated divergence (73/998) assuming no indels occurred, and (2) we calculated divergence (59/998) assuming that all adjacent nucleotide changes in noncoding DNA represent one insertion and one deletion rather than nucleotide substitutions. This difference did not affect any of our conclusions, so only the former analysis is reported.

Within *M. m. domesticus* (*N* = 10 alleles), no polymorphisms were observed for the 998 bp of coding and noncoding *Abpa* sequence. Comparisons of polymorphism and divergence for *Abpa*, *Glra2*, and *Amg* are given in table 1. All three loci experience similar rates of recombination (*Abpa*, 0.54 cM/Mb; *Amg*, 0.44 cM/Mb; *Glra2*, 0.51 cM/Mb), suggesting that the effects of selection at linked sites may not be very different for these loci. Observed and expected values in HKA tests are reported in table 2. *Abpa* shows a significant departure from neutral expectations in comparison with *Amg* ($\chi^2 = 11.95, P < 0.001$) or with *Glra2* ($\chi^2 = 7.58, P < 0.01$). The direction of the departure in both cases is toward a deficiency of polymorphism and an excess of divergence at *Abpa* relative to the other loci. No departure from neutral expectations is seen in the comparison between *Glra2* and *Amg* (*P > 0.1*). We also compared *Abpa* to *Amg* and *Glra2* using only noncoding (intron) sites. Both of these HKA tests showed a significant deficiency of polymorphism at *Abpa* (*Amg* vs. *Abpa*: $\chi^2 = 9.28, P < 0.01$; *Glra2* vs. *Abpa*: $\chi^2 = 5.98, P < 0.05$). The HKA test is based on randomly drawn alleles from a population, yet two alleles drawn from one individual may not represent random draws. For example, inbreeding would reduce heterozygosity and could thus bias the
Fig. 1.—Aligned nucleotide and amino acid sequences of Abpa for M. m. domesticus (Mmd) and M. caroli (Mc). Coding sequence is in lowercase letters, and intron sequence is in uppercase letters.

Table 1
Levels of Polymorphism and Divergence at Abpa, Glra2, and Amg

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosome</th>
<th>Recombination Rate (cM/Mb)</th>
<th>Length (bp)</th>
<th>No. of Polymorphic Base Substitutions</th>
<th>No. of Polymorphic Insertions/Deletions</th>
<th>π (%)</th>
<th>θ (%)</th>
<th>Divergence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abpa</td>
<td>X</td>
<td>0.54</td>
<td>998</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>7.7</td>
</tr>
<tr>
<td>Glra2</td>
<td>X</td>
<td>0.51</td>
<td>1,996</td>
<td>8</td>
<td>1</td>
<td>0.135</td>
<td>0.142</td>
<td>2.9</td>
</tr>
<tr>
<td>Amg</td>
<td>X</td>
<td>0.44</td>
<td>1,141</td>
<td>4</td>
<td>4</td>
<td>0.160</td>
<td>0.124</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Note.—Polymorphism for each locus is based on a sample of 10 Mus musculus domesticus alleles. Divergence (Kimura 1980) is between a randomly chosen allele from M. m. domesticus and a randomly chosen allele from Mus caroli.

HKA test in comparisons between Abpa (N = 10 alleles from 5 individuals) and Amg or Glra2 (N = 10 alleles from 10 individuals). We recalculated the HKA tests using a sample size of 5 for Abpa and obtained similar results (Abpa vs. Amg: χ² = 8.3, P < 0.01; Abpa vs. Glra2: χ² = 5.0, P < 0.05). The absence of variation at Abpa is striking in view of the fact that the five individuals are from Egypt, Bulgaria, France, Italy, and Senegal. In contrast, the 10 individuals surveyed for Amg and Glra2 are all from Italy.
K₀ and K₁ values in comparisons between *Abpa* variants are shown in table 3. The phylogenetic relationships of the six taxa are shown in figure 2. Of the 15 pairwise comparisons among these taxa, 11 comparisons show values of K₀/K₁ > 1, and all comparisons showed large values of K₁/K₀. For example, *M. m. domesticus* and *M. m. musculus* differ by six nonsynonymous and zero synonymous substitutions. The lowest value of K₀/K₁ was 0.85 (M. m. castaneus vs. M. m. musculus). These observations stand in contrast to an average value of K₀/K₁ of 0.143 for 363 homologous genes compared between mouse and rat (Wolfe and Sharp 1993). In fact, of the 363 genes compared by Wolfe and Sharp (1993), only one revealed K₀/K₁ > 1 (for interleukin-3, K₀/K₁ = 1.12). Thus, the K₀/K₁ values reported here are well above average.

**Discussion**

The results presented here provide evidence that selection has shaped the evolution of *Abpa* in house mice. In particular, reduced variability in *M. m. domesticus* at *Abpa* relative to *Amg* or *Gfra2* is consistent with a recent adaptive fixation (a selective sweep) at or near *Abpa*.

Previous work has provided some evidence for a positive correlation between heterozygosity and recombination rate in *Drosophila* (Begun and Aquadro 1992; Moriyama and Powell 1996; Stephan et al. 1998), mice (Nachman 1997), humans (Nachman et al. 1998), beetles (Kraft et al. 1998), tomatos (Stephan and Langley 1998), and goatgrasses (Dvorák, Luo, and Yang 1998). In these diverse organisms, regions of low recombination typically exhibit reduced nucleotide variability, presumably as a consequence of either positive or negative selection at linked sites (e.g., Maynard Smith and Haigh 1974; Charlesworth, Morgan, and Charlesworth 1993). Thus, a significant HKA statistic may result from selection at the locus under consideration, or it may result from selection at linked sites.

It is noteworthy that the rates of recombination estimated for *Abpa*, *Amg*, and *Gfra2* are all approximately 0.50 cM/Mb. This indicates that the reduction in variability at *Abpa* relative to *Amg* or *Gfra2* is not attributable to different recombinational environments for these genes. It is still possible that the significant HKA result for *Abpa* is due to selection at a closely linked locus, although two observations argue against this. First, the high K₀/K₁ ratios at *Abpa* are consistent with strong directional selection at *Abpa* itself. Second, experimental studies have shown a role for *Abpa* in mate choice between subspecies of *M. musculus* (Laukaitis, Critser, and Karn 1997). The combined observations of low polymorphism at *Abpa*, high recombination rate, high K₀/K₁ ratios, and a functional role of *Abpa* in mate choice suggest that *Abpa* itself, rather than a linked locus, has been the target of recent directional selection.

It is surprising that the level of divergence (K) at *Abpa* between *M. m. domesticus* and *M. caroli* (K = 7.7%, SE = 0.9%) is over twice the average value (K = 3.2%, SE = 0.2%) from introns of four X-linked genes for this species pair. Moreover, the high level of divergence at *Abpa* is observed for both the coding and the noncoding portions of the gene: K₀ = 7.2% (SE = 3.8%) and K₁ = 5.8% (SE = 4.5%), and for intron sites, K₀ = 7.8% (SE = 1.0%). The deviation observed in the HKA test may, in principle, be due to both an excess of divergence and a deficiency of polymorphism. Selection at linked sites may reduce polymorphism but is not expected to increase divergence (Birky and Walsh 1988). What might explain the high divergence? It may be partly due to a higher mutation rate for the autosomal *Abpa* locus than for the X-linked loci. A higher mutation rate

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**Table 2**

<table>
<thead>
<tr>
<th>Loci</th>
<th>θ₁ (%)</th>
<th>θ₂ (%)</th>
<th>Sₑ/ES₁</th>
<th>Sₑ/ES₂</th>
<th>Dₑ/ED₁</th>
<th>Dₑ/ED₂</th>
<th>T</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abpa</em>-Amg . . . . . .</td>
<td>0.111</td>
<td>0.036</td>
<td>0.31</td>
<td>0.049</td>
<td>0.13</td>
<td>0.09</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><em>Abpa</em>-Gfra2 . . . . .</td>
<td>0.169</td>
<td>0.076</td>
<td>0.48</td>
<td>0.032</td>
<td>0.04</td>
<td>0.03</td>
<td>0.00</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

Note.—Subscripts denote locus 1 and locus 2, respectively. S = observed segregating sites; ES = expected segregating sites; D = observed divergence; ED = expected divergence; T = divergence time as in Hudson, Kreitman, and Aguadé (1987).

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**Table 3**

<table>
<thead>
<tr>
<th>Values of K₀ and K₁ in Comparisons Among <em>Abpa</em> Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison</td>
</tr>
<tr>
<td>domestincus-musculus . . . . . . . .</td>
</tr>
<tr>
<td>domestincus-castaneus . . . . . . .</td>
</tr>
<tr>
<td>domestincus-spicilegus . . . . . . .</td>
</tr>
<tr>
<td>domestincus-spretus . . . . . . . .</td>
</tr>
<tr>
<td>domestincus-caroli . . . . . . . .</td>
</tr>
<tr>
<td>musculus-castaneus . . . . . . . .</td>
</tr>
<tr>
<td>musculus-spicilegus . . . . . . . .</td>
</tr>
<tr>
<td>musculus-spretus . . . . . . . . .</td>
</tr>
<tr>
<td>musculus-caroli . . . . . . . . .</td>
</tr>
<tr>
<td>castaneus-castaneus . . . . . . . .</td>
</tr>
<tr>
<td>castaneus-spretus . . . . . . . . .</td>
</tr>
<tr>
<td>castaneus-caroli . . . . . . . . .</td>
</tr>
<tr>
<td>spicilegus-spretus . . . . . . . .</td>
</tr>
<tr>
<td>spicilegus-caroli . . . . . . . . .</td>
</tr>
<tr>
<td>spreus-caroli . . . . . . . . . . .</td>
</tr>
</tbody>
</table>

**Fig. 2.—**Phylogenetic relationships and geographic distributions of the six taxa in this study. This phylogeny is supported by multiple molecular and morphological data sets (reviewed in Boursot et al. 1993; Silver 1995).
could be locus-specific, it might reflect differences due to male-driven molecular evolution (Li 1997), or it may result from an adaptively lower mutation rate on the X chromosome (McVean and Hurst 1997). Alternatively, it is possible that selection has driven some of the mutations to fixation. Selection appears to be a likely explanation for some of the nonsynonymous substitutions (in light of the $K_a/K_s$ ratios in table 3) but seems less probable for the synonymous or intron sites.

Can we estimate the rate of adaptive fixations at Abpa in *M. musculus* from these data? Six nonsynonymous substitutions distinguish the Abpa alleles of *M. m. musculus* and *M. m. domesticus*, and these taxa have been separated for approximately 500,000 years (e.g., Boursot et al. 1993). If substitutions occurred at a constant rate, this would result in one fixation every 167,000 years. If, on the other hand, these differences are all due to selection for premating isolation following secondary contact (approximately 10,000 years ago), then the rate would be substantially higher (one fixation every 3,300 years).

The data in table 3 indicate that $K_a/K_s$ ratios are uniformly high in comparisons between pairs of taxa. The observation that female *M. m. domesticus* in the laboratory discriminate among Abpa alleles during mate choice suggests that Abpa may function in premating isolation between subspecies in nature. Several of the taxa in table 3 are sympatric over parts of their ranges. For example, *M. spretus* and *M. m. domesticus*, while not sister taxa, are broadly sympatric in Spain and North Africa. In laboratory crosses between these species, male F1's are sterile while female F1's are fertile. Under many conditions, the cost of production of such low-fitness hybrids is expected to lead to assortative mating (e.g., Butlin 1995). It is noteworthy that the $K_a/K_s$ ratio for this pair is quite high (3.08). If selection for premating isolation is driving divergence at this locus, then interactions between sympatric species (even if they are not sister taxa) may influence rates of molecular evolution.

The geographic distribution of Abpa alleles among subspecies of *M. musculus* is also consistent with the idea that selection has acted recently at Abpa to fix distinct alleles in each subspecies (Karn and Dlouhy 1991). It is unusual to find a biochemical polymorphism that segregates discretely along taxonomic lines for the different subspecies of *M. musculus*.

The picture of Abpa evolution which is developing from behavioral experiments (Laukaitis, Critser, and Karn 1997), geographic variation (Karn and Dlouhy 1991), molecular evolution (Hwang et al. 1997), and the population genetic data presented here is one of recurrent selective sweeps fixing unique amino acid replacements in different species and subspecies of house mice. These observations are well in accord with the suggestion that sexual selection may drive the molecular evolution of genes involved in prezygotic reproductive isolation (e.g., Tsaur and Wu 1997; Civetta and Singh 1998).

Several mammalian proteins related to ABP may function in similar ways. Rat prostatic steroid binding protein (PBP) has a common subunit (C3) disulfide-bridged to one of two variable subunits (C1 or C2) (Heyns and DeMoor 1977; Parker, Needham, and White 1982), essentially the same quaternary structure as ABP (Dlouhy, Taylor, and Karn 1987). Sequence data show that PBP is evolutionarily related to ABP (Dominguez 1995). In a large search for genes with elevated rates of nonsynonymous substitution, Endo, Ikeo, and Gojobori (1996) identified the rat PBP gene as a likely target of positive selection. Since the function of PBP is unknown, the structural relationship with ABP does not shed more light on the function of either one. Karn (1994) first reported that the amino acid sequence of Abpa was 50% identical to chain 1 of cat Fel dI, a third protein in this family. Laukaitis, Critser, and Karn (1997) showed that mice coat their pelts with ABP which apparently finds its way from there to the animals’ environment, a characteristic also described for cat Fel dI (Morgenstern et al. 1991). Whether cat Fel dI is involved in pheromonal activity is as yet unknown.

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