Convergent spectral shifts to blue-green vision in mammals extends the known sensitivity of vertebrate M/LWS pigments

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Daylight vision in most mammals is mediated predominantly by a middle/long wavelength-sensitive (M/LWS) pigment. Although spectral sensitivity and associated shifts in M/LWS are mainly determined by five critical sites, predicted phenotypic variation is rarely validated, and its ecological significance is unclear. We experimentally determine spectral tuning of M/LWS pigments and show that two highly divergent taxa, the gerbil and the elephant-shrew, have undergone independent dramatic blue-green shifts to 490 nm. By generating mutant proteins, we identify additional critical sites contributing to these shifts. Our results, which extend the known range of spectral tuning of vertebrate M/LWS, provide a compelling case of functional convergence, likely related to parallel adaptive shifts from nocturnal to brighter light conditions in similar habitats.

Evidence, to date, indicates that the spectral tuning of M/LWS pigments (499 nm to 571 nm) (4), measured by the wavelength of maximum sensitivity ($\lambda_{\text{max}}$), is mainly controlled by amino acid identity at five critical sites (positions 180, 197, 277, 285, and 308) (5), although other sites (e.g., 213 and 294) may also affect spectral tuning via epistatic interactions (6). Predicted $\lambda_{\text{max}}$ values from known critical sites imply spectral shifts in M/LWS pigments during the diversification of mammals (7), yet these have rarely been validated (5), and their ecological significance is not known.

Here we determined the spectral phenotypes of M/LWS pigments in the highly divergent mammalian clades Rodentia (superorder Euararchontoglires) and Afrotheria, for which sequence-based predictions have suggested marked short-wavelength spectral shifts (7). Our results indicate that five rodents—the alpine marmot, degu, golden hamster (“hamster”), lesser Egyptian jerboa (“jerboa”), and Mongolian gerbil (“gerbil”)—are all sensitive to shorter wavelengths of light (516, 502, 504, 501, and 490 nm, respectively) than the predicted values (Dataset S1). We also found that the pigment of one species, the Damaraland mole-rat, showed no spectral shifts in $\lambda_{\text{max}}$ (positions 180, 197, 277, 285, and 308) (5). We also performed M/L opsin in vitro expression assays for mouse/rat in Fig. 1) (10). Thus the observed spectral changes are likely to be associated with transitions from a nocturnal niche to one characterized by a wider range of light conditions.

To determine the molecular basis of spectral tuning, we performed in vitro expression of ancestral proteins (Fig. 1), and, using site-directed mutagenesis, generated mutants containing derived substitutions. For the degu, hamster, and jerboa, differences in spectral sensitivity between the expressed derived and respective ancestral proteins could be explained by observed substitutions at known critical sites (Y277F, T285A, and A308S). In contrast, the gerbil and elephant-shrew were each characterized by an amino acid substitution at a single critical site (T285A and A308S, respectively). Introducing these residues into the ancestral proteins did not recover the recorded $\lambda_{\text{max}}$ value of the wild types. We therefore searched the protein sequences of each taxon for other potentially important substitutions on the basis of changes in the presence of a hydroxyl group (11). For gerbil M/LWS pigment, we found that S107G and S134A gave rise to spectral shifts in $\lambda_{\text{max}}$ (−3 and −6 nm, respectively), and that the combination of S107G, S134A, and T285A recovered the $\lambda_{\text{max}}$ shift to 490 nm seen in the wild-type gerbil (Dataset S2).

We repeated this approach for the elephant-shrew, and found a critical site (position 48), with an A48T mutation producing a −9-nm $\lambda_{\text{max}}$ shift. This species also showed an amino acid substitution (H294Y) at a known critical site (6) that resulted in a −3-nm shift. When combined with the A308S critical replacement, these three substitutions caused a 44-nm $\lambda_{\text{max}}$ shift toward blue-green range, which is still 16 nm short of the observed $\lambda_{\text{max}}$ (Dataset S2). Our findings thus indicate that lineages of two distantly related superorders of mammals have undergone

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The authors declare no competing interest.

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tuning to blue-green light via nonidentical molecular routes, and that shifts in the latter stem, in part, from unknown molecular mechanisms.

Both identified critical sites associated with large spectral shifts (i.e., S134A in gerbil and A48T in elephant-shrew) occur in exon 2. By comparing orthologous exonic sequence across vertebrates, we found the same S134A replacement in some ray-finned fishes, lobe-finned fishes, and jawless fishes (Dataset S3). Thus the mechanism underlying the ~6-nm $\lambda_{\text{max}}$ shift in the gerbil may have evolved independently in multiple vertebrate lineages. Indeed, the S134A replacement may also explain tuning in the cave fish, where a reported shift from 564 nm to 558 nm could not be fully explained by the five critical sites (5).

While our results from the gerbil and elephant-shrew M/LWS reveal a possible link between molecular adaptations and ecological shifts, their daylight color vision is also mediated by SWS1, and possibly by RH1 pigment under mesopic conditions (1). To gain a more complete picture of visual phenotypes in these taxa, we also expressed these two pigments in vitro. We found that both of the SWS1 pigments are ultraviolet sensitive ($\lambda_{\text{max}}$ 360 and 359 nm, respectively) and that RH1 has near-identical sensitivities (502 and 503 nm) (Fig. 1). Moreover, neither SWS1 nor RH1 shows clear changes since the ancestral state (12, 13). Thus we conclude that parallel transitions in color vision range of these two highly divergent mammals have been driven by functionally convergent changes in their M/LWS opsins, and may reflect ecological or temporal convergence on similar photic niches.

**Materials and Methods**

To interrogate reported shifts in M/LWS spectral sensitivity in rodents (Rodentia, Euarchontoglires) and some lineages of Afrotheria, we expressed pigments in vitro using published sequences (Dataset S1) and directly measured spectral sensitivity following previous experimental protocols (7). We confirmed extreme phenotypes by repeating these steps based on either newly generated sequences (Damaraland mole-rat and gerbil) or additional published sequence from elephant-shrew (Datasets S1 and S2). For the latter two taxa, we also determined the phenotypes for SWS1 and RH1 pigments.

To elucidate the molecular basis of phenotypic change in the Rodentia and Afrotheria clades, we generated variants of ancestral pigments (A to E in Fig. 1) in which we introduced one or more of the derived residues by PCR-based site-directed mutagenesis (7). We identified more critical sites and screened these in other vertebrate species in National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/).

**Data Availability.** All data discussed are available in Datasets S1–S3.

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