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**Prevalence and Potential Evolutionary Significance of Color
Mutations in Freshwater Crayfish (Decapoda: Astacidea)**

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ABSTRACT

Color mutations may lead to the evolution and diversification of color phenotypes in animals. However, much of the research on this topic has been conducted on vertebrates. Here, I compile records of color mutations in freshwater crayfish (Decapoda: Astacidea). I found color mutations to be surprisingly common, with 103 documented occurrences. The overwhelming majority of these mutations resulted in blue coloration (72.81%), although I also documented red and orange mutations, among others. Whether or not these mutations can lead to adaptive evolution in crayfishes is unknown. Naturally, some crayfish species exhibit conspicuous colors like blue, orange, and red. Many of these conspicuously colored species are also semi-terrestrial burrowers, which have limited migration potential, smaller population sizes, and thus reduced gene flow compared to aquatic burrowing crayfishes. I speculate that in at least some species, color mutations may be a neutral color trait which faces little to no selection pressure. This is one potential explanation as to why semi-terrestrial burrowing species are more likely to be conspicuously colored. Genetic studies and behavioral experiments are required to test this hypothesis.

Keywords: blue mutation, color abnormality, color morph, mutant, pigment

NOTE

Every few years, news outlets report the discovery of a conspicuously colored blue lobster (*Homarus* sp. Weber, 1795), which is presumed to be the result of a one in two or one in three million chance mutation. This phenomenon has been reported over a dozen times throughout the range of both *Homarus* species (Aiken and Waddy 1995; Beale et al. 1998). White, yellow, orange; and in rare cases, bilateral phenotypes and unique mottled patterns have also been reported. Interestingly, similar color mutations are reported in other decapods, like freshwater crayfish (Momot and Gall 1971; Fitzpatrick 1995; Secker 2013) and spiny lobsters (Landa-Jaime et al. 2018). Although a majority of these reports are anecdotal and not validated through peer-review, color mutations in decapods appear to be common.

Across animals, mutations are thought to play a dominant role in the evolution of color phenotypes (Bennett and Lamoreux 2003; Rosenblum et al. 2004; Hoekstra 2006); although most of this research has been conducted on vertebrates. In many cases, a single mutation can lead to a drastic change in color (Orteu and Jiggins 2020). For example, mutations that alter melanin production (both pheomelanin and eumelanin) in vertebrates such as rodents have been studied for decades. In mice, there are over 800 alleles known to influence color, with many of these alleles having been cloned or sequenced (Bennett and Lamoreux 2003). In some cases, mutations or mutation loci are initially identified in lab populations and then investigated in natural systems. For example, in the deer mouse (*Peromyscus polionotus* Wagner, 1843), a single amino acid mutation in the melanocortin-1 receptor *Mcl1r* generates a lighter coat color which aids in crypsis in some populations of this species (Hoekstra et al. 2006). Work on vertebrates demonstrates the benefits of documenting color mutations, as this can allow researchers to understand how mutations can result in phenotypic change in natural populations

(Orteu and Jiggins 2020). Although the prevalence and potential evolutionary significance of color mutations is well studied in model organisms like rodents, research on this topic in other groups, like crustaceans, is sparse.

Here, I compile records of color abnormalities which are putative genetic mutations throughout freshwater crayfish (Decapoda: Astacidea). I chose to focus on freshwater crayfish for several reasons. First, crayfish are present on all continents aside from Antarctica and mainland Africa (Longshaw and Stebbing 2016), where they are amongst the most abundant freshwater macroinvertebrates (Momot 1978, 1995). Second, natural colors in crayfish varies widely both within and between species. Although crayfish are most often associated with cryptic colors (i.e., brown, tan) that presumably aid in crypsis, with over 700 species, crayfish possess a range of conspicuous colors and patterns—with many species naturally exhibiting blue, red, orange, or purple phenotypes (Schuster 2020). Lastly, color abnormalities and color mutations appear to be relatively common in crayfish, with many reports of these mutations scattered throughout the literature (Momot and Gall 1971; Fitzpatrick 1995; Secker 2013; Hamr et al. 2019). However, a comprehensive list of these mutations and a discussion of their potential evolutionary significance in crayfish has yet to be conducted.

I compiled reports of color abnormalities in crayfish by searching literature and other relevant sources. I presumed these color abnormalities are the result of pigment-related mutations (Black 1976; Walker et al. 2000), although I acknowledge that diet or other environmental factors could influence color. For literature searches, I performed Google Scholar searches using the keyword “crayfish” combined one additional keyword, including: “color”, “mutation”, “morph”, “phase”, “phenotype”, “blue”, “orange”, “red”, “white”, “leucistic”, and “albino”, Literature searches were performed in March 2023. I also compiled records of

mutations on the citizen science platform iNaturalist using the same search keywords as above within Infraorder Astacidea (excluding family Nephropidae). I also searched throughout crayfish-specific literature, including the journal *Freshwater Crayfish* and the associated newsletter for the International Association of Astacology, *Crayfish News*. Lastly, I searched Google, Google Images and Flickr. I identified all crayfish with color abnormalities down to the lowest taxonomic level possible. Photographs were excluded if there was any evidence of recent molting or if the crayfish was not alive and therefore prone to color degradation. If the photographed crayfish was suspected to have been bred in captivity or purchased from the pet trade, I also excluded it. A complete list of mutations is presented in Supplemental Data 1, which contains references and sources for all photographs included in the study. I did not request unpublished observations from crayfish biologists as these observations may not be rigorously recorded and documented.

In total, I recorded 103 putative color mutations. Of these, 19 (18.45%) were found from scientific literature, 57 (55.34%) were found from iNaturalist, whereas the remaining 27 (26.21%) were found from other sources, such as the *Crayfish News* Newsletter. 86 (83.50%) observations were identified down to the species level, whereas the remaining 17 (16.5%) were identified to genus level. All four extant crayfish families were recorded with these mutations, with a majority of these coming from Cambaridae (76; 73.79%), followed by Astacidae (16; 15.54%), Parastacidae (10; 9.71%), and Cambaroididae (1; < 0.01%).

Blue was the most common color mutation, with 75 occurrences. 12 orange mutations were documented also documented; whereas there were 7 white mutations, and three red mutations. I recorded 5 accounts of bilateral color phenotypes crayfish, with different color

patterns split down the center line of their carapace. Pink was recorded only a single time, in *Lacunicambarus freudensteini* (Glon et al. 2020).

Blue mutations comprised the overwhelming majority of mutations I found. Therefore, because of their prevalence, much of my discussion focuses on blue mutations, although the same arguments and rationale I discuss could equally apply to other color mutations. For example, the genes responsible for blue color mutations in crayfishes are unknown, but inheritance studies have been conducted on blue mutations in two species: *Cherax destructor* Clark, 1936, and *Procambarus alleni* Faxon, 1884. Despite being from separate families, blue mutations in both species are the result of a single autosomal recessive mutation (Black 1976; Walker et al. 2000). The simple mendelian inheritance of these mutations allows for the phenotype to spread rapidly, as these species are produced for the pet trade purposes (Chucholl 2013; Faulkes 2015). Although speculative, both blue mutations could be the result of mutations to the same loci, as has been reported throughout other animals (Martin and Orgogozo 2013). In other taxa, repeat mutations on the same loci, resulting in similar phenotypes are well known (Martin and Orgogozo 2013). In crayfishes, the prevalence of blue mutations I recorded throughout the literature could be result from mutations repeatedly occurring on the same loci, resulting in commonality of blue color across distantly related species. However, genetic studies are required to substantiate these claims. Luckily, pigment-altering candidate genes from other crustaceans make the testing the hypothesis of repeated occurrence of blue mutations possible (Huang et al. 2022; Lin et al. 2022).

The physiological mechanisms that control decapod pigmentation are well-understood (Fox 1973; Wade et al. 2015). Decapod coloration results from the expression of carotenoid pigments (i.e., astaxanthin) and carotenoid-protein complexes (crustochrin. α -crustacyanin, β -

crustacyanin; Cianci et al. 2002; Tlusty and Hyland 2005; Begum et al. 2015). Each pigment has its own spectral properties which relate to specific reflectance of light. For example, astaxanthin reflects red wavelengths, whereas α -crustacyanin reflects blue wavelengths (Tlusty and Hyland 2005). Since these pigments are expressed in different layers of the cuticle and epidermis, their layering can result in a diversity of color phenotypes (Tlusty and Hyland 2005). In a cryptic colored species, the combined reflectance of all pigment's relates to generally cryptic colors, whereas in color mutants, there is a presumed under- or over-expression of a pigment or carotenoid-pigment (Gaeta et al. 2021). Regarding blue color mutations, these individuals are likely to express blue coloration from some mechanisms that either 1) upregulates the expression of α -crustacyanin, or 2) downregulates the expression of other non- α -crustacyanin pigments (Gaeta et al. 2021). The same logic can be applied to other color mutations, such as red mutations that either result in the over-expression of astaxanthin, or the under-expression of other pigments. Future, physiological studies should confirm these hypotheses and can also explore whether blue mutant crayfishes are the result of the same or different mechanisms (Hsiung et al. 2015).

Evolutionarily, it is unknown whether color mutations in crayfish can lead to adaptive evolution in natural populations, as is it known in other taxa (Bennett and Lamoreux 2003; Rosenblum et al. 2004; Hoekstra 2006). Natural color variation in crayfishes is surprisingly understudied, with no evidence regarding the adaptive value of conspicuous colors in these species (Schuster 2020). Paradoxically, many of the conspicuously colored crayfish are also semi-terrestrial burrowing species (Schuster 2020), which are nocturnal and reside within burrows (i.e., primary and secondary burrowers; Hobbs 1981; Bearden et al. 2021). Although future research may show that conspicuous color in these species is adaptive, an alternative to

this theory is that color in these species may be neutral. Recently, research on Coconut crabs, *Birgus latro* Linnaeus, 1767 has speculated that their red:blue color polymorphism is non-adaptive and may be a neutral color trait that has arisen from mutations (Caro 2021). In theory, the neutral color theory can similarly apply to conspicuous colors in semi-terrestrial burrowing crayfishes, as there is no evidence suggest that these colors serve a function (Schuster 2020). This idea is further supported by our data, as color mutations appear to be relatively common in crayfish. If a color mutation appears in a semi-terrestrial burrowing species which rarely leaves their burrow, the trait may not be selected against, and remain evolutionary neutral. Most semi-terrestrial burrowing crayfish have small geographic ranges due to poor dispersal abilities over land, resulting in lower population sizes, genetic diversity, and gene flow compared to aquatic burrowing crayfish (Ponniah and Hughes 2004; Stern et al. 2017; Hurt et al. 2019). And since in at least blue color mutations can result from simple genetic mutations (Black 1976; Walker et al. 2000), once a mutation arises, it may quickly spread throughout the population. However, the neutral theory of color evolution remains poorly tested. Crayfish may be an ideal group to pursue this provocative, but potentially information theory.

In summary, I hope that by documenting the widespread occurrence and commonality of color mutations across crayfishes, that this will stimulate research on both proximate and ultimate studies of coloration throughout crayfishes and other crustaceans. Such work should pursue the genetic architecture of color in crayfishes, as well as studies that test for the adaptive value (or lack thereof) of conspicuous color in semi-terrestrial burrowing species.

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Figure 1. Color mutations throughout freshwater crayfish. Orange mutations in A) *Faxonius nana* and B) *Cambarus bartonii*. Blue color mutations in C) *Faxonius rusticus*, D) *Pacifastacus leniusculus*, and E) *Spinastacoides inermis*. F) White color mutation in *Faxonius propinquus*. G) Red color mutation in *Cambarus asperimanus*. Photo credits A) Dustin Lynch, Arkansas Natural Heritage Commission, B) Emilio Concari, C) Wisconsin Department of Natural Resources, D) John Schaefer, E) Alex2713 on iNaturalist, F) Premek Hamr, G) Benjamin Lowe.

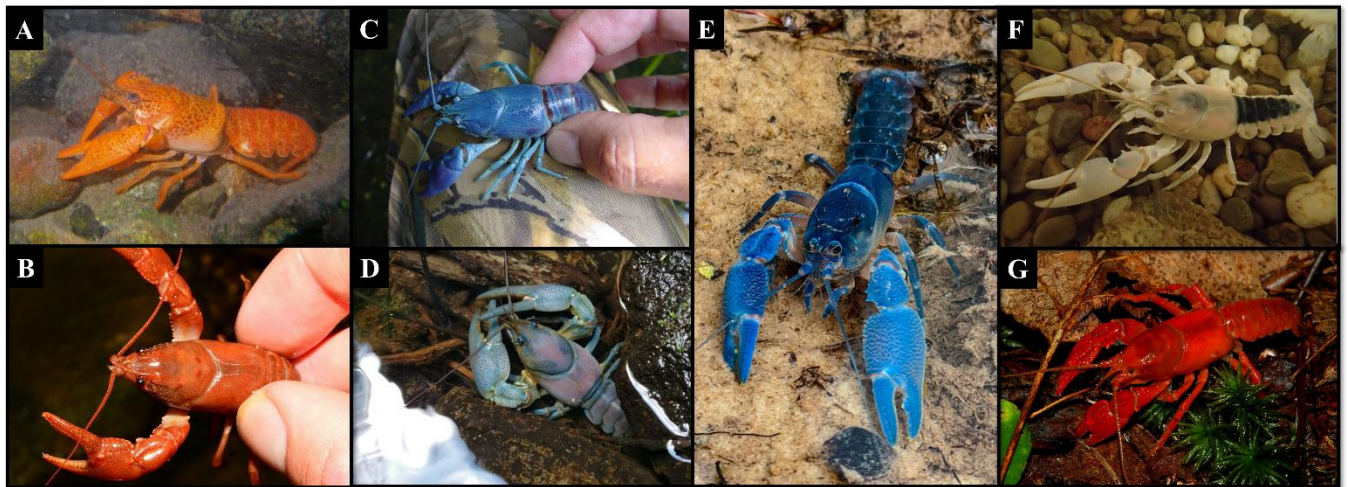


Figure 2. Distribution in color of color mutations across freshwater crayfishes. Colors are coded based on the observed phenotype, aside from bilateral color phenotypes, which are coded as green. 103 occurrences of color mutations were used to create this figure. Blue is overwhelmingly the most common color mutation phenotype recorded.

