Evolutionary relationships of the inter/intraspecific color variations on the pereopods of the intertidal hermit crab *Clibanarius* Dana, 1852

Akihiro Yoshikawa

Abstract

Introduction

Intertidal hermit crabs belonging to the genera *Clibanarius* Dana, 1852 have a remarkable inter-specific color variation without distinctive morphological differences. The coloration on the pereopods, therefore, can be one of the reliable characteristics to distinguish *Clibanarius* species. In contrast, distinctive intra-specific color variations are also reported in *Clibanarius* species. Thus, the questions of my doctoral thesis are how such various inter-specific color pattern on exoskeletons evolved in the *Clibanarius* species, and how intra-specific variation of colorations are generated/maintained among the intra-populations. Since these questions are still unrevealed, I conducted the phylogenetic and ecological study to elucidate the evolutionarily relationships of the color patterns and the cause of intra-specific color variations on *Clibanarius* species.

Materials & methods

In Chapter 1, detailed live coloration of shield and cephalic appendages, chelipeds and ambulatory pereopods of species used in this study are described based on freshly collected specimens. In Chapter 2, the phylogenetic relationships among 19 species of *Clibanarius* were reconstructed to understand evolutionarily relationships of each coloration on pereopods based on mitochondrial [12S rRNA, 16S rRNA and cytochrome oxidase I (COI)] and nuclear (histone H3) DNA markers. Then, the ancestral state reconstruction analysis was made focused on four coloration [stripe(s) (ST), bright color spots (SP), solid color (*i.e.,* color pattern without conspicuous strips nor bright spots) (SC) and much brighter color than the color of propodi (BC)]. In Chapter 3, the occurrence of color variations of *C. virescens* were examined with quantitative data regarding color transformation with growth stages, sex, and regional differences of the frequency of the color variations; total 6 color variations were

investigated: 3 variations on the dactyls and 3 variations of pereopod coloration (propodus, carpus, and merus). For statistical analysis, pair-wise t-test with Bonferroni adjustments was conducted to elucidate the size differences in dactyl length/body size (shield length is used as an index of body size) between each dactyl-coloration. Besides, we also conducted the analysis for SL size between each pereopod color variation. In addition, the phylogenetic and population genetic analyses were conducted by using COI region on the population of Indo-West pacific area to test whether intra-specific color variations indicate the initial stages of speciation or not.

Results

In total, nine teen species from *Clibanarius* and three species from three four genera were analyzed using the combined DNA dataset (1,438-bp) that included the four genes. According to the present ML phylogenetic analysis, the most recent common ancestor of the genus *Clibanarius* may have bright color spots, which were probably similar to those found on the ambulatory pereopods of *C. cruentatus* and *C. snelliusi*. In addition, the monophyly of the striped and solid color elements were not supported in this analysis.

By the study on the intra-specific color variations of *C. virescens*, I clarified here that the coloration on the pereopod dactyls gradually changed from solid color (yellow or white) to having dark colored area(s) or transverse band(s) with the growth stage and size at maturity. In addition, the frequency of the occurrence of those color-types differed among regions. The frequency of occurrence of the solid color-type was higher than those of other color-types in the tropical regions. The present results also indicated that the white ambulatory leg type of *C. virescens* was coloration frequently seen in the juvenile stage. Molecular analysis showed that there were no genetic differences among each coloration but detected the genetic differences between the populations from Indian Ocean and those of Pacific Ocean by phylogeographical analysis using mitochondrial COI region.

Discussion

The present results suggested that the striped and solid color elements have evolved multiple times independently, with the ancestral color pattern potentially being scattered, bright color spots with a bright color band. The present findings also suggested that evolutionary adaptation from hard substrates to mudflats and soft sediments might have occurred at least twice. Moreover, during this survey, I recorded the color variation of *C. virescens* which shows the whitish pereopods legs (W-PCM). I also clarified the color variation of *C. virescens* on pereopods were related to their growth stages. Molecular analysis, then, showed that there were no genetic differences among each coloration but detected the genetic differences between the population from Indian Ocean and those of Pacific Ocean by phylogeographical analysis using mitochondrial COI region.

Conclusion

The present study suggested that the striped and solid color elements evolved multiple times independently, with the ancestral color pattern potentially being scattered, bright color spots with a brilliant color band on the ambulatory pereopods. The present results also suggested that evolutionary adaptation from hard substrates to mudflats and soft sediments may have occurred at least twice. In addition, I elucidated that the color variations on dactyls of *C. virescens* was transferred from solid color to dark-colored spots or band depends on the growth stage and size at maturity. Besides, the frequencies of the occurrence of those color-types differed between regions; the appearance of the solid color-type is higher than other color-types in the tropical or sub-tropical regions. The present results also indicated that the white color type was frequently found in the juvenile stage. Color variations on dactyls, therefore, might depend on the shield size or growth stage.