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Evolution of Decoration in Majoid Crabs: A Comparative Phylogenetic Analysis of the Role of Body Size and Alternative Defensive Strategies

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abstract: Although experimental studies have demonstrated the antipredatory advantages of camouflage and its associated costs, few studies have examined the evolution of camouflage in a phylogenetic context. We use phylogenetic comparative methods to examine evolutionary trade-offs associated with camouflage in the crab superfamily Majoidea. The majoids, or spider crabs, are known for their decoration behavior in which they attach materials from their environment to hooked setae on their carapace. We found that coverage of hooked setae on a crab (morphology) strongly predicts decoration cover in the field (behavior). Half of the species examined exhibited decreases in the coverage of hooked setae with ontogeny, and we also found a strong negative correlation between the extent of hooked setae and adult body size among species using independent contrasts, suggesting that size may constrain the evolution of camouflage. Finally, using a well-resolved clade of epialtids (kelp crabs)—many of which decorate little but use color change as an alternative camouflage strategy—we found a negative correlation between utilization of decoration versus color camouflage strategies. Our findings suggest that the costs of hook production and decoration maintenance and/or the lowered adaptive value of camouflage for larger species may limit the evolutionary distribution of decoration camouflage among the majoids.

Keywords: Majoidea, camouflage, independent contrasts, decoration, color change.

Introduction

Although a wide range of animal taxa use concealment strategies such as crypsis or mimicry to avoid predators, relatively little is known about the evolution of these strategies compared with other forms of adaptive coloration, such as aposematism (Cott 1940; Endler 1978; Wicksten 1983; Ruxton et al. 2004; Caro 2005). While there has

been theoretical work modeling the evolution of adaptive coloration across different environments (Merilaita and Tullberg 2005; Berke et al. 2006; Merilaita and Ruxton 2007) and some experimental tests of these models (Merilaita and Lind 2005; Cuthill et al. 2006), phylogenetic comparative analyses have focused primarily on the evolution of conspicuous coloration (i.e., aposematism) and its positive association with traits such as toxicity, gregariousness, and size (Tullberg and Hunter 1996; Summers and Clough 2001; Hagman and Forsman 2003; Nilsson and Forsman 2003; Summers 2003). Curiously, few studies have examined the distribution of cryptic coloration or other concealment strategies across a well-resolved phylogeny (Ortolani 1999; Stoner et al. 2003). Such comparative phylogenetic analyses could help us understand, for example, why species have evolved to use crypsis versus other forms of adaptive coloration such as mimicry and the extent to which ecological and morphological factors affect the evolution of such concealment strategies (Stamp and Wilkens 1993; Ruxton et al. 2004; Grant 2007). Here we use camouflage as a general term encompassing both crypsis (a strategy designed to avoid detection) and mimicry or masquerade (strategies decreasing the risk of recognition) as methods of concealment (Ruxton et al. 2004).

Camouflage is dependent on matching a portion of the background (Endler 1978) and consequently limits animals to particular habitats where their phenotype most closely matches the environment (Cott 1940; Stamp and Wilkens 1993; Ruxton et al. 2004). Using this reasoning, phylogenetically controlled studies of vertebrates have found strong correlations between animal coloration and environment and concluded that coloration patterns in these taxa primarily evolved for camouflage (Ortolani 1999; Stoner et al. 2003; Gomez and Thery 2004; Caro 2005). Studies focusing on single species have documented substantial antipredatory benefits to camouflage (King 1992; Stachowicz and Hay 1999*b*; Johannesson and Ek-

endahl 2002; Thanh et al. 2003; Merilaita and Lind 2005; Hultgren and Stachowicz 2008*a*). However, camouflage can also be costly to produce or maintain (Berke and Woodin 2008) and results in opportunity costs due to restrictions on habitat use. These costs could constrain the evolution of camouflage (Stamp and Wilkens 1993; Carrascal et al. 2001; Ruxton et al. 2004; Hultgren and Stachowicz 2008*a*). For example, studies on individual species have also demonstrated that reliance on camouflage is often greater in juvenile or smaller individuals (Stachowicz and Hay 1999*b*; Palma and Steneck 2001; Grant 2007; Berke and Woodin 2008), possibly because the costs of camouflage outweigh the benefits for larger individuals that may have outgrown predators or are too large to effectively conceal (Cott 1940). However, it is less well understood whether adult body size may similarly constrain the evolution of camouflage across species (Nilsson and Forsman 2003).

While studies focusing on individual species are crucial for elucidating the mechanisms by which camouflage could be costly, larger-scale comparative approaches can complement such studies by examining how these costs (e.g., costs associated with size) may limit the evolution of camouflage over evolutionary timescales. Phylogenetic comparative approaches can be effective tools to examine broadscale patterns in the evolution of defensive traits such as camouflage, in particular, how such defenses may be correlated with other fitness and environmental traits across closely related species. In this study, we use comparative methods to examine broadscale patterns of camouflage within the brachyuran crab superfamily Majoidea to better understand the trade-offs and other factors associated with the evolution of camouflage.

Many crabs in the superfamily Majoidea (also known as spider crabs or decorator crabs) exhibit a unique behavior in which they cover themselves with materials from their environment as camouflage (Wicksten 1993). Although this habit of "decoration" (also called covering or masking) is widespread across many invertebrate phyla (Berke et al. 2006), only majoids attach materials from their environment to specialized hooked setae on their carapace (Wicksten 1979, 1993). Decoration behavior thus requires a morphological adaptation—hooked setae (Wicksten 1979, 1993; Berke et al. 2006)—and hook coverage should be strongly positively correlated with decoration coverage, although crabs may have hooks and choose not to decorate. Decoration has been demonstrated to reduce predation on these crabs (Stachowicz and Hay 1999*b*; Thanh et al. 2003; Hultgren and Stachowicz 2008*a*). However, despite its clear adaptive value, decoration behavior varies widely among the majoids (0%–100% of the body covered) both within and among species. Unlike the majority of brachyuran crabs, majoid crabs have a terminal molt (which corresponds with sexual maturity for many if not all species) and do not undergo indeterminate growth (Rathbun 1925; Hinsch 1972). Many majoid species have been reported to cease or decrease decoration as they approach or reach maturity (Wicksten 1979; Fernandez et al. 1998; Stachowicz and Hay 1999*b*; Cruz-Rivera 2001; Berke and Woodin 2008). For example, Stachowicz and Hay (1999*b*) demonstrated that the majoid *Libinia dubia* generally stops decorating when it reaches a size exceeding the gape width of predatory fish in the system. These data, along with experimental work demonstrating energetic costs to carrying decoration (Berke and Woodin 2008), suggest that intraspecific decreases in decoration cover with increased size may occur because the costs imposed by decoration exceed the antipredatory benefits for larger animals that are less susceptible to predators (Stachowicz and Hay 1999*b*) or for whom camouflage would be visually less effective (Cott 1940). Furthermore, some majoids that do not decorate change the color of their carapace by sequestering pigments from their algal diets (Wilson 1987; Hultgren and Stachowicz 2008*a*) or adopting cryptic lifestyles or morphologies (Patton 1979; Wicksten 1983; Hay et al. 1990; Stachowicz and Hay 1996, 1999*a*) to avoid predators. However, these correlations, while intriguing, are largely anecdotal and limited to a few taxa. Phylogenetically controlled tests of association between decoration, alternative camouflage strategies, and organism size have not been previously conducted. Such tests would provide an important evolutionary perspective in examining whether costs or other factors limit the evolution of decoration behavior in the superfamily Majoidea and, more generally, the evolution of camouflage in other taxa.

In this study, we use a recent phylogeny (Hultgren and Stachowicz 2008*b*) and a combination of new and existing data on decoration behavior and other traits to examine several factors that may influence the evolution of camouflage behavior in the superfamily Majoidea. Using a phylogenetic comparative approach, we examine (1) the degree to which morphology (i.e., hook cover) influences decoration behavior in the field, (2) how decoration extent and associated morphological traits are associated with crab size, and (3) whether there are evolutionary trade-offs between decoration and alternate camouflage strategies such as color change.

Methods

Majoid Phylogeny

We obtained two best estimates of a majoid phylogeny using trees constructed with Bayesian analyses of a combined data set that included sequence data from three loci:

nuclear 28S ribosomal RNA, mitochondrial 16S ribosomal RNA, and the mitochondrial protein coding gene cytochrome oxidase I (Hultgren and Stachowicz 2008b). These included (1) a tree constructed using all individuals sequenced for more than two-thirds of the loci ("complete taxa" tree, $N = 37$ species) and (2) a tree constructed using only individuals with all three loci sequenced ("complete loci" tree, $N = 26$ species; figs. 2 and 3, respectively, of Hultgren and Stachowicz 2008b). Although these trees are topographically similar, we use both in comparative analyses to examine whether the correlations we tested were robust to using trees that maximized either taxon sampling (complete taxa tree) or character data (complete loci tree). Trees included multiple individuals of a species, but species were always monophyletic, so we pruned the phylogeny to include a single exemplar individual from each species ("species" taxa set; Hultgren and Stachowicz 2008b) to avoid phylogenetic biases resulting from unequal replication within species. Species for which decoration data were unavailable were also removed from the phylogeny, but this still left 32 species from seven families, allowing us to broadly test associations between size and decoration. We checked that our sample did not overestimate incidence of decoration across majoids by comparing the percent of "decorated" species (species that decorated during at least part of their lifetime) in our sample (78%) to the percent of such species (∼74%) across 214 North American majoids (Rathbun 1925) for which decoration status could be assigned (K. M. Hultgren, unpublished data). We also calculated contrasts separately for only "decorated" species in our sample ($N = 25$ taxa). Taxa sampled in this study are primarily composed of common majoids from North America and Japan (for further details, see Hultgren and Stachowicz 2008b), spanning 12% of the estimated ∼170 majoid genera. Although logistical considerations prevented sampling of the entire majoid tree (800+ species), simulation studies demonstrate that incomplete taxon sampling can provide statistically satisfactory trait correlations if taxon sampling is random or based on character states of a third independent trait, for example, geography (Ackerly 2000). However, we also tested correlations in this study using two more well-sampled, monophyletic subsets of the tree (the Oregoniidae clade and a clade of epialtid crabs). In addition, our trees included nearly all described eastern Pacific *Pugettia* species (epialtid or "kelp" crabs: *Pugettia producta*, *Pugettia richii*, *Pugettia gracilis*, *Pugettia dalli*, and *Mimulus foliatus*), which formed a well-supported clade with western Pacific *Pugettia* (*Pugettia quadridens* and *Pugettia minor*) as an outgroup. Crabs in this group (also known as kelp crabs) decorate minimally but sequester pigments from the algae they consume, and some change color to match their algal backgrounds in a form of color camouflage

(Hines 1982; Hultgren and Stachowicz 2008a), allowing us to test how alternative defensive strategies might constrain the evolution of decoration behavior in this group.

Quantifying Variability in Decoration

To measure variation in decoration among species in the Majoidea, we assessed hook cover and decoration cover for multiple individuals of 32 different majoid species (mean = 26 individuals per species) from museum and field collections (app. A in the online edition of the *American Naturalist*). For each individual, we measured crab size using maximum carapace width (CW), a standard measurement used in the taxonomic literature. To assess patterns of intraspecific variation in decoration across species that varied in absolute size, we standardized crab size as the percentage of maximum observed size (CW_{st}) using the formula $CW_{st} = (100 \times CW) \times CW_{max}^{-1}$, where CW_{max} is the maximum male or female CW for that species. Maximum CW for each species was obtained from the taxonomic literature (app. A); in the cases when individuals we surveyed were larger than maximum sizes reported from the literature, we used these observed maximum values.

We quantified two measures of decoration cover. First, because hooked setae typically occurred in distinct and dense patches on the carapace, we were able to quantify the fraction of the area of the carapace covered by hooked setae. Since crabs attach decoration to their body using only these hooked setae, hook area represents the morphological component of decoration, or the amount of "potential" decoration area available for crabs. Although multiple types of hooked setae have been identified for a few species (Wicksten 1979, 1993; Rorandelli et al. 2007; Berke and Woodin 2008), such hook variation has not been described for the vast majority of majoids, and we classified all setae that functioned to hold decoration in field and museum specimens as hooked setae. Second, for a subset of species for which we had collected individuals in the field with decoration intact ($N = 20$ species, mean = 13 individuals per species), we measured "actual" decoration—that is, the area of the carapace actually covered by decoration materials—in addition to hook area. We used ImageJ (ver. 1.37; Abramoff et al. 2004) to measure the proportion of the carapace covered by hooked setae or actual decoration on digital images of carapaces. Because we found strong correlations between hook area and actual decoration using both uncorrected species means and phylogenetically independent contrasts (see "Results"), for the remainder of comparative analyses in this study, we use hook cover as a primary index of decoration (and refer to hook cover as decoration). Hook cover likely reflects realized decoration and its associated costs over

longer time periods better than instantaneous measures of actual decoration cover. Although many crabs decorate their legs and chelae, we report decoration values from the carapace only, since legs are often autotomized and at least one leg was missing from many individuals. To quantify ontogenetic variation in decoration, we ran linear models testing for the effects of relative size (CW_{st}), sex, and size × sex interactions on decoration ($\log_{10}$ hook cover) for all species that decorated for at least part of their lifetime. We used one-tailed tests to test whether decoration was negatively correlated with size.

Because many crabs showed decreases in hook area and realized decoration with increasing size (see "Results"), we calculated species-specific values of decoration using three methods. First, to capture ontogenetic patterns into a single metric, we estimated "lifetime" hook cover by calculating the area under the regression curves of hook cover ($\log_{10}$ transformed) and size (CW_{st} from 1%–100%) for each species. For species that showed differences in hook cover between sexes, we calculated the area under the hook cover–size curve for males and females separately and used the average of these two values. Because one species in our study (*Libinia dubia*) varies geographically in its preferences for materials used for decoration (Stachowicz and Hay 2000), we surveyed populations of this species from multiple locations and performed additional analyses to ensure that there were no geographic differences in hook cover (app. B in the online edition of the *American Naturalist*). Second, we also calculated a single mean value of "juvenile" hook cover for each species, since juveniles often exhibited more consistent hook cover, and hook cover at this life stage represented decoration potential when individuals of a species were typically the most vulnerable (and decorated the most). We empirically defined juveniles as individuals with $CW_{st} < 40\%$ (the size at which hook cover began to decline for many species; see "Results") and calculated mean hook cover for all individuals in this category. For species that did not differ in hook cover with carapace size ($P > .05$), we used mean hook cover values across all size classes. Unless otherwise noted, we use both lifetime and juvenile hook cover in phylogenetic comparative analyses examining correlations between a species' decoration ability and other factors. Finally, we coded decoration as a set of categorical variables by dividing the carapace into five different regions (fig. 2) and assessing the presence or absence of hooked setae in these regions in juveniles of each species (for discussion of how these body regions were delineated, see "Results").

Phylogenetic Effects

If shared history influenced decoration cover, closely related species should be more similar in decoration cover than expected by chance. We used both categorical (see fig. 2) and continuous (juvenile and lifetime hook cover) decoration characters to test for phylogenetic effects on the evolution of decoration behavior and size variation in the Majoidea. For these analyses, we used only the complete taxa tree because this tree had the largest number of taxa and many closely related congeners were missing from the complete loci tree (Hultgren and Stachowicz 2008*b*). We first tested categorical decoration characters. Using the program Mesquite (ver. 2.01; Maddison and Maddison 2007), we simulated 10,000 random trees using an equiprobable method that generates random trees such that each possible topology is equally likely (Maddison and Maddison 2007). To test whether decoration was phylogenetically constrained, we first coded decoration for each species (1) using the five discrete decoration characters described previously (unordered) and (2) considering decoration as a single character (presence/absence), and we then examined whether the number of steps in the complete taxa tree was significantly shorter than the number of steps in simulated trees. We ran a similar analysis coding decoration as one linearly ordered character with values ranging from 0 to 5 (0 = no decoration, 5 = all areas covered), used MacClade (ver. 4.08; Maddison and Maddison 2005) to create 10,000 random trees (equiprobable method), and compared the number of steps in the actual complete taxa tree with the simulated trees. Second, we examined whether there was a phylogenetic signal in decoration coded as a continuous character (lifetime decoration) or crab size, using the analysis of traits (AOT) module of the program Phylocom (ver. 3.41; Webb et al. 2007), since this method can accommodate polytomies (nodes with more than two branches) in a tree. The AOT module measures phylogenetic signal using a method modified from Blomberg et al. (2003), which uses the average magnitude of independent contrasts over the tree but incorporates polytomies by using the standard deviation of the descendent trait means instead of the independent contrast (Webb et al. 2007). If a certain trait is phylogenetically conserved (i.e., related species are similar), mean divergence deviation will be smaller than in null model simulations in which trait values are randomized over the tips of a tree. For these analyses, we ran 10,000 null model simulations and compared those with the mean value of divergence deviations in the complete taxa tree. We used two-tailed tests to evaluate whether there was a phylogenetic signal for all analyses.

Character Correlations and Independent Contrasts

We used both majoid trees (complete taxa and complete loci) to test two hypotheses about the evolution of decoration using phylogenetically independent contrasts

(PICs) calculated with the phenotypic diversity analysis program (PDAP) module (Midford et al. 2003) implemented in Mesquite. First, we tested whether morphology was a good proxy for behavior by testing whether hook cover of field-collected specimens was positively correlated with decoration cover of field-collected specimens. Second, across all majoids in our study, we examined whether smaller species decorated more by testing whether hook cover (lifetime and juvenile) was negatively correlated with crab size (CW_{max}). For size–hook cover analyses, in addition to using both majoid trees (complete taxa and complete loci), we tested correlations using a complete taxa tree trimmed of all nondecorating species (e.g., using only species that decorated at some point in their life; $N = 25$ taxa). All trait values used in the analyses were log-transformed unless otherwise noted. Before calculating correlations, we ensured that the absolute value of each contrast was not correlated with the square root of the corrected branch lengths (Garland et al. 1992; Midford et al. 2003) and used branch length transformations if needed (Garland et al. 1992). Results were similar if all branch lengths were set to 1. Because our trees contained "soft" polytomies—nodes giving rise to more than two descendant taxa, likely as a result of lack of information about the true bifurcating pattern of speciation rather than simultaneous speciation events—we subtracted 1 df for each polytomy. This correction has been suggested by Garland and Diaz-Uriarte (1999) to reduce Type I error rates while still retaining power by using the full set of contrasts.

Color Change and Decoration in the Epialtidae

To assess whether decoration camouflage was negatively correlated with the alternative camouflage strategy of color change in kelp crabs, we experimentally quantified the magnitude of color change and decoration cover for five species using data from 3-month laboratory assays. Data for *P. producta*, *P. richii*, and *M. foliatus* from Bodega Bay, California, were obtained in a previous study (Hultgren and Stachowicz 2008*a*), and we used the same methods to obtain data for *P. gracilis* and *P. dalli* (summarized in app. C in the online edition of the *American Naturalist*). Briefly, red individuals of each species were fed algae of that color (red) or a different color (amber), and the degree to which individuals in each treatment changed color upon molting was measured by assessing color change in digital photographs. In addition to using lifetime hook cover values to assess decoration, we experimentally assessed actual decoration cover during these experiments using decoration assays (app. C). Using these data, we tested for a negative correlation between net color change and decoration (lifetime hook cover and actual decoration) using one-tailed tests. We also tested whether decoration de-

creased with crab size (CW_{max}) in this clade and used the Akaike Information Criterion (AIC) to determine whether a model including crab size was more informative in predicting decoration than a model with color change alone (Burnham and Anderson 2002).

Results

Variation in Decoration

Of the species that we examined for intraspecific patterns in decoration cover with size (standardized within species; i.e., CW_{st}), 13 of 24 showed significant decreases in hook cover with increasing size ($P < .0456$, mean $R^2 = 0.55$; app. A; species with significant regressions are shown in fig. 1). In three species (*Hyas coarctatus*, *Oregonia gracilis*, and *Pugettia producta*), males decorated less than females ($P < .0066$), and there was a significant size × sex interaction in *H. coarctatus* ($P = .0013$) and *O. gracilis* ($P = .0013$), in which males exhibited stronger decreases in decoration with ontogeny than females.

In addition to intraspecific ontogenetic variation in decoration, juvenile decoration cover varied quantitatively among species from 0% to 100% (fig. 2) and was tightly correlated with lifetime decoration cover ($F_{1,30} = 432.4718$, $P < .0001$, $R^2 = 0.9351$; data not shown). Categorical decoration characters fell into six nested groupings that corresponded to juvenile hook cover (fig. 2). Although there were exceptions in some species, juvenile crabs typically possessed hooked setae in (1) no portions of their body; (2) their rostrum only (low hook cover = 0.5%–3%); (3) their rostrum, the sides of their body, and some epibranchial regions (minimal hook cover = 6%–18%); (4) their rostrum, sides, epibranchial, and protogastric regions (medium hook cover = 25%–39%); (5) their entire body, excepting some cardiac and metabranchial regions (medium-high hook cover = 55%–70%); or (6) the majority of their carapace ("high" hook cover = 83%–100%). These data were used to divide the crab carapace into five different regions (fig. 2) used to code categorical decoration characters.

Phylogenetic Effects

Decoration, whether coded categorically or continuously, was a strongly conserved character across the majoid tree (fig. 3). When decoration was coded categorically, the length of the actual tree was significantly shorter than lengths of simulated trees, whether we coded decoration as an unordered set of five characters (actual tree length = 27, median tree length of random trees = 37, $P = .0062$), as a single ordered character (actual tree length = 29 steps, median tree length in random trees = 37, $P = .0268$) or as pres-
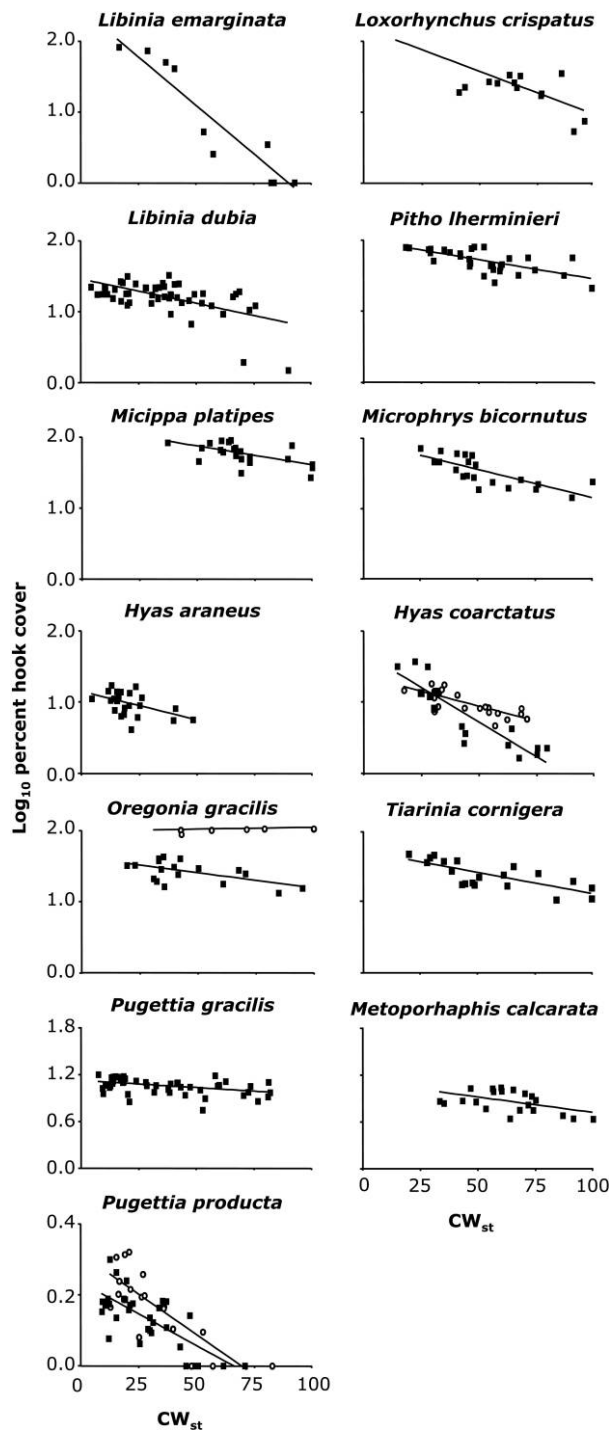
Figure 1: Majoid species exhibiting significant variation in decoration ($\log_{10}$ hook cover, *Y*-axis; note different scales) with size (CW$_{st}$, or percent of maximum recorded size) or sex. Lifetime hook cover is calculated for each species as the area under the regression curve. For *Oregonia gracilis*, *Hyas coarctatus*, and *Pugettia producta*, circles indicate females and squares indicate males. Statistics for individual figures are given in appendix A in the online edition of the *American Naturalist*.

ence/absence (actual tree length = 4, median tree length of random trees = 7, $P = .0238$). Analyses using the AOT module in Phylocom yielded similar patterns using traits coded as continuous characters. Crab size ($\log_{10}$ CW$_{max}$, $P = .0011$) and lifetime decoration ($\log_{10}$ lifetime hook cover, $P = .0309$) showed significantly lower values of divergence deviation than null models, indicating that these characters were phylogenetically conserved.

Character Correlations and Independent Contrasts

Across the full majoid tree, we found that crabs with higher values of hook cover covered a higher proportion of their carapace with decoration, using raw species means ($P < .0001$) and PICs (complete taxa tree $P < .0001$; complete loci $P < .0001$; table 1; fig. 4*a*, 4*b*). Hook cover explained 78%–91% of variation for actual decoration cover.

We found support for the hypothesis that species with a smaller maximum size had more decoration (i.e., hook cover) than larger species (table 1; fig. 4*c*, 4*d*), using both lifetime and juvenile hook cover as decoration variables. There was a significant negative correlation between crab size and decoration using the raw species means (lifetime hook cover $P = .0026$; fig. 4*c*) and PICs, using both the complete taxa tree (log-transformed branch lengths; lifetime hook cover $P = .0007$; fig. 4*d*; juvenile hook cover $P = .0024$) and the complete loci tree (lifetime hook cover $P = .0045$, juvenile hook cover $P = .0064$). There was still a significant negative correlation when all nondecorating taxa were removed (log-transformed branch lengths; lifetime hook cover $P = .0231$). There was also a negative correlation between lifetime hook cover and size in one of the more well-sampled clades within our phylogeny (partial epialtid clade; *Pugettia* + *Mimulus* + *Taliepus*, $P = .0181$). In another well-sampled clade, the Oregoniidae, larger crabs tended to decorate less, but there was no significant negative correlation using PICs (log-transformed branch lengths, $P = .0808$).

Within the east-Pacific *Pugettia* clade, there was a significant negative correlation between decoration and net color change using raw values ($P = .0206$; fig. 4*e*) and PICs using either decoration character (actual decoration $P = .0016$; fig. 4*f*; lifetime hook cover $P = .0138$). Larger crabs tended to rely on color change while smaller crabs relied on decoration, such that there was a positive correlation between crab size and color change ($F_{1,3} = 9.8619$, $P = .0258$) and a negative correlation between crab size and lifetime hook cover ($F_{1,3} = 5.607$, $P = .0493$). AIC testing indicated that a model with color change alone (Akaike weight = 0.66) was more optimal (i.e., more informative, given the number of variables) than a model including only size (Akaike weight = 0.00)
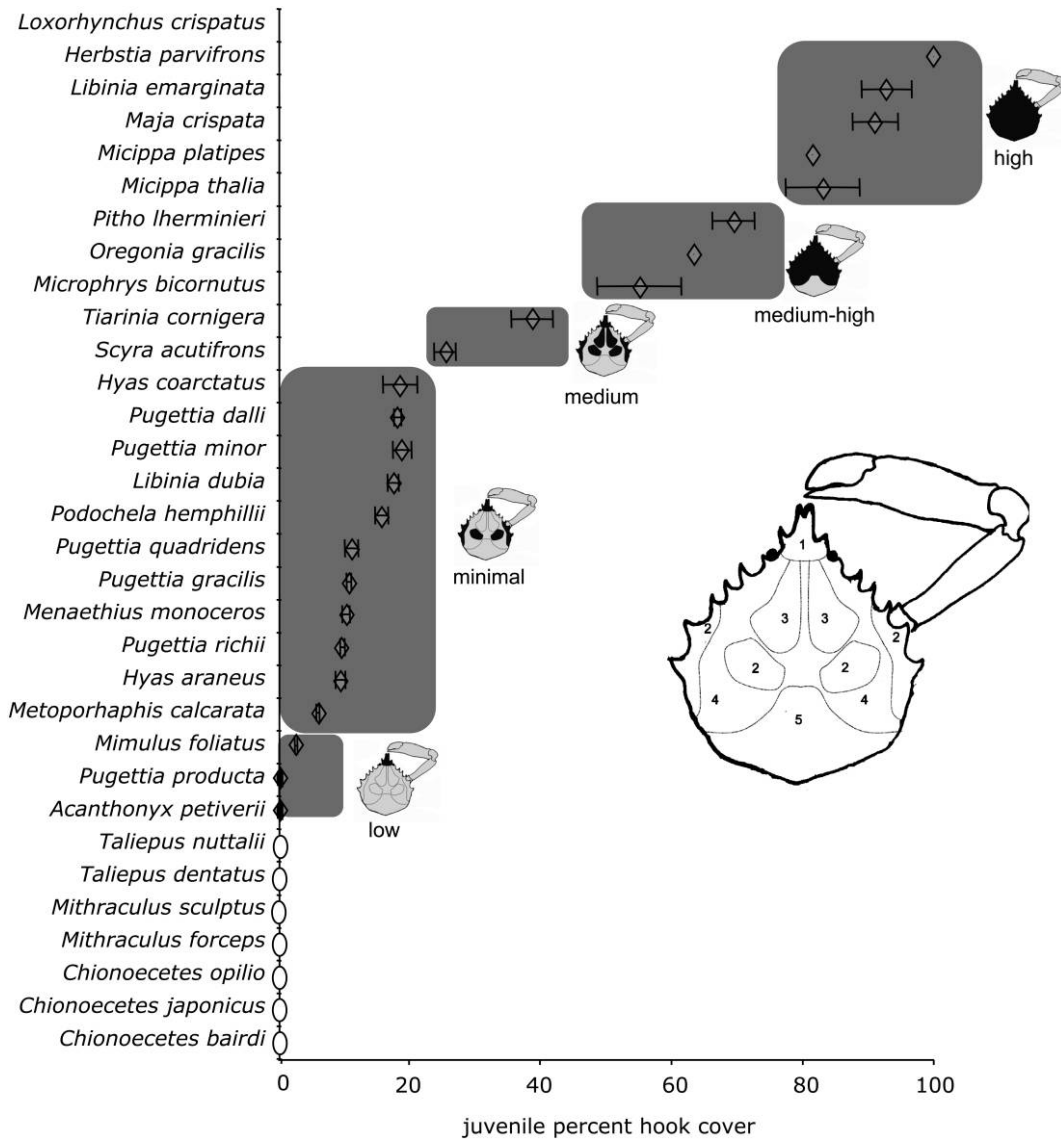
Figure 2: Mean juvenile hook cover and portions of the body covered for different species used in the phylogeny. Large crab illustration represents a generalized majoid (modified from Rathbun 1925), with portions of the body outlined in the order in which they were decorated (*1* = rostrum; *2* = epibranchial areas and sides of the body; *3* = protogastric regions; *4* = mesobranchial areas; *5* = metabranchial and cardiac areas). In the graph, bars represent standard errors, and open ovals indicate nondecorators. Shaded areas indicate majoids belonging to different groupings of categorical decoration characters, and crab diagrams indicate approximate portions of the body covered by decorators in each grouping.

or a model including both variables (Akaike weight = 0.34, $F_{2,2}$ = 36.71, P = .116).

Discussion

This study, representing the first phylogenetic investigation of camouflage morphology in the superfamily Majoidea, shows that decreased decoration is associated with larger body size and the evolution of alternative defenses such as color change. Although ontogenetic decreases in decoration cover have been documented for many individual species of majoid, this study is the first to demonstrate that decoration and size are also negatively correlated across many species over evolutionary scales. This may be in part because larger species and individuals may derive less benefit (relative to costs) from camouflage as a result of their reduced risk of predation and/or the reduced effectiveness of camouflage for larger animals. Energetic
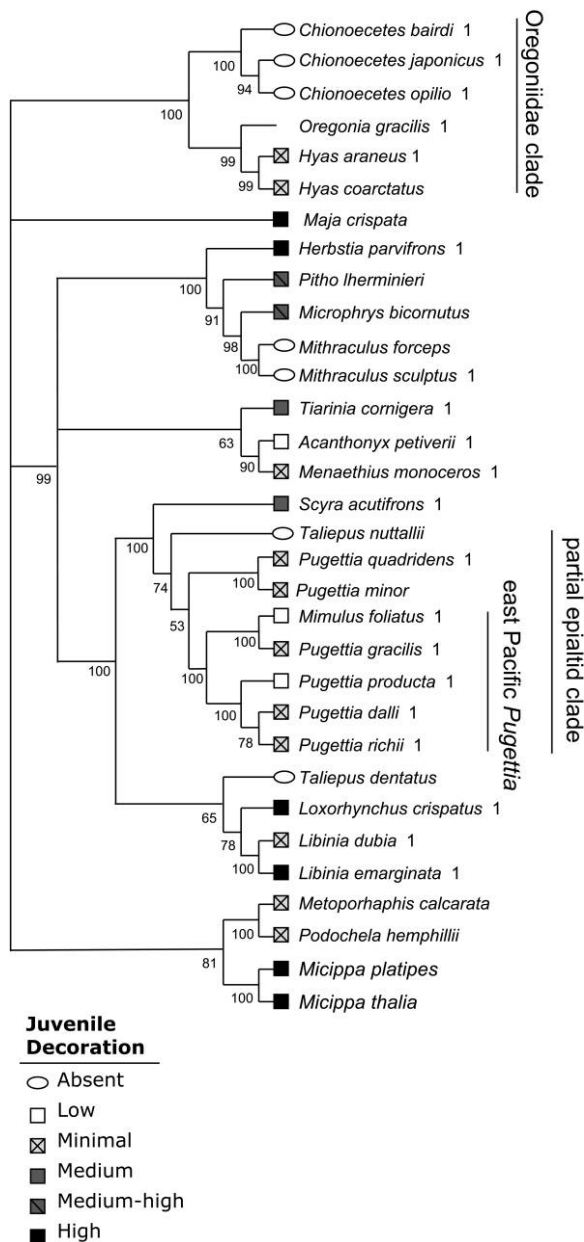
Figure 3: Bayesian consensus tree of majoids used in the study using the complete taxa tree (species names and tree modified from Hultgren and Stachowicz 2008*b*; fig. 2). Numbers under each node indicate Bayesian posterior probabilities for that node; icons mapped to terminal taxa indicate juvenile decoration category groupings. Juvenile decoration for *Oregonia gracilis* is not figured because decoration varied between sexes. Vertical lines delineate smaller clades used in comparative analyses.

costs of decoration or functional constraints may also help explain the strong negative correlation between decoration and color change in east-Pacific *Pugettia* species, suggesting that multiple factors may limit the evolution of dec-

oration behavior. Together with recent experimental evidence for energetic costs of decoration (Berke and Woodin 2008), these findings provide strong comparative support for the idea that costs of decorating may limit the evolution and expression of this camouflage behavior within and among species.

Decoration cover was lost and gained within and among species in a distinctly nested pattern (fig. 2). These data suggest possible developmental constraints on evolution of decoration, that is, that decoration could evolve via addition or deletion of the expression of hooked setae on certain portions of the body. More complete taxon sampling, especially of closely related species, together with a better understanding of the developmental pathways that lead to the production of hooked setae, is needed to adequately examine this idea. Alternately, decoration of certain portions of the body may have different adaptive consequences. For example, all crabs that decorated covered their rostrum, suggesting that concealment of the rostrum (and possibly moving appendages in that area, such as stalked eyes) is especially important for effective camouflage. Even minimal decoration on the rostrum area is effective in reducing predation in field experiments (Hultgren and Stachowicz 2008*a*), and crabs stripped of decoration typically decorate the rostrum area first (K. M. Hultgren and J. J. Stachowicz, personal observation). In contrast, the rear of the carapace was typically decorated only in animals that also decorated all other parts of their body (fig. 2), suggesting that crabs might be limited to placing decoration there by reach of the claws or that their camouflage value is low. Complete decoration including this part of the carapace could serve alternative functions, such as food storage (Woods and McLay 1994; Rorandelli et al. 2007) or protection against nonvisual (e.g., olfactory, tactile) predators. Resolving the degree to which these patterns are driven by developmental constraints or the adaptive consequences of decorating certain portions of the body will require additional studies that integrate ecology, behavior, morphology, and development.

Nondecorating majoids were found in three unrelated genera (fig. 3). Because of the limited taxonomic sampling in this study, we did not perform tests of the directionality of character change, that is, whether nondecorators represent independent losses or gains of camouflage (Harlin and Harlin 2003), though multiple losses may be a more parsimonious explanation, given the high prevalence of decoration in species across our tree and in wider taxonomic surveys of crab decoration (75%–80% of species are decorators; see "Methods"). Loss of decoration in these genera may be influenced by multiple factors. For example, all crabs in the *Mithraculus* genus (Mithracidae) lack hooked setae needed to decorate (Rathbun 1925), but many species are crevice dwellers or are known to form

Table 1: Factors correlated with hooked setae cover across different sets of majoid species, using raw species correlations and phylogenetically independent contrasts (PICs; linear regression model)

Hypothesis, species used in analysis, and tree/clade used for PIC	Linear regression					
	y	x	df	F	P	R^2
1. Actual decoration is positively correlated with hook cover:						
Field collected Majoidea:						
None (raw species means)	Actual decoration	Hook cover	18	192.19	<.0001	.91
Complete taxa	Actual decoration	Hook cover	16	64.84	<.0001	.78
Complete loci	Actual decoration	Hook cover	13	170.02	<.0001	.91
2. Smaller species decorate more than larger species:						
Majoidea:						
None (raw species means)	Lifetime hook cover	Maximum CW	30	9.06	.0026	.23
Complete taxa	Lifetime hook cover	Maximum CW	27	12.71	.0007	.30
Complete taxa	Juvenile hook cover	Maximum CW	27	9.48	.0024	.24
Complete loci	Lifetime hook cover	Maximum CW	20	8.38	.0045	.27
Complete loci	Juvenile hook cover	Maximum CW	20	7.40	.0064	.24
Decorating species only:						
Complete taxa	Lifetime hook cover	Maximum CW	20	4.51	.0231	.16
Oregoniidae:						
Oregoniidae	Lifetime hook cover	Maximum CW	4	2.94	.0808	.42
Pugettia, Mimulus, Taliepus:						
Partial Epialtidae	Lifetime hook cover	Maximum CW	6	7.22	.0181	.55
3. Decoration is negatively correlated with color camouflage:						
East-Pacific *Pugettia*:						
None (raw species means)	Lifetime hook cover	Net color change	3	11.83	.0206	.80
East-Pacific *Pugettia*	Actual decoration	Net color change	3	77.10	.0016	.96
East-Pacific *Pugettia*	Lifetime hook cover	Net color change	3	16.14	.0138	.84

Note: All values used in analysis were $\log_{10}$ transformed. CW = carapace width.

associations with structurally or chemically defended hosts (Patton 1979; Wicksten 1983; Stachowicz and Hay 1996, 1999a), and utilization of such refuges may function as an alternative defense strategy that minimizes the need for decoration camouflage. Decoration could even be a hindrance in such cases either by making the crab contrast with the host coloration or by making it more difficult to squeeze among tight branches for protection, resulting in selection against decoration in these species.

In this study, we found evidence for ontogenetic decreases in decoration cover (for half of the majoids tested) that were paralleled by interspecific decreases in decoration with size. Previous workers have attributed ontogenetic decreases in decoration to result from some combination of the costs of hook production and decoration maintenance, the need to divert energy to reproduction or increased claw size in adulthood, and the lowered adaptive value of camouflage for larger individuals or species that have reached a size refuge from predation (Stachowicz and Hay 1999b; Berke and Woodin 2008). Although our study focused on the morphological component of decoration (i.e., hook cover), decoration also has a strong behavioral component that increases flexibility and may mitigate costs. For example, majoids can behaviorally adjust decoration quantity and species composition in response to predation (Thanh et al. 2003). While we were not able to sample the entire majoid tree (800+ species), consistent negative correlations between size and hook cover across our broad subsample suggest that there may be widespread costs to producing hooked setae.

An important alternative hypothesis that may explain negative correlations between size and decoration is that cryptic decoration camouflage could be more visually effective against predators for smaller relative to larger species or individuals (Cott 1940). While there is some experimental evidence that individual cryptic prey are more difficult to detect than groups of cryptic individuals (Jackson et al. 2005), no work thus far has specifically investigated the influence of organism size on effectiveness of cryptic coloration or patterning. However, comparative studies suggest that the converse is true: conspicuous coloration is associated with increased body size in dendrobatid frog species (Hagman and Forsman 2003), confirming experimental work showing that detectability and effectiveness of aposematic coloration increases with increasing body size (Gamberale and Tullberg 1996) and group size (Riipi et al. 2001). In addition, adult individuals may use different habitats than juveniles where alternate concealment or defensive strategies may be more effective. For example, the kelp crab *Pugettia producta* (included in this study) lives in red algae as a juvenile but shifts to kelp forests as an adult, where strategies such as color camouflage are more effective (Hines 1982; Hultgren and Stachowicz 2008a). Either way, these studies suggest the idea
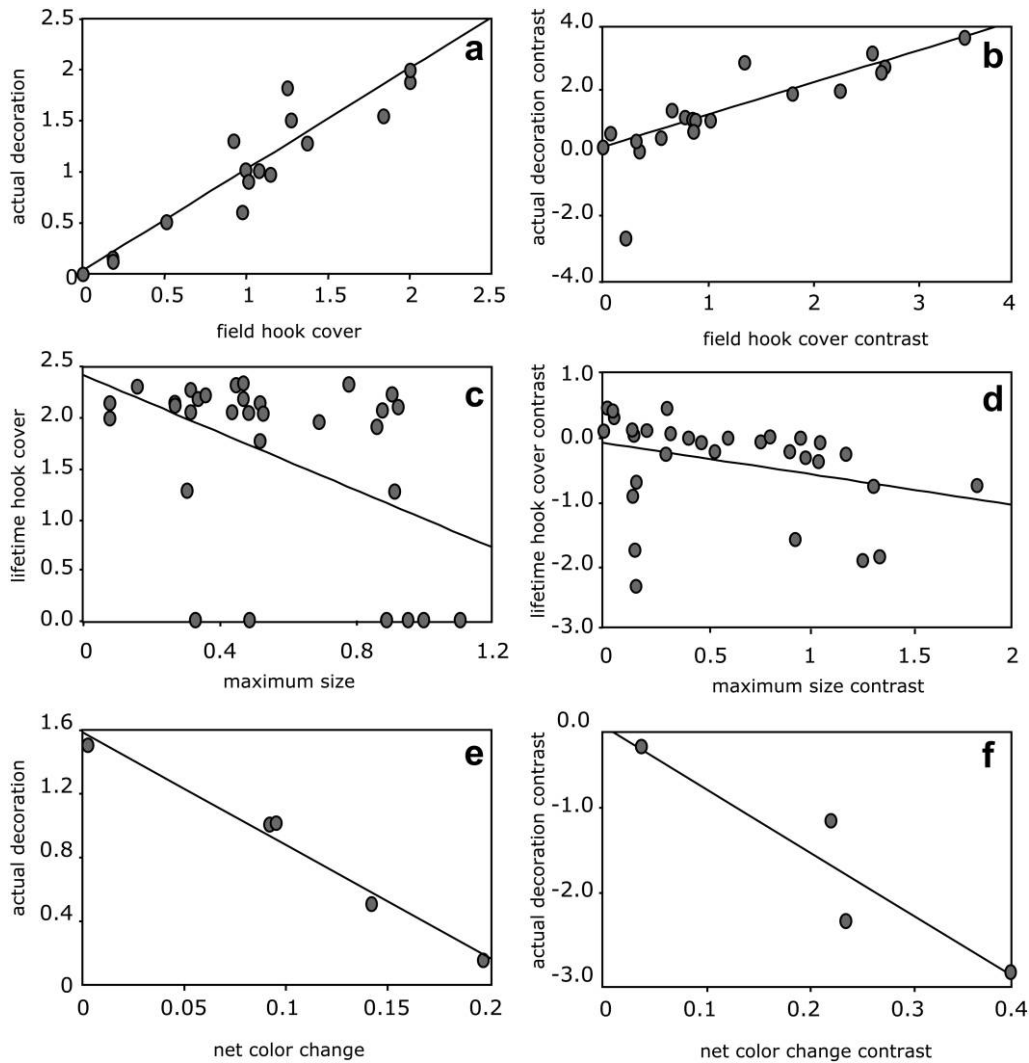
Figure 4: Character correlations using raw species means (*a, c, e*) and phylogenetically independent contrasts (*b, d, f*). All values are $\log_{10}$ transformed. Panels indicate positive correlations between amount of actual decoration cover and hook cover in the field (*a, b*), negative correlations between decoration cover and crab size (*c, d*), and negative correlations between decoration cover and net color change (*e, f*). Statistics for individual correlations are given in table 1.

that the value of adaptive coloration or concealment strategies is strongly influenced by size.

Body size tended to explain more variation in hook cover within smaller, more well-resolved clades such as the Epialtidae ($R^2 = 0.55$) than over the entire majoid superfamily (mean $R^2 = 0.28$). Differences in crab size and decoration among clades are likely driven by several ecological and evolutionary factors, and as such, it may be more appropriate to test specific hypotheses about evolution of decoration camouflage in clades that share a more recent common ancestor. For example, in the eastern Pacific *Pugettia* clade, our experimental assays demonstrated

that the magnitude of color change (as well as size) was negatively correlated with decoration. *Pugettia dalli* (one of the smallest crabs in our study) decorated more and changed color the least, while the largest species in this clade, *P. producta,* decorated the least and changed color the most. Negative correlations between decoration and color change in this group may be caused by allocation trade-offs between color and decoration and/or the potential ineffectiveness of combining these two types of camouflage in algal habitats (for more details, see Hultgren and Stachowicz 2008*a*). Either way, this pattern suggests that simultaneously optimizing multiple defensive strate-

gies may be difficult, as many other studies have noted (Paul and Hay 1986; Stachowicz and Lindquist 2000; Kicklighter and Hay 2007). Additional ecological and comparative studies of closely related majoids using other types of alternate defensive strategies (e.g., body morphology or coloration that matches their habitat) would be useful to elucidate if similar trade-offs occur in other groups.

Our study clearly demonstrates that decoration camouflage is negatively correlated with body size in majoid crabs, both within and between species. While phylogenetic comparative analyses provide a powerful approach to examining large-scale trade-offs between defensive strategies and other factors, experiments examining the adaptive costs of camouflage behavior and other defensive strategies are needed to understand the mechanisms driving such associations. In particular, it is important to understand whether decreases in camouflage with size are due solely to decreased benefits of camouflage for larger individuals relative to the energetic costs (Berke and Woodin 2008) or to decreased visual effectiveness of camouflage in larger individuals. Together with studies in terrestrial systems (Cott 1940; King 1992; Stamp and Wilkens 1993; Hagman and Forsman 2003; Grant 2007) and marine systems (Hacker and Madin 1991; Lindquist and Hay 1996; Stachowicz and Hay 1999*b*; Palma and Steneck 2001; Berke and Woodin 2008), this work supports the idea that the adaptive value of camouflage and other defenses is strongly influenced by body size. More specifically, camouflage may be more adaptive for species and life stages with small body size, whereas conspicuous warning coloration is associated with larger body size (Hagman and Forsman 2003). The associations between camouflage, body size, and other behavioral and habitat changes occurring across ontogeny suggest that the evolution of these traits should be considered in concert.

Acknowledgments

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