

Molecular phylogeny analysis of fiddler crabs: Test of the hypothesis of increasing behavioral complexity in evolution

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ABSTRACT The current phylogenetic hypothesis for the evolution and biogeography of fiddler crabs relies on the assumption that complex behavioral traits are assumed to also be evolutionary derived. Indo-west Pacific fiddler crabs have simpler reproductive social behavior and are more marine and were thought to be ancestral to the more behaviorally complex and more terrestrial American species. It was also hypothesized that the evolution of more complex social and reproductive behavior was associated with the colonization of the higher intertidal zones. Our phylogenetic analysis, based upon a set of independent molecular characters, however, demonstrates how widely entrenched ideas about evolution and biogeography led to a reasonable, but apparently incorrect, conclusion about the evolutionary trends within this pantropical group of crustaceans. Species bearing the set of “derived traits” are phylogenetically ancestral, suggesting an alternative evolutionary scenario: the evolution of reproductive behavioral complexity in fiddler crabs may have arisen multiple times during their evolution. The evolution of behavioral complexity may have arisen by coopting of a series of other adaptations for high intertidal living and antipredator escape. A calibration of rates of molecular evolution from populations on either side of the Isthmus of Panama suggest a sequence divergence rate for 16S rRNA of 0.9% per million years. The divergence between the ancestral clade and derived forms is estimated to be ≈ 22 million years ago, whereas the divergence between the American and Indo-west Pacific is estimated to be ≈ 17 million years ago.

It is commonly supposed that species with complex traits evolve from “simpler” ancestors (1–4). Groups with complex behavior are thought to derive from antecedents with simpler systems (5). This sort of presumption is quite reasonable, given that a sequence of behaviors could have been constructed in evolution by accretion of individual behavioral modules. Such a temporal increase in complexity is not inevitable, though, and recent studies, for example, show strong conservation of social/behavioral traits within primate lineages (6). An important question is whether the evolution of behavioral complexity is always a consequence of selection on behavior itself or whether it might evolve as a byproduct of natural selection, enforced by ecological parameters not directly related to the behavior. Behavior originating as responses to predators, for example, can be incorporated by evolution into reproductive behavioral repertoires (7).

Fiddler crabs (genus *Uca*, family Ocypodidae, subphylum Crustacea) provide an impressive instance of the evolution of complex breeding behavior linked with terrestrialization. Their adaptations to terrestrial life include efficient water retention, extraordinary running ability, excellent vision for perceiving predators at the horizon, and the ability to build high intertidal breeding burrows. Foraging and reproductive

activities occur mostly in air, and vision in air is intimately a part of territorial and mate recognition behavior (8).

The most striking characteristic of male fiddler crabs is a tremendously enlarged and often colorful master claw that is used for species-specific displays, threats, and intermale combat—it most probably evolved under the control of sexual selection (8–11). Species differ in their complexity of reproductive behavior (9). The Indo-west Pacific fiddler crabs have been characterized as less terrestrial and behaviorally simple, in that mating in most species occurs on the surface and the female typically incubates eggs at the surface or in her own burrow. American species tend to be more terrestrial and also display more complex breeding behavior, including more complex waving displays, approach signals between males and females, and mate-attracting burrow structures, such as pillars (7, 8). In nearly all American species, males guard a burrow, and the waving display leads the female to the burrow within which mating usually takes place. Females are then guarded by the male during egg incubation. In American species, males usually wave in dense groups and breeding burrows occur in great densities during the reproductive season (9). Most American subgenera are also morphologically differentiated in relation to the Indo-west Pacific species (8). A single subgenus, *Celuca*, violates the Indo-west Pacific versus American dichotomy: the “American mating style” occurs in both American and Indo-west Pacific representatives of *Celuca* (8).

Two alternative hypotheses for the phylogeny of fiddler crabs reflect the more general debate focusing on the evolution of complex breeding behavior. The more marine, less behaviorally complex Indo-west Pacific fiddler crabs were hypothesized to be phylogenetically ancestral to the American forms, which are morphologically differentiated (8). The subgenus *Celuca* conflicts with the east–west biogeographic dichotomy, as it bears many of the American traits, yet it occurs on both sides of the Pacific. This was explained by recolonization of the Indo-west Pacific from the Americas after the complex traits had evolved (8). An alternative hypothesis proposed by Salmon and Zucker (10) suggested parallel and independent origins of complex breeding behavior in both faunal regions. Under this argument, ecological circumstances in the two biogeographic regions might have selected for different mating systems and degrees of social interaction during breeding.

METHODS

We tested these hypotheses by sequencing 16S ribosomal DNA for all nine subgenera of fiddler crabs (28 species) using standard methods (12, 13). The phylogenetic analysis was performed in three steps, as indicated by break bars on the cladogram (see Fig. 1). First, only transversions and indels were used to identify the most ancestral splits within the fiddler crabs (10 most parsimonious trees, tree length = 337 steps, consistency index excluding uninformative characters = 0.40). Second, only transversions were used in regions of high variability, identified by a sliding window analysis, and both transitions and transversions with equal weights were used in

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regions of lower variability (11 most parsimonious trees, tree length = 681 steps, consistency index excluding uninformative sites = 0.42). Third, all observed substitutions were weighted equally (three most parsimonious trees, length = 576 substitutions, consistency index excluding uninformative sites = 0.48).

We used the sequence divergence between populations of *Uca vocator* collected on either side of the Isthmus of Panama to estimate divergences of deeper splits in the *Uca* clade. We calculated modified Kimura DNA distances using the DNADIST program (14). The time calibration depends upon the estimate of 3–3.5 million years ago for the development of the Isthmus as a terrestrial barrier between marine populations (15), although it has been argued that subtidal species on either side of the Isthmus may have been isolated much earlier (16).

RESULTS

Our results suggest a surprising phylogeny. Fig. 1 shows the phylogeny obtained by parsimony and neighbor joining using the ghost crab (*Ocypode quadrata*) as outgroup, as it is regarded as the closest relative (8, 19). The most ancestral clade among fiddler crabs is represented by the American subgenus *Uca*, combined with the only West African species (*U. Afruca tangeri*). Crane (8) previously thought these two groups to be closely related. Because the most ancestral subgenera of fiddler crabs have their current distribution on either side of the Atlantic, we suggest that the ancestors of all fiddler crabs may have arisen in the Proto-Atlantic and were separated by continental drift. Alternatively, the single West African species may have arisen through a rare migration across the Atlantic, which is known to have happened in other invertebrate groups (20). The relatively large genetic distance between the subgenera *Uca* and *Afruca* militate against a recent dispersal event, however. Unfortunately, the fossil record of the genus *Uca* is sparse, but the oldest occurrence is in Brazil (21) and belongs to the subgenus *Uca*, which is at least consistent with the ancestral position of the subgenus on the molecular tree.

The remaining species of the genus *Uca* comprise two derived sister clades—one containing all Indo-west Pacific

species, the second containing all other American species. The subgenus *Celuca*, which is found in both biogeographic regions, appears to be polyphyletic, which resolves the unlikely hypothesis of multiple cross-Pacific larval transport.

Fig. 2 illustrates the genetic distances associated with important divergence times in the history of the *Uca* clade. The time calibration was based upon the genetic distance between populations of *U. vocator* collected on either side of the Isthmus, which suggested a divergence rate of $\approx 0.9\%$ per million years. Based upon this calibration, the split between American and Indo-west Pacific clades was ≈ 17 million years ago, whereas the split between the ancestral subgenus *Uca* and derived clades occurred ≈ 22 million years ago, or in the early Miocene.

DISCUSSION

The ancestral part of the *Uca* clade is found in the subgenus *Uca*, which is closely related to the ghost crab genus *Ocypode*. The split between this subgenus and the derived clades probably occurred in the Lower Miocene, and the isolation between American and Indo-west Pacific clades occurred soon thereafter, also in the Lower Miocene. The eastern Pacific has therefore been a longstanding biogeographic barrier for the genus *Uca*, as it likely has been for many other marine invertebrates (22).

The most derived and complex breeding behavior, involving male care of incubating females, is therefore found in the most ancestral part of the phylogeny. Thus, the behaviorally simple Indo-west Pacific males may have either lost the “derived” traits, or complex breeding behavior has evolved at least three times independently: the first time in the group including the phylogenetically ancestral American subgenus *Uca* and its close eastern Atlantic ally *U. tangeri*, a second time in the phylogenetically derived American clade, and a third time in the Indo-west Pacific representatives of the subgenus *Celuca*. In recent years, more and more variability in reproductive behavior has been found among the species of *Uca* (10, 23, 24), and it is therefore likely that past characterizations have masked a great deal of variability and convergent evolution. For example, the supposedly derived form of male waving, followed by attraction to the female into a burrow and copu-

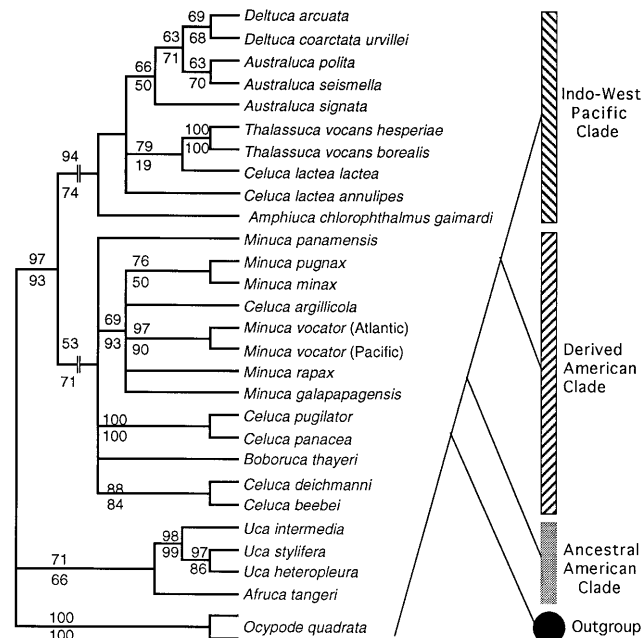


FIG. 1. Strict consensus tree of the genus *Uca* obtained by parsimony (17) and neighbor joining (18). Numbers above the branches are bootstrap values obtained by parsimony, those below the branches are from neighbor joining.

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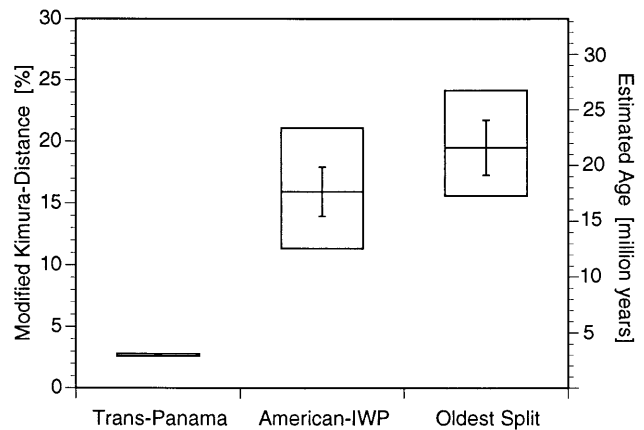


FIG. 2. Modified Kimura DNA distances among fiddler crabs as compared with calculated times of divergence. Calibration is based upon a divergence time of 3 million years for trans-Isthmus of Panama populations of *U. vocator* (left column). The middle column gives the split between the Indo-west Pacific (IWP)- and Atlantic-derived clades, and the right column gives the time of divergence between the subgenus *Uca* (ancestral group) and derived clades. Minimum, maximum, and average Kimura distances (left ordinate) with standard deviations are presented.

lation, has recently been found in *Uca tetragonon*, a member of the supposedly ancestral Indo-west Pacific group (25).

The mitochondrial phylogeny suggests that adaptations to higher intertidal life, such as excellent vision, deep burrowing, rapid locomotion, and water retention preceded and allowed the rise of extensive periods of subaerial reproductive displays. Sexual selection on morphology and behavior may also have been directional, resulting in strikingly similar solutions irrespective of the phylogenetic position. The outgroup genus, *Ocypode*, which from our larger molecular data set on crabs (19) is *Uca*'s closest relative, bears all of the so-called advanced characters, including high intertidal occurrence, deep burrowing, excellent vision geared toward high intertidal life, resemblance to the so-called broad-front American-style fiddler crabs, and even claw waving during courtship in at least one species (26). The eyes of *Uca* and *Ocypode* are quite similar, with a concentration of ommatidia in the center, which was interpreted as an adaptation to detect predators on the horizon (27). It is probably no accident that the waving display occurs above the horizon. It is likely a design whose evolution arose to coopt the visual reaction of semiterrestrial crabs to predators (7, 28, 29). Thus, the evolution of reproductive displays need not have arisen from a "primitive" lower intertidal ancestor. Instead, the relatively high intertidal occurrence on broad mud flats may have been a prerequisite for the evolution of the complex reproductive behavior of *Uca*. It is still possible that the more terrestrialized species of *Uca* predominate in the Americas, as originally suggested by Crane. This may relate to the radiation of the genus into a region where there was a paucity of higher intertidal deposit feeders, leaving an ecological "vacuum" (8).

The molecular phylogeny therefore reverses a traditional view that associates behavioral complexity with an evolutionarily derived status and suggests instead an ecologically consistent picture of an ancestor with traits associated with higher intertidal life that could have been coopted into the evolution of the fiddler crab's complex mating system, allowing the evolution of the extreme dimorphism. While loss of complex structures occurs often in evolution, it would seem to be reasonable to infer that a complex set of behavioral traits might have been built up from simpler sets in ancestors. The hypothesis of an evolutionary trend toward complexity and invasion of the physiologically extreme high intertidal environment, however, seems to be an illusion in this group; many of the so-called advanced features were at the ready in the fiddler crab's ancestors. Our results, furthermore, do not support the IndoPacific center of origin notion and, rather, support a large degree of isolation between the Americas and the Indo-west Pacific, as suggested by Ekman's classic book (30).

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