HYBRIDIZATION OF THE GREEN TURTLE (*CHЕLONИA MYDАS*) AND HAWKSBILL TURTLE (*ERЕTMΟCHЕLΥS IMBРИCATA*) IN THE PACIFIC OCEAN: INDICATION OF AN ABSENCE OF GENDER BIAS IN THE DIRECTIONALITY OF CROSSES

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**ABSTRACT**

On 5 September 1999 a juvenile sea turtle (BLA-428) was captured near Bahía de los Angeles, Gulf of California, Mexico. The presence of intermediate morphological characteristics suggested this turtle was a hybrid between a green turtle (*Chelonia mydas*) and hawksbill turtle (*Eretmochelys imbricata*). BLA-428 exhibited intermediate morphology with respect to number of post-orbital scales, number of prefrontal scales, presence of a median ridge on the lower mandible, carapace scute imbrication, marginal scute dentication, and number of claws on the front flippers. To determine the genotype of BLA-428, we amplified a single-copy nuclear locus CM-14A known to contain species-specific restriction site polymorphisms. Restriction enzyme digests (*Dra I* and *Nde I*) of the CM-14A fragment indicated this individual was a cross between *C. mydas* and either *E. imbricata* or *Caretta caretta*. Sequence of the mitochondrial DNA control region indicated the mother was *E. imbricata* with a common Pacific haplotype. This is the first known case of a *C. mydas* × *E. imbricata* cross in the Pacific Ocean Basin. Further, it provides the first clear evidence for bi-directional hybridization in marine turtles.

Hybridization among marine turtles has been reported from several areas around the world. The general inaccessibility of adults and scarcity of information on mating behavior, however, has hindered the elucidation of specific factors contributing to the occurrence of marine turtle hybrids or to the final outcome of such crosses. Although five of the six hard-shelled species have been reported to interbreed (Table 1), several of these reports are based solely on equivocal morphological data. Crosses of *Caretta caretta* × *Eretmochelys imbricata* (Kamezaki, 1983; Frazier, 1988) and *C. caretta* × *Chelonia mydas* in the Pacific (Kamezaki et al., 1996; C. Limpus, Queensland Dept. of the Environment, pers. comm.) and *C. mydas* × *Lepidochelys olivacea* in the Atlantic (M. Marcovaldi, Project Tamar, pers. comm.) have been described based on the presence of intermediate features in otherwise diagnostic morphological characters. However, these accounts should be accepted with caution due to the inherent highly variable marine turtle morphology.

The application of molecular genetic techniques has significantly facilitated scientists’ ability to confirm incidences of suspected hybridization. Wood et al. (1983) and Conceição et al. (1990) were the first to employ protein electrophoresis to confirm the status of suspected marine turtle hybrids. In the process of describing global phylogeny of several marine turtle species (Bowen et al., 1992, 1994; Karl et al., 1992), a series of mitochondrial (mt) DNA and single-copy nuclear (scn) DNA markers were identified that distinguish marine turtle species using restriction site polymorphisms (RSPs) and/or DNA sequence data. Since it is assumed that mtDNA is primarily, if not exclusively, maternally inherited in marine turtles, the species of the female parent of an individual hybrid can be identified by characterizing the mtDNA. The genotype at a scnDNA locus will be a combination of both parental species alleles. The paternal species, therefore, can be deter-
Table 1. Summary of known or suspected marine turtle hybrids.

<table>
<thead>
<tr>
<th>Hybrid cross</th>
<th>Collection site</th>
<th>Analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chelonia mydas</em>/Eretmochelys imbricata</td>
<td>Western Atlantic (Suriname&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>alozymes</td>
<td>Wood et al., 1983</td>
</tr>
<tr>
<td></td>
<td>Western Atlantic (Suriname&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>mtDNA, scnDNA</td>
<td>Karl et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Eastern Pacific (Mexico)</td>
<td>mtDNA, scnDNA, morphology</td>
<td>this study</td>
</tr>
<tr>
<td><em>Chelonia mydas</em>/Caretta caretta</td>
<td>Western Pacific (Australia)</td>
<td>morphology</td>
<td>C. Limpus, pers. comm.</td>
</tr>
<tr>
<td></td>
<td>Western Pacific (Japan)</td>
<td>morphology</td>
<td>Kamezaki et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Western Atlantic (Brazil)</td>
<td>morphology</td>
<td>Karl et al., 1995</td>
</tr>
<tr>
<td><em>Caretta caretta</em>/Eretmochelys imbricata</td>
<td>Western Pacific (Japan)</td>
<td>morphology</td>
<td>Kamezaki, 1983</td>
</tr>
<tr>
<td></td>
<td>Western Pacific (?)</td>
<td>morphology</td>
<td>Frazier, 1988</td>
</tr>
<tr>
<td></td>
<td>Western Atlantic (Brazil)</td>
<td>alozymes</td>
<td>Conceição et al., 1990</td>
</tr>
<tr>
<td></td>
<td>Western Atlantic (U.S.A.)</td>
<td>mtDNA, scnDNA</td>
<td>Karl et al., 1995</td>
</tr>
<tr>
<td><em>Lepidochelys kempii</em>/Caretta caretta</td>
<td>Western Atlantic (U.S.A.)</td>
<td>mtDNA, scnDNA</td>
<td>Karl et al., 1995</td>
</tr>
<tr>
<td><em>Lepidochelys olivacea</em>/Chelonia mydas</td>
<td>Western Atlantic (Brazil)</td>
<td>morphology</td>
<td>M. Marcovaldi, pers. comm.</td>
</tr>
</tbody>
</table>

<sup>a</sup>Hatchlings housed at the Grand Cayman Turtle Farm, Grand Cayman Islands.
determined by subtracting the mitochondrially determined maternal species from the biparental nuclear data. This type of analysis has confirmed the hybrid status and parental gender directionality of a number of marine turtle F₁ hybrids (e.g., *E. imbricata* [male] × *C. caretta* [female], *C. mydas* [male] × *C. caretta* [female], *C. caretta* [male] × *Lepidochelys kempii* [female]) and a likely F₂ *E. imbricata* × *C. mydas* hybrid (Karl et al., 1995).

Here, we report an additional occurrence of hybridization in marine turtles found in Gulf of California, Mexico: a male green turtle crossed with a female hawksbill turtle. This is the first example of a *C. mydas* × *E. imbricata* cross in the Pacific Ocean Basin. Moreover, when considered with the previously described female *C. mydas* crossed with a male *E. imbricata*, this report provides the first indication for bi-directionality in parental species gender in marine turtle hybrids.

**METHODS**

On 5 September 1999 a commercial fishermen captured a juvenile sea turtle by hand near Bahía de los Angeles, Gulf of California, Mexico (individual number BLA-428). At the time of capture, this individual measured 35.9 cm straight carapace length and weighed 5.4 kg. The presence of intermediate, putatively species-specific morphological characteristics suggested this turtle was a hybrid between *C. mydas* and *E. imbricata*. Based on this suspicion, several morphological traits were characterized and a sample of skin tissue was collected and preserved in NaCl/DMSO solution for subsequent genetic analysis.

The morphological traits of individual BLA-428 measured included: (1) number of post orbital scales, (2) number of prefrontal scales, (3) presence of a median ridge on the lower mandible, (4) carapace scute imbrication, (5) marginal scute dentation, (6) and number of claws on the front flippers.

To determine the genotype of BLA-428, we conducted both mtDNA and scnDNA analyses. Half of the tissue sample was used to isolate total DNA with a modified phenol/chloroform protocol (Karl et al., 1992). We used the polymerase chain reaction (PCR) to amplify the single-copy nuclear locus CM-14A known to contain species-specific restriction site polymorphisms at *Nde* I and *Dra* I recognition sites (Karl et al., 1995). CM-14A is a subfragment of a longer DNA fragment described as CM-14 in Karl et al. (1992). An internal primer (CM14R.1: Ô5Ô ¬ Ô3Õ) was designed to flank the polymorphic restriction sites and used in conjunction with the CM14L primer previously described (Karl et al., 1992). Each restriction enzyme cuts (or does not cut) the PCR fragment at particular sites resulting in the number and lengths of DNA fragments being distinctive for each species. Mitochondrial control region DNA sequences were determined using primers TCR5 and TCR6 and protocols from Norman et al. (1994) with minor modification. Amplification and cycling parameters were as in Norman et al. (1994). Free nucleotides and primers were removed from successful amplifications by centrifugal filtration with Millipore Ultrafree-MC (30,000 NMWL) filter units. The purified and concentrated DNA was sequenced using an ET Terminator sequencing kit (Perkin-Elmer, Co.) and run on an ABI Prism 310 Genetic Analyzer.

**RESULTS**

**MORPHOLOGY.**—The dorsal and lateral views of the heads and carapace are presented for *E. imbricata*, BLA-428, and *C. mydas* in Figure 1. Physical characteristics of *E. imbricata*, BLA-428, and *C. mydas* are summarized in Table 2. Cranial scale patterns of BLA-428 were a mix of *C. mydas* and *E. imbricata* scellation with one pair of prefrontal scales similar to *C. mydas* (Fig. 1A) and three post-orbital scales per lateral surface simi-
lar to *E. imbricata* (Fig. 1B). Mandibular dentation possessed a median ridge similar to that found in *C. mydas*. The carapace scutes were imbricated as in *E. imbricata*; however, the degree of overlap was notably less than that characterizing hawksbill turtles of equivalent size (Fig. 1C). Marginal scute dentation was present and apparently intermediate between *E. imbricata* and *C. mydas* (Fig. 1C). Individual BLA-428 possessed a single claw on each flipper characteristic of *C. mydas*.

**GENETICS.**—The locus CM-14A digested with the restriction enzyme *Dra I* (Fig. 2A) indicated that individual BLA-428 was indeed a hybrid. The entire length of the CM-14A PCR product is approximately 600 bp. The restriction digestion with enzyme *Dra I* cuts the *C. mydas* PCR product at one site resulting in two fragments approximately 330 bp and 270 bp in size (filled triangles in Fig. 2A). *Dra I* does not cut *E. imbricata* or *C. caretta* PCR products (open triangle in Fig. 2A). The *Dra I* digestion of the BLA-428
PCR product resulted in three fragments. One allele remained uncut resulting in the 600 bp fragment as in *E. imbricata* or *C. caretta*. The other allele was cut at one site resulting in two fragments approximately 330 bp and 270 bp as in *C. mydas*.

Locus CM-14A digested with the restriction enzyme *Nde I* (Fig. 2B) does not cut the *C. mydas* PCR product (open triangle). *Nde I* does, however, cut both the *E. imbricata* and *C. caretta* PCR fragments at one site resulting in two fragments (filled triangles). One fragment was approximately 530 bp and the other was approximately 70 bp that is nearly too small to be detected on this gel. The *Nde I* digestion of the DNA fragment from sample BLA-428 resulted in three fragments. One allele remained uncut whereas the other allele was cut at one site, identically to *E. imbricata* and *C. caretta* (Fig. 2). Together these digestions indicate that individual BLA-428 is the result of hybridization between *C. mydas* and either *E. imbricata* or *C. caretta*.

The mtDNA control region sequence was identical to that of *E. imbricata* haplotype A1 described in Broderick and Moritz (1996). This indicates that the female parent of BLA-428 was a hawksbill turtle. Haplotype A1 is widespread at high frequencies in *E. imbricata* populations from the Indo-Pacific and has been found in Malaysia, Saudi Arabia, Seychelles, Solomon Islands, Australia, and Mexico (Broderick and Moritz, 1996; D. Broderick, pers. comm.).

**DISCUSSION**

Hybridization between *C. mydas* and *E. imbricata* has been described previously (Wood et al., 1983; Karl et al., 1995); however, to our knowledge this is the first example of a hybrid cross between these species in the Pacific Ocean. Although we cannot rule out that this is a F₁ (or later) hybrid, the intermediate morphology and heterozygosity at the first, arbitrarily chosen nuclear locus point in the direction of at least an early generation hybrid.

The precise region from which this turtle originated is difficult to ascertain due to the high frequency and widespread nature of the specific haplotype in the Pacific Ocean Basin (Broderick et al., 1994; Broderick and Moritz, 1996, pers. comm.). The closest concentrated (i.e., large) nesting area of *E. imbricata* is located in Hawaii (Balazs, 1982). To access the Gulf of California, a post-hatchling dispersing from nesting beaches at this central Pacific locality could use the easterly flowing North Pacific Current to end up in the eastern Pacific. Such a trajectory, however, would require traversing a pelagic zone exceeding 5000 km (Lagerloef et al., 1999). Based on the small size of this juvenile turtle (SCL = 35.9 cm), we believe that a pelagic journey of this magnitude, coupled with an
800 km northward movement up the Gulf of California (to arrive in Bahía de los Angeles), is unlikely. Further, such a trajectory would carry this turtle across a pelagic region throughout which hawksbill presence is rare to non-existent. Of 2534 turtle sightings during pelagic surface faunal transects in the eastern Pacific Ocean, not a single *E. imbricata* of any life stage was observed (Olson et al., 2000, 2001).

Spatial and temporal sympatry for nesting of these two species in the eastern Pacific provides the opportunity for *E. imbricata* × *C. mydas* courtship in this region. A major *C. mydas* rookery is located in Michoacán, Mexico, and casual nesting has been reported throughout the Pacific coast from Mexico to Central America (Alvarado and Figueroa, 1989). Widespread, albeit rare, nesting of *E. imbricata* has been documented in western Mexico and islands of the eastern Pacific Ocean (Cliffton et al., 1982; Briseño, R., pers. comm.). Hawksbills have been observed to nest occasionally at the largest green turtle rookery along the Pacific coast of Mexico (J. Alvarado, pers. comm.). Further, the May–November nesting season for *E. imbricata* along the Pacific coast of Mexico (Márquez, 1970) partially overlaps with the September–December nesting season for *C. mydas* in the same region (Alvarado and Figueroa, 1989). Finally, the mtDNA haplotype of BLA-428 (A1) occurs with high frequency in the Mexican nesting populations. Of a total of three individuals surveyed from Michoacán, Mexico, Broderick (unpubl. data) identified two as haplotype A1 and one as a closely related haplotype (A2). Although it would be incorrect to draw specific frequency conclusions from this limited sampling, it is highly unlikely that two of three individuals would possess a moderate to low frequency haplotype by chance alone. Nonetheless, further sampling (both of individuals and nesting beaches) is necessary to draw specific conclusions on haplotype frequency in the eastern Pacific.

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**Figure 2.** Restriction digestion pattern of scnDNA locus CM-14A for the enzymes A) *Dra* I and B) *Nde* I. Both restriction patterns are consistent with sample BLA-428 being a hybrid between *C. mydas* and *E. imbricata*. Open triangles indicate uncut amplified fragment from the locus and filled triangles indicate digested fragments. Note that BLA-428 is a mixture of the two patterns.
Our identification of a *C. mydas* [male] × *E. imbricata* [female] cross provides the reciprocal example of the *E. imbricata* [male] × *C. mydas* [female] hybrid offspring reported by Karl et al. (1995) and further emphasizes that post-zygotic isolating mechanisms (i.e., molecular) may not exist in marine turtles despite the large evolutionary divergence between species. Previously, it has been suggested that mechanical differences (i.e., size) between the species may act as a prezygotic isolating barrier to hybridization. Since males must grasp and hold onto a female to copulate, insufficient male size has been suggested as a physical isolating mechanism among sea turtles (Limpus, 1993). This present case, however, indicates that putative mechanical isolation does not necessarily hinder bi-directional interspecific mating and weakens arguments of inherent gender bias in the directionality of marine turtle hybrid crosses. Although information on adult male size is lacking, the mean size of nesting females for the respective populations can provide a relative measure. In the *E. imbricata* [male] × *C. mydas* [female] cross from Suriname (Karl et al., 1995), the mean size of adult female *E. imbricata* (SCL = 83.8 cm; Pritchard, 1969) is considerably smaller than the mean size adult female *C. mydas* (SCL = 111.8 cm; Pritchard, 1969). Thus, small male size could play a limiting role in the prevalence of *E. imbricata* [male] × *C. mydas* [female] hybrids. Therefore, the example described by Karl et al. (1995) may have resulted from courtship between an exceedingly large male *E. imbricata* and a newly mature female *C. mydas*. By contrast, mean size of adult female *C. mydas* (SCL = 77.3 cm; Alvarado and Figueroa, 1989) in the eastern Pacific Ocean and female *E. imbricata* (SCL = 68.6 cm; Witzell and Banner, 1980) in American Samoa (the closest rookery with mean size data available) is similar. Given the relatively large temporal, spatial, and developmental stage variance in size, it seems clear that mean size considerations are a rough measure of mating compatibility at best and likely are not a controlling force in marine turtle species separation.

Unfortunately, behavioral mechanisms facilitating hybridization in marine turtles also are poorly understood. It is likely, however, that rarity of mature *E. imbricata* in this region facilitates a behavioral mechanism for interspecific hybridization similar to that described for *Lepomis* sp. sunfish (Avise and Saunders, 1984). As a female *E. imbricata* completes vitallogenesis, the absence of adult male conspecifics may result in a greater receptivity toward heterospecific suitors. While adult male *C. mydas* have been documented in this region (Alvarado and Figueroa, 1989), all life stages of *E. imbricata* and particularly adults, remain exceedingly rare (Cliftton et al., 1982; Seminoff et al., 2003).

Hybridization among marine turtles provides a compelling example of interbreeding between ancient lineages. In addition to providing new information on the interbreeding potential and gender specificity, this report re-emphasizes the interbreeding potential of ancient marine turtle lineages. Separation between the tribes Caretini (represented by *Caretta*, *Eretomchelys*, *Lepidochelys*) and Chelonini (represented by *Chelonia* and *Natator*) may have occurred as long as 50 mya (Bowen et al., 1993; Ernst and Barbour, 1989). In contrast, the oldest natural hybridizations known for birds and frogs are estimated to be from lineages separated for 20–25 million years (Wilson et al., 1974; Prager and Wilson, 1975) and the oldest known mammal hybridization involves species separated for about six million years (Wilson et al., 1974).

The fact that there is crossbreeding between the Caretini and Chelonini raises interesting questions about the evolutionary relationship between the two groups. How could members of these tribes hybridize after tens of millions of years of divergent evolution? The answer is undoubtedly due, at least in part, to the slow rate of genomic and morpho-
logical evolution of sea turtles (Bowen et al., 1993; Pritchard, 1997). Further, standard definitions of the term species include the presence of a fertility barrier (i.e., pre- and/or post-zygotic isolation; Freeman and Herron, 2001). The present and previous examples indicate that sea turtles somehow avoid this barrier both pre-zygotically (e.g., physical, behavioral, etc.) and post-zygotically (i.e., molecular). Nonetheless, we are unable to ascertain the fertility of BLA-428; therefore, the evolutionary significance of this finding is unknown.

Despite the steady accumulation of accounts of marine turtle hybridization, this phenomenon remains relatively uncommon. In Mexico, all sea turtles are protected under federal law (Anonymous, 1990). However, in countries where sea turtles are afforded protection on a species by species basis, the presence of hybrid animals presents a challenge to the interpretation and enforcement of protective legislation, particularly if hybridization is more common than we are currently aware of (Carr and Dodd, 1983). From a conservation standpoint, to effectively protect sea turtle populations anywhere in the world, a better understanding of mechanisms operating to separate the species is necessary.

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LITERATURE CITED


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