Journal of Experimental Marine Biology and Ecology 463 (2015) 63–71

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

High frequency of multiple paternity in a solitary population of olive ridley sea turtles in Honduras

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ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form 24 October 2014
Accepted 25 October 2014
Available online 22 November 2014

Keywords:
Inter-beach movement
Lepidochelys olivacea
Maternal samples
Microsatellites
Nest-site fidelity
Pooled samples

ABSTRACT

Females of all seven living species of sea turtles are known to be polyandrous and show multiple paternity. The frequency of multiple paternity varies among species, and among populations of the same species. In the olive ridley sea turtle (*Lepidochelys olivacea*), multiple paternity levels correlate with the abundance of individuals in the mating system, being much higher in arribada rookeries than in solitary nesting sites. We used two highly polymorphic microsatellite markers (Cm84 and Or1) to assess the level of multiple paternity in an olive ridley solitary population nesting in the Gulf of Fonseca, Honduras. We found evidence of multiple paternity in 6 out of 8 clutches (75%), with a minimum number of two fathers in four clutches, and a minimum of three in the remaining two clutches. This high level of multiple paternity in a small solitary population suggests that some of the females nesting in Honduras may be coming from proximal Nicaraguan arribada nesting beaches. Historical evidences and recent satellite telemetry data support this hypothesis. In addition, we show that multiple paternity studies can be effectively performed in the absence of maternal samples, and that pooled DNA samples can be used with results comparable to individual hatchling sampling in multiple paternity analyses.

1. Introduction

In order to make effective management decisions and improve current conservation projects on nesting beaches, it is important to accurately estimate population size, population structure, and reproductive behavior. In populations where polyandry occurs, multiple paternity influences the effective population size (Sugg and Chesser, 1994) and the genetic variability within a population (Baer and Schmid-Hempel, 1999). Multiple paternity studies yield valuable information regarding mating patterns, and help in understanding population structure (Jensen et al., 2006). Recent studies have shown evidence of multiple paternity in all sea turtle species: green (*Chelonia midas*) (FitzSimmons, 1998; Lee and Hays, 2004), loggerhead (*Caretta caretta*) (Moore and Ball, 2002; Zbinden et al., 2007b), leatherback (*Dermochelys coriacea*) (Crim et al., 2002; Stewart and Dutton, 2011), hawksbill (*Eretmochelys imbricata*) (Joseph and Shaw, 2011), flatback (*Natator depressus*) (Theissinger et al., 2009), olive ridley (*Lepidochelys olivacea*) (Hoeckert et al., 1996; Jensen et al., 2006) and Kemp's ridley (*Lepidochelys kempi*) (Kichler et al., 1999), with high inter- and intraspecific variability (Uller and Olsson, 2008). In the case of olive ridleys, Jensen et al. (2006) showed that multiple paternity strongly depends on reproductive patterns, with arribada nesters showing much higher rates than solitary nesters. They suggested that the frequency of multiple paternity depends primarily on the abundance of individuals in the mating system, and calculated the relationship between population size and multiple paternity levels for the genus *Lepidochelys*.

Because of their abundance, high polymorphism content, codominance, easy detection, and transferability among studies, microsatellites are ideal molecular markers for paternity studies (Aggarwal et al., 2004). For assessing multiple paternity in sea turtle clutches, it is not unusual to analyze both the mother and the offspring. Once the maternal alleles for each microsatellite marker have been identified in the hatchlings, the remaining alleles constitute the paternal contribution. Multiple paternity can be inferred in egg clutches laid by a single mother from the presence of more than two paternal alleles, with three alleles meaning at least two fathers, and five alleles meaning at least three fathers. The analysis of individual offspring genotypes allows estimating the paternal contribution of each father, and in some cases, identifying actual multiple paternity with less than five total alleles, when the observed distribution of alleles is not compatible with only one father (Jensen et al., 2006).

Although determination of multiple paternity in single clutches based on knowledge of the maternal and individual offspring genotypes is the ideal and most informative procedure for multiple paternity studies, this protocol may sometimes be impractical or impossible. In some cases, samples from mothers may be unavailable, such as in

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http://dx.doi.org/10.1016/j.jembe.2014.10.023

conservation projects where beaches are monitored and nests are collected during morning patrols (de Santos Loureiro, 2008; Godgenger et al., 2009; Zbinden et al., 2007a). In other cases, the high number of samples required in typical multiple paternity studies (Hoeckert et al., 1996; Hoekert et al., 2002; Jensen et al., 2006; Stewart and Dutton, 2011), become cost prohibitive. Taking into account that it is the total number of different alleles in each clutch that establishes both presence and frequency of multiple paternity in a population, maternal samples are not strictly necessary for detecting multiple paternity. In the absence of female samples, the presence of five or more alleles in a single nest evidences at least two contributing males, and thus, multiple paternity. Pearse et al. (2002), Theissinger et al. (2009), and Valenzuela (2000) have previously applied this criterion in turtle multiple paternity studies that lacked maternal samples for a number of clutches. The specific genotypes of individual offspring are not essential information either. Given that multiple paternity can be correctly assessed knowing the total number of alleles in a clutch, it may be advantageous in terms of time and budget to obtain this information via pooled samples from hatchlings in a clutch, rather than by individually analyzing each hatchling sample. The use of pooled samples for microsatellite analysis has been successful in human forensic and epidemiological studies with high numbers of individuals (Pacek et al., 1993; Sham et al., 2002). However, due to the frequent presence of PCR artifacts, the electrophoretic patterns of pooled samples tend to be complex and difficult to interpret (Schnack et al., 2004). Preliminary DNA pooling is recommended for paternity studies, to reduce the number of tests required to identify potential parents for an individual progeny (Curnow and Morris, 1998), but is rarely used in multiple paternity studies. Gosselin et al. (2005) pooled eggs from individual pleopods in a multiple paternity study on the American lobster, *Homarus americanus*, following Urbani et al. (1998), who previously used this method with the snow crab, *Chionoecetes opilio*. To our knowledge, the use of pooled DNA samples has not been reported to date in multiple paternity studies on sea turtles or any other vertebrates.

Olive ridley sea turtle eggs have been economically exploited in the South coast of Honduras since the 1940s (Campbell, 2007). Significant population declines led to the implementation of conservation measures by the Honduran government in 1975, which established an ongoing yearly protected period when the collection of eggs is forbidden and the eggs are relocated to hatcheries (Minarik, 1985). However, studies on the olive ridley population nesting in Honduras are scarce. The environmental NGO *Protective Turtle Ecology Center for Training, Outreach, and Research, Inc.* (ProTECTOR) has been monitoring the nesting beaches since 2007 (Dunbar and Salinas, 2008; Dunbar et al., 2010), yet until now, no genetic studies have been performed.

Olive ridley nesting beaches in Honduras are located within the Gulf of Fonseca, a shallow-water inlet of the Pacific Ocean, 80 km long and 50 km wide, sheltered by islands at its entrance (Lemay et al., 2007) (Fig. 1). Punta Ratón (13.26570 N, 87.51228 W) is the main nesting beach in the country, with an estimated number of 400–500 nests per season (Dunbar, personal communication). Three other nesting beaches are known along the east coast of the Gulf: El Venado (13.11581 N, 087.42725 W), which receives approximately 200–250 nests per season; along with smaller sites at Boca del Río Viejo and Cedeño, with approximately 80–140 nests each per season (Dunbar, personal communication). According to historical reports, 100% of eggs from Punta Ratón were consumed for more than three decades (1940s–1970s) (Campbell, 2007) before the establishment of protection measures. The fact that the turtle population nevertheless persisted caused Pritchard (2007b) to speculate that females nesting at Punta Ratón may, in reality, come from arribada populations at the Chacocente and La Flor beaches in Nicaragua (Pritchard, 2007b).

The main goals of our study were: 1) to assess the levels of multiple paternity in the olive ridley sea turtle population nesting in the South

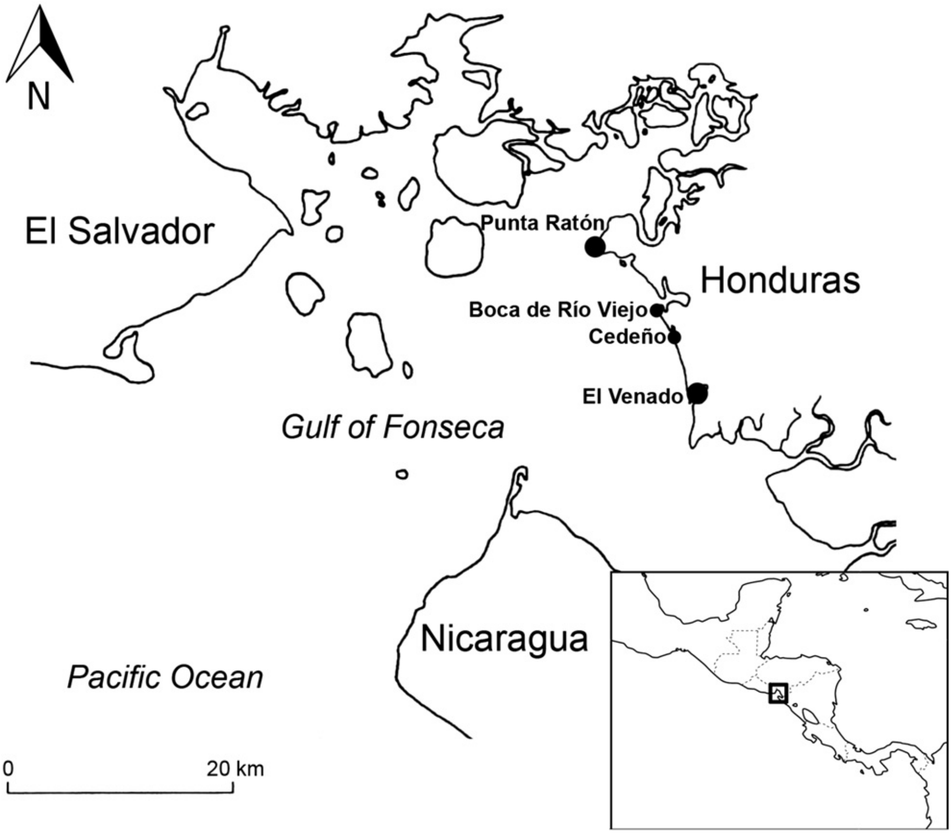

Fig. 1. The Gulf of Fonseca. Circles indicate the four main nesting beaches for *L. olivacea* on the South coast of Honduras. Large circles indicate the two main *L. olivacea* nesting beaches at Punta Ratón and El Venado, the field sites for this study.