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A genetic assessment of polyandry and breeding-site fidelity in lemon sharks

JOSEPH D. DiBATTISTA,* KEVIN A. FELDHEIM,† XAVIER THIBERT-PLANTE,* SAMUEL H. GRUBER‡ and ANDREW P. HENDRY*

Redpath Museum and Department of Biology, McGill University, 859 Sherbrooke Street West, Montréal, Québec, Canada H3A 2K6, †Field Museum, Pritzker Laboratory for Molecular Systematics and Evolution, 1400 South Lake Shore Drive, Chicago, IL 60605, USA, ‡Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, FL 33149, USA

Abstract

We here employ 11 microsatellite markers and recently developed litter reconstruction methods to infer mating system parameters (i.e. polyandry and breeding-site fidelity) at a lemon shark nursery site in Marquesas Key, Florida. Four hundred and eight juvenile or subadult sharks were genotyped over eight complete breeding seasons. Using this information, we were able to infer family structure, as well as fully or partially reconstruct genotypes of 46 mothers and 163 fathers. Multiple litter reconstruction methods were used, and novel simulations helped define apparent bias and precision of at least some mating system parameters. For Marquesas Key, we find that adult female lemon sharks display high levels of polyandry (81% of all litters sampled) and stronger fidelity to the nursery site than do males. Indeed, few male sharks sired offspring from more than one litter during the course of the study. These findings were quite similar to previous results from another lemon shark nursery site (Bimini, Bahamas), suggesting conserved mating system parameters despite significant variation in early life-history traits (i.e. body size and growth) among sites. The finding of at least some site fidelity in females also supports the need for careful conservation of each nursery.

Keywords: litter reconstruction, mating system, microsatellite, philopatry, polyandry, shark

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Introduction

Most long-term studies of animal mating systems in the wild have focused on just a few model species, or have only examined a single population within a species (but see Luyten & Liley 1991; Bollmer *et al*. 2003). This is unfortunate because inter- and intraspecific variation in mating system characteristics can be crucial to effective long-term conservation and management, particularly if mating is tailored to local conditions owing to either selection or plasticity. For example, the degree of polyandry (i.e. females mating with multiple males) can influence population-level processes, such as population growth rate and extinction risk, by altering genetic variability, the level of inbreeding, and adaptive potential (for review see Frankham 2005). Polyandry can also have individual-level effects by altering average offspring viability and reproductive success (Zeh & Zeh 2001). As another example, fidelity to specific mating or breeding grounds has implications for the degree of genetic (and demographic) connectivity among populations, and therefore the spatial scale of their management in nature (Avise 2004; Waples & Gaggiotti 2006). We address these questions for a taxonomic group (i.e. sharks) where mating systems have almost never been examined among multiple nursery sites of a single species.

Our work focuses on the lemon shark, a large, placentally viviparous coastal species found throughout the western Atlantic, on the west coast of Africa, and in the Pacific near Baja California (Compagno 1984). Adult females of this species use shallow, estuarine nursery areas for both mating and parturition (Feldheim *et al*. 2002a). We have previously described the mating system of this species at one nursery site, Bimini, Bahamas. In brief, Feldheim *et al*. (2002a, 2004)

Correspondence: Joseph D. DiBattista, Fax: (514) 398 3185; E-mail: joseph.dibattista@mail.mcgill.ca

found that females mated polyandrously and usually returned to Bimini biennially to give birth. The present study makes a similar analysis for another lemon shark nursery at Marquesas Key (MK), Florida.

A comparison of mating system parameters between these nurseries is particularly informative given that they are ecologically similar, and yet juvenile lemon sharks have very different life histories (Barker *et al*. 2005). Compared to Bimini, Marquesas sharks are significantly larger at age (length: 74 cm vs. 54 cm at age 1) and grow much faster (20 cm vs. 6 cm between age 0 and age 1). This is surprising given that these sites were found to form part of one large panmictic population based on microsatellite analyses (Feldheim *et al*. 2001). From an evolutionary perspective, however, these two nursery sites likely represent separate populations. In support of this, there appears to be little contemporary exchange of breeding individuals among nurseries (J. DiBattista, unpublished data). Large and fast-growing individuals are also strongly selected against at Bimini, Bahamas (DiBattista *et al*. 2007). This argues for local adaptation in the lemon shark; strong selection may be maintaining considerable divergence in juvenile size and growth despite substantial gene flow (e.g. Hendry *et al*. 2002; Saint-Laurent *et al*. 2003). Thus, the present study provides a rare opportunity to examine the extent to which mating systems are consistent between nursery sites, despite dramatic differences in traits that might influence selection on mating parameters.

We aim to specifically examine the two mating system parameters emphasized above: polyandry and breeding-site fidelity. With respect to polyandry, this mating strategy has been identified in several shark species to date. Some authors have suggested that this behaviour provides indirect genetic benefits to offspring (Feldheim *et al*. 2004; Daly Engel *et al*. 2007), although a recent explicit test failed to find supporting evidence (DiBattista *et al*. 2008). With respect to nursery-site fidelity, little is known for sharks, but inferences from recent genetic work suggest that at least some females return in multiple years to the same nursery sites (Feldheim *et al*. 2002a; Feldheim *et al*. 2004; Keeney *et al*. 2005). If this were indeed a general pattern, it would suggest the need for local conservation and management of each nursery — because these sites are important for offspring survival and growth (Branstetter 1990; Heupel *et al*. 2007).

Size and growth differences at juvenile life stages will influence the length and age at which indeterminately growing animals mature (see Hutchings 1993). Work in other taxa has found that early life-history traits, as well as experience as a juvenile, influence both mating strategies (e.g. sneaker vs. dominant males, Gross 1991; monogamous vs. polygamous females, Valimaki & Kaitala 2007), and subsequent reproductive success (e.g. Emlen 1994; Fleming *et al*. 1997). Polyandry in particular appears to be dependent on body size and age, with offspring achieving larger size and maturing faster, subsequently mating with more individuals each year (see Valimaki & Kaitala 2007). Thus, based on this, we might expect a higher proportion of adult sharks at Marquesas to exhibit polyandrous behaviour compared to Bimini sharks. Similarly, the observed morphological differences among nursery sites may be indicative of site-specific adaptations to local environmental conditions (i.e. local adaptation; Taylor 1991), and so in this case philopatry should be prominent, and site specific.

Genetic methods are particularly important for characterizing the mating system of sharks. Here, direct observations of mating are often difficult because male and female sharks do not remain associated following copulation, and the mother does not remain with her offspring after they are born (for review, see Pratt & Carrier 2001). Furthermore, long-term sperm storage in paired oviducal glands suggests the potential for sperm competition (Pratt 1993; Pratt & Tanaka 1994). Despite the likely utility of genetic methods however, they have only been applied to a few shark species, including nurse sharks (*Ginglymostoma cirratum*, Saville *et al*. 2002), lemon sharks (*Negaprion brevirostris*, Feldheim *et al*. 2002a; Feldheim *et al*. 2004), sandbar sharks (*Carcharhinus plumbeus*, Daly Engel *et al*. 2006; Daly Engel *et al*. 2007; Portnoy *et al*. 2007), and bonnethead sharks (*Sphyrna tiburo*, Chapman *et al*. 2004). Moreover, these studies have mostly been based on only a few litters sampled within a single year (but see Feldheim *et al*. 2002a; Feldheim *et al*. 2004), and just one study has examined mating systems at more than one site (Chapman *et al*. 2004).

With few exceptions (see Saville *et al*. 2002), any genetic analyses of shark mating systems are faced with the logistic constraint of sampling putative parents, owing to their high vagility. Instead, one must intensively sample and genotype the offspring cohort, and use these to reconstruct parental genotypes in order to deduce mating system parameters (for example see Feldheim *et al*. 2002a; Feldheim *et al*. 2004). Until recently, these analyses were based on manual litter reconstruction methods or pairwise likelihood approaches (i.e. KINSHIP, Goodnight & Queller 1999). New genetic algorithms (e.g. COLONY Wang 2004) have been developed that use genetic information from all sampled offspring simultaneously (group-likelihood approach) to infer family structure, and thus mating system parameters, even without sampled adults.

In this study, we genetically characterize lemon shark mating patterns at the MK nursery site using microsatellite markers and multiple litter reconstruction methods. We also make comparisons to results for lemon sharks at Bimini, Bahamas, to determine whether mating systems are conserved species-wide, or tailored to individual environmental and selective conditions. These analyses are aided by simulations that inform the ability of our methods to recover known reproductive parameters (i.e. the proportion

of polyandrous litters in this case), which also allow, for the first time, robust comparisons between nursery sites by placing statistical confidence in our findings.

Materials and methods

Sample collection

Marquesas Key (MK; 24°34.13′N, 82°07.40′W) is a mangrove-fringed island found in the Florida Keys, approximately 25 km west of Key West. MK encloses a shallow seagrass lagoon (approximate area: 22 km^2), subdivided by several deep channels, that serves as a nursery for between 75 and 100 juvenile lemon sharks in any given year (S. Gruber, unpublished data). MK is also a National Wildlife Refuge and part of the Florida Keys National Marine Sanctuary; our study thus provides an opportunity to characterize the genetic mating patterns of lemon sharks at a relatively pristine nursery site.

Annual sampling (1998–2000 and 2002–2006) always took place soon after pupping by adult females (i.e. between July and September), and effort varied among years (between 7 and 30 consecutive days). Although sampling was thus not exhaustive, most juveniles were likely captured in at least some years given that increased sampling effort did not lead to increased catches (S. Gruber, unpublished data). Newborn and juvenile sharks were captured in the lagoon by using gill nets (180 m long × 2 m deep), as previously developed and used at our comparison site, Bimini, Bahamas (see Manire & Gruber 1991). Subadult sharks were captured with rod and reel fishing gear on the flats, just outside of the main nursery lagoon. Adult sharks were rarely caught. When feasible, each shark was weighed (kilograms), measured for precaudal length (PCL, tip of snout to precaudal pit in millimetres; Compagno 1984), and tagged intramuscularly with an individually coded passive integrated transponder (PIT) tag. Each subsequent time a shark was captured, we recorded its tag number and again measured its length and mass. A small (2 mm^2) piece of fin tissue was taken from every shark for subsequent DNA extraction.

Age classification

Most age-0 sharks could be identified based on the presence of an open umbilical scar, which slowly closes during the first few months of life. The age of sharks without obvious umbilical scars was determined based on body length — because length frequency distributions are almost non-overlapping between ages (for more details see Barker *et al*. 2005). For the few individuals near the size threshold between ages, or suspected to be from older age classes (i.e. age 3 and up), microsatellite analyses were used to match individuals of uncertain age to their siblings of known age (for details see below). Note that age-1 individuals sampled in 1998 were used to provide information on family structure the year before sampling (1997), just as age-1 individuals sampled in 2002 were used to make some inferences about the year in which sampling was skipped (2001).

Microsatellite isolation, characterization, and genotyping procedures

Total genomic DNA was extracted from all fin samples following a salting-out protocol (Sunnucks & Hales 1996) and genotyped using a combination of six dinucleotide microsatellite primer pairs described elsewhere (LS22, LS30, LS48, LS52, LS54, LS75; Feldheim *et al*. 2002a, b) and five new tetranucleotide microsatellite loci (see Appendix S1 and Table S1, Supplementary material). Multilocus genotypes were generated for a total of 408 lemon sharks captured during the study period, with all individuals being typed for at least 10 of 11 loci.

Genotyping errors can be problematic for litter reconstruction (Hoffman & Amos 2005; but also see Wang 2004). To minimize such errors, (i) an allelic mobility reference was constructed for every locus and included in each run to ensure accurate and consistent scoring (Feldheim *et al*. 2001), and (ii) any failed polymerase chain reactions (PCR), or samples with weak banding intensity or homozygous genotypes, were repeated up to three additional times to avoid errors due to allelic dropout or false alleles (Taberlet *et al*. 1996). We also estimated the rate of potential genotyping errors as per Hoffman & Amos (2005) by independently re-genotyping 55 randomly selected individuals at each locus (13% of all samples), and comparing them to the original genotypes (Table S2, Supplementary material). The incidence of detectable PCR amplification errors when averaging over all 11 loci was 0.0018 errors per reaction (range: 0–0.02), 0.0045 errors per allele (range: 0–0.05), or 0.0017 errors per single locus genotype (range: 0–0.018). Furthermore, an independent observer re-scored these samples in blind fashion, identifying 0.0018 typing errors per reaction (range: 0–0.02), 0.0045 errors per allele (range: 0–0.05), or 0.0017 errors per single locus genotype (range: 0–0.018). It should be noted that there was a clear bias among loci; all amplification and scoring errors occurred at a single locus (LS30). Possible reasons include (i) it was a dinucleotide repeat, (ii) amplification was inconsistent, and (iii) scoring was the most difficult. These values are still considered low however (see Hoffman & Amos 2005) and therefore used as a reference in later pedigree analyses described below.

Identification of unintentionally resampled individuals

Of the 419 initially sampled sharks, we found 11 pairs that had identical composite genotypes, as identified by the program IDENTITY version 1.1 (Amos 2000). It is unlikely

that the members of each pair were different individuals who were genetically identical by chance; the probability of identity (P_{ID}; Paetkau & Strobeck 1994) across all individuals and loci was very low in our data set ($P_{ID} = 1.11 \times 10^{-15}$, single locus range: 0.005–0.23; for comparison see Hoffman & Amos 2005). Moreover, our power to uniquely identify related individuals (PI_{sib}, Paetkau *et al*. 1995; calculated using GIMLET version 1.3, Valière 2002) was very high ($PI_{sib} = 1.40 \times 10^{-5}$, single locus range: 0.28–0.51). Our set of markers is therefore sufficient to discriminate among potential unique or duplicate genotypes, and to even distinguish siblings with high confidence. Thus, the 11 pairs of genetically identical samples were probably sharks who had been captured twice but had lost their tags between sampling periods — which occurs at a frequency of about 12% in lemon sharks at the Bimini site (Feldheim *et al*. 2002b). This inference was supported by the fact that all genetically identical individuals were also phenotypically similar (sex, size, age). Thus, we had 408 unique individuals for pedigree analysis.

Litter reconstruction

We inferred sibling groups based on maximum likelihood as implemented in COLONY version 1.2 (Wang 2004). This approach uses group-likelihood ratios to partition individuals into full- and half-sibling families based on multilocus gene arrays. It also accounts for genotyping errors — we assumed a rate of 0.02 per locus for both allelic dropouts and typing errors. Although conservative, given our low average rate of observed genotyping (0.0017) and scoring error (0.0017), we feel this is prudent as one of the loci (LS30) approached 2% error (see Table S2). Inferred sibling groups, however, were identical when LS30 was removed from analysis, and so it was included here. In brief, we ran groups of age-0 sharks in COLONY, separated by year of birth (i.e. cohorts), to identify possible within-year sibling groups. Age-0 sharks from each cohort were also run separately with cohorts from every other year to identify potential between-year sibling groups. Because convergence problems can be common with maximum-likelihood estimation, we performed each analysis three times using the same information, and obtained identical family structures in all cases.

COLONY also reconstructs the genotypes of parents for sibling groups. Here, we accepted parental genotypes reconstructed with greater than 95% confidence at a locus — a level based on pilot studies conducted with known pedigrees (data not shown). Reconstruction also assumed that maternally related half-siblings were more likely than half-siblings through the father. Although this may potentially bias our results, or overestimate the level of female polyandry at MK, previous work at another site (Bimini, Bahamas) indicates that this is likely not the case (for more details see Feldheim *et al*. 2004). Using parentage assignment rather than genotype reconstruction, Feldheim *et al*. (2004) identified within and between-year maternally related half-siblings for all five physically sampled adult females. Furthermore, complete litters were directly sampled from two of these females, which again confirmed multiple paternity (resulting in maternally related half-sibling groups across years). It should also be noted that our approach does not preclude the identification of paternal half-sibling groups, as several were identified during this study. Indeed, manual litter reconstruction methods that do not make these assumptions recovered similar patterns (see Results).

We also estimated the degree of polyandry in each year at MK based on the inferred family structure from COLONY. We here define the degree of polyandry as the proportion of litters with more than one genetic father; this was used as a reference in all subsequent analyses (see below). At least three offspring must be analysed to even detect multiple paternity within litters, and so litters with two or fewer offspring were excluded from analysis (see Neff & Pitcher 2002).

Validation of litter reconstruction methods with COLONY

To validate COLONY for our study system, and to determine the robustness of polyandry estimates calculated from our inferred family structure, we ran two types of simulations. First, we evaluated the effects of sample size on the measurement of polyandry; that is, the difference between polyandry estimated for an entire simulated population and polyandry estimated for a subset of that population. Second, we evaluated the ability of COLONY to identify the correct level of polyandry for a set of 'known' family groups. These simulations directly inform the probability of detecting polyandry with our molecular markers, given a set of realistic population parameters (see below). This is critical given that the probability of actually detecting multiple paternity in nature is a function of the sample size, the power of the markers used, and the possible unequal genetic contribution of putative sires (see Neff & Pitcher 2002).

Each simulation used the same basic approach. In brief, we generated 16 litters (i.e. the maximum observed in a year at MK), and thus 16 females, by first constructing 11-locus maternal genotypes from the observed allele frequencies in our population. Each female was then mated to males whose genotypes were also constructed from the same allele frequency distribution. In simulations considering monoandry, each female was mated to only a single male. In simulations considering polyandry, each female was mated to between one and four males; the proportion of females mated with multiple males corresponded to the proportion of polyandrous litters set for the simulation. Furthermore, for all litters sired by multiple males, the

degree of polyandry for each litter (i.e. two, three, or four fathers) corresponded to the distribution observed in a previously studied lemon shark population (see Feldheim *et al*. 2004). It should be noted that a new male was generated for each mating event, thus male genotypes were never used more than once in a simulation. Each mating also resulted in a number of offspring following a Poisson distribution (and thus a range of litter sizes), which were generated from the parental genotypes through Mendelian inheritance (error was introduced to offspring genotypes at a rate of 1% to simulate both mutation and genotyping errors). The exact family structure, and thus all sibling relationships, was recorded for the entire population in each simulation, and the 'true' level of polyandry was therefore known.

To first assess the effects of sample size on the inferred level polyandry (where sample size refers to the number of sampled offspring), we generated 100 simulated populations for each different level of polyandry (i.e. the proportion of litters with multiple sires) ranging from 0% to 100%, increasing by increments of 10%. Each of these populations was sampled 100 times at all sample sizes as well, ranging from 10 to 110, increasing by increments of 10. This resulted in 1 210 000 different comparisons of the level of polyandry in the whole simulated population vs. the level of polyandry in the subsample.

To next assess the relative performance of COLONY in recovering the correct level of polyandry for known family groups, we took a similar approach to the above. However, not all of the simulated populations generated previously were tested because of an obvious computational time issue when using COLONY. Instead, eight sample sizes ranging from 10 to 80 (similar to those from our MK data set), increasing by increments of 10, were surveyed. For each sample size, 100 simulated populations for each of the 11 previously defined levels of polyandry were generated using the methods described above. The resulting 8800 data sets were analysed by COLONY, and the inferred family structure was then used to estimate the proportion of polyandrous litters. In rare cases where the population size was smaller than the requested sample size (38 out of 8800 data sets), the population size was used as the sample size. The performance of COLONY was approximated by the variance of the mean polyandry estimate at different sample sizes, and the deviation of inferred polyandry from the 'true' level of polyandry for known family groups.

Congruence with manual litter reconstruction methods

To validate comparisons between our study and previous work on lemon sharks (i.e. Feldheim *et al*. 2002a; Feldheim *et al*. 2004), we also performed litter construction for MK using the earlier methods. Specifically, we used KINSHIP version 1.3 (Goodnight & Queller 1999) to group age-0 sharks into within- and between-year sibling groups. Often times, half-siblings matched with their full-sibling groups in the KINSHIP matrix output, at a confidence level of 99%. These putative groups were further explored manually to see whether the genotypes of all members were consistent. When a maximum of four alleles was found per locus, full-sibship was assigned to the group. When five or more alleles were seen at a minimum of two loci, then two or more groups of half-siblings were distinguished. The inferred family structure was then used to manually reconstruct the genotypes of parents that were not physically sampled (for more details see Feldheim *et al*. 2002a; Feldheim *et al*. 2004). Maternal genotypes were manually reconstructed based first on two or more groups of half-siblings, followed by paternal genotypes, which were inferred by splitting these same half-sibling groups into full-sibling groups based on shared alleles. Because male genotypes from MK were thus reconstructed from relatively few newborns, more than 90% were only partially reconstructed in this case. CERVUS version 2.0 (Marshall *et al*. 1998) was also used to assign any remaining offspring to reconstructed parents (only adults reconstructed at a minimum of six loci were considered) with both relaxed (80%) and strict (95%) confidence; however, these analyses provided no additional information (but were consistent with our results). Results from COLONY were compared to the above manual method by calculating the proportion of offspring assigned to the same father or mother in each year (i.e. the same full or half-sibling group).

Results

Genetic mating patterns inferred from COLONY

Table 1 presents summary details of our family structure as inferred by COLONY (for more detail see Table S3, Supplementary material). We were unable to assign 15 newborns to either a parent or family group, probably because we failed to sample any of their siblings. We also failed to assign families to 13 sampled subadult sharks, which may have been transient individuals from neighbouring keys. These 28 individuals were excluded from further analyses; we now highlight several patterns apparent in the pedigree.

Mean litter size was 4.29 ± 0.24 pups (range: 1–13 pups), with 46 unique mothers of whom 20 had fully reconstructed genotypes. We also found 163 unique fathers, of whom only eight had fully reconstructed genotypes. The greater difficulty in reconstructing paternal rather than maternal genotypes was due to the few offspring assigned to individual males (2.17 ± 0.12). Interestingly, a number of newborn sharks sampled only a few days apart on opposite sides of the MK nursery lagoon grouped as full-siblings. This result suggests that either (i) newborns are quickly capable of

Table 1 Summary of lemon shark mating system characteristics in the Marquesas Key nursery. All adults identified in this study were genetically reconstructed from genotypes of sampled offspring, and not physically sampled. The number of adult females and mean offspring per female can be inferred from the number of litters and mean litter size, respectively. Means values are ± 1 SEM

Year	Number of sampled offspring	Number of litters	Mean litter size	Adult males	Mean offspring per male
1997	20	5	1.60 ± 0.24	7	1.14 ± 0.14
1998	44	10	4.40 ± 0.60	18	2.44 ± 0.36
1999	68	15	4.47 ± 0.58	34	1.97 ± 0.24
2000	69	12	5.50 ± 0.70	33	2.063 ± 0.32
2001	16	7	2.28 ± 0.29	13	1.23 ± 0.12
2002	47	11	4.27 ± 0.38	18	2.47 ± 0.31
2003	34	6	5.16 ± 0.73	12	2.58 ± 0.51
2004	44	10	4.40 ± 0.67	17	2.69 ± 0.38
2005	18	4	3.75 ± 0.85	6	2.50 ± 0.34
2006	20	5	4.00 ± 0.83	8	2.50 ± 0.42
All years	380	85	4.29 ± 0.24	166	2.17 ± 0.12

using the whole nursery lagoon, despite several deep channels; or (ii) pregnant females drop pups from the same litter in different locations.

Multiple paternity of a given litter was evident in a large number of within-year half-sibling groups related through the mother. Excluding litters with two or fewer offspring, an average of 81% (95% confidence interval: 56–100%) of all the litters sampled had multiple fathers with an average of 1.95 ± 0.091 sires per litter (range: 1–4 sires). The estimated level of polyandry (number of litters with multiple fathers) was highly variable (Fig. 1), although in most years, measurement error was quite large, and so this may not reflect the true range of values. The 3 years (1999, 2000, 2003) in which error was considerably lower (and confidence higher) show that polyandry was high in general.

Females often gave birth in multiple years (22 of 46 inferred mothers), as evidenced by a number of between-year half-sibling groups related through the mother (Table S3). Ten of these mothers produced litters in 2 years, seven produced litters in 3 years, and five produced litters in 4 years. Of the 22 females that produced litters in multiple years, almost all did so on a 2-year cycle. One type of exception was represented by three females who had an odd number of years between litters (14REC, 11REC, 40 REC). Another type of exception was represented by females who always had an even number of years between litters, but this was sometimes more than 2 years (13REC, 3REC, 9REC, 21REC, and 8REC). The apparent missing years in the normal 2-year cycle may reflect females that did not produce litters in some years, sometimes produced litters elsewhere, or produced litters that we failed to sample. Regardless, the large number of females producing litters at MK in more than 1 year provides evidence that at least some females return in multiple years to the same nursery site (i.e. philopatry).

In contrast to females, males rarely sired offspring in more than one litter either within or between years (only 1.8% of all males identified; Table S3). Exceptions included

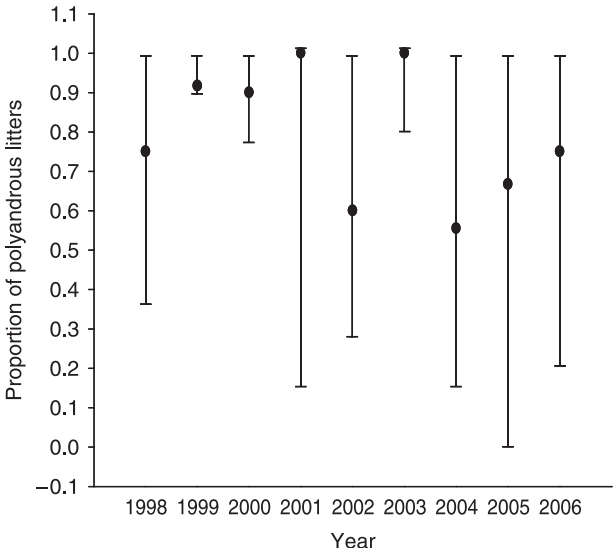

Fig. 1 The degree of polyandrous lemon shark litters in each year was assessed using the inferred family structure from COLONY. Polyandry could not be inferred for litters of two or fewer sampled juveniles and so these were not considered here, which includes all litters from 1997. The number of litters considered in each year are as follows: 1998, 8 (50 offspring); 1999, 11 (71 offspring); 2000, 10 (66 offspring); 2001, 3 (17 offspring); 2002, 10 (49 offspring); 2003, 5 (33 offspring); 2004, 9 (45 offspring); 2005, 3 (15 offspring); 2006, 4 (20 offspring). Error bars represent the 95% confidence intervals of the mean, which accounts for both the uncertainty in incomplete sampling of offspring and pedigree reconstruction with COLONY; these were estimated from our simulation results based on the sample size for each year.

one male (RECMale31) who sired pups with female 30REC (in both 1998 and 2000) and female 16REC (in 1999), and two males who sired pups with the same female (42REC) twice (RECMale 98 in 2002 and 2004 and RECMale130 in 1998 and 2004). These results suggest that males either move more often among nursery sites or are much more

abundant than females (i.e. skewed operational sex ratio), which may influence reproductive success. It also appears that the few males that did sire offspring on more than one occasion always did so with the same female, suggesting that sperm storage behaviour and postcopulatory female choice may play a role in this system. This is the most parsimonious explanation given that female lemon sharks probably do not pair bond with males, or mate with the same male in multiple years. This idea is also supported by pedigree data (i.e. 1991–2007) from our comparison study site (Bimini, Bahamas; J. DiBattista, unpublished data), where 10 adult females had offspring with the same male on more than one occasion.

Validation of litter reconstruction methods with COLONY

How does sample size influence the estimation of polyandry? As the sampled proportion of the population increased, so too did the agreement between polyandry estimated in the whole simulated population vs. that in the sample (Fig. 2). In all cases, reduced sampling led to the expectation of underestimated polyandry, with the extent of underestimation increasing with increasing polyandry. A likely reason is that a reduction in sampling decreases the number of sampled offspring per litter, which subsequently decreases the chance of identifying siblings. Furthermore, as the level of polyandry in the simulated population increases, so too does the variance in polyandry estimates among samples — and this effect is greatest for the smallest sample sizes. In short, both the bias and precision of polyandry estimates decreases with increasing sample size and increasing polyandry levels.

To what extent can COLONY recover the correct level of polyandry? More precisely, how well does COLONY reconstruct the population pedigree from which polyandry is then inferred? To address this question, we compared the specified level of polyandry in our simulated populations, vs. the level of polyandry estimated by COLONY. These analyses show that COLONY tends to overestimate the 'true' level of polyandry in our simulated populations (Fig. 3). These problems were less acute with an increase in sampling effort and with an increase in polyandry.

Overall, our simulations directly inform the probability of detecting multiple paternity with COLONY based on our molecular markers, and considering a realistic range of population parameters (i.e. number of litters, males, litter size). Thus, it appears that the microsatellite loci selected here had sufficient genetic resolution to detect polyandry when present in our study population.

Congruence with manual litter reconstruction methods

Reconstructed family structures based on COLONY vs. those based on manual litter reconstruction methods (i.e. along with KINSHIP) were generally similar but with some key differences. For within-year comparisons, the proportion of offspring assigned to the same maternal sibling-group by the two methods was high in each year (mean: 93%; Fig. 4). The proportion of offspring assigned to the same paternal sibling group by the two methods was lower (mean: 78%; Fig. 4). For between-year comparisons, the corresponding values were 83% for maternal sibling groups but only 38% for paternal sibling groups (results not shown). The reduced success for paternal relationships is probably because COLONY assumes polygamy in one sex only, in this case through the female. This assumption does not preclude the identification of between-year paternal links (three were found), although it probably makes their detection more difficult. Previous work also suggests that COLONY has a tendency to split larger families into smaller ones (Wang 2004; Jones *et al*. 2007), possibly resulting in some full-siblings being incorrectly assigned as half-siblings. This would inflate the number of males identified for each litter, and could also explain the inconsistency in paternal relationships between COLONY vs. manual methods.

Discussion

Female lemon sharks display high levels of polyandry and at least some fidelity to the MK nursery site. Male sharks, on the other hand, rarely contributed to more than one litter over the 10 breeding seasons. These general results are consistent with those documented for another nursery site in the western Atlantic (Bimini, Bahamas; Feldheim *et al*. 2002a; Feldheim *et al*. 2004). Although this suggests little geographical variation in mating systems, the level of confidence in these comparisons requires a consideration of the biases and precision inherent in the various litter reconstruction methods. After addressing these considerations, we discuss implications for the conservation and management of large coastal shark populations.

Validation and congruence of methods

The genetic evaluation of mating systems is difficult when few adults can be sampled, such as in the case of the lemon shark. Here, one must rely on the reconstruction of full- and half-sibling groups based on offspring genotypes. We used simulations to evaluate how this approach is influenced by limited sampling and uncertainty in pedigree reconstruction. With respect to incomplete sampling of progeny, we found that polyandry was generally underestimated, particularly when sample sizes were smallest and 'true' polyandry was greatest (Fig. 2). With respect to pedigree reconstruction in COLONY, polyandry is generally overestimated, particularly when sample sizes are smallest and 'true' polyandry is lowest (Fig. 3). Although we do not know the actual number of sharks at our study site, we can

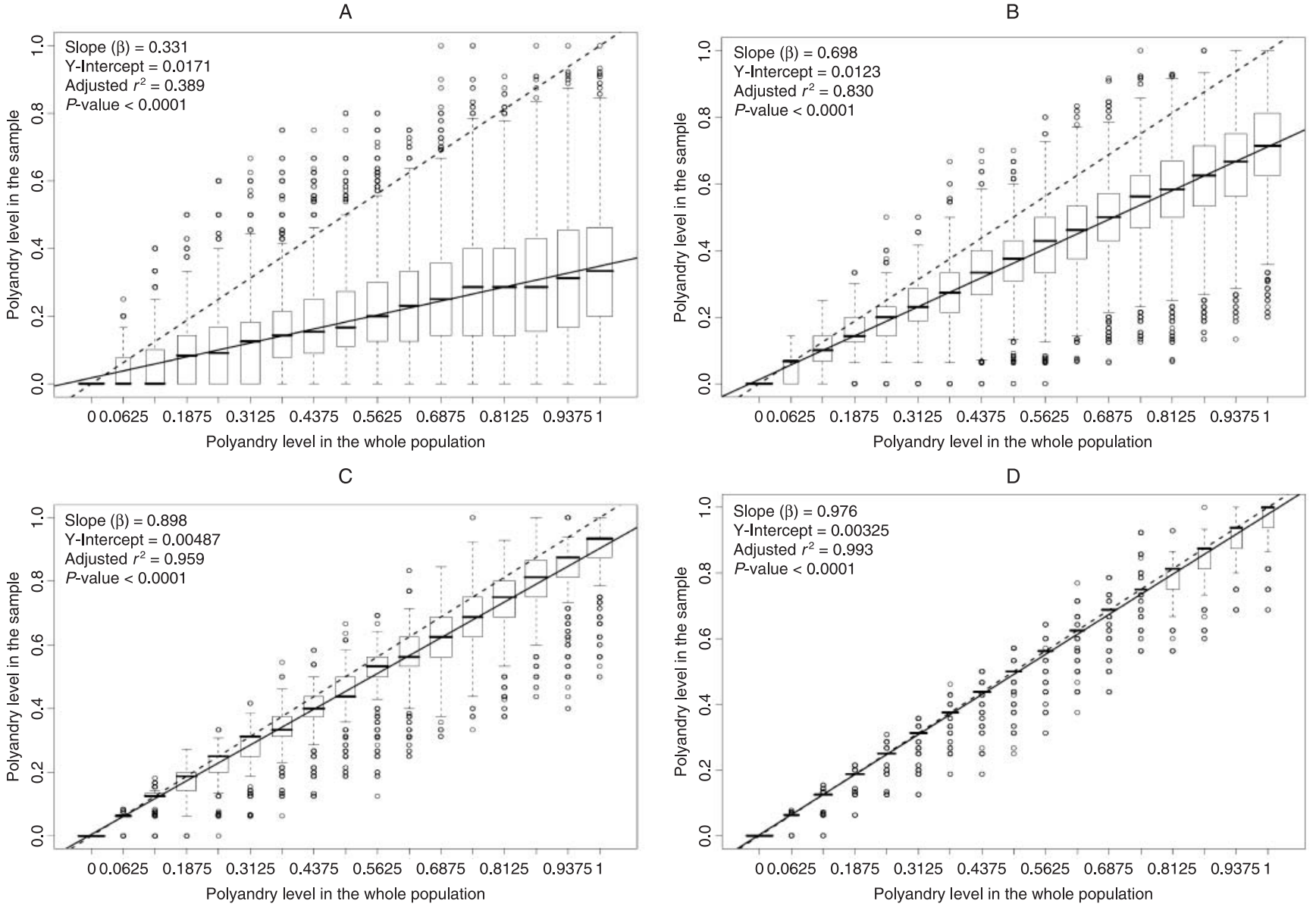

Fig. 2 A comparison of the level of polyandry in each simulated population vs. that in the sample only, as a function of the proportion of the population sampled (A, 0–25% sampled; B, 25–50% sampled; C, 50–75% sampled; D, 75–100% sampled). The solid line is the regression, whereas the dashed line is the ideal case where one would find exactly the level of polyandry that is present in the population. Box plots represent medians and quartiles, whiskers signify the 5th and 95th percentiles. The width of each box indicates sample size, and all data points shown are outliers.

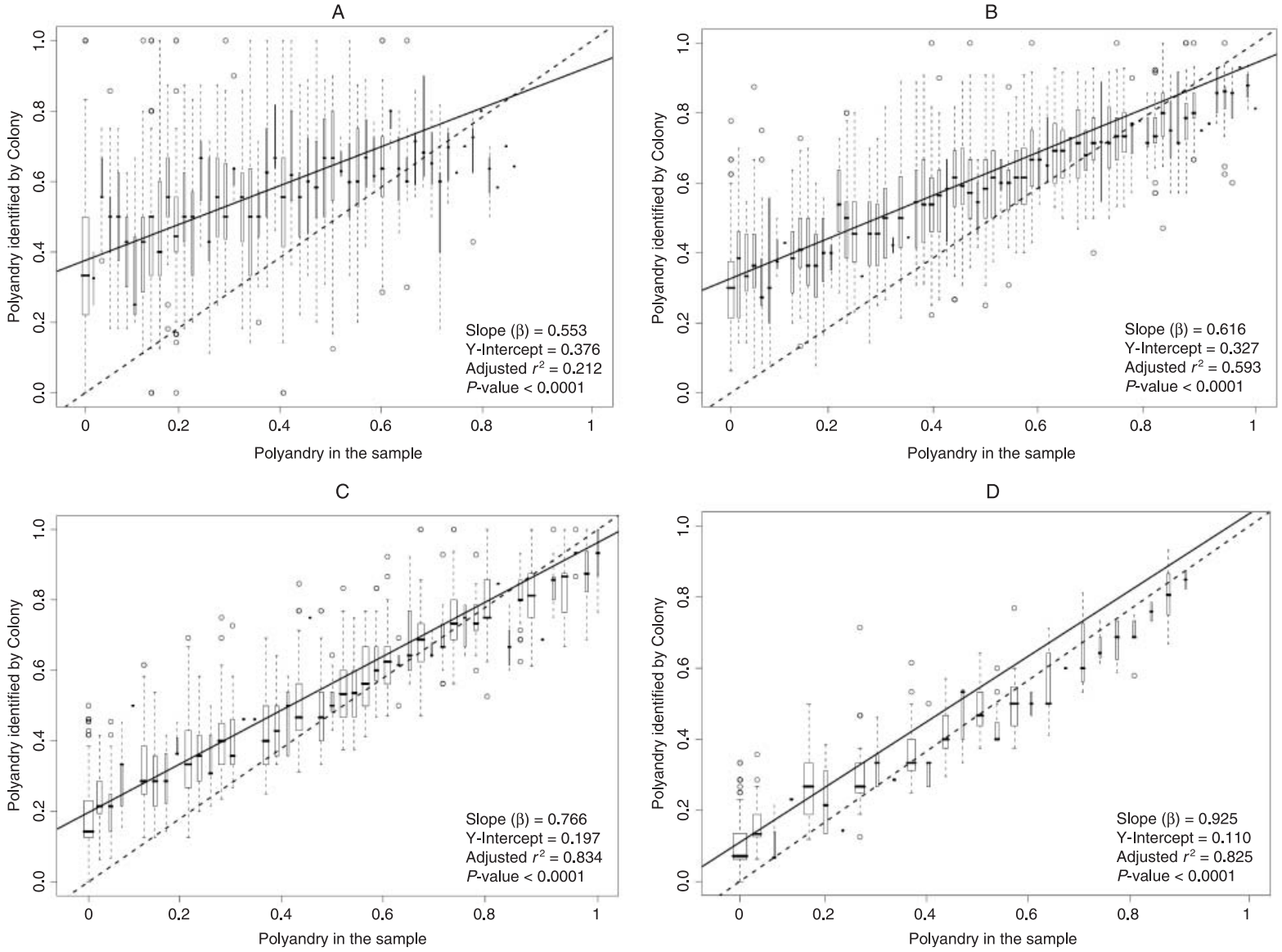

Fig. 3 A comparison of the level of polyandry estimated from pedigrees generated in COLONY using simulated populations vs. the 'known' level of polyandry in the sample, as a function of the proportion of the population sampled (A, 0–25% sampled; B, 25–50% sampled; C, 50–75% sampled; D, 75–100% sampled). The solid line is the regression, whereas the dashed line is the ideal case where one would find exactly the level of polyandry that is present in the sample. Box plots represent medians and quartiles, whiskers signify the 5th and 95th percentiles. The width of each box indicates sample size, and all data points shown are outliers.

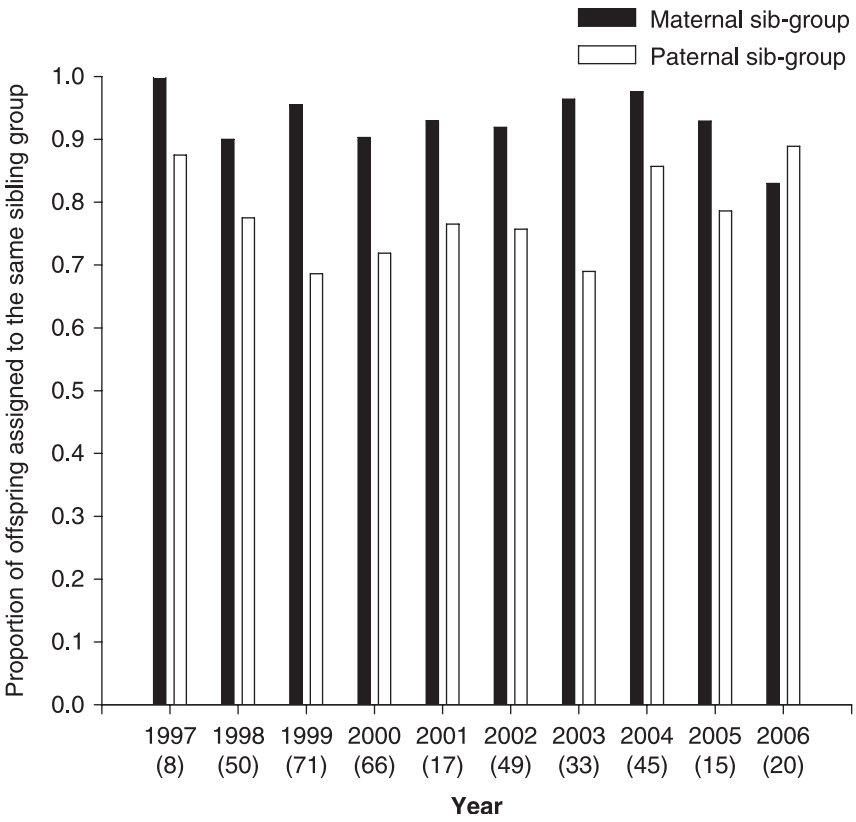

Fig. 4 Similarity of newborn lemon shark family structure generated from methods using COLONY vs. older manual reconstruction methods using KINSHIP 1.3 (Feldheim *et al*. 2004). Similarity is based on the proportion of offspring within each year assigned to the same full- or half-sibling group (and thus the same mother or father). Numbers in bracket are the number of offspring sampled (*N*).

approximate this in order to facilitate the interpretation of our simulations. If we assume that the year in which we sampled exhaustively (1999) and identified 77 newborns represents the maximum number of sharks, and this value remains constant from year to year, then in all other years we sampled between 10% and 86% of all offspring at our site. Furthermore, given that the bias and precision from our simulations appear to be small when at least 75% of the site is sampled (Figs 2D and 3D, although there always remains an upward bias in polyandry due to pedigree reconstruction in COLONY), we can be most confident about polyandry estimates inferred from 1999 and 2000 (i.e. 100% and 93% of the population sampled). This suggests that polyandry at MK approached 90% in at least some years, but when averaged over all years, the proportion of polyandrous litters was 81%.

Given the above, comparisons among sites will depend on the proportion of individuals sampled and the actual level of polyandry. Such comparisons may also depend on the precise estimation method, such as when using COLONY vs. manual reconstruction. Here, we found that both methods generated similar maternal family structure but increasingly deviated for paternal family structure, particularly between years (Fig. 4). This was not surprising given that our success in reconstructing adult male genotypes was low owing to the few offspring sired by each male. In general, paternal genotype reconstruction should improve with nested half-sibling families, increasing sample size, more markers, and more variable markers (Wang 2004). Given these uncertainties, we therefore used both methods for MK and for Bimini. With COLONY, polyandry was estimated to be 89% at Bimini (data not shown) and 81% at MK. With manual reconstruction, polyandry was estimated to be 86% at Bimini (see Feldheim *et al*. 2004) and 43% at MK (data not shown). Thus, regardless of the method, lemon sharks are clearly polyandrous to an extent that is roughly similar between the sites. It should be noted that at Bimini sampling is considered exhaustive, with 99% of the offspring sampled in each year, and some adults are also sampled (Gruber *et al*. 2001). Thus, given the lower power of manual reconstruction methods when sample sizes are more limited (i.e. MK), we expect that true polyandry levels approach those estimated by COLONY, although again, this method may yield some upward bias.

Genetic polyandry in sharks

Our study adds to previous work in documenting polyandry in sharks (Saville *et al*. 2002; Chapman *et al*. 2004; Daly Engel *et al*. 2007). As other examples, the percentage of multiply sired litters was 19% for a population of bonnetheads (*Sphyrna tiburo*) off the coast of Florida (Chapman *et al*. 2004) and 40% for a population of sandbar sharks (*Carcharhinus plumbeus*) in Hawaii (Daly Engel *et al*. 2007).

These results suggest differences in mating systems among species, although some of the variation may stem from different estimation methods, as we have here shown. And yet, some real variation seems likely given that mating system variability is common in nature (Endler & Houde 1995), and sharks exhibit a wide array of reproductive modes ranging from oviparity to viviparity (Dulvy & Reynolds 1997).

Intraspecific differences in mating systems likely have a different basis, given that the members of a given species have (by definition) compatible, and therefore similar, mating behaviours. Variation among nursery sites may instead depend on differences in the spatial (for review, see Westneat & Sherman 1997) and temporal (for review, see Stutchbury & Morton 1995) availability and quality of males. Intraspecific variation could also be due to environmental variation in factors such as resources (Travis *et al*. 1995) or predation (Lodé *et al*. 2004), although previous studies have found conflicting evidence. For example, extra-pair fertilization is influenced by breeding density in some studies (Rätti *et al*. 2001), but not others (Soucy & Travis 2003), and influenced by breeding synchrony in some studies (Chuang *et al*. 1999) but not others (Westneat & Mays 2005). Our study provides an interesting counterpoint to this previous work in that we did not find noteworthy differences in polyandry between Bimini and MK, despite divergence in juvenile size and growth rates (both higher at MK, which is indicative of local adaptation; Barker *et al*. 2005). We therefore suggest that it may be just as interesting to examine the causes of mating system *similarity*, despite differences in life history.

One might expect differences in the level of polyandry between our comparison sites, given the marked divergence in early life-history traits (i.e. size and growth; Barker *et al*. 2005), and strong selection acting on these traits in at least one of the nurseries (DiBattista *et al*. 2007). It remains possible, however, that these morphological differences are stage dependent, only appearing in juvenile sharks. In fact, the same characteristics that impede the recovery of the lemon shark from sustained fishing pressure (long generation times and late age-at-maturity) may also allow for compensatory growth once juvenile sharks become older, expand their home range, and leave the nursery site altogether. Thus, small, slow-growing Bimini sharks may grow much faster once free of the selective pressures inherent to the Bimini nursery. Similarly, large, fast-growing sharks from MK may grow slowly once outside the confines of the nursery lagoon. Mark–recapture data for subadult and adult lemon sharks from each site would obviously be critical in this regard by providing growth rate estimates from older age classes.

Another question, then, is why polyandry is so consistently high in lemon sharks? Commonly considered possibilities in other taxa include direct benefits (increased parental care, nuptial gifts, or resource allocation; Arnqvist & Nilsson 2000) or indirect benefits (increased genetic compatibility, good genes, or genetic variability; Neff & Pitcher 2005). Direct benefits seem unlikely in lemon sharks given the lack of parental care, nuptial gifts, or male resource defence (Pratt & Carrier 2001). Moreover, any indirect benefits would have to be strong because mating females are often injured when resisting males (Pratt & Carrier 2001). We have failed to find evidence, however, for indirect benefits at Bimini based on juvenile survivorship or neutral genetic diversity, although benefits at other life-history stages or those based on coding genes cannot be ruled out (DiBattista *et al*. 2008). Some of our results also suggest that sperm storage and postcopulatory female choice may occur in this species to some degree. It seems more likely, however, that the majority of female lemon sharks are polyandrous as a matter of convenience, thus mating multiply simply to avoid harassment from other aggressive males. This idea is also indirectly supported by previous genetic work in sharks (Portnoy *et al*. 2007), and other vertebrate taxa (Lee & Hays 2004; Fitze *et al*. 2005), but has yet to be tested directly in the lemon shark.

Philopatry to Marquesas Key nursery

Whether or not large coastal sharks show philopatry to particular nursery sites has important implications for the spatial scale of management and conservation. One way to examine such fidelity is through mark–recapture or manual tracking methods. Data for juvenile sharks, including those at Bimini (Morrissey & Gruber 1993), indicate they generally remain in restricted areas (for review see Wiley & Simpendorfer 2007) and return to these areas after experimental displacement (Edrén & Gruber 2005). Older juveniles, however, eventually leave nursery sites and take up a much more vagile existence, often ranging over vast areas of ocean (Feldheim *et al*. 2001). It therefore remains uncertain whether these adults return to their natal sites for reproduction, and whether or not they keep returning to a particular site over multiple years. Tagging data are of little assistance in this respect because tagged adults are rarely encountered, and so genetic data are the most practical solution.

One genetic approach used to infer philopatry is to examine sex-specific dispersal with mtDNA (e.g. Fitzsimmons *et al*. 1997; Rosel *et al*. 1999); unfortunately, variation in this marker is too low to be of use for lemon sharks (Schultz *et al*. in review). We therefore argue for the value of individual-based pedigree analyses in this case, which can inform philopatry in general by determining whether particular males or females produce offspring in multiple reproductive episodes at a single site. Such analyses are feasible for other shark species found at enclosed sites, amenable to efficient and repeated standardized sampling.

Most coastal nursery sites fit these characteristics, and juveniles from other shark species tend to show nursery-site fidelity for at least the first year (see Wiley & Simpendorfer 2007). This approach can, and has (e.g. Garant *et al*. 2004), been widely applied to other animal systems as well, which generally do not face the logistic constraint of long generation times and few sampled adults. Based on our pedigree analysis, we found that roughly half of the females, but few of the males, reproduced in multiple years at MK, and some of these did so many times. These results parallel those for lemon sharks at Bimini (Feldheim *et al*. 2002a; Feldheim *et al*. 2004) and suggest that at least some females show fidelity to sites for parturition, which means that different nurseries may be independent in an evolutionary sense. It is important to recognize, however, that whereas our results infer philopatry in general (i.e. females returning to sites they used for parturition before), they do not indicate natal philopatry (i.e. females returning to sites where they were born to give birth).

Natal philopatry, however, is quite a reasonable expectation for marine organisms given its prevalence in other species, such as whales (Goerlitz *et al*. 2003), turtles (Peare & Parker 1996), and salmon (Quinn & Dittman 1990), and its potential fitness advantages for sharks. Indeed, natal philopatry should evolve if pupping grounds are 'selected for' because they are more successful in producing animals that survive and reproduce than other sites (Hueter *et al*. 2005). In such cases, high homing accuracy may facilitate 'rapid' evolutionary divergence (Hendry & Kinnison 1999). One factor influencing whether a female shark returns to her natal site to breed or give birth is habitat patchiness. In such cases, natal philopatry would be beneficial if high quality breeding or birthing grounds are scarce, or at least patchy and spatially dispersed (e.g. Hastings 1983). Alternatively, the degree of natal philopatry may instead regulate the optimal level of inbreeding at a site (Weatherhead & Forbes 1994). Consider, however, that although selection may favour females that return to sites that were clearly suitable in the past, if conditions change, natal philopatry may become maladaptive. Given the highly vagile nature of the adult lemon shark, it remains to be seen how they might assess the current ecological sustainability of their natal site (i.e. predation risk, resource availability, environmental quality), and whether it pays to return there for parturition, or if it is better to seek out other sites. Either way, additional information about natal philopatry in sharks will be important to the determination of the appropriate spatial scale for management.

We would like to particularly emphasize the importance of evaluating natal philopatry in the lemon shark. In this case, natural restocking of females from surrounding areas would not be expected over ecological timescales following localized depletion, or damage to a nursery site. Some recent authors have even suggested that precise natal homing, combined with local extinctions, can act as an 'evolutionary handicap' by leading to permanent loss of genetic variation within a species (see Lee *et al*. 2007). That said, we here assume that females are the vehicles that transmit genes at MK nursery site (Avise 1995), and so their fidelity should define reproductive units, regardless of male behaviour. We therefore favour local vs. broad-scale management of lemon shark nurseries in the western Atlantic, with the emphasis on habitat preservation. Protection of nursery habitats (see Heupel *et al*. 2007) is critical as human activities have drastically altered or degraded a number of coastal areas commonly used by sharks for pupping (Hueter *et al*. 2005).

Conclusion

This long-term study advances our knowledge of shark mating systems. In our case, we found sex-specific breeding patterns, whereby females returned biennially to MK nursery to give birth but males were rarely detected more than once over a 10-year period. We strongly encourage a localized management approach for lemon shark nurseries based on these findings. Our comparison between the two nursery sites also leads us to believe that lemon shark mating systems are conserved, which is surprising given the substantial early life-history differences in this case. A more rigorous treatment of variability in mating patterns between sites is clearly needed however, as well as an investigation of the possible role for natal philopatry in this species.

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References

Amos W (2000) IDENTITY, a general match-finding program V1.1. Available from URL: http://www.zoo.cam.ac.uk/zoostaff/amos/newpat.htm.

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*, **60**, 145–164.

Avise JC (1995) Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. *Conservation Biology*, **9**, 686–690.

Avise JC (2004) *Molecular Markers, Natural History, and Evolution*. Sinauer & Associates, Sunderland, Massachusetts.

Barker MJ, Gruber SH, Newman SP, Schluessel V (2005) Spatial and ontogenetic variation in growth of nursery-bound juvenile lemon sharks, *Negaprion brevirostris*: a comparison of two age-assigning techniques. *Environmental Biology of Fishes*, **72**, 343–355.

Bollmer JL, Sanchez T, Cannon M *et al*. (2003) Variation in morphology and mating system among island populations of Galapagos hawks. *Condor*, **105**, 428–438.

Branstetter S (1990) Early life-history implications of selected carcharhinoid and lamnoid sharks of the northwest Atlantic. *NOAA Technical Reports, National Marine Fisheries Service*, **90**, 17–28.

Chapman DD, Prodhöl PA, Gelsleichter J, Manire C, Shivji MS (2004) Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. *Molecular Ecology*, **13**, 1965–1974.

Chuang HC, Webster MS, Holmes RT (1999) Extra-pair paternity and local synchrony in the balck-throated blue warbler. *Auk*, **116**, 726–736.

Compagno LJV (1984) *FAO Species Catalogue: Sharks of the World*. Food and Agriculture Organization of the United Nations, Rome.

Daly-Engel TS, Grubbs RD, Holland KN, Toonen RJ, Bowen BW (2006) Assessment of multiple paternity in single litters from three species of carcharhinid sharks in Hawaii. *Environmental Biology of Fishes*, **76**, 419–424.

Daly-Engel TS, Grubbs RD, Bowen BW, Toonen RJ (2007) Frequency of multiple paternity in an unexploited tropical population of snadbar sharks (*Carcharhinus plumbeus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **64**, 198–204.

DiBattista JD, Feldheim KA, Gruber SH, Hendry AP (2007) When bigger is not better: selection against large size, high condition and fast growth in juvenile lemon sharks. *Journal of Evolutionary Biology*, **20**, 201–212.

DiBattista JD, Feldheim KA, Gruber SH, Hendry AP (2008) Are indirect genetic benefits associated with polyandry? Testing predictions in a natural population of lemon sharks. *Molecular Ecology*, **17**, 783–795.

Dulvy NK, Reynolds JD (1997) Evolutionary transitions among egg-laying, live-bearing and maternal inputs in sharks and rays. *Proceedings of the Royal Society B: Biological Sciences*, **264**, 1309–1315.

Edrén SMC, Gruber SH (2005) Homing ability of young lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes*, **72**, 267–281.

Emlen DJ (1994) Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society B: Biological Sciences*, **256**, 131–136.

Endler JA, Houde AE (1995) Geographic variation in female preferences for male traits in *Poecilia reticulate*. *Evolution*, **49**, 456–468.

Feldheim KA, Gruber SH, Ashley MV (2001) Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology*, **10**, 295–303.

Feldheim KA, Gruber SH, Ashley MV (2002a) The breeding biology of lemon sharks at a tropical nursery lagoon. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 1655–1661.

Feldheim KA, Gruber SH, De Marignac JRC, Ashley MV (2002b) Genetic tagging to determine passive integrated transponder tag loss in lemon sharks. *Journal of Fish Biology*, **61**, 1309–1313.

Feldheim KA, Gruber SH, Ashley MV (2004) Reconstruction of parental microsatellite genotypes reveals female polyandry and philopatry in the lemon shark, *Negaprion brevirostris*. *Evolution*, **10**, 2332–2342.

Fitze PS, Le Galliard JF, Federici P, Richard M, Clobert J (2005) Conflict over multiple-partner mating between males and females of the polygynandrous common lizards. *Evolution*, **59**, 2451–2459.

Fitzsimmons NN, Limpus CJ, Norman JA *et al*. (1997) Philopatry of male marine turtles inferred from mitochondrial DNA markers. *Proceedings of the National Academy of Sciences, USA*, **94**, 8912–8917.

Fleming IA, Lamberg A, Jonsson B (1997) Effects of early experience on the reproductive performance of Atlantic salmon. *Behavioral Ecology*, **8**, 470–480.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

Garant D, Kruuk LE, McCleery RH, Sheldon BC (2004) Evolution in a changing environment: a case study with great tit fledging mass. *American Naturalist*, **164**, E115–E129.

Goerlitz DS, Urbán J, Rojas-Bracho L, Belson M, Schaeff CM (2003) Mitochondrial DNA variation among Eastern North Pacific gray whales (*Eschrichtius robustus*) on winter breeding grounds in Baja California. *Canadian Journal of Zoology*, **81**, 1965–1972.

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, **8**, 1231–1234.

Gross MR (1991) Salmon breeding behavior and life history evolution in changing environments. *Ecology*, **72**, 1180–1186.

Gruber SH, De Marignac JRC, Hoenig JM (2001) Survival of juvenile lemon sharks at Bimini, Bahamas, estimated by mark-depletion experiments. *Transactions of the American Fisheries Society*, **130**, 376–384.

Hastings A (1983) Can spatial variation alone lead to selection for dispersal? *Theoretical Population Biology*, **24**, 244–251.

Hendry AP, Kinnison MT (1999) The pace of modern life: measuring rates of microevolution. *Evolution*, **53**, 1637–1653.

Hendry AP, Taylor EB, McPhail JD (2002) Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the misty system. *Evolution*, **56**, 1199–1216.

Heupel MR, Carlson JK, Simpendorfer CA (2007) Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series*, **337**, 287–297.

Hoffman JI, Amos W (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology*, **14**, 599–612.

Hueter R, Heupel MR, Heist EJ, Keeney DB (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science*, **35**, 239–247.

Hutchings JA (1993) Adaptive life histories effected by age-specific survival and growth rate. *Ecology*, **74**, 673–684.

Jones B, Grossman GD, Walsh DCI *et al*. (2007) Estimating differential reproductive success from nests of related individuals, with application to a study of the mottled sculpin, *Cottus bairdi*. *Genetics*, **176**, 2427–2439.

Keeney DB, Heupel MR, Hueter R, Heist EJ (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. *Molecular Ecology*, **14**, 1911–1923.

Lee PLM, Hays GC (2004) Polyandry in a marine turtle: females make the best of a bad job. *Proceedings of the National Academy of Sciences, USA*, **101**, 6530–6535.

Lee PLM, Luschi P, Hays GC (2007) Detecting female precise natal philopatry in green turtles using assignment methods. *Molecular Ecology*, **16**, 61–74.

Lodé T, Holveck M, Lesbarrères D, Pagano A (2004) Sex-biased predation by polecats influences the mating system of frogs. *Proceedings of the Royal Society B: Biological Sciences (Supplement)*, **271**, S399–S401.

Luyten PH, Liley NR (1991) Sexual selection and competitive mating success of males guppies (*Poecilia reticulata*) from four Trinidad populations. *Behavioral Ecology and Sociobiology*, **28**, 329–336.

Manire CH, Gruber SH (1991) Effect of M-type dart tags on field growth of juvenile lemon sharks. *Transactions of the American Fisheries Society*, **120**, 776–780.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Morrissey JF, Gruber SH (1993) Habitat selection by juvenile lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes*, **38**, 311–319.

Neff BD, Pitcher TE (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fishes. *Journal of Fish Biology*, **61**, 739–750.

Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19–38.

Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 489–495.

Paetkau D, Calvert W, Sterling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Peare T, Parker PG (1996) Local genetic structure within two rookeries of *Chelonia mydas* (the green turtle). *Heredity*, **77**, 619–628.

Portnoy DS, Piercy AN, Musick JA, Burgess GH, Graves JE (2007) Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic and Gulf of Mexico. *Molecular Ecology*, **16**, 187–197.

Pratt HL Jr (1993) The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. *Environmental Biology of Fishes*, **38**, 139–149.

Pratt HL Jr, Carrier JC (2001) A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. *Environmental Biology of Fishes*, **60**, 157–188.

Pratt HL Jr, Tanaka S (1994) Sperm storage in male elasmobranchs: a description and survey. *Journal of Morphology*, **219**, 297–308.

Quinn TP, Dittman AH (1990) Pacific salmon migrations and homing: mechanisms and adaptive significance. *Trends in Ecology & Evolution*, **5**, 174–177.

Rätti O, Lundberg A, Tegelström H, Alatalo RV (2001) No evidence for effects of breeding density and male removal on extrapair paternity in the pied flycatcher. *Auk*, **118**, 147–155.

Rosel PE, France SC, Wang JY, Kocher TD (1999) Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers. *Molecular Ecology Suppl*., **8**, S41–S54.

Saint-Laurent R, Legault M, Bernatckez L (2003) Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill). *Molecular Ecology*, **12**, 315–330.

Saville KJ, Lindley AM, Maries EG, Carrier JC, Pratt HL (2002) Multiple paternity in the nurse shark, *Ginglymostoma cirratum*. *Environmental Biology of Fishes*, **63**, 347–351.

Schultz JK, Feldheim KA, Gruber SH, Ashley MV, Bowen BW (in press) Habitat preference-not distance-isolates transoceanic lemon shark populations. *Molecular Ecology*, in review.

Soucy S, Travis J (2003) Multiple paternity and population genetic structure in natural populations of the poeciliid fish, *Heterandria formosa*. *Journal of Evolutionary Biology*, **16**, 1328–1336.

Stutchbury BJ, Morton ES (1995) The effect of breeding synchrony on extra-pair mating systems in songbirds. *Behaviour*, **132**, 675–690.

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, **13**, 510–524.

Taberlet P, Griffin S, Goosens B *et al*. (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, **24**, 3189–3194.

Taylor EB (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, **98**, 185–207.

Travis SE, Slobodchikoff CN, Keim P (1995) Ecological and demographic effects on intraspecific variation in the social system of prairie dogs. *Ecology*, **76**, 1794–1803.

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Molecular Ecology Notes*, **2**, 377–379.

Valimaki P, Kaitala A (2007) Life history tradeoffs in relation to the degree of polyandry and developmental pathway in *Pieris napi* (Lepidoptera, Pieridae). *Oikos*, **116**, 1569–1580.

Wang J (2004) Sibship reconstruction from genetic data with typing errors. *Genetics*, **166**, 1963–1979.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.

Weatherhead PJ, Forbes MRL (1994) Natal philopatry in passerine birds: genetic or ecological influences? *Behavioral Ecology*, **5**, 426–433.

Westneat DF, Mays HL (2005) Tests of spatial and temporal factors influencing extra-pair paternity in red-winged blackbirds. *Molecular Ecology*, **14**, 2155–2167.

Westneat DF, Sherman PW (1997) Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioral Ecology and Sociobiology*, **41**, 205–215.

Wiley TR, Simpendorfer CA (2007) The ecology of elasmobranchs occurring in the everglades national park, Florida: implications for conservation and management. *Bulletin of Marine Science*, **80**, 171–189.

Zeh JA, Zeh GW (2001) Reproduction mode and the genetic benefits of polyandry. *Animal Behaviour*, **61**, 1051–1063.

This study forms part of Joseph DiBattista's PhD thesis on quantitative genetics and the evolution of mating systems, using a natural population of lemon sharks as a model system. Kevin Feldheim is interested in shark population genetics. He is also involved in a number of other projects employing microsatellite markers to infer relatedness and parentage. Xavier Thibert-Plante is a computer guru interested in addressing ecological questions on speciation using individual-based modeling approaches. Andrew Hendry is currently investigating factors that influence the evolution of biological diversity, including natural selection, gene flow, adaptation, and reproductive isolation. He conducts research in a number of study systems, at such exotic places as the Galapagos islands (Darwin's Finches), Trinidad and Tobago (Guppies), British Columbia (Sticklebacks), and Alaska (Sockeye Salmon). Samuel Gruber has been doing elasmobranch research for the past 40 years, and his current focus is on the ecology and conservation biology of sharks. He founded both the American Elasmobranch Society and the Bimini Biological Field Station, and is enjoying his recent retirement from the University of Miami.

Supplementary material

The following supplementary material is available for this article:

Appendix S1 Microsatellite markers

Table S1 Primer sequences and summary characteristics for five microsatellite loci isolated in the lemon shark (*Negaprion brevirostris*) based on the analysis of 408 individuals sampled from Marquesas Key, Florida (T_a, annealing temperature; N_a, number of alleles scored; H_O and H_E, observed and expected heterozygosities, respectively; P_E, exclusion probability for candidate parents)

Table S2 Locus-specific genotyping error based on 55 randomly selected lemon shark samples, expressed as the error per allele or per reaction

Table S3 Maternity (and paternity) reconstruction in the Marquesas nursery using offspring genotypes from lemon sharks captured between 1998 and 2006. All mothers and fathers have been inferred through genotype reconstruction (see text) and are therefore denoted 'REC'; male ID numbers are arbitrary and do not reflect the actual number of parental males in this population. Columns 4 and 5 give the total number of offspring in each litter and the identity of all inferred males siring a litter, respectively. Numbers in brackets after each inferred male represent the number of offspring sired by that particular male. Text in bold represents males or females detected in multiple years

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