

# Social and genetic mating system of Ridgway's hawk (*Buteo ridgwayi*), an endemic raptor on Hispaniola

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**Abstract:** Patterns of social organization and mating systems have been shown to be functions of ecological factors such as resource allocation and breeding density. In some species, particularly birds, social organization and genetic mating systems differ with molecular studies providing evidence of extra-pair young frequently occurring within broods of socially monogamous species. Here we examine the social and genetic mating system of an ecologically little-known forest raptor endemic to the island of Hispaniola in the Caribbean. From 2005–2009, our field observations of over 60 breeding pairs verified a social mating system of monogamy for the species. During the same time period, we collected blood samples ( $n = 146$  birds, 48 nests) and used microsatellite profiles from 10 loci to estimate genetic relatedness among nestlings in a brood and assign putative fathers. We found no evidence of extra-pair paternity in 41 broods. We had one instance where a social male was not assigned as the putative father, however, the confidence level of this assignment was not significant since the genotypes of the social and assigned males were very similar. Our results support our hypothesis that genetic monogamy would be exhibited by Ridgway's hawk, an island-endemic tropical raptor.

**Key Words:** *Buteo ridgwayi*, extra-pair paternity (EPP), monogamy, nestling relatedness, paternity exclusion, tropics

## INTRODUCTION

Mating systems and patterns of social organization have been shown to be functions of ecological factors such as resource distribution and breeding density (Lin *et al.* 2009, Macdonald *et al.* 1997). Recently, it has become evident that social and genetic mating systems differ in many taxa, particularly in birds (Griffith *et al.* 2002, Westneat & Sherman 1997). Molecular studies of avian breeding systems have revealed that many socially monogamous species are not genetically monogamous, with extra-pair paternity (EPP) occurring in the majority of broods in some populations (Westneat & Sherman 1997).

Although differences in life-history strategies and ecological factors have generally been proposed as the main factor in the variation of EPPs among species, there has been little evidence explaining this variation (Griffith *et al.* 2002). Phylogenetic analysis has shown

that more than 50% of interspecific variation in EPP can be explained by phylogeny (Arnold & Owens 2002). In raptors, EPP is reported to occur in low frequency in socially monogamous species (Alcaide *et al.* 2005, Korpimäki *et al.* 1996, Rutz 2005).

Stutchbury & Morton (1995) proposed the breeding synchrony hypothesis to explain low EPP in tropical regions. In the tropics, the majority of species tend to breed asynchronously (thus fewer females are fertile at the same time) resulting in fewer opportunities for EPP. The limited number of tropical bird species for which mating system studies have been carried out show relatively low levels of EPP (Douglas *et al.* 2012, Krueger *et al.* 2008, Stutchbury & Morton 2001).

Griffith (2000) found lower rates of EPP for species on islands compared with mainland counterparts. The author mentions several non-mutually exclusive hypotheses to explain lower EPP on islands including: reduced genetic variability in island species, reduced breeding densities, lower food abundance, phenotypic plasticity to harsher environments, and the cost of mate abandonment on islands.

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The level of male parental care also appears to be a predictor of EPP level (Neudorf 2004). Males involved in parenting activities may be limited in obtaining extra-pair matings because of constraints imposed by caring for young. In taxa that display male parental care, such as Strigiformes and Falconiformes (Müller *et al.* 2001), EPP occurs infrequently or is sometimes entirely absent.

The social mating system of diurnal raptors can vary, and cooperative breeding has been reported in up to 14% of species (Kimball *et al.* 2003). Certain populations of the Galapagos hawk *Buteo galapagoensis* are polyandrous and produce broods with multiple paternity (Faaborg *et al.* 1995), but the majority of species in the genus *Buteo* are reported to be socially monogamous (Ferguson-Lees & Christie 2001). The genetic mating system is documented for only one other socially monogamous *Buteo* species, Swainson's hawk *Buteo swainsoni* (Briggs & Collopy 2012) which has a very low percentage (5%) of broods with extra-pair young.

Ridgway's hawk is reported to exhibit a social mating system of monogamy (Wiley & Wiley 1981). To corroborate this, we examined the detailed spatial organization of breeding adults over five breeding seasons. Further, considering Ridgway's hawk is a tropical island raptor with attributes of parental care, we hypothesized the mating system to be genetically monogamous. To test our hypothesis, we used microsatellite profiles and examined the level of EPP via paternity exclusion analysis and by determining within-brood nestling relatedness. We predicted the frequency of broods with EPP would be very low or absent.

## METHODS

### Study area

The island of Hispaniola (19°0'N, 71°0'W) is located in the Caribbean, and consists of the nations of Haiti and the Dominican Republic (Figure 1). Less than 1.5% of Haiti's original forest is left, most of which is in the inaccessible uplands of the island and is highly degraded (Rimmer *et al.* 2005). The Dominican Republic has not fared much better with only 10% of its original forest cover remaining and under threat to further loss from unregulated logging, slash-and-burn agriculture and charcoal production (Latta *et al.* 2006). We conducted our study in Los Haitises National Park (19°N, 70°W) which ranges from 0–380 m asl in altitude and is located on the north-east coast of the Dominican Republic (Figure 1). It is a platform karst (eroded limestone) formation, with dense clusters of steep conical hills, or mogotes, of nearly uniform height (200–300 m) separated by sinkhole valleys. The Los Haitises region consists of thousands of such mogotes.



**Figure 1.** Map showing relative location of Hispaniola in the Caribbean, the nations of Haiti and Dominican Republic and their respective capital cities: Port-au-Prince (1) and Santo Domingo (2); and the study area of Los Haitises National Park (3).

### Study species

Ridgway's hawk (*Buteo ridgwayi*) is a forest raptor endemic to the island of Hispaniola in the Caribbean. The species is limited to moist low-elevation rain-forest habitats that host high reptile abundance, as reptiles constitute ~80% of its diet (Woolaver *et al.* 2013a). The species was locally common in areas of Haiti and the Dominican Republic at the turn of the century (Cory 1885, Wetmore & Lincoln 1934), but is now listed as Critically Endangered (BirdLife International 2011). The current global population size is estimated at <110 pairs, limited to an area of 1600 km<sup>2</sup> of rain forest in Los Haitises National Park on the north-east coast of the Dominican Republic (Woolaver 2011).

### Nest monitoring

Breeding pairs of *Buteo ridgwayi* were studied over five breeding seasons, 2005–2009. Early-season observations for breeding pairs were made from vantage points on hillsides overlooking valleys to identify nest locations. Due to the topography of the study area, nesting sites were easy to distinguish for each pair as nests were always located in separate valleys. Once found, nests were visited every 1–3 d for easily accessible nests, or every 1–2 wk for sites that were more difficult to access. During each visit, the following behavioural observations were recorded during standardized 4-h sessions: nest-building activities, brooding and incubation behaviour and duration, copulations, territorial defence or displays, pair interactions. Nest observations were carried out with binoculars and a spotting scope from a covered vantage point 10–25 m away.

### Trapping, measuring and ringing Ridgway's hawk

Nestlings were accessed at the nest when 15–40 d old. Nestlings were placed in cotton bags and lowered to the ground below the nest, where they were measured, ringed and their blood sampled for DNA. Handling time of nestlings did not exceed 20 min per individual. Adults were captured using bal-chatri noose traps baited with white domestic mice *Mus musculus* (Thorstrom 1996). Adults were not trapped when the pair was incubating eggs. Ridgway's hawks were ringed with colour anodized Acraft<sup>®</sup> aluminium rivet bands. The bands were coloured and individually numbered so that different colour combinations could be used to identify individual birds. No more than one band was ever placed on a leg.

### DNA collection and extraction

Whole blood was collected from 146 Ridgway's Hawks within the study period from 2005–2008: 34 adults and 112 nestlings. Approximately 0.2 ml of blood was drawn via capillary tube from a patagial vein puncture, half of which was stored in 1.6 ml of Queen's lysis buffer (Seutin *et al.* 1991). The other 0.1 ml of blood was stored in 1.8 ml of 95% ethanol. All samples were stored at ambient temperature until delivered to laboratory facilities where they were preserved at –20 °C.

Total cell DNA was isolated by blood cell lysis, followed by DNA precipitation using ammonium acetate and isopropanol (L. de Sousa, B. Woolfenden and S. Tarof, unpublished protocol). This involved the addition of 50  $\mu$ l of blood/Queen's lysis buffer to 600  $\mu$ l of cell lysis buffer and 5  $\mu$ l of ice-cold Proteinase K (40 ng  $\mu$ l<sup>-1</sup>). This solution was then incubated at 55–60 °C for 5 h and then at 37 °C overnight. Ice cold ammonium acetate (200  $\mu$ l) was then added, mixed gently, and centrifuged to precipitate protein. The aqueous phase, including the dissolved genomic DNA was removed and placed in a fresh tube. Ice-cold isopropanol (600  $\mu$ l) was added and the solution inverted until DNA was visible as a white floating string or flake. This solution was then centrifuged to collect genomic DNA as a pellet at the bottom of the tube. The supernatant was removed and the DNA pellet washed with ice-cold 70% ethanol. This solution was then centrifuged and the ethanol then removed. This ethanol wash was repeated a second time. The tube was then left open and inverted overnight to allow the DNA pellet to dry completely. The DNA pellet was then suspended in 100–200  $\mu$ l of TE buffer (10 mM Tris-HCl, 1 mM EDTA) at 37 °C for 24 h. DNA was stored at 4 °C while in use, and at –20 °C for longer-term storage.

DNA was visualized under ultraviolet radiation on a 1% agarose test gel, pre-stained with ethidium bromide.

Samples were visualized next to a MassRuler high range DNA ladder mix (Fermentas O'GeneRuler<sup>™</sup>).

### Microsatellite genotyping

Eleven microsatellite loci isolated from common buzzard *Buteo buteo* (Johnson *et al.* 2005) and Swainson's hawk *Buteo swainsoni* (Hull *et al.* 2007) were tested for examining allelic variation in *B. ridgwayi* (Woolaver *et al.* 2013b). Polymerase Chain Reaction (PCR) protocols for each set of primers were optimized for *B. ridgwayi* using blood from 24 individuals (6 adult females, 6 adult males and 12 nestlings) sampled in 2005–2007. Non-radioactive, fluorescently labelled (Black, Blue and Green) microsatellite primers were provided by Integrated DNA Technologies (IDT<sup>™</sup>). Optimal reagent volumes and annealing temperatures varied by primer sets. In general, genomic DNA was amplified for each individual in 10  $\mu$ l reactions containing 5.3–6.4  $\mu$ l distilled water, 1.0  $\mu$ l of PCR reaction buffer (10 $\times$  TSG), 0.6–1.4  $\mu$ l of 20mM MgSO<sub>4</sub>, 0.4  $\mu$ l of 10mM dNTPs, 0.2  $\mu$ l of fluorescently dyed 10  $\mu$ M forward and reverse primers, 0.2  $\mu$ l of Taq DNA polymerase (TSG), and 1  $\mu$ l of DNA template (*c.* 15 ng DNA in TE buffer). PCR reactions were carried out in an Eppendorf MasterCycler<sup>™</sup> thermal cycler.

For the Bbu primer pairs: an initial 2-min denaturing step at 94 °C was followed by 12 cycles of 45 s at 94 °C, 45 s at the primer specific annealing temperature, and a 50 s extension step at 72 °C. This was followed by a further 22 cycles of 30 s at 89 °C, 45 s at the primer specific annealing temperature, and a 50-s extension step at 72 °C. The PCR reaction finished with a final 5-min extension step at 72 °C, and samples were then held at 4 °C until taken from the thermal cycler. Primer specific annealing temperatures were as follows: Bbu51 50 °C, Bbu17 and Bbu34 53 °C, Bbu46 54 °C, Bbu42 55 °C, Bbu03 56 °C, Bbu33 58 °C and Bbu59 59 °C. For the Bsw primer pairs (Bsw107, 122, 207, 234, 310 and 324): An initial 2-min denaturing step at 94 °C was followed by 30 cycles of 30 s at 94 °C, 45 s at 58 °C and a 45 s extension step at 72 °C. This reaction finished with a 30-min extension step at 72 °C and PCR products were then held at 15 °C until removed from the thermal cycler.

Each locus was amplified separately but since primers had been fluorescently labelled, loci were pooled post-PCR in Poolplex reactions. PCR products were visualized using a CEQ 8000<sup>™</sup> DNA sequencer, and allele sizes were assigned using the Beckman Coulter CEQ 8000 Genetic Analysis System<sup>™</sup> software.

The genetic mating system of Ridgway's hawk was examined using two complementary analyses of microsatellite profiles: the first was a likelihood ratio method to identify and assign putative fathers, and the second was a method for estimating genetic relatedness

**Table 1.** Characteristics of microsatellite loci of Ridgway's hawk used for assigning paternity and estimating relatedness. Individuals were sampled within Los Haitises National Park, Hispaniola, between 2005 and 2008. Parameters include sample size (n), expected heterozygosity ( $H_e$ ), Polymorphic Information Content (PIC) and probability of exclusion ( $P_{\text{exclusion}}$ ).

Locus	Alleles	n	$H_e$	PIC	$P_{\text{exclusion}}$
Bbu33	4	142	0.538	0.432	0.855
Bbu34	4	135	0.617	0.544	0.802
Bbu42	8	147	0.799	0.769	0.572
Bbu46	9	142	0.823	0.799	0.521
Bbu51	6	136	0.755	0.718	0.638
Bsw107	11	144	0.809	0.783	0.543
Bsw122	12	139	0.828	0.812	0.493
Bsw207	8	142	0.783	0.747	0.598
Bsw234	8	138	0.820	0.794	0.533
Bsw310	13	142	0.883	0.869	0.786
Total	83	–	–	–	0.0001 <sup>a</sup>
Mean	8.3	–	0.766	0.718	–
SD	3.1	–	0.106	0.134	–

<sup>a</sup>Total probability of exclusion using all loci.

among nestlings in a brood. Paternity and nestling relatedness were examined using 10 polymorphic loci (Table 1).

## Data analysis

**Paternity exclusion.** Paternity assignment was performed using the microsatellite profiles and the likelihood-based method in the computer program CERVUS 3.0 (Kalinowski *et al.* 2007, 2010). To identify the putative father and to assess the statistical confidence of this identification, CERVUS calculates an LOD score (natural logarithm of the likelihood-odds ratio) for each candidate male (Kalinowski *et al.* 2007, 2010). A positive LOD indicates that a candidate is more likely to be the father than one randomly drawn from the population. The male with the highest score is assigned as the putative father (Kalinowski *et al.* 2007). The statistical confidence of this estimate is determined by measuring the difference between the LOD scores (Delta,  $\Delta$ ) of the males with the two highest scores (Kalinowski *et al.* 2007). The larger the difference, the more confidence that the male with the highest LOD score is the actual father. Critical values for  $\Delta$  are generated in CERVUS by bootstrapping from the experimental population (Kalinowski *et al.* 2007). CERVUS takes into account genotyping errors, and limits the number of extra-pair paternities according to the estimated proportion of potential fathers that have been sampled (Kalinowski *et al.* 2007).

Parameters used in CERVUS for maximum likelihood paternity assignment included 100 000 simulation cycles

with relaxed and strict confidence levels set at 80% and 95%, respectively. The proportion of candidate parents sampled was 0.20. The proportion of loci typed and loci mistyped were 0.90 and 0.01, respectively. The minimum number of typed loci used for the analysis was seven.

**Relatedness.** Relatedness among nestlings in a brood was carried out using the genetic software program KINGROUP v2 (Konovalov *et al.* 2004). Relatedness estimators using co-dominant markers are unbiased even when allele frequencies are estimated from relatively small samples (Queller & Goodnight 1989), making them the best available markers for estimating relatedness (Blouin 2003, Blouin *et al.* 1996, DeWoody 2005). They have been shown to be a valuable tool for inferring relationships among individuals with unknown ancestry, in the absence of pedigree information (Russello & Amato 2004).

Relatedness was determined using pairwise relatedness coefficients ( $r$ ), which can detect relationships between significantly related individuals including that of parent–offspring, full siblings and half-siblings. This method was chosen for its proven accuracy in detecting relatedness among individuals (Gautschi *et al.* 2003). The pairwise relatedness coefficients were calculated based on Queller & Goodnight (1989). This relatedness estimator ranges from  $-1$  to  $+1$ . A positive value indicates that two individuals share more alleles by descent than expected by chance. First-order relatives such as full siblings should have an  $r$  value of approximately 0.5, and half-siblings an  $r$  of 0.25. Pairwise relatedness coefficients within broods were compared to theoretical values using  $t$ -tests. A genetic relationship between two nestlings was considered significant ( $P < 0.05$ ) by KINGROUP (Konovalov *et al.* 2004), if the pairwise relatedness coefficient was  $\geq 0.25$ , similar to that of half-sibling relatedness or greater.

## RESULTS

### Social mating system

During all observation periods during the 5-y study ( $>2000$  observation hours) no more than two adults were ever observed interacting non-aggressively within a territory. Aggressive interactions between adjacent individuals or pairs were observed during aerial territorial displays, but these sightings were rare ( $n = 15$ ). Whenever individual birds were identified from band combinations (23 of 35 adults re-sighted over the study period), identification confirmed the attending female and male at nests to be the social pair within the territory. No non-paired males were observed provisioning food or seen near

**Table 2.** Number of copulations of Ridgway's hawk breeding pairs recorded by month and breeding stage in Los Haitises National Park, Hispaniola, from 2005–2009.

	January	February	March	April	May	June
Nest building	15	22	9	1	0	0
Incubation	0	2	2	1	0	0
Young nestling	0	0	1 <sup>a</sup>	0	0	0
Old nestling	0	0	0	3	2	1
Fledgling	0	0	0	0	1	0
Recently failed nest	0	0	1 <sup>b</sup>	7 <sup>c</sup>	7 <sup>d</sup>	0
Total	15	24	13	12	10	1

<sup>a</sup>Nest with 6 d-old nestlings.

<sup>b</sup>Failed during incubation.

<sup>c</sup>Three failed with young chicks, others failed at unknown stage.

<sup>d</sup>Five failed with nestlings.

the resident female in an active territory and extra-pair copulation attempts were not observed.

Copulations were observed during all stages of the breeding episode over a 6-mo period ( $n = 74$ , Table 2). Copulations were observed from January to June during nest building ( $n = 47$ ), incubation stage ( $n = 5$ ), nestling stage ( $n = 7$ ), fledgling of young ( $n = 1$ ) and after recent nest failures ( $n = 15$ ). Peaks in copulation frequencies occurred just prior to egg-laying (January–March) and to a lesser extent after a nest had failed (April–May). Copulation behaviour between two individuals may be indicative of whether copulations are within-pair or extra-pair. Female solicitation followed by male mounting without interruption by the female can be suggestive of a within-pair copulation. Of 62 copulation attempts observed to completion, 60 were carried out without any interruption by the female. On two occasions, a male mounted the female but the female did not lift her tail to allow the copulation to continue. In both instances the male dismounted and remained perched next to the female and was identified as the social male.

### Microsatellite genotyping

A total of 34 adults and 112 nestlings were used for the microsatellite genotype profiling. Of these samples, 19 adults and 86 nestlings from 48 broods were used for the paternity exclusion and brood relatedness analysis (samples with only one individual per family unit were not applicable). We were able to catch and sample seven nests with full family units (social male, social female, + all nestlings), 12 nests with social male + nestlings, eight nests with social female + nestlings, and 19 nests where only the nestlings could be sampled.

Eighty-three different alleles were found from 10 loci, with an observed heterozygosity of  $0.766 \pm 0.106$

(Table 1). The mean polymorphic information content of all loci was  $0.718 \pm 0.134$  (Table 1). The combined probability of non-exclusion for the marker set was 0.001 (Table 1).

### Genetic mating system

**Paternity assignment.** The attending male was assigned as the putative father for 29 out of 30 nestlings from 19 broods (Appendix 1). From 2005–2007, none of the broods showed evidence of extra-pair young. In 2008 there was one instance of an attending male not being assigned as the actual father to one of two nestlings. However, the confidence level of the assignment was not significant (Delta Value of 0), with both the attending male and the assigned male having similar genotypes, and found to be closely related similar to that of a parent-offspring or full sibling (relatedness coefficient  $r = 0.52$ ,  $P < 0.00$ ). Thus it is probable that this one case was not an instance of extra-pair paternity but an incorrect assignment of parentage due to the similar genotypes.

**Brood relatedness.** Brood relatedness values were used to assess potential EPP in cases where one or both parents were not genotyped. The relatedness coefficient among nestlings in the 41 broods sampled over the study period ranged from 0.32 to 0.65 and averaged  $0.48 \pm 0.08$  (Appendix 1). These values did not significantly differ from the expected 0.50 ( $t_{1,40} = -0.35$ ,  $P = 0.73$ ) and indicated full-sibling relatedness on average for all broods. For comparison, relatedness coefficients were obtained from known half-siblings (based on social pairings); nestlings from nests of different years where one parent was identified from the previous year and the other parent was a new mate. Half-sibling relatedness coefficients ranged from 0.16 to 0.29 and averaged  $0.23 \pm 0.04$  ( $n = 12$ ). Relatedness coefficients of half-siblings did not differ significantly from the expected 0.25 ( $t_{1,11} = -1.58$ ,  $P = 0.09$ ) and were significantly lower compared with relatedness coefficients of nestlings within the same brood ( $F_{1,40} = -11.1$ ,  $P < 0.001$ ). This suggests that all 41 broods sampled over the study period consisted of full siblings with no cases of extra-pair young.

### DISCUSSION

Nest site and territory observations verified a socially monogamous mating system for Ridgway's hawk, as originally noted by Wiley & Wiley (1981). The majority of *Buteo* species appear to hold socially monogamous pair bonds (Newton 1979) however cooperative breeding systems are not uncommon in diurnal raptor species

(Kimball *et al.* 2003). The Galapagos hawk, which consists of several island subpopulations that range from social monogamy to polyandry (Bollmer *et al.* 2003, Faaborg *et al.* 1995), is the only documented *Buteo* species to have a clear cooperative breeding system. Polyandry has been suggested for the Puna subspecies of Red-backed hawk *Buteo polyosoma* (Kimball *et al.* 2003), but this has not been confirmed.

For the closest taxonomic relative to Ridgway's hawk, the red-shouldered hawk *B. lineatus* (Amaral *et al.* 2009), there is a single record of three adults attending a nest in Florida (<http://bna.birds.cornell.edu/BNA/species/107>). For the geographically closest forest *Buteo* species, the Puerto Rican broad-winged hawk *B. platypterus brunnescens*, there is one report of a nesting female accepting food from an intruding male in the absence of the attending male (Delannoy & Cruz 1988). Similar documented cases have been reported for *B. buteo* (Barrientos & López-Darias 2006), *B. swainsoni* (Cash 1989) and *B. jamaicensis* (Santana *et al.* 1986) but these anecdotal sightings are rare and not suggestive of a cooperative social breeding system.

We found no evidence of extra-paternity in 41 broods, with nestlings showing a genetic similarity level congruent with those expected among full siblings, implying that the nestlings were produced by the social parents. Since there was one instance of a social male not being assigned paternity (even though the confidence level of the assignment was very low), it is not possible to rule out the possibility of EPP in the species. However, our results indicate the species is effectively genetically monogamous.

Ridgway's hawk is a socially monogamous species, exhibiting male parental care (Woolaver 2011). These traits are assumed to be associated with low EPP levels. Male Ridgway's hawks were observed incubating albeit less often than females, and were the main food provider to the nestlings, and therefore would have reduced time to pursue EPCs. In addition, the valley landscape of each territory would allow for easy detection of intruding males, making it easy for social males to limit EPCs within their territory. Within avian taxa, socially monogamous males appear to have evolved two strategies to ensure paternity: mate guarding (Birkhead 1979) and/or frequent within-pair copulations (Birkhead *et al.* 1987). Ridgway's hawks seem to have employed the latter strategy. During the study period, copulations were observed between breeding pairs during all stages of the breeding episode, and were frequent during late nest-building and early incubation stages. Males were often away from their nests for extended periods of time to hunt and provide food to the female and nestlings, and would not be able to effectively guard their mates.

Other contemporary ecological factors, such as breeding synchrony, have also been suggested to be

correlated to EPP levels (Griffith *et al.* 2002). Species that breed over a short period of time with most females in breeding condition at the same time, allow for EPC opportunity; whereas species that breed over a longer period with females in breeding condition at different times would support much fewer opportunities. Tropical species may have fewer cases of EPP because most species tend to breed asynchronously, with fewer females fertile at the same time (as few as 8%), resulting in less opportunity for EPC (Stutchbury & Morton 1995). During the present study, Ridgway's hawk females were observed breeding over a 6-mo period with a large temporal variation in copulation activity and egg-laying, from January until late June (Woolaver 2011).

The challenge in applying hypotheses to a given taxon is that often certain life-history traits co-occur (Westneat & Stewart 2003) and it is difficult to determine to what extent each trait may contribute to EPP rates. For example, longevity and male parental care are both life-history traits for several taxa including seabirds (Arnold & Owens 2002), owls (Arsenault *et al.* 2002, Saladin *et al.* 2007), parrots (Masello *et al.* 2002) and vultures (Decker *et al.* 1993). The phylogenetic component also adds to the challenge of trying to explain or predict EPP. As a general rule, EPP tends to be higher in passerines and lower in raptors and seabirds (Westneat & Sherman 1997). However recent studies have reported species which appear to be exceptions to the rule. Genetic monogamy or very low EPP has been reported in several passerines (Kleven *et al.* 2008, Maguire & Mulder 2008, Taylor *et al.* 2008), and high levels of EPP have been found in albatross (Huyvaert *et al.* 2000). At the same time, many species appear to be conforming to original predictions, including low EPP in raptors (Arsenault *et al.* 2002, Hogan & Cooke 2010, Saladin *et al.* 2007, this study), seabirds (Anker-Nilssen *et al.* 2008, 2010; Wojczulanis-Jakubas *et al.* 2009) and higher EPP in passerines (Stewart *et al.* 2010). It is not surprising that factors contributing to the frequency of EP mating are challenging to resolve since just a few ecological or biological differences between populations of closely related species can result in very large variation in EPP (Kingma *et al.* 2009, Labarbera *et al.* 2012, Stutchbury *et al.* 2007).

Overall, our genetic results, along with our observations of the species spatial organization, support our hypothesis that the social and genetic mating system for Ridgway's hawk is monogamy. However, there are other species that do not follow these general predictions (Huyvaert *et al.* 2000, Macedo *et al.* 2008, Taylor *et al.* 2008, Townsend *et al.* 2011), thus more molecular studies of tropical species are needed for a better understanding, not only of general genetic mating systems, but also of the contributing evolutionary and ecological factors that determine EPP frequency (Stutchbury & Morton 2008).

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**Appendix 1.** Results of genetic mating system analysis of Ridgway's hawks (breeding pairs and nestlings) sampled within their breeding territories in Los Haitises National Park, Hispaniola, between 2005 and 2008. Microsatellite profiles were used from 10 loci to estimate genetic relatedness among nestlings in a brood (KINGROUP Nestling Relatedness Coefficients) and assignment of putative fathers (CERVUS paternity assignment and LOD Score). Social male (SM) and female (SF) were the adults observed attending the nest. Assigned male refers to the male identified by the CERVUS program as the most likely candidate to be the genetic father. NS refers to not sampled, + denotes a new mate, and LOD Score is reported with strict confidence \*\* (95%) and relaxed confidence \* (80%).

Year	Breeding territory	Social female ID/ Social male ID	Nestling ID	No. of loci mismatch with SF	No. of loci mismatch with SM	LOD score confidence	Assigned male	Nestling Relatedness Coefficients, P-value
2005	Britos 1	5005 / 5006	5007	0 / 10	0 / 10	**	5006	0.46, 0.005
			5008	0 / 10	0 / 10	*	5006	
2005	Cacata 1	5009 / NS	5010	0 / 9	–	–	–	0.65, 0.000
			5011	0 / 9	–	–	–	
2005	Indios 1	6041 / 7035	5013	0 / 10	0 / 10	*	7035	–
2005	Casa Grande 1	5016 / 5017	5018	0 / 9	0 / 10	*	5017	0.50, 0.003
			5019	0 / 9	1 / 10 <sup>a</sup>	*	5017	0.39, 0.022
			5020	0 / 9	0 / 10	**	5017	0.48, 0.005
2005	Cotorra 1	NS / 5022	5023	–	0 / 10	**	5022	0.57, 0.001
			5024	–	0 / 10	**	5022	
2005	Malena 1	5027 / 5028	5029	0/9	0 / 7	**	5028	–
2005	Mata Limon 1	NS / NS	5032	–	–	–	–	0.53, 0.002
			5033	–	–	–	–	
2006	Arrollitos 1	NS / 6011	6012	–	0 / 9	**	6011	0.41, 0.013
			6013	–	1 / 10 <sup>a</sup>	*	6011	
2006	Britos 2	NS / NS	6019	–	–	–	–	0.33, 0.010
			6020	–	–	–	–	
2006	Cacata 2	NS+ / NS	6025	–	–	–	–	0.48, 0.001
			6026	–	–	–	–	
2006	Cacata 3	NS / NS	6027	–	–	–	–	0.39, 0.016
			6028	–	–	–	–	
2006	Caimonis 1	NS / 6030	6031	–	0 / 10	**	6030	0.49, 0.010
			6032	–	0 / 10	**	6030	
2006	Casa Grande 2	NS / NS	6033	–	–	–	–	0.51, 0.001
			6034	–	–	–	–	
2006	Malena 1	5027 / 5028	6045	0 / 9	0 / 7	**	5028	0.54, 0.000
			6046	0 / 10	0 / 7	**	5028	
2006	Mata Limon 1	NS / NS	6048	–	–	–	–	0.48, 0.001
			6049	–	–	–	–	
2006	Mata Limon 2	NS / NS	6050	–	–	–	–	0.45, 0.001
			6051	–	–	–	–	
2006	Tiladora 1	6053 / NS	6054	1 / 10 <sup>a</sup>	–	–	–	0.38, 0.002
			6055	0 / 9	–	–	–	

## Appendix 1. Continued.

Year	Breeding territory	Social female ID/ Social male ID	Nestling ID	No. of loci mismatch with SF	No. of loci mismatch with SM	LOD score confidence	Assigned male	Nestling Relatedness Coefficients, P-value
2007	Aguatico 1	NS / NS	7001	–	–	–	–	0.48, 0.001
			7002	–	–	–	–	
2007	Aguatico 2	NS / 7004	7005	0 / 10	0 / 10	*	7004	0.55, 0.000
			7006	0 / 10	0 / 10	*	7004	
2007	Casa Grande 1	NS+ / 5017	7008	–	0 / 10	**	5017	–
2007	Calle Sol 1	6039 / NS	7009	1 / 10 <sup>a</sup>	–	–	–	0.40, 0.010
			7010	0 / 9	–	–	–	
2007	Cacata 4	NS / NS	7011	–	–	–	–	0.51, 0.001
			7012	–	–	–	–	
2007	Cacata 3	NS / NS	7013	–	–	–	–	0.62, 0.000
			7014	–	–	–	–	
2007	Cacata 2	7029 / NS	7015	0 / 9	–	–	–	0.64, 0.001
			7016	0 / 9	–	–	–	
2007	Arrollitos 2	NS / NS	7017	–	–	–	–	0.47, 0.001
			7018	–	–	–	–	
2007	Indios 1	6041 / 7035	7021	0 / 10	0 / 10	*	7035	–
2007	Tiladora 1	6053 / NS	7022	0 / 10	–	–	–	0.61, 0.000
			7023	0 / 9	–	–	–	
2007	Britos 1	NS+ / 5006	7024	–	0 / 10	**	5006	0.48, 0.005
			7025	–	0 / 10	*	5006	
2007	Britos 2	NS / NS	7026	–	–	–	–	0.32, 0.060
			7027	–	–	–	–	
2007	Casa Grande 1	NS+ / 5017	7040	–	0 / 10	*	5017	–
2008	Guallullos 1	7028 / NS	8001	0 / 9	–	–	–	0.45, 0.001
			8002	0 / 10	–	–	–	
2008	Llalla 1	8043 / 6042	8003	0 / 8	0 / 10	**	6042	0.55, 0.000
			8004	0 / 8	0 / 10	**	6042	
2008	Cacata 2	7029 / NS	8005	0 / 9	–	–	–	0.63, 0.000
			8006	0 / 9	–	–	–	
2008	DeVio 1	NS / NS	8008	–	–	–	–	0.43, 0.003
			8009	–	–	–	–	
2008	Arrollitos 3	NS / NS	8010	–	–	–	–	0.56, 0.000
			8011	–	–	–	–	
2008	Aguatico 2	NS / 7004	8012	–	1 / 10	ns	6042	0.62, 0.000
			8013	–	0 / 10	**	7004	
2008	Caimonis 1	NS / 6030	8014	–	0 / 9	**	6030	0.46, 0.005
			8015	–	1 / 9 <sup>a</sup>	*	6030	
2008	Mata Limon 3	NS / 7033	8016	–	0 / 10	**	7033	–
2008	Britos 3	NS / NS	8019	–	–	–	–	0.43, 0.005
			8020	–	–	–	–	
2008	Tiladora 1	6053 / NS	8021	1 / 10 <sup>a</sup>	–	–	–	0.56, 0.000
			8022	0 / 10	–	–	–	
2008	Arrollitos 4	NS / NS	8025	–	–	–	–	0.54, 0.000
			8026	–	–	–	–	
2008	Arrollitos 5	NS / NS	8027	–	–	–	–	0.47, 0.001
			8028	–	–	–	–	
2008	Arrollitos 1	NS / 6011	8029	–	1 / 10 <sup>a</sup>	*	6011	0.39, 0.033
			8030	–	0 / 10	**	6011	
2008	Arrollitos 2	NS / NS	8031	–	–	–	–	0.61, 0.000
			8032	–	–	–	–	
2008	Calle Sol 1	NS+ / NS	8037	–	–	–	–	0.49, 0.001
			8038	–	–	–	–	
2008	Mata Limon 1	NS / 7033	8044	–	1 / 10 <sup>a</sup>	*	7033	–

<sup>a</sup> Mismatch likely due to genotyping error.