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RESEARCH ARTICLE

A non-invasive genetic evaluation of population size, natal philopatry, and roosting behavior of non-breeding eastern imperial eagles (*Aquila heliaca*) in central Asia

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Abstract Roughly one-third of all eagle species are considered to be threatened or endangered, but the ecology of most eagles remains poorly understood. While the pronounced territoriality of breeding adults facilitates behavioral studies, the demography of non-breeding individuals (pre-adults and non-territorial floaters) is almost completely unknown. Traditionally, limited data on pre-adult and floater movement come from wing-tagging and/or telemetry studies. As an alternative to these methods we used genetic analyses of non-invasively collected feathers to investigate the population biology of non-breeding eastern imperial eagles (*Aquila heliaca*) in Kazakhstan. Microsatellite profiles of shed feathers indicate that eastern imperial eagles roost communally with other raptors. Furthermore, roosts are large and dynamic: 287 non-breeding eastern imperial eagles were detected in our sample, and a mark-recapture analysis estimated the total number to be 308 ± 8. The natal origins of these individuals were investigated by comparing their microsatellite profiles to those available for >90% of the eastern imperial eagle chicks hatched at the study site over the six previous breeding seasons. Only 4% of the individuals genetically matched a chick, suggesting that the reserve may serve as a critical refugium for pre-adults and itinerant floaters. Feathers have long been recognized as a suitable source of DNA, but few studies have used wide-scale, non-invasive collections of feathers (>1,000 samples) to investigate fundamental aspects of avian biology. Our research demonstrates that non-invasive genetic analyses of feathers can be used to evaluate population size, natal philopatry, and local movements of birds that are difficult to study using traditional means.

Keywords Feather · Floater · Juvenile · Mark-recapture · Non-invasive sampling

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Introduction

Roughly one-third of all eagles are listed as threatened or endangered (IUCN 2006), but the basic ecology of most species is still poorly understood. For eagles and many other raptors, direct evaluations of abundance, natal philopatry, and dispersal are particularly challenging because individuals are not only difficult to capture and mark, but they can also be nearly impossible to re-sight. Furthermore, wing-tagging and telemetry studies generally collect information on only a limited number of individuals due to financial and logistical constraints, so when ecological data are available for an eagle species it is usually sparse. As an alternative to traditional monitoring techniques, we used naturally shed feathers to tag eagles genetically in central Asia. Here, we report the results of

the first direct enumeration of eagles using genetic tags and subsequently use those tags to investigate natal origin (i.e., to address natal philopatry and dispersal). Our research is particularly novel because we focus on non-breeding individuals (pre-adults and non-territorial floaters) instead of breeding adults. Because eagles are long-lived and exhibit delayed sexual maturity non-breeding individuals represent a significant, but understudied, demographic component of the population.

The eastern imperial eagle (*Aquila heliaca*) is a large raptor found throughout central Europe and Asia. While once common in many parts of their range, eastern imperial eagles experienced rapid population declines during the middle of the twentieth century (del Hoyo et al. 1994) and are now either extirpated or very rare in many areas (CITES Appendix I; IUCN 2006; UNEP-WCMC 2006). Like many raptors, eastern imperial eagles are difficult to study because males and females cannot be easily distinguished from a distance (they are sexually dimorphic only in size, not plumage), most individuals participate in long annual migrations, and adults are exceedingly difficult to capture and mark. Until now, research on eastern imperial eagles has focused on the breeding portion of populations because, despite their migratory nature, adults exhibit high fidelity to territories across breeding seasons (del Hoyo et al. 1994; Rudnick et al. 2005). Pre-adults and floaters (i.e., non-breeders), on the other hand, are more difficult to study because they are not affiliated with a particular territory.

Our research focuses on an eastern imperial eagle population located in a zapovednik (national nature reserve) in north-central Kazakhstan. The Naurzum Zapovednik and its associated buffer zone encompass ~307,000 ha and represent a diverse ecosystem where southern fragments of Siberian forest meet central Asian steppe (Katzner et al. 2006a). Today, over two dozen raptor species annually breed within the reserve (some at remarkably high densities; Bragin 1989) and this extraordinary assemblage includes three eagle species in addition to the eastern imperial eagle; the steppe eagle (*A. nipalensis*), the golden eagle (*A. chrysaetos*), and the white-tailed eagle (*Haliaeetus albicilla*). There are currently ~40 active eastern imperial eagle nesting territories within the reserve, ~20 white-tailed eagle territories, ~13 steppe eagle territories, and ~4 golden eagle territories. The Naurzum Zapovednik is ideal for studying eastern imperial eagles because, with 40 territories in just under 28,000 ha of forest area, this population of eastern imperial eagles is likely the largest and densest breeding population in the world. The Naurzum Zapovednik is also an excellent location for studying non-breeding eastern imperial eagles because many pre-adults also congregate at the reserve. The exact number of non-breeders is unknown, but past field surveys have revealed as many as 37 simultaneously airborne individuals in regions of the reserve interstitial to known breeding territories (Table 1).

In northern Kazakhstan, eastern imperial eagles typically hatch in May or June and depart by September, presumably migrating to wintering grounds in southwestern Asia and the Middle East (del Hoyo et al. 1994; Katzner 2003). Whereas breeding adults are philopatric and return to the same nesting territories each year (Rudnick et al. 2005), the fate of non-breeders is unknown. Pre-adults cannot return to the territory from which they hatched because their natal territories are usually occupied by their parents, who can hold a territory for over 20 years. Non-breeders may, however, return to reserve lands outside of established breeding territories. Here, we use genetic analyses of non-invasively collected feathers to study the non-breeding eastern imperial eagles located in the Naurzum Zapovednik. Specifically, we investigate: (1) population size, (2) natal origins, and (3) patterns of roost usage. Conventional investigation of these demographic and behavioral questions requires invasive methods (e.g., wing-tagging, radio and satellite telemetry, etc.). We show that these same topics can be investigated not only non-invasively, but also at much larger spatial scales and sample sizes than is generally possible in traditional field studies.

Material and methods

Sample collection

During July 2004, naturally shed feathers were collected at the Naurzum Zapovednik in the Kostanay Oblast of north-central Kazakhstan. Feathers were placed in paper

Table 1 The number of eastern imperial eagles simultaneously observed in areas of the Naurzum Zapovednik not ascribed to a breeding territory

Date of observation	Number of individuals
June 19, 1978	37
May 20, 1981	24
May 27, 1982	25
July 13, 1985	19
June 23, 1993	32
July 10, 1994	7
July 06, 1996	10
June 11, 1997	13
June 07, 1998	>25
July 03, 2003	17
June 18, 2004	21

Data were collected by E.A. Bragin and T.E. Katzner during routine population monitoring

envelopes and stored dry at room temperature. A total of 1,822 feathers were collected over four sampling occasions, from four roosting areas. The maximum distance between neighboring roosts was 1.82 km, the minimum was 0.43 km, and the mean distance between neighboring roosts was 1.02 km. These roosting areas were also several kilometers from the nearest eastern imperial eagle breeding territory. Sampling locations (i.e., roosting areas) were chosen because they were in areas where significant numbers of pre-adult eastern imperial eagles had been observed.

Sampling occasions were 5 days apart, all four roosting areas were sampled on each sampling occasion, and a concerted effort was always made to collect all eastern imperial eagle feathers present. Adult eastern imperial eagles molt flight feathers fairly continuously throughout the year, thus, they do not exhibit a single molting event characteristic of some other avian species (e.g., waterfowl). Essentially no data are available on the molting of pre-adults, but we have no evidence to suggest molting is significantly different for these individuals and newly shed feathers were consistently available at roosting sites throughout sample collection. Feathers collected during the first sampling occasion had accumulated for an unknown period of time, however, feathers deposited prior to the previous winter were easily identified and discarded due to the harsh environmental conditions they experienced. Thus, while the feathers collected during the first sampling occasion potentially represented a larger number of individuals, the feathers collected were still representative of individuals present at the Naurzum Zapovednik during the spring and early summer of 2004.

Chicks hatched at the Naurzum Zapovednik were used to investigate the natal origins of the non-breeders that were sampled via feather collections. In other words, were the non-breeders we sampled hatched in the Naurzum Zapovednik or elsewhere? As part of earlier efforts to monitor breeding success (Rudnick et al. 2005), developing blood feathers were directly plucked from eagle chicks hatched at the Naurzum Zapovednik between 1998 and 2003. A total of 230 eastern imperial eagle chicks were sampled from ~90% of the nesting territories occupied in the reserve during each year of sample collection (from 1998 to 2003 there were, respectively, 28, 37, 61, 31, 44, and 29 chicks sampled per year). Developing blood feathers were placed in a lysis buffer (100 mM Tris–HCl pH 8.0, 100 mM EDTA, 10 mM NaCl, 2% SDS) immediately upon collection, and ultimately stored at −80°C.

DNA methods

DNA was isolated from both sample types (shed feathers and blood) as described in Rudnick et al. (2005). A negative control containing no eagle tissue was included in each set of DNA extractions, and a no-template negative control was included in each set of PCR. Our previous work on eastern imperial eagles utilized a suite of nine microsatellite markers, for which we demonstrated a global (i.e., across loci) genotyping error rate of less than 1.5% (Rudnick et al. 2005) under the same sample collection methods and analysis techniques employed here. A subset of the seven microsatellites that provided the most robust PCR amplifications (*IEAAAG-4, Aa02, Aa35, Aa36, Aa39, Aa43*, and *Aa49*) were used for the current analyses, and all samples were genotyped as in Rudnick et al. (2005) which should be consulted for more details regarding methodology and genotyping error rates. Microsatellite profiles for non-invasively collected feathers were used to group genetically identical samples into individuals (see below for additional details), and species identification was corroborated using the mitochondrial cytochrome *c* oxidase I gene (Rudnick et al. 2007). GENEPOP (Raymond and Rousset 1995) was used to calculate allele frequencies and observed heterozygosities for all eastern imperial eagles identified from the non-invasively collected feathers, as well as to assess gametic phase disequilibria. Samples collected from white-tailed eagle and steppe eagle chicks were used to estimate allele frequencies for those species, which helped verify that non-invasively collected feathers were eastern imperial eagle in origin.

Individual identification

Multi-locus microsatellite profiles for non-invasively collected feathers were used to group genetically identical samples into individuals. Numerous methods have been suggested for limiting errors in microsatellite data generated from non-invasively collected samples (Taberlet et al. 1996; Morin et al. 2001; Paetkau 2003; McKelvey and Schwartz 2004; Waits and Paetkau 2005). Given the large size of our dataset, we chose to employ a method of "sample culling" similar to that suggested by Paetkau (2003) to ensure high quality data. All shed feathers were initially genotyped at four microsatellite loci. Any sample that failed to amplify at a minimum of two loci was immediately discarded. Samples that were not discarded were genotyped at three other loci. Up to two additional amplifications were then attempted for any locus that was missing data. After genotyping was complete, any sample that failed to amplify at a minimum of five loci was discarded.

The probability that two different individuals shared the same multi-locus genotype strictly by chance was calculated from the unbiased estimator of the probability of identity (P_{ID}) provided by Paetkau et al. (1998). Only

samples missing no more than one microsatellite genotype (i.e., samples that amplified at 6 or 7 loci) were initially used to define individuals as the multi-locus P_{ID} of the five least variable microsatellite loci was 2.3×10^{-5}. Samples missing only two microsatellite genotypes (i.e., samples that amplified at 5 loci) were then matched to the individuals defined in the previous step. At this stage, any sample that was missing two microsatellite genotypes and did not match one of the previously defined individuals was discarded because we considered the P_{ID} of the three least variable microsatellite loci (6.2×10^{-3}) too high for accurate individual identification (see Waits and Paetkau 2005). During both steps of individual identification, any two samples exhibiting microsatellite profiles that differed by only one or two loci were flagged. All samples flagged in this manner were re-genotyped at the "mismatched" loci to confirm the true genotype and guard against possible genotyping error. To validate the claim that individuals identified from the non-invasively collected feathers were indeed non-breeding individuals, multi-locus microsatellite profiles were compared to those of 82 adult eastern imperial eagles that bred in the Naurzum Zapovednik between 1999 and 2002 (Rudnick et al. 2005 and unpublished data).

Population size

The number of individuals identified from the non-invasively collected feathers provided a minimum estimate of abundance. To improve upon that estimate, data were analyzed in a mark-recapture framework. Because sample collection occurred over only 15 days (four sampling periods, each 5 days apart) and a concerted effort was made to collect all putative eastern imperial eagle feathers present each sampling occasion, the population was assumed to be closed (i.e., no birth, death, immigration, or emigration occurred during the sampling period) for the purpose of abundance estimation. All data collected from all four roosts during a single sampling occasion were pooled so that abundance was estimated from the total number of individuals captured on each occasion, regardless of where capture occurred. Multiple feathers collected from the same individual during a single sampling occasion were collapsed into a single capture (i.e., an individual could be captured only once during a sampling occasion). Program MARK (White and Burnham 1999) was used to estimate abundance under a series of closed-capture models. All models were run under the Closed Population Estimation option in MARK (models from Otis et al. 1978) and abundance was estimated directly. Closed-capture models were ranked by AICc values (Akaike's Information Criterion corrected for small sample size) and the model with the smallest AICc was used to estimate abundance.

Natal origins

Non-breeding individuals include both pre-adults and floaters. While the proportion of each type of non-breeding individual at the Naurzum Zapovednik is unclear, the bulk of the feathers collected in the field were clearly from birds that had not yet attained adult plumage, corroborating field observations that indicated a significant number of pre-adults are present. Eastern imperial eagles attain adult plumage between 5 and 6 years of age, meaning pre-adults present at the Naurzum Zapovednik in 2004 were hatched between 1998 and 2003. Previous monitoring efforts sampled 230 eastern imperial eagle chicks (>90%) hatched at the reserve during this time period (Rudnick et al. 2005). Natal philopatry was investigated by comparing the microsatellite profiles of non-breeding individuals sampled in 2004 to those available for chicks hatched at the Naurzum Zapovednik between 1998 and 2003. Thus, non-breeding individuals hatched at the Naurzum Zapovednik were identified.

We investigated the possibility that eastern imperial eagles are congregating at the Naurzum Zapovednik from multiple, genetically distinct populations by calculating F_{IS} (SPAGeDi Version 1.1; Hardy and Vekemans 2002) for the dataset of all pre-adults and floaters identified from the naturally shed feathers. The statistical significance of F_{IS} was evaluated by comparing the observed value to those obtained by permuting genes among individuals 10,000 times. We also analyzed this dataset of individuals with the software program STRUCTURE (Version 2.1; Pritchard et al. 2000; Falush et al. 2003; Pritchard and Wen 2003; Evanno et al. 2005). STRUCTURE uses a Bayesian clustering algorithm to infer population structure from mutlilocus genotypes and it provides an ad hoc method for identifying the number of populations (K) present in a dataset. We analyzed the dataset of all non-breeding eastern imperial eagles for K values 1 through 10, with each K being run 10 independent times. Analysis at a given K included 100,000 replicates, following a burn-in of 50,000 replicates. The ancestry model was set to admixture and allele frequencies were allowed to be correlated.

Roost usage

Naturally shed feathers were collected over 15 days at four roosting areas. After genetically identical feathers were assigned to individuals, the roost usage of specific individuals was monitored over time. This information was used to quantify the number of individuals present at each roosting site and the number of roosting sites a given individual visited.

Results

Individual identification

DNA was successfully extracted from 1,676 feathers (i.e., feathers amplified at a minimum of two out of the four loci used for initial screening) and of these, 1,146 (68%) amplified at the minimum number of microsatellite loci to be initially assigned to individuals; a total of 1,005 feathers (88%) amplified at six or seven loci, while 141 (12%) amplified at five loci. During individual identification, any two samples exhibiting microsatellite profiles that differed by only one or two loci were flagged and re-genotyped. "Mismatched" genotypes could not be resolved for 30 feathers, and these samples were discarded. Although we initially opted to discard any sample that amplified at less than five microsatellite loci, a few feathers (60) that amplified at only four loci were ultimately included in the individual identification analysis because they exhibited alleles consistent with white-tailed eagle allele frequency distributions (and inconsistent with eastern imperial eagles; unpublished data). Thus, to provide an absolute minimum number of white-tailed eagles present, feathers that amplified at only four microsatellite loci were included in the individual identification process if they exhibited alleles characteristic of white-tailed eagles. Known white-tailed eagle samples (chicks) were used to generate the white-tailed eagle allele frequency distributions.

In the final analysis, a total of 1,176 feathers were assigned to 314 individual eagles and a PCR-based assay of the mitochondrial cytochrome *c* oxidase I gene indicated these feathers were derived from at least three different eagle species (Rudnick et al. 2007). A total of 268 individuals were identified as eastern imperial eagles, 13 individuals as white-tailed eagles, and 1 individual as a golden eagle (Rudnick et al. 2007). Thus, 95% of the successfully identified individuals were eastern imperial eagles and fewer than 5% were white-tailed eagles or golden eagles. The species identity of 32 individuals could not be determined via the mtDNA assay due to insufficient DNA quantities. Rather than discarding these individuals, their species identity was ascertained from microsatellite data. Eastern imperial eagles and white-tailed eagles have fixed differences in their microsatellite allele frequency profiles, and individuals were classified as either an eastern imperial eagle or a white-tailed eagle based on their multilocus genotypes. Of the 32 individuals that were not typed using the mtDNA assay, 26 were identified as eastern imperial eagles, 3 were identified as white-tailed eagles, and 3 individuals exhibited alleles that were characteristic of neither species but whose alleles were consistent with those observed in the golden eagle that was identified from the mtDNA assay (golden eagle allele frequencies are not available for these microsatellites).

As a further verification of species identity, microsatellite profiles for the eastern imperial eagles identified through the mtDNA assay were compared to the eastern imperial eagle allele frequency distributions established from known (chick) samples. The microsatellite profiles of nearly all presumptive eastern imperial eagle samples matched the mtDNA haplotype data, but seven individuals were ambiguous and subsequently removed from all analyses because their species identity could not be satisfactorily resolved. The possibility exists that these seven individuals were hybrids, but we find this explanation unlikely because no hybrid breeding pairs have been observed at the Naurzum Zapovednik in over 30 years of monitoring and we know of no evidence documenting hybridization elsewhere.

After all species identification was complete, 287 eastern imperial eagles, 16 white-tailed eagles, and 4 golden eagles (307 total individuals) were identified from 1,161 feather samples. We assigned a mean of 3.8 feathers (range 1–39) to each individual using the microsatellite data, with over 65% of the individuals being identified on the basis of two or more feathers (Fig. 1). Because we knew the genotypes of 82 territorial eastern imperial eagles that were present in the Naurzum Zapovednik between 1999 and 2002 (Rudnick et al. 2005), these genotypes were compared to those of the 287 non-breeding eastern imperial eagles identified in the current study. There were no matches between known breeders and presumptive non-breeders; thus, although there was a very small chance that a few individuals were unsampled breeders recruited in 2003 or 2004, for the purposes of this study all 287 individuals were considered pre-adults or floaters. When all 287 eastern imperial eagles were considered, the seven microsatellites used in the current analyses averaged 10

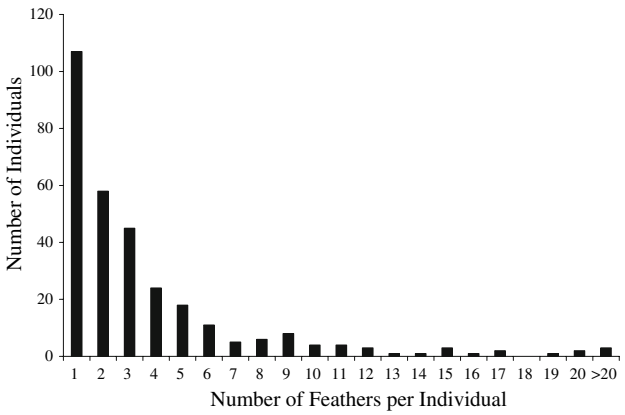

Fig. 1 Frequency distribution for the number of feathers assigned to each of 307 individuals

alleles per locus. The mean observed heterozygosity was 0.71, and no significant single-locus deviations from Hardy-Weinberg expectations were detected. Gametic phase disequilibria were non-significant after performing a sequential Bonferroni correction for multiple comparisons (Rice 1989).

Population size

Our tally of 287 non-breeding eastern imperial eagles identified from the non-invasively collected feathers provided a minimum estimate of abundance. Because a noticeable reduction in the total number of individuals captured was observed during the final sampling occasion (only half as many individuals were captured; Table 2), abundance was estimated from only the first three sampling occasions. A concerted effort was made to collect all putative eastern imperial eagle feathers present each sampling occasion thus, it was unlikely that the reduction in the total number of individuals observed during the final sampling occasion was an artifact of sampling. Eastern imperial eagles generally begin emigrating from the Naurzum Zapovednik near the end of July and, as the final sampling occasion was July 21, the decrease in the number of sampled individuals was likely due to birds leaving the study site. To avoid violating the assumption of population closure, abundance was estimated from only the first three sampling occasions. Program MARK was used to evaluate three closed-capture models that investigated the effect of incorporating temporal and behavioral variation in capture probability on estimated abundance (Table 3). Based on ranked AICc values, the best of these models for estimating abundance incorporated temporal variation in capture probability (Table 3). This model estimated the abundance of non-breeding eastern imperial eagles at the Zapovednik to be 308 ± 8 individuals. Across all three models, estimated capture (p) and recapture (c) probabilities ranged from 0.44 to 0.55 (standard errors ranged from 0.02 to 0.04).

Natal origins

Microsatellite profiles for the 287 non-breeding eastern imperial eagles were compared to those available for 230 chicks hatched at the Zapovednik between 1998 and 2003. Only 11 of the non-breeding individuals (3.8%) were found to match a chick genetically (4 in 2000, 2 in 2001, 4 in 2002, and 1 chick hatched in 2003). All chicks were hatched in different nesting territories (i.e., no siblings were sampled).

F_{IS} for the dataset of pre-adults and floaters was 0.0337, and this was statistically significant ($p = 0.0032$; one-sided test). The STRUCTURE analysis, however, detected no population genetic structure (e.g., a Wahlund effect). In other words, it failed to partition the non-breeding eastern imperial eagles into genetically distinct populations. As a series of K's are evaluated, likelihoods for K are expected to increase and either peak or plateau as K approaches the true K (Evanno et al. 2005 and references therein). When the eastern imperial eagle data were evaluated for K values ranging from 1 through 10, likelihoods for K failed to exhibit this trend (Fig. 2a). Furthermore, ΔK is expected to exhibit a clear maximum when the true K is >1 (Evanno et al. 2005) but, again, this trend was not observed in the eastern imperial eagle data (Fig. 2b). When individual results were considered, all individuals were always assigned to K populations (1–10) with equal probabilities.

Roost usage

Between 11 and 182 eastern imperial eagles visited a given roosting area over all sampling occasions, while between 2 and 84 eastern imperial eagles visited a given roosting area

Table 2 Summary of the number of individuals that visited each roosting area

	Sampling occasions				Individuals
	July 06	July 11	July 16	July 21	
Roost 1	74	55 (29)	70 (29)	34 (12)	144
Roost 2	84	83 (49)	80 (35)	44 (14)	182
Roost 3	33	24 (13)	37 (24)	6 (3)	73
Roost 4	11	2 (0)	0	0	11
Individuals	169	137 (55)	157 (47)	80 (16)	

Across a row, numbers in parentheses are the number of new individuals captured each sampling occasion. "Individuals" refers to the total number of genetically unique individuals non-invasively captured either at a roost (rows) or during a sampling occasion (columns). Because an individual could be sampled at more than one roosting area within a single sampling occasion, the numbers in the "individuals" row are not the sum of their columns

Table 3 Closed-capture models estimated in program MARK

Model description	ΔAICc	AICc Weight	AICc Value	Parameters	Abundance
Temporal variation (M_t)	0.0000	0.7255	−1,440.4760	4	308 ± 8
Null model (M_o)[a]	2.7393	0.1844	−1,437.7367	2	309 ± 8
Behavioral variation (M_b)	4.1722	0.0901	−1,436.3038	3	302 ± 11

[a] No temporal or behavioral variation

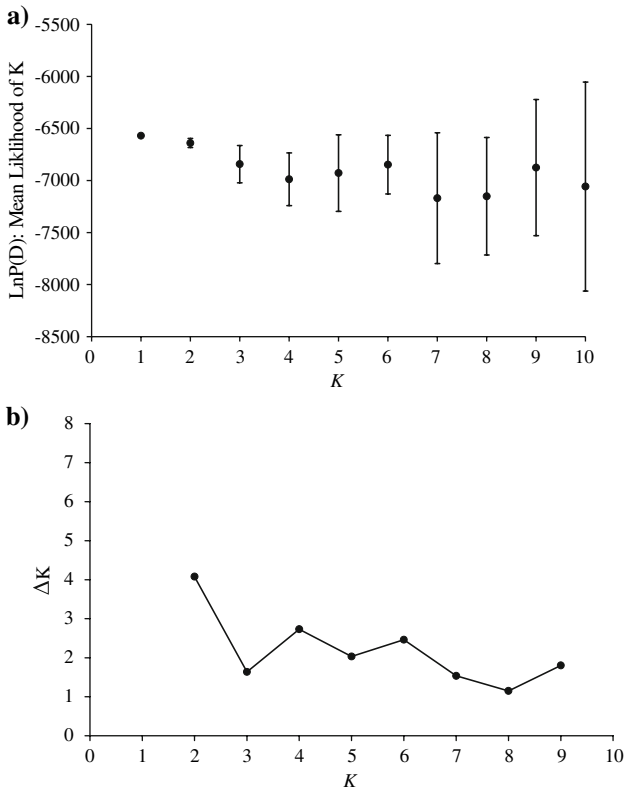

Fig. 2 Results from the STRUCTURE analysis, where *K* values (i.e., number of populations in the dataset) 1 through 10 were investigated. (**a**) Mean likelihood values (±SD) for 10 separate STRUCTURE runs at each value of *K*. (**b**) Δ*K* values for *K* 2 though 9 (Δ*K* cannot be calculated for the first or final values in a series). Neither dataset indicates *K* is >1

sometime during the 5-day period between two sampling occasions (Table 2). Individuals visited an average of 1.4 ± 0.6 roosting areas over the entire study. While we intended to collect only eastern imperial eagle feathers, a small number of white-tailed eagles and golden eagles were genetically identified among the sampled individuals. Incidental collection of white-tailed eagle and golden eagle feathers occurred at all roosting areas, over all sampling occasions.

Discussion

The basic population ecology of most eagles, including eastern imperial eagles, is poorly understood. While population data are available for a small number of species, these data are generally severely limited in scope because traditional techniques for estimating parameters such as abundance, natal philopatry, and dispersal (e.g., wing-tagging and radio or satellite telemetry) often provide information on only a few individuals. Because eagles exhibit delayed sexual maturity, research on eagle ecology is further complicated by the presence of pre-reproductive non-breeders. Limited data suggest that pre-adults often exhibit wide-ranging itinerant behavior prior to their recruitment into a breeding population (Watson 1997; Harmata et al. 1999; Ferrer 2001; Real and Manosa 2001; McGrady et al. 2003). Further evidence also suggests that, following this exploratory period, some individuals ultimately return to breed near their natal territories (Watson 1997). While there have been no published data on the movements of pre-adult eastern imperial eagles, the extended pre-adult phase in their life history suggests they can be expected to exhibit behavior similar to that described for other eagle species.

Field observations initially suggested that several dozen pre-adult eastern imperial eagles congregated at the Naurzum Zapovednik in the early breeding season, but our genetic analyses revealed that at least 287 non-breeders were present at the reserve in July 2004 (a maximum of 21 individuals were simultaneously observed by visual observation in 2004; Table 1). This number (287) might seem high at first glance, but we have demonstrated that methodological errors which artificially inflate population size (e.g., allelic dropout, false alleles; Waits and Leberg 2000) are rare; quality control procedures indicate the genotyping error rate is less than 0.04% per locus (Rudnick et al. 2005). Furthermore, the simple fact that we collected over 1,800 shed feathers in just a few days during the summer of 2004 underscores the number of eagles that utilize the Naurzum Zapovednik (collections in 2005 and 2006 yielded similar number of feathers). The minimum genetic estimate of population size (287) yields a mean of roughly 6.3 feathers collected per eagle—in our view, a very reasonable number—compared to the roughly 86 feathers per eagle that would be required if we were to assume the 2004 population size to be 21 (Table 1). Together, the 1,800 feathers we collected and our previous demonstration of low error rates (coupled with the extensive sample culling whereby suspect genotypes were

discarded) give us confidence that our minimum estimate of 287 non-breeding eastern imperial eagles at the Naurzum Zapovednik in 2004 is quite reasonable. Mark-recapture modeling increased the number of individuals only slightly to ~308 (Table 3). Capture heterogeneity confounds virtually all mark-recapture studies (Link 2003; Lukacs and Burnham 2005) and in theory could have artificially inflated or deflated our estimates of eagle abundance. If so, the inflation bias was minor because the mark-recapture estimates (Table 3) are only ~7% greater than our empirical (genetic) observation of 287 individuals. A deflation bias could be larger, but if so means that our abundance estimates are conservative because we are underestimating the true number of eagles. Regardless of the precise number of eagles sampled (e.g., 287, 302 or 309), the genetic data clearly indicate the census population size is an order of magnitude greater than previously suspected (Table 1).

Beyond dramatically improving the estimate of absolute eastern imperial eagle numbers provided by our molecular data, the non-breeder genotypes were also used to investigate several aspects of eastern imperial eagle ecology. Because numerous eagle species exhibit a nomadic pre-adult phase (see earlier citations), we did not expect all of the non-breeding eastern imperial eagles we sampled to match chicks hatched in the Naurzum Zapovednik. Nevertheless, we were surprised to find that only 11 out of 287 non-breeders matched one of the chicks hatched in the Naurzum Zapovednik over the six previous breeding seasons. Given that the chick samples represented ~90% of nesting territories occupied in the reserve during each year of sample collection (Rudnick et al. 2005), it appears that the majority of non-breeding individuals present in 2004 either had natal origins outside the Naurzum Zapovednik or were older than we suspected.

If a significant proportion of the non-breeding individuals present in 2004 were older than we suspected, they might indeed have been hatched at the Naurzum Zapovednik. However, if non-breeding eastern imperial eagles with natal origins outside the Naurzum Zapovednik are congregating at the reserve, they may be derived from one or a few highly productive breeding populations (like the breeding population present at the Naurzum Zapovednik), or from a large number of less productive breeding populations. Either way, non-breeding eastern imperial eagles could be gathering from a wide geographic area that encompasses multiple, demographic populations that may or may not be genetically distinct. Microsatellite data have indicated that a closely related species, the Spanish imperial eagle (*Aquila adalberti*), exhibits low levels of genetic differentiation among populations (Martinez-Cruz et al. 2004), but this result may not be indicative of trends expected in other eagle species because the Spanish imperial eagle is critically endangered and known to occupy fragmented habitat (Collar and Andrews 1988; Gonzalez et al. 1989a). Mitochondrial data suggest that white-bellied sea-eagles (*Haliaeetus leucogaster*) and white-tailed eagles are genetically homogenous across vast geographic distances (Shephard et al. 2005; Hailer et al. 2007), and this paucity of genetic structure is probably more typical of eagle populations. The statistically significant F_{IS} value observed for the pre-adults and floaters suggests these individuals are gathering at the Naurzum Zapovednik from multiple, genetically distinct breeding populations (F_{IS} for the Naurzum Zapovednik's breeding adults was non-significant; data not shown). While significant, the F_{IS} value is quite small and the STRUCTURE analysis found no support for alternatives to the null hypothesis that all the Naurzum Zapovednik's floaters belong to a single genetic population. Still, these results do not preclude the possibility that individuals are congregating from across the species' range; if gene flow is high among breeding populations, genetic homogenization is likely and assignment methods generally perform poorly under these conditions (Evanno et al. 2005; Latch et al. 2006). Genetic data from additional eastern imperial eagle populations are necessary to further elucidate the natal origins of the non-breeding eastern imperial eagles congregating at the Naurzum Zapovednik.

Between 1998 and 2003, the breeding population of eastern imperial eagles at the Naurzum Zapovednik produced ~38 chicks per year. If only 3.8% (11 individuals) of the non-breeding eastern imperial eagles present at the Naurzum Zapovednik actually hatched there, where are the rest of the chicks that were produced during this time period? Pre-adult eastern imperial eagles could be experiencing high mortality rates, but this explanation seems unlikely given the large number of non-breeding eastern imperial eagles that are present at the Naurzum Zapovednik. Furthermore, it is difficult to evaluate because estimates of pre-adult survival are not available and yearly pre-adult survival estimates for other eagle species are varied, ranging anywhere from 0.19 ± 0.07 (golden eagle; McIntyre et al. 2006) to 0.81 ± 0.05 (bald eagles (*Haliaeetus leucocephalus*); Millsap et al. 2004). An alternate explanation is that chicks hatched at the Naurzum Zapovednik are dispersing (either temporarily or permanently) to other parts of the species' range. If true, this would suggest that there may be other, possibly undiscovered locations that are supporting significant numbers of both non-breeding and breeding eastern imperial eagles.

Eagles are vagile organisms and non-breeders in particular are not tied to any particular geographic site. Thus, a reasonable explanation for the large number of non-breeding eastern imperial eagles present at the Naurzum Zapovednik is the quality of the habitat. The Naurzum

Zapovednik has long been recognized as a critical resource that provides habitat for many taxa. The protected lands of the reserve accommodate an extraordinary collection of raptor species, as well as one of the densest populations of eastern imperial eagles in the world (Bragin 1989). Why might such a large number of non-breeding eastern imperial eagles also be gathering at the Naurzum Zapovednik? Like the pre-adult eastern imperial eagles at the Naurzum Zapovednik, pre-adult Bonelli's eagles (*Hieraaetus fasciatus*) are found to gather in areas devoid of adult nesting territories (Manosa et al. 1998 and references therein). Manosa et al. (1998) concluded that pre-adult Bonelli's eagles were selecting temporary settlement areas based on prey abundance, which has also been suggested to influence the selection of temporary dispersal areas by pre-adult Spanish imperial eagles (Gonzalez et al. 1989b) and bald eagles (Harmata et al. 1999). The Naurzum Zapovednik offers a wide variety of abundant prey, including waterfowl (Anatidae), hares (*Lepus* spp.), and rodents (Katzner et al. 2006b). In particular, steppe marmots (*Marmota bobac*) and ground squirrels (*Spermophilus* spp.) form large colonies outside adult breeding territories, and pre-adults may be congregating at the Naurzum Zapovednik to take advantage of prime roosting habitat coupled with plentiful food resources.

Conclusions

Research on eagle movements and behavior has historically been conducted through wing-tagging and telemetry. The same is also true for most other raptor species. As an alternative to these techniques, we used non-invasively collected feathers to genetically identify and mark non-breeding eastern imperial eagles. Ultimately, these genetic tags allowed us to characterize eastern imperial eagle roost usage and estimate eastern imperial eagle abundance at a national nature reserve in Kazakhstan. Furthermore, genetic tags of non-breeding eastern imperial eagles were compared to an existing database of chick genotypes to investigate natal origins and identify the proportion of individuals that hatched at our study site. The techniques we utilized do not replace wing-tagging and telemetry data, but provide data that are both comparable and complementary. While naturally shed feathers have long been identified as a suitable source of DNA (Morin et al. 1994; Taberlet and Bouvet 1991; Pearce et al. 1997), few genetic studies have used wide-scale collections (>1,000 samples) to investigate fundamental aspects of avian biology. Our efforts in this regard have revealed several unexpected aspects of eagle biology, most notably the large number of non-breeders sampled from our study site and the small number of non-breeders that actually hatched there. Collectively, these results suggest that the Naurzum Zapovednik serves as critical summer habitat for non-breeding eastern imperial eagles and that future research on the fate and distribution of hatchlings is needed to provide a comprehensive characterization of natal philopatry.

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