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RESEARCH ARTICLE

Recruitment Rates, Natal and Breeding Dispersal of Montagu's Harriers (*Circus Pygargus)* by Means of Microsatellite Analysis

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Abstract:

Introduction:

Adult philopatry as well as juvenile dispersal and recruitment rates are key factors for population development. We investigated these questions for the first time in an increasing German population of Montagu's harrier in Frankonia using microsatellite markers.

Methods:

By means of 16 loci, we genotyped 2265 samples from juvenile and adult female Montagu's harriers. Parentage and identity tests were used to reconstruct life histories of birds for a 10 year period. Most of the birds were breeding in one or two years. The longest life history was eight years.

Results:

Adult philopatry was quite high and differed significantly between sexes. We found 73.5% of females to breed < 5 km around the previous nest site (80.4% < 10 km, median nesting distance 2.1 km). All investigated males (n=18) were breeding in a distance of < 5 km (median nesting distance 1.3 km) to the previous nest. Juveniles showed a low recruitment rate (females: 2.9%, males: 4.9%, together 4%). Median natal dispersal distance was 19.1 km for females and 12.3 km for males. We found 29.4% of females and 41.2% of males to be philopatric, as the distance between hatching and first breeding site was < 10 km. Philopatry results mostly agree with data from other European countries.

Discussion:

Due to strict marker and data selection we received high quality life histories of Montagu's harriers, which demonstrate that microsatellite analyses are valuable tools in ornithology.

Conclusion:

Nevertheless, comparison of philopatry and recruitment rates depend directly on the scale used and investigation method and therefore remain a challenge.

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1. INTRODUCTION

Montagu's harrier (*Circus pygargus*, Linnaeus, 1758) is one of the most flexible and adaptive migrating raptors in our world and hence in focus of scientific interest. It breeds in Europe and western Asia, but winters six to eight months in semi-arid open habitats of West, East and South Africa, south of the Saharan desert as well as on the Indian sub-continent [1, 2]. The species is a traditional wetland breeder and used to build its nest in steppe-like grasslands [3], salt meadows [4] and heathlands [1]. Since the beginning of the 20th century, breeding in grain fields was observed [5], which has become the most preferred vegetation type in Europe today [6]. In Europe, the species breeds patchily distributed and is regionally altogether absent [3]. BirdLife International [7] classifies Montagu's harrier population as "least concern". Nevertheless, many European populations suffer from extensive agriculture, which includes nest destruction, loss of prey, over-use of pesticides as well as improved locust control in wintering areas [8]. In Europe, the survival of the ground nesting Montagu's harrier strongly depends on human conservation management, especially nest protection.

Conservation of a migrating species is a challenge, since research in breeding, wintering and migration areas need to be combined. Montagu's harrier has been studied by several research teams over the last decades *e.g*. [6, 9 - 22]. Research mainly focused on ecology and breeding biology. Especially migration routes have been extensively investigated [23 - 28]. To study population dynamics, which is important for effective conservation management, many different aspects like spatial distribution, genetic structure and connectivity between breeding areas need to be considered. Key ecological questions include natal and breeding dispersal and philopatry as well as juvenile recruitment rates. The recruitment rate of a population describes the proportion of juvenile birds that return to their breeding area for breeding. Natal dispersal is defined as the movement of juveniles from the place of birth to the place of their first reproduction attempt. Breeding dispersal (or adult philopatry) concerns the movement of adults that had reproduced in one year to a breeding site in the following years [29, 30]. Juvenile recruitment, as well as natal and breeding dispersal can influence the genetic structure of populations substantially, since a more or less intensive geographic exchange of individuals lead to connection or isolation of neighbouring groups. Dispersal prevents inbreeding by gene flow, plays a key role in range expansion of metapopulations and influences source-sink dynamics in patchily distributed populations [30, 31]. Both, natal and breeding dispersal differs between species in its specification, because it is influenced by habitat requirements, social system, geographical range and migratory status of the discussed species [31]. Consequently, knowledge about dispersal and philopatry behaviour is of paramount importance to evaluate conservation status and management strategies of declining or threatened species.

Adult philopatry and natal dispersal data are still missing for many species, including the Montagu's harrier. Long-term and large-scale observations are needed, which traditionally require mark and recapture methods (ringing, wing-tagging, radio and satellite telemetry). In recent times, the analysis of genetic markers has been established as powerful tools for scientific research including conservation issues [32 - 36].

In this study, we provide evidence from microsatellite analysis for dispersal, philopatry and recruitment in an expanding population of Montagu's harriers in Mainfranken, Germany. Genetic analyses were conducted by means of 16 recently isolated highly informative microsatellite loci (STRs - short-tandem-repeats) (Janowski *et al*., 2014). Paternity and identity analyses were used to reveal life histories for individual birds.

2. MATERIALS AND METHODS

2.1. The Breeding Population in Mainfranken, Germany

Montagu's harrier is a regionally threatened bird species in Germany and therefore included in the Red List of Threatened Species to category 2 [37]. In a long-term trend, the German breeding population is apparently growing, primarily due to effective nest protection regimes [38, 39]. Illner 2017 [40] summarises that the number of breeding females increased by 15% from an average of 400 in 2004-2007 to an average of 485 in 2011-2014 (estimates of undiscovered broods are included).

Among German populations of Montagu's harrier, the breeding population in Mainfranken (Bavarian administrative district Unterfranken) is the largest and most successful one with 143 breeding pairs and 254 fledged chicks in 2016 [41]. The breeding population is patchily distributed in a radius of about 40 km around the village Volkach 49°52′N,

10°14′E between the centres Würzburg 49°46′N, 09°56′E in the south and Schweinfurt 50°03′N, 10°14′E in the north. The landscape is characterized by intensively used agricultural habitats, with open and large grain fields. The exact locations of individual breeding sites vary in consecutive years. Since 1999, a conservation program for Montagu's harriers has been organized by the Bavarian Environment Agency, the Bavarian Association for Bird Protection, the Bavarian State Ministry for Environment and Health and many volunteering bird conservationists. This conservation program is apparently successful as breeding pair numbers are increasing. Montagu's harrier is now listed in the Red List of Threatened Species of Bavaria to category "extreme rare species and species with geographical restriction" [42].

Montagu's harriers mainly breed on the ground in fields used to grow cereals. Similar to the situation in other European countries, nest protection can be ensured by leaving an undisturbed area of 50 by 50 m around a nest (*i.e.* no mowing activity) [43 - 47]. Additionally to conservation, a research program was initiated in 2000. Since then, all nests were searched, chicks ringed and marked with wing-tags for identification in future years. Furthermore, blood samples from chicks were collected during the ringing process for genetic analyses and GPS data were recorded of each nest.

2.2. Sample Collection

When visiting harrier nests for ringing between 2000 and 2012, we also took blood samples from chicks by puncturing their brachial vein on one of the two wings. Samples were stored in EDTA buffer {10% EDTA, 0.5% NaF, 0.5% thymol, 1%, Tris-HCL, pH = 7.5 [48]} at 4°C until DNA extraction. Overall, 2068 blood samples were obtained.

For paternity analyses, we furthermore collected blood samples from incubating adult females between May and end of July in the years 2009 to 2012. To minimize disturbance of incubating birds, we did not catch them but obtained blood samples *via* the "bug method": Dummy eggs were picked with triatomine bugs to collect blood from an incubating female. Larvae of *Dipetalogaster maxima* Uhler, 1894 (Heteroptera, Reduviidae) were obtained commercially from G. Schaub (Ruhr University Bochum, Germany). A single third instar larva was placed in an artificial harrier egg (made by R. Nagel, Institute of Avian Research (Vogelwarte Helgoland) Wilhelmshaven, Germany) and put for at least 4 h in a harrier clutch Incubating females quickly return for incubation. As eggs come into close contact with the skin, the bugs can target blood vessels of the female through small holes in the egg. This non-invasive method is widely used to sample blood of sensitive, wild or captured animals [49 - 55]. For more information about this method see description in Janowski *et al.* 2014 [56]. Blood was removed from the bug with a small syringe and stored in an EDTA buffer and cooled to 4°C for later use. DNA remained intact and was not contaminated by insect DNA in this procedure. Altogether 197 blood samples from adult females were collected in that way.

2.3. Sample Processing and Genotyping

DNA was isolated from blood following a standard protocol with proteinase K digestion (Merck, Darmstadt) and phenol-chloroform extraction [57] for genotyping.

Altogether, 2265 samples from chicks and adult females were genotyped with 19 STR primer pairs that were recently isolated for Montagu's harrier using next-generation sequencing [56]. These 19 microsatellite loci were amplified with three multiplex PCR sets for high throughput genotyping by capillary array electrophoresis using the MegaBACE 1000 system of Amersham Biosciences (detailed description in Janowski *et al.*, 2014) [56]. In the process of primer development and multiplex arrangement, 10 samples of the same juveniles were genotyped several times (see primer development in Janowski *et al.*, 2014) [56]. Furthermore, 192 samples from adult females were genotyped two times. This repetition was carried out as a quality check for amplification consistency in multiplex PCR and reproducibility. Rounding of decimal values (manual binning of peaks to allele-units) was performed as described in Janowski *et al.*, 2014 [56].

All juvenile Montagu's harriers were genetically sexed using the primers 1237L (sequence 5'-3': GAGAAACTGTGCAAAACAG) and 1272H (sequence 5'-3': TCCAGAATATCTTCTGCTCC) to amplify an intron region in the CHD-gene [58]. Males show one and females two sex-specific alleles. PCR was conducted with radioactively labelled nucleotides (^{33}P-α-dATP) under the following conditions: A 25 µL reaction volume contained 60 ng of isolated DNA, 10 pmol/µL of each forward and reverse primer, 2.5 µL of a 10x PCR buffer (Bioron), a nucleotide mix containing 0.1 mM of dGTP, dCTP and dTTP, as well as 45 µM of dATP, 0.15 units of Top-Taq DNA polymerase (Bioron, Ludwigshafen), 1 µCi [α-^{33}P]-dATP (Perkin Elmer) and a variable amount of mono distilled water to reach the end volume of 25 µL. Thermocycling was performed in a Tgradient ThermoCycler (Biometra, Göttingen) under the following conditions: Initial denaturing step for 2 min at 94°C, 38 cycles of 30 sec at 94°C, 60 sec at 56°C and 2 min at 72°C, followed by a final extension step for 10 min at 72°C and a pause step at 16°C for storage. After denaturation of

PCR products at 95 °C for 5 min, they were separated by vertical high-resolution Polyacrylamide Gel Electrophoresis (PAGE) (containing 5% urea) at 65 W for 1.5 h (run length approximately 40 cm). The gel was dried and analysed by autoradiography using an X-ray film (Fujifilm Super RX).

2.4. Data Selection, Identity Analyses and Parentage Assessments

In order to evaluate the reliability of paternity and identity tests, all 19 loci had to be characterized using the software Cervus 3.0 [59]. We randomly chose 444 samples from juvenile Montagu's harriers for characterization tests. Only loci with best assessed values were selected for paternity and identity analyses. Furthermore, a most conservative approach of strict data selection was applied to allow for accurate identification of individuals: All identity and parentage analyses were performed only with fully genotyped samples. Moreover, only families consisting of at least three nest mates (number of eggs per nest in Mainfranken is between two and seven, mostly four to six) were included in parentage tests. These criteria should ascertain correct assignment results. Altogether 166 adult females and 1290 juvenile Montagu's harriers remained for paternity and identity tests after samples were removed which did not fulfil our quality standards.

Cervus 3.0 [59] was used to identify identical adult females that could have been sampled repeatedly 2009-2012 by chance (*via* the bug method). We found 126 adult females for which we had matching genotypes sampled in different years. Furthermore, genotypes of adult females were compared with all female chicks to find individuals which had been sampled previously as chicks. Consequently, discovered hatching years could be used for life history assessment and philopatry analyses. Only 100% identical genotypes were treated as identical.

Parentage analyses were conducted by software Colony 2.0 [60]. The remaining 1290 samples from chicks comprised 585 females, 691 males and 14 individuals without successful genetic sexing. Our 126 adult females and all juvenile females represented potential mothers in parentage assignments. Since lack of samples from adult males, juvenile males were treated as potential fathers. The following adjustments were considered for a Colony run: We assumed a polygamous mating system without inbreeding, because samples had been collected successively over years. Hence, parents could have produced chicks in different breeding seasons. We chose a medium run length, a full-likelihood method and no sibship prior. We neither gave information about known paternal or maternal sibs, nor excluded paternity, maternity and paternal or maternal sibs, respectively. Consequently, parentage was assigned only by genotyping data. Results for parentage assignment were taken from the "Best Configuration" output-file. An important advantage of Colony programme is the possibility of assigning parentage to sibs, without a corresponding parental genotype. If Colony determines that two or more siblings share the same parent, but a corresponding genotype is missing in the parent data file, the reconstructed genotype of mother and/or father appears with synonym. An allocated mother is presented with a '#' followed by a serial number and a father with a '*' and a serial number, respectively. Thus, it is possible to identify full and half sibs. To check parentage results for assignment mistakes, we compared them with expected full sibs, according to nest number and hatching year.

After paternity tests, we continued with strict data selection. Only clutches, where calculated relationships matched expected ones (checking for ring numbers) and where at least one parent was unambiguously identified were used for further analyses. Furthermore, families were controlled for allele-mismatches between chicks and assigned parents. Allele-mismatches which were not related to interpretation mistakes of peaks or transcription errors led to exclusion of the corresponding sample. Comparison of known mother-chick relationships (where adult females were sampled *via* the bug-method) revealed no assignment mistakes. This very conservative way of data selection reduced the number of samples to 1276 chicks and 123 adult females.

2.5. Analyses and Statistics

GPS data from nest locations and life histories of individual birds were used to assess philopatry and dispersal. Distances between nests were directly calculated by GPS data in Excel. Life histories were reconstructed for single birds by a '01-matrix', where parentage proofs appeared with '1' (else '0'). Of course, life histories include times with frequent breeding observations and times where one or more years were without breeding evidence. Since we lack information about 'missing years' (brood damage, intermission, breeding outside sampling area or detection failure of broods), adult philopatry was only calculated for birds breeding in consecutive years. An F-test was calculated to test for unequal variance in nesting distances within the sample group, due to unequal sample size of both sexes. Comparison of adult philopatry (nesting distances) between both sexes was hence calculated with a two sided t-test with unequal variances.

Mean age of first breeding was compared between males and females by first testing for unequal variances (F-test) followed by a two sided t-test with unequal variances. We only took broods of the Mainfranken population into account. Birds that performed their first brood outside the investigation area or lost their first brood before sample collection, may distort the values. Therefore, we always refer to the 'first recognized breeding attempt' instead of the true first one.

Concerning juvenile dispersal, we calculated distances between the sampled or assigned hatching site and the sampled or assigned nest site of first recognized breeding. Natal dispersal was compared between sexes by first testing for unequal variances (F-test) followed by a two sided t-test with unequal variances.

By referring to Liminana *et al.,* 2012 [61], we called juvenile birds to be philopatric, when they were found as breeders < 10 km away from their hatching site. In order to investigate sex specific philopatry rates of returners, percentages of birds, returned to the 10 km area, were compared *via* χ^2-homogenity-test. We also performed a χ^2-homogenity-test to compare philopatry in the 10 km area for all analysed 1276 chicks, no matter if they were seen again in Mainfranken or not. Juvenile recruitment was calculated as the number of chicks that were sampled in Mainfranken and returned for breeding.

3. RESULTS

3.1. STR Locus Characteristics

From the initial 19 loci used for genotyping, three had to be excluded from STR analyses: Decimal alleles at locus MS_Cpyg19 could not be rounded to full allele units, MS_Cpyg39 showed indication of null alleles and locus IEAAAG15 [62] showed amplification problems during the genotyping process. Consequently, parentage and identity tests were based on 16 loci. Table **1** summarizes parentage and identity characteristics for the whole marker set, while Table **2** gives detailed characterization results for each individual locus.

Table 1. Parentage and identity statistics across 16 loci used for STR analyses of Montagu's harriers.
N_{Aall}: mean number of alleles across all loci; PIC*all*: polymorphism information content across all loci; NE-1P and NE-2P: combined non-exclusion probability for two possible parents when the genotype of the correct parent is unknown (1P) or known (2P). NE-PP: combined non-exclusion probability for parent pairs. NE-I and NE-SI: probability of mistaken identity between two randomly-chosen individuals (I) or full-sibs (SI).

Parameter	Value/Probability
N_{Aall}	9.8
PIC_{all}	0.7
NE-1P	$5.9*10^{-4}$
NE-2P	$3.8*10^{-6}$
NE-PP	$5.5*10^{-10}$
NE-I	$1.7*10^{-16}$
NE-SI	$1.2*10^{-6}$

Table 2. Characterization of the 16 STR loci used for genotyping Montagu's harriers.
N_A: number of alleles per locus; H_{obs}: observed heterozygosity; H_{exp}: Expected heterozygosity; PIC: Polymorphism information content; origin of STR loci: see Janowski *et al.* (2014); [56] [63]; [64].

Locus	Allele Range (bp)	N_A	H_{obs}	H_{exp}	PIC
MS_Cpyg01	305–321	9	0.8	0.8	0.7
MS_Cpyg04	220–300	16	0.9	0.8	0.8
MS_Cpyg05	127–202	14	0.9	0.9	0.9
MS_Cpyg06	376–446	14	0.8	0.9	0.9
MS_Cpyg07	310–354	12	0.7	0.7	0.7
MS_Cpyg16	147–153	4	0.5	0.5	0.4
MS_Cpyg23	287–303	8	0.7	0.7	0.6
MS_Cpyg25	162–184	12	0.8	0.8	0.8
MS_Cpyg26	239–255	8	0.6	0.6	0.6
MS_Cpyg29	350–395	10	0.8	0.8	0.8
MS_Cpyg30	282–322	11	0.9	0.9	0.9

(Table 2) contd.....

Locus	Allele Range (bp)	N_A	H_{obs}	H_{exp}	PIC
MS_Cpyg31	147–175	8	0.6	0.6	0.6
MS_Cpyg33	137–152	6	0.5	0.5	0.5
MS_Cpyg42	314–346	8	0.5	0.6	0.5
Age5[1]	158–185	10	0.8	0.8	0.7
Hvo-02[2]	148–160	7	0.3	0.3	0.2

According to the results, our marker set is proven as highly informative. High information content is revealed by PIC values > 0.5 for 14 out of 16 loci and a PIC value of 0.7 for combined loci. Deviation of Hardy-Weinberg equilibrium could not be detected for any locus. There was no evidence for apparent null alleles in any of these loci, since frequency was always less than 0.05 [59].

3.2. Life Histories

Reconstruction of individual life histories provides the basis for dispersal and philopatry estimations. Life histories for 123 adult females (sampled *via* the bug method), and 40 for males (parentage assessment) were reconstructed. Most of the females were identified in a single year only (Table **3**), and only a few females could be identified in up to eight years. Likewise, most of the males were detected in a single year only (altogether ranging between one and five years).

Table 3. Individual detection frequencies of females and males in Montagu's harriers between 2002 and 2012, Analysis includes 123 adult females (100%) and 40 males (100%).

Detection in population [minimum number of years]	Frequency (% of total)	
	Females	Males
1	54 (43.9)	23 (57.5)
2	33 (26.8)	10 (25)
3	17 (13.8)	5 (12.5)
4	6 (4.9)	1 (2.5)
5	6 (4.9)	1 (2.5)
6	4 (3.3)	0
7	2 (1.6)	0
8	1 (0.8)	0
Mean Detection Rate	2	1.6
Median Detection Rate	2	1

3.3. Adult Philopatry

For 102 female and 18 male harriers we could determine distances between their nest sites in two consecutive years. Of them, 80.4% of females could be considered as philopatric, since nesting sites were located in a distance of < 10 km to the one of the previous year (73.5% < 5 km, 37.3% < 1 km) (Fig. **1**). The smallest distance was 80 m and the longest 44.7 km within the investigation area (Table **4**). All analysed males were apparently breeding in a radius of < 5 km to the nest site of the previous year (44.4% < 1 km) (Fig. **1**). Hence, all of males could be classified as philopatric. Distances ranged between 170 m and 4.4 km (Table **4**). Nesting distances differed significantly between sexes (F-test: $p < 0.01$, t-test: $p < 0.01$). Adult males are thus more philopatric than adult females.

Table 4. Nesting distances of female and male Montagu's harriers.

-	Female	Male
Investigation Period	2002–2012	2002–2012
Mean Nesting Distance	6.4 km	1.7 km
Median Nesting Distance	2.1	1.3
SD	9.8	1.4
Number of comparisons	102	18
Distances ≤ 10 km	80.4%	100%
Distances ≤ 5 km	73.5%	100%
Distances ≤ 1 km	37.3%	44.4%
Distance Range	80 m–44.7 km	170 m–4.4 km

SD: standard deviation; number of comparisons: number of calculated distances

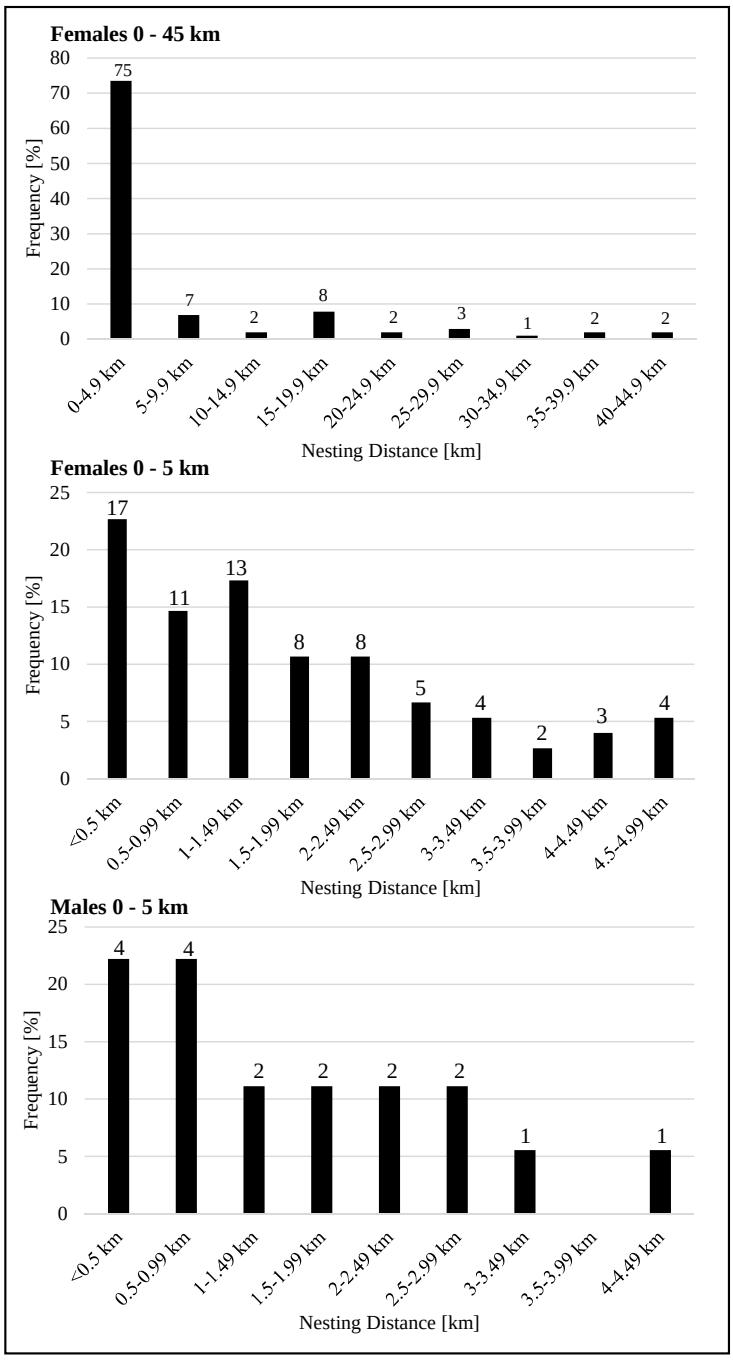

Fig. (1). Adult philopatry of female and male Montagu's harriers.

Nesting distances between two consecutive years are given in intervals of 5 km for both sexes (each interval counted without the upper value). Additionally, the interval 0-5 km is detailed for both sexes. Numbers on top of each bar represent numbers of individuals.

There is a slight but not significant trend visible, concerning detection frequency and mean nesting distances (Fig. **2**). Mean nesting distances were lowest for a single female that was recorded in eight breeding seasons. Females that were detected in seven years (2 individuals) and four years (6 individuals) also settled close to the previous nesting site. Females that could only be detected in two years (19 individuals) were breeding further away from the previous site.

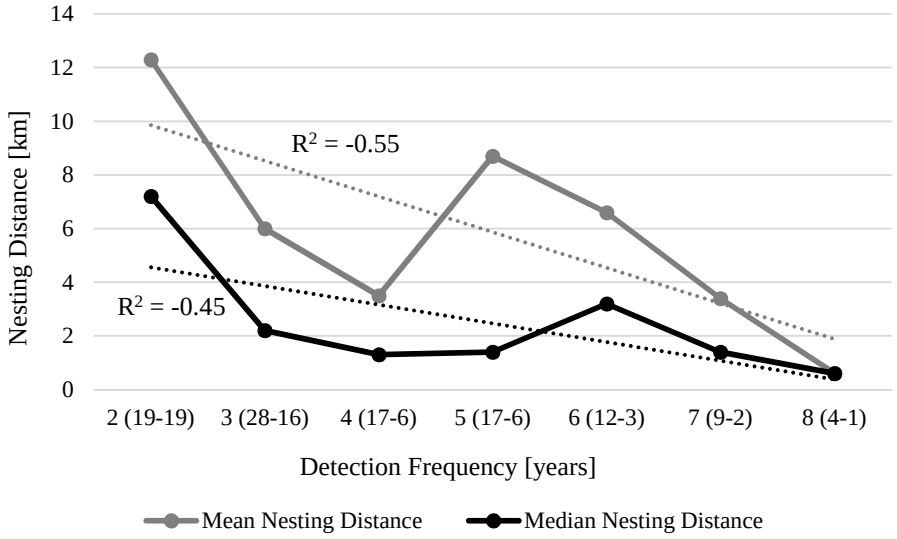

Fig. (2). Nesting Distances of females in two consecutive years depending on detection frequency.

Mean and median nesting distances are given for females which were observed breeding two to eight times. X-axis show detection number with number of distances counted and number of individuals in brackets. R^2 for trend curve (dotted lines) is shown.

Fig. (**3**) shows mean and median nesting distances for females depending on how often they bred consecutively. It need to be considered, that some individuals are counted more than once in the calculation, since their life histories showed for example times where a single individual bred two years in a row (one distance) followed by a pause year and a three year breeding sequence (another two distances) afterwards. Distances spanned over pause years are not included. There is no trend visible that show a relationship between breeding sequence length and nesting distance.

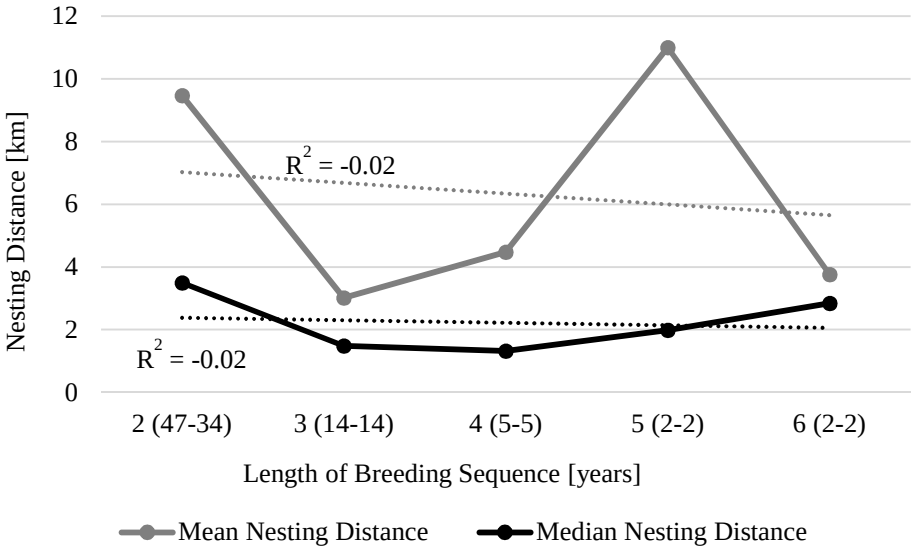

Fig. (3). 3: Nesting Distances of females depending on breeding sequence length.

Mean and median nesting distances are given for females which were observed breeding in two to six years consecutively. X-axis show length of breeding sequence with number of distances counted and number of individuals included in brackets. R_2 for trend curve (dotted lines) is shown.

3.4. Age of First Breeding, Natal Dispersal and Recruitment Rate of Juveniles

Reconstructed life histories revealed hatching year and several breeding attempts for 17 females (11 have been sampled as adults *via* the bug method) and 34 males.

Time between hatching and first recognized breeding attempt ranged between one year and five years for females and between one year and seven years for males. Especially the very long time spans might be incorrect due to incomplete sampling or absence of the respective birds in those years. However, we found a mean age of first breeding of about two years for females and three years for males (differences not significant; F-test: $p = 0.44$, t-test: $p = 0.10$). Moreover, we identified three females and one male which started breeding as one year old birds. (Fig. **4**) illustrates the natal dispersal distances for females and males. Table **5** compares age and distances between hatching and first recognized breeding for both sexes.

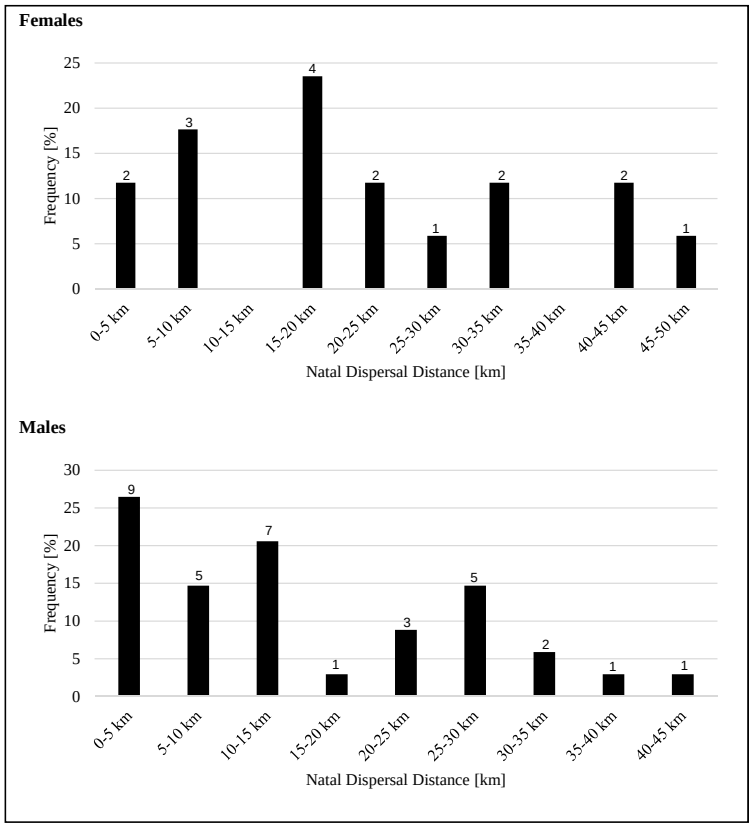

Fig. (4). Natal dispersal distances for female and male Montagu's harriers.

Distances between hatching site and place of first recorded breeding attempt is given in intervals of 5 km (each interval counted without the upper value). Numbers on top of each bar represent absolute numbers for each distance.

Table 5. Age of first breeding and natal dispersal distances of Montagu's harriers

–	Females		Males	
–	**Age**	**Distance**	**Age**	**Distance**
Mean	2 years	21.6 km	3 years	15.2 km
Median	2 years	19.1 km	3 years	12.3 km
Range	1-5 years	1.9-46.8 km	1-7 years	0.4-44.1 km
SD	1	13.7	1.2	11.8

SD: standard deviation of the mean.

Distances between hatching site and place of first recorded breeding attempt is given in intervals of 5 km (each interval counted without the upper value). Numbers on top of each bar represent absolute numbers for each distance.

Distances between hatching site and first breeding attempt did not differ between sexes (F-test: $p = 0.46$, t-test: $p = 0.09$) (Table **5**). We found 29.4% of returned females (5 individuals) and 41.2% of returned males (14 individuals) to be philopatric in our definition (< 10 km). There was no significant difference between the amount of males and females that bred in the 10 km area: $\chi^2=3.03$, $df = 1$, $p = 0.08$. Concerning all analysed 1276 chicks, philopatry rates (percentage of chicks that were detected breeding < 10 km apart from their hatching site) between females (0.8%) and males (0.6%) were even smaller and not significantly different: $\chi^2 = 0.03$, $df = 1$, $p = 0.86$. Furthermore, calculated minimal recruitment rate of juveniles amounted 2.9% (17 individuals) for females and 4.9% for males (34 individuals). Hence, minimal overall recruitment rate of chicks that had hatched in Mainfranken and had been analysed (1276 individuals) was 4%.

4. DISCUSSION AND CONCLUSION

4.1. Reconstruction of Life Histories with Microsatellite Markers

Traditionally, the reconstruction of life histories of birds relies on recoveries from ringed or sightings from otherwise marked birds. These methods can work quite well in species which are easy to find and trap. For many other birds it might be difficult to obtain a sufficiently large sample size which allows the calculation of life history variables.

Genetic studies in ornithology are still quite new compared to traditional methods [99]. In order to be useful, we need methods of high resolution which allow the identification of individuals. Starting with multilocus DNA fingerprinting with labelled probes in the 1980s, the development of microsatellite markers is presently the method of choice for the identification of individuals and for parentage studies [35, 65 - 68]. Also SNP markers can be useful in this context [99]. Although microsatellite analyses are widely used today, they are not without inherent problems and much care is needed to correctly establish the genotype of an individual. Therefore, we decided to carry out a most conservative approach of strict data selection and controlling steps. This included characterization and selection of the most informative STR markers, exclusion of non-fully genotyped samples or of families which were too small for parentage analyses.

According to the achieved characterization results, our marker set is of high quality and thus suitable for parentage and identity tests. Neither deviation of Hardy-Weinberg equilibrium, nor evidence for apparent null alleles could be detected for any of the used loci. Accurate identification of individuals and correct assignment results were our highest aim. This led to quite a high reduction of the original sample size. However, our life history of Montagu's harriers are of high quality and demonstrate that microsatellite analyses are valuable tools in ornithology. As we were able to obtain blood samples from incubating female harriers *via* the bug method, the STR analysis was much easier and more straightforward for females than for males, whose genotypes could only be reconstructed indirectly *via* parentage assignment.

4.2. Adult Philopatry

Dispersal depends on age and sex. In most species, juveniles disperse more than adults which have established a breeding territory. Both, female biased natal and breeding dispersal has been reported for many bird species [30]. Factors influencing such a sex-biased dispersal could be resource competition (between age classes and sexes), intrasexual competition for mates and inbreeding avoidance [30, 69, 70]. Moreover, female-biased dispersal is seen as a result of a monogamous mating system. Most of avian raptors are monogamous {only one breeding partner per season or even the same partner for many years [71 - 73]}, which should favour philopatric males. These males gain advantages when staying in or returning to their natal area to acquire or defend a territory and resources to attract females. Philopatric males could benefit from familiarity with competitors and predators, territorial circumstances (breeding and hunting habitat) and resource availability [30, 74]. Also in raptors, females disperse more than males [75 - 77], but exceptions towards males or sex-consistent dispersal behaviour also exist [78]. Most raptors return to their breeding sites of the previous year and hence are quite philopatric.

Since male Montagu's harriers provide food for their females during breeding and for their offspring during rearing and post-fledging period [6, 13], being philopatric might also improve their ability of gaining the required resources. Moreover, the species is a semi-colonial breeder. Colonies can be maintained over years and may provide advantages regarding predator defence [9, 79].

In our study, adult males were more philopatric than adult females and nesting site distances differed significantly

between both sexes. This result might be somehow biased by the large difference of sample sizes between males and females.

There was a slight but not significant trend visible, concerning detection frequency and mean nesting distances for females. Females that breed more than two years in Mainfranken tend to breed closer to the nest in the previous year. Although we don't have knowledge about the age for most of the birds, the group of two-year breeders might be characterized by young and inexperienced females, which are still searching for a (new) breeding territory. Moreover, it is likely that young birds cannot compete with older ones for good breeding territories due to their deficiency in habitat familiarity. Older birds are more experienced in resource acquisition and predator defence, since they already bred for several years in the area. On the other hand, nesting distance between two consecutive years does not depend on length of breeding sequence. Females that bred in three consecutive years were not found to breed closer to their previous nest than those ones that bred in five or six years successively. This finding reflects the species flexibility and mobility to react on ecological and environmental changes. Montagu's harriers depend on vole abundance in the breeding area. Voles are their main prey during breeding season in Germany [80, 81], as in other European regions [82 - 87]. Vole abundance fluctuates from year to year and can vary between different localities. Even during breeding season change of vole abundance is possible, so that birds can change their breeding site in reaction to that (after nest predation). Agricultural environment also influences adult philopatry. Rotating crop cultivation directly navigate adequate breeding sites. Nevertheless, our results agree with the few data in the literature: Breeding adults seem to be relatively philopatric to their previous breeding site M. Salamolard, A. Butet, A. Leroux, V. Bretagnolle *unpublished data* in [20]. For hen harriers *Circus cyaneus* similar data have been published [88].

4.3. Philopatry and Recruitment Rate of Juveniles

4.3.1. Age of First Breeding

For the first time, we could provide evidence for the age of mean first breeding of Montagu's harriers in Mainfranken. Age of recognized first breeding is two years for females and three years for males. This finding is in agreement with data from Spain (female: two years, male: three years) and France (female: Three years, male: four years) [89]. Moreover, first-year breeders (three females and one male in Mainfranken) are also reported in Spain for males [90]) and probably also for females [20].

4.3.2. Recruitment Rate

In agreement with published data, the overall recruitment rates of juvenile Montagu's harriers in Mainfranken were quite low: Only 2.9% of females and 4.9% of males, which had been sampled as chicks, were found to breed in Mainfranken in later years (all together only 4% of 1,276 sampled individuals). This value must be regarded as a minimum estimate, as we could not assess harriers which had bred outside the investigation area. Similar findings are known from France and Spain: In France, a mean recruitment rate of juveniles of about 8% (2.7-20.6% in different years) was estimated [91] using ringing and wing-tag data. In Spain, 6.6% of 217 tagged juvenile birds were observed near the natal area in the next years, but only 4.2% started their first breeding attempt [6]. Moreover, Arroyo and Bretagnolle [92] reported that 15% of returned birds were breeding in a distance of more than 50 km away from the natal nest. Juvenile recruitment, of course, is affected directly by juvenile survival and dispersal. The juvenile survival rate is assumed between 31% and 69% [61] and 67-75% after the second winter [93], respectively. Furthermore, return rates are also influenced by the migration behaviour of a species. Wintering ranges of Montagu's harriers originating from different breeding populations partly overlap. Birds migrate between different home ranges, tracking seasonal changes in food availability [94]. During foraging migration, they get in contact with individuals from other populations and hence, potential breeding partners [21], which might also influence dispersal and recruitment.

4.3.3. Natal Dispersal

In general, a very low recruitment rate and natal philopatry have been assumed [61, 95]. Our present study confirms the investigations using wing-tags and PVC-rings. Natal dispersal distances in Mainfranken did not differ between sexes, which is in agreement with findings in different Spanish breeding areas [61]. In Spain, only 7% of 1,662 tagged juvenile birds could be identified as breeders in later years. Only 4.2% of tagged females and 3.2% of males were detected breeding within 10 km of their natal nest and hence were considered to be philopatric. However, results varied clearly between regions, monitoring intensity and marking technique, respectively, leading to an overall recruitment rate between 0-25%.

In Mainfranken, the philopatry rate of 1276 juvenile birds was comparatively low, with 0.8% for females and 0.6% for males. Reports of juvenile birds, that had been wing-tagged in Mainfranken and observed as breeders *e.g.* in Tattendorf, Austria; Račiněves, Czech Republic and Éguilly-sous-Bois, France (*unpublished data*), also indicate a pronounced dispersal and low philopatry. Discussing and comparing philopatry rates is always difficult, since results depend directly on the scale used. We have restricted our genetic sampling to the Mainfranken breeding population, although it is clear that birds easily migrate between Mainfranken and close neighbouring areas, *e.g.* Nördlinger Ries (administrative districts Donau-Ries, Bavaria and Ostalb, Baden-Württemberg).

Differences in regional and sex specific philopatry rates may be due to different carrying capacities of the environment, lower survival rates of adult birds or differences in sex-specific survival of juveniles (recruits) [61, 96, 97]. The small sex specific difference (though not significant) in philopatry rates in Spanish and French studies by Leroux and Bretagnolle [16] and Arroyo [6] are interpreted in the context with self-regulation of breeding colonies. The authors suggested that especially small breeding colonies favour the production of the more philopatric sex that would return to the colony and hence, would contribute to colony preservation: In France, males dominate juvenile sex ratios with 55.2% and are considered to be more philopatric [16], while in Spain this is found for females (54% quota in sex ratios) [98]. Our data from Mainfranken appear to agree with this hypothesis: more returned males (41.2%) bred in a distance of less than 10 km to the hatching site than returned females (29.4%). Additionally, males were the predominant sex of chicks between 2000-2012 (53.1% males vs. 46.9% females) (*unpublished data*).

For the first time microsatellite analysis offered important basic information concerning natal and breeding dispersal in German Montagu's harriers. Nevertheless, more studies are needed to understand the biology of the species and to develop strategies of its conservation [99].

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was analyzed and approved by: Regierung von Unterfranken, D-97064 Würzburg, no.: 55.2-2531.01 -46/11.

HUMAN AND ANIMAL RIGHTS

No Laboratory Animals were used for the studies that on the bases of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest. Financial support was provided by the Bayerisches Landesamt für Umwelt. S. Janowski thanks the Landesgraduiertenförderung Baden-Württemberg and the Gerhard und Ellen Zeidler-Stiftung for scholarships.

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